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# Evidence of proatherogenic inflammation in polycystic ovary syndrome

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**Abstract**

Women with polycystic ovary syndrome (PCOS) have chronic low-level inflammation that can increase the risk of atherogenesis. We measured circulating proatherogenic inflammatory mediators in women with PCOS (8 lean: body mass index, 18-25 kg/m$^2$; 8 obese: body mass index, 30-40 kg/m$^2$) and weight-matched controls (8 lean, 8 obese). Blood samples were obtained fasting and 2 hours after glucose ingestion to measure interleukin-6 (IL-6), soluble intercellular adhesion molecule–1 (sICAM-1), monocyte chemotactic protein–1 (MCP-1), C-reactive protein (CRP), matrix metalloproteinase–2, plasminogen activator inhibitor–1 (PAI-1), and activated nuclear factor κB in mononuclear cells. Truncal fat was determined by dual-energy x-ray absorptiometry. Fasting MCP-1 levels were elevated in lean women with PCOS compared with lean controls (159.9 ± 14.1 vs 121.2 ± 5.4 pg/mL, $P < .02$). Hyperglycemia failed to suppress matrix metalloproteinase–2 in lean women with PCOS compared with lean controls (1.7 ± 1.2 vs −4.8 ± 1.6 pg/mL, $P < .002$). Among women with PCOS, obese individuals exhibited higher fasting sICAM-1 (16.1 ± 0.8 vs 10.5 ± 1.0 ng/mL, $P < .03$) and PAI-1 (6.1 ± 0.7 vs 3.4 ± 0.8 ng/mL, $P < .03$) levels. Trend analysis revealed higher ($P < .005$) IL-6, sICAM-1, CRP, PAI-1, systolic and diastolic blood pressures, triglycerides, fasting insulin, and homeostasis model assessment of insulin resistance index in women with PCOS compared with weight-matched controls, and the highest levels in the obese regardless of PCOS status. Fasting MCP-1 levels correlated with activated nuclear factor κB during hyperglycemia ($P < .05$) and androstenedione ($P < .004$). Truncal fat correlated with fasting IL-6 ($P < .004$), sICAM-1 ($P < .006$), CRP ($P < .0009$), and PAI-1 ($P < .02$). We conclude that both PCOS and obesity contribute to a proatherogenic state; but in women with PCOS, abdominal adiposity and hyperandrogenism may exacerbate the risk of atherosclerosis.


## 1. Introduction

The polycystic ovary syndrome (PCOS) is one of the most common female endocrinopathies, affecting between 4% and 10% of reproductive-age women [1,2]. The disorder is characterized by hyperandrogenism, chronic oligo- or anovulation, and polycystic ovaries, with 2 out of these 3 findings required to diagnose PCOS [3,4]. As many as 70% of women with PCOS exhibit insulin resistance, with the compensatory hyperinsulinemia considered to be the cause of the hyperandrogenism [4-7]. Insulin resistance is also associated with accelerated atherogenesis [8]. Indeed, women with PCOS have a higher prevalence of coronary artery calcification, a radiographic marker of atherosclerosis [9,10]. Women with PCOS are often obese, which is another risk factor for developing atherosclerosis and hyperglycemia [11,12].

Polycystic ovary syndrome is a proinflammatory state as evidenced by elevated plasma concentrations of a number of inflammatory mediators of atherogenesis. High levels of interleukin-6 (IL-6), soluble intercellular adhesion molecule–1 (sICAM-1), monocyte chemotactic protein–1 (MCP-1), C-reactive protein (CRP), matrix metalloproteinase–2 (MMP-2), and plasminogen activator inhibitor–1 (PAI-1) have all been independently reported in the disorder [13-18]. It remains controversial whether the elevated levels of IL-6, sICAM-1, CRP, and MMP-2 observed in women with PCOS are a function of obesity [14,17,19,20].

Hyperglycemia is proinflammatory because of its ability to generate reactive oxygen species (ROS) from peripheral blood mononuclear cells (MNC). Reactive oxygen species–induced oxidative stress activates a transcription factor

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known as *nuclear factor κB* (NFκB), the cardinal signal of inflammation that promotes atherogenesis [21-23]. We have recently shown that, in PCOS, ROS generation and NFκB activation are increased after oral glucose ingestion independent of obesity and that both are related to circulating androgens [24,25]. Nuclear factor κB regulates gene transcription of IL-6, a proinflammatory cytokine capable of inducing the endothelial expression of sICAM-1 and MCP-1. Soluble intercellular adhesion molecule–1 causes attachment of MNC to the endothelial layer of the blood vessel wall, and MCP-1 facilitates migration of MNC into the vascular interstitium [26,27]. Interleukin-6 also stimulates CRP synthesis in the liver. C-reactive protein is a major predictor of atherosclerosis in asymptomatic individuals and may also play a functional role by promoting the uptake of lipids into MNC-derived foamy macrophages within atherosclerotic plaques [28-30]. Subsequent plaque rupture and thrombosis during a cardiovascular event are promoted by MMP-2 and PAI-1, respectively [31,32]. The collective action of all of these inflammatory mediators is required for atherogenesis. To our knowledge, these mediators have never been simultaneously measured in plasma in a single cohort of women with PCOS after controlling for obesity.

We embarked on a study to determine the status of circulating levels of IL-6, CRP, sICAM-1, MCP-1, MMP-2, and PAI-1 in women with PCOS. We also examined the relationship of these inflammatory mediators with body composition, hyperglycemia-induced NFκB activation in MNC, and circulating androgens. We hypothesized that circulating IL-6, CRP, sICAM-1, MCP-1, MMP-2, and PAI-1 are increased in PCOS and that these inflammatory mediators are related to measures of adiposity, NFκB activation, and circulating androgens.

## 2. Materials and methods

### 2.1. Subjects

Sixteen women with PCOS (8 lean and 8 obese) between 20 and 33 years of age and 16 weight-matched control subjects (8 lean and 8 obese) between 20 and 40 years of age volunteered to participate in the study. Subjects in the present report represent part of a larger cohort that is involved in our studies on PCOS and insulin resistance, and data from some of these subjects have been presented in previous publications [24,33]. *Obesity* was defined as a body mass index (BMI) between 30 and 40 kg/m$^2$. Lean subjects had a BMI between 18 and 25 kg/m$^2$. The women with PCOS were diagnosed on the basis of oligomenorrhea and hyperandrogenemia after excluding nonclassic congenital adrenal hyperplasia, Cushing syndrome, hyperprolactinemia, and thyroid disease. Polycystic ovaries were present on ultrasound in all subjects with PCOS. All control subjects were ovulatory as evidenced by regular menses and a luteal phase serum progesterone level greater than 5 ng/mL. All control subjects exhibited normal circulating androgen levels and the absence of polycystic ovaries on ultrasound.

All subjects were screened for diabetes or inflammatory illnesses, and none were taking medications that would affect carbohydrate metabolism or immune function for at least 6 weeks before study participation. The metabolic syndrome was diagnosed by the Adult Treatment Panel III guidelines [34]. None of the subjects were involved in any regular exercise program for at least 6 months before the time of testing. All of the subjects provided written informed consent in accordance with Institutional Review Board guidelines for the protection of human subjects.

### 2.2. Study design

All study subjects underwent an oral glucose tolerance test (OGTT) between days 5 and 8 after the onset of menses, and an overnight fast of approximately 12 hours. The women were provided with a healthy diet consisting of 50% carbohydrate, 35% fat, and 15% protein for 3 consecutive days before the test. All subjects also underwent body composition assessment on the same day the OGTT was performed.

### 2.3. Oral glucose tolerance test

All subjects ingested a 75-g glucose beverage. Blood samples were drawn while fasting for glucose and insulin determination, and 2 hours after ingestion of the glucose beverage to measure glucose. Plasma glucose concentrations were assayed immediately, and additional plasma isolated from the fasting and 2-hour post–glucose ingestion blood samples was stored at −80°C until assayed for IL-6, sICAM-1, MCP-1, CRP, MMP-2, and PAI-1. Glucose tolerance was assessed by the World Health Organization criteria, with *normal glucose tolerance* defined as a 2-hour glucose-stimulated value less than 140 mg/dL [35]. Insulin resistance was estimated by homeostasis model assessment (HOMA-IR) using the following formula: fasting glucose (millimoles per liter) × fasting insulin (microunits per milliliter)/22.5 [36].

### 2.4. Body composition assessment

Height without shoes was measured to the nearest 1.0 cm. Body weight was measured to the nearest 0.1 kg. Waist circumference was measured at the level of the umbilicus and used to estimate abdominal adiposity [37]. In addition, all subjects underwent dual-energy x-ray absorptiometry to determine percentage total body fat and percentage truncal fat using the QDR 4500 Elite model scanner (Hologic, Waltham, MA) as previously described [24,38].

### 2.5. Plasma measurements

Plasma glucose concentrations were measured by the glucose oxidase method (YSI, Yellow Springs, OH), whereas plasma insulin concentrations were measured by a double-antibody radioimmunoassay (Linco Research, St Charles, MO). Luteinizing hormone (LH), testosterone, androstenedione, and dehydroepiandrosterone-sulfate (DHEA-S) levels

were measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Plasma IL-6 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (eBioscience, San Diego, CA). The plasma concentrations of sICAM-1, MCP-1, MMP-2, and PAI-1 were also measured by ELISA (R&D Systems, Minneapolis, MN). Plasma CRP concentrations were measured by a high-sensitivity ELISA (Alpha Diagnostics International, San Antonio, TX). All samples from each subject were measured in duplicate in the same assay. The interassay and intraassay coefficients of variation for all assays were 7% and 12%, respectively.

### 2.6. NFκB electrophoretic mobility shift assay

Nuclear extracts of DNA-binding protein were prepared from MNC using the method described by Andrews and Faller [39]. Total protein concentrations were determined using the bicinchoninic acid protein assay (Pierce Chemical, Rockville, IL). An NFκB gel retardation assay was performed using the NFκB-binding protein detection kit (Life Technologies, Inc., Long Island, NY). The double-stranded oligonucleotide containing a tandem repeat of the consensus sequence for the NFκB-binding site (Santa Cruz Biotechnology, Santa Cruz, CA) was radiolabeled with $\gamma$-$^{32}$P [32] (GE Healthcare Bio-Sciences, Piscataway, NJ) using T4 kinase (Invitrogen, Carlsbad, CA). Nuclear extract (7.5 $\mu$g) was mixed with the incubation buffer, and the mixture was preincubated at 4°C for 15 minutes. Labeled oligonucleotide (60 000 cpm) was added, and the mixture was incubated at room temperature for 20 minutes. The samples were electrophoresed on 6% nondenaturing polyacrylamide gels. The gels were dried under vacuum and exposed to x-ray film. Densitometry was performed using Kodak (Rochester, NY) 1D Image Analysis software version 3.6.

### 2.7. Statistics

The StatView statistical package (SAS Institute, Cary, NC) was used for data analysis. Descriptive data and primary dependent variables were compared using analysis of variance for multiple group comparisons. Detection of significance by analysis of variance was followed by a post hoc analysis using unpaired Student $t$ tests between groups to identify the source of significance. Differences between pre– and post–glucose challenge variables within groups were analyzed using the paired Student $t$ test. Regression analyses were performed using Pearson ($r$) correlation for parametric data and Spearman rank order ($\rho$) correlation for nonparametric data. A trend analysis was performed for variables demonstrating an ascending or descending pattern in mean values among groups. The trend analysis used Spearman rank order ($\rho$) correlation between the variable and a number between 1 and 4 assigned to each group, in the order of the observed pattern. All values are expressed as means ± SE. An $\alpha$ level of .05 was used to determine statistical significance.

## 3. Results

### 3.1. Age, body composition, blood pressure, and lipids

Obese women with PCOS were similar in age compared with obese controls, whereas lean women with PCOS were slightly younger than lean controls (Table 1). Weight, BMI, percentage total body fat, percentage truncal fat, and waist circumference were significantly ($P < .04$) greater in obese subjects compared with those who were lean whether or not they had PCOS, but were similar when women with PCOS were compared with weight-matched controls.

Table 1
Age, body composition, blood pressure, and fasting lipid levels of subjects

| | PCOS | | Control | |
|---|---|---|---|---|
| | Lean (n = 8) | Obese (n = 8) | Lean (n = 8) | Obese (n = 8) |
| Age, y | 27 ± 2* | 25 ± 2$^{\dagger}$ | 33 ± 2 | 30 ± 3 |
| Height, cm | 162.6 ± 3.8 | 164.6 ± 2.8 | 165.1 ± 1.0 | 163.9 ± 2.7 |
| Body weight, kg | 63.2 ± 2.3 | 92.1 ± 3.5$^{\dagger,\ddagger}$ | 61.2 ± 2.2 | 94.2 ± 4.1$^{\S,\|\|}$ |
| BMI, kg/m$^2$ | 23.0 ± 0.9 | 36.1 ± 0.6$^{\dagger,\ddagger}$ | 22.4 ± 0.9 | 35.0 ± 1.0$^{\S,\|\|}$ |
| Total body fat, % | 30.1 ± 1.9 | 42.6 ± 1.0 $^{\dagger,\ddagger}$ | 29.3 ± 2.0 | 42.5 ± 1.0$^{\S,\|\|}$ |
| Truncal fat, % | 28.7 ± 2.6 | 43.8 ± 1.0$^{\dagger,\ddagger}$ | 26.3 ± 2.7 | 42.3 ± 0.9$^{\S,\|\|}$ |
| Waist circumference, cm | 76.8 ± 2.8 | 104.8 ± 4.4$^{\dagger,\ddagger}$ | 74.9 ± 3.0 | 102.1 ± 3.0$^{\S,\|\|}$ |
| Systolic blood pressure, mm Hg | 112 ± 3 | 130 ± 6$^{\dagger,\ddagger}$ | 104 ± 3 | 118 ± 5$^{\|\|}$ |
| Diastolic blood pressure, mm Hg | 70 ± 4* | 78 ± 5$^{\dagger}$ | 58 ± 3 | 76 ± 3$^{\|\|}$ |
| Total cholesterol, mg/dL | 164 ± 13 | 179 ± 11 | 174 ± 7 | 190 ± 23 |
| Triglycerides, mg/dL | 99 ± 33 | 120 ± 25 | 53 ± 6 | 111 ± 39 |
| HDL cholesterol, mg/dL | 51 ± 4 | 39 ± 2$^{\dagger,\|\|}$ | 55 ± 4 | 48 ± 4 |
| LDL cholesterol, mg/dL | 100 ± 11 | 120 ± 10 | 112 ± 7 | 115 ± 18 |

Values are expressed as means ± SE.

* Lean PCOS vs lean control; $P$ less than .05.

$^{\dagger}$ Obese PCOS vs lean PCOS; $P$ less than .03.

$^{\ddagger}$ Obese PCOS vs lean control; $P$ less than .009.

$^{\S}$ Obese control vs lean PCOS; $P$ less than .0001.

$^{\|\|}$ Obese control vs lean control; $P$ less than .04.

Systolic and diastolic blood pressures were significantly ($P < .04$) higher in obese individuals whether or not they had PCOS and in lean women with PCOS compared with lean controls, but mean values were in the normotensive range The levels of total cholesterol and low-density lipoprotein cholesterol were similar among groups. Triglyceride levels were higher in lean women with PCOS compared with lean controls, but not significantly. High-density lipoprotein (HDL) cholesterol was significantly ($P < .03$) lower in obese women with PCOS compared with lean individuals whether or not they had PCOS. There was a significant trend in systolic ($\rho = 0.61$, $P < .0008$) and diastolic ($\rho = 0.60$, $P < .0009$) blood pressures and in triglycerides ($\rho = 0.48$, $P < .008$), in which mean levels were higher in lean women with PCOS compared with lean controls and in obese controls compared with lean women with PCOS, with the highest levels evident in obese women with PCOS. High-density lipoprotein also exhibited a significant trend among groups, but in the opposite direction ($\rho = -0.49$, $P < .0007$).

Two obese women with PCOS met the Adult Treatment Panel III guidelines for the metabolic syndrome by exhibiting increases in waist circumference (98 and 109 cm), systolic blood pressure (140 and 148 mm Hg), and plasma triglycerides (159 and 232 mg/dL).

### 3.2. Plasma hormone levels, glycemic status, and insulin resistance

Circulating levels of LH, testosterone, and androstenedione were significantly ($P < .04$) elevated in women with PCOS compared with control subjects independent of body mass (Table 2). Circulating DHEA-S levels were significantly ($P < .02$) elevated in lean and obese women with PCOS compared with obese controls, but were similar in lean women with PCOS compared with lean controls.

Table 3
Fasting plasma levels of inflammatory mediators

| | PCOS | | Control | |
|---|---|---|---|---|
| | Lean (n = 8) | Obese (n = 8) | Lean (n = 8) | Obese (n = 8) |
| IL-6, pg/mL | 1.2 ± 0.3 | 3.6 ± 1.4*,† | 0.6 ± 0.1 | 2.1 ± 0.5 |
| sICAM-1, ng/mL | 10.5 ± 1.0 | 16.1 ± 0.8*,†,‡ | 8.2 ± 1.0 | 13.0 ± 1.3§ |
| MCP-1, pg/mL | 159.9 ± 14.1‖ | 145.0 ± 12.8 | 121.2 ± 5.4 | 123.3 ± 9.8¶ |
| CRP, mg/L | 1.2 ± 0.4 | 5.7 ± 1.7*,† | 0.3 ± 0.1 | 6.5 ± 1.1§,¶ |
| MMP-2, ng/mL | 20.0 ± 1.1 | 17.6 ± 1.3† | 23.0 ± 1.9 | 19.0 ± 0.9§ |
| PAI-1, ng/mL | 3.4 ± 0.8‡ | 6.1 ± 0.7† | 3.5 ± 0.9 | 5.5 ± 0.7 |

Values are expressed as means ± SE.
* Obese PCOS vs lean PCOS; $P$ less than .03.
† Obese PCOS vs lean control; $P$ less than .02.
‡ Obese PCOS vs obese control; $P$ less than .04.
§ Obese control vs lean control; $P$ less than .05.
‖ Lean PCOS vs lean control; $P$ less than .02.
¶ Obese control vs lean PCOS; $P$ less than .03.

Glucose levels while fasting and 2 hours post–glucose ingestion were similar in women with PCOS compared with controls independent of body mass. All 16 control subjects had a normal glucose response during the OGTT, with 2-hour glucose levels ranging between 62 and 138 mg/dL. Two-hour glucose values were consistent with impaired glucose tolerance in 1 lean woman with PCOS (148 mg/dL) and 1 obese woman with PCOS (192 mg/dL). Fasting insulin levels and HOMA-IR were significantly higher ($P < .05$) in the obese whether or not they had PCOS compared with lean controls. The HOMA-IR in lean women with PCOS was similar to that in obese controls, but was significantly ($P < .02$) lower compared with that in obese women with PCOS. There was a significant increasing trend in fasting

Table 2
Plasma hormone, glucose, and insulin levels and HOMA-IR

| | PCOS | | Control | |
|---|---|---|---|---|
| | Lean (n = 8) | Obese (n = 8) | Lean (n = 8) | Obese (n = 8) |
| LH, mIU/mL | 13.6 ± 1.5*,† | 8.8 ± 1.5‡,§,‖ | 3.2 ± 0.5 | 2.7 ± 0.4 |
| Testosterone, ng/dL | 70.6 ± 9.2*,† | 87.4 ± 10.9§,‖ | 43.8 ± 4.5 | 32.3 ± 4.8 |
| Androstenedione, ng/mL | 3.5 ± 0.3*,† | 3.4 ± 0.2§,‖ | 1.6 ± 0.1 | 1.9 ± 0.2 |
| DHEA-S, μg/dL | 318 ± 42† | 333 ± 46§,‖ | 215 ± 31 | 173 ± 31 |
| Fasting glucose, mg/dL | 86.3 ± 2.7 | 88.9 ± 1.2 | 84.6 ± 1.4 | 84.1 ± 4.1 |
| 2-h glucose, mg/dL | 102.4 ± 8.4 | 122.0 ± 13.0 | 96.3 ± 9.2 | 118.0 ± 3.7 |
| Fasting insulin, μIU/mL | 10.8 ± 1.6 | 18.8 ± 3.1‡,§ | 7.0 ± 1.2 | 13.4 ± 2.1¶ |
| HOMA-IR, mmol/L-μU/mL | 2.3 ± 0.4 | 4.1 ± 0.7‡,‖ | 1.5 ± 0.2 | 2.9 ± 0.5¶ |

Values are expressed as means ± SE. Conversion factors to SI units: testosterone, ×3.467 (nanomoles per liter); androstenedione, ×3.492 (nanomoles per liter); DHEA-S, ×0.002714 (micromoles per liter); glucose, ×0.0551 (millimoles per liter); insulin, ×.175 (picomoles per liter).
* Lean PCOS vs lean control; $P$ less than .03.
† Lean PCOS vs obese control; $P$ less than .02.
‡ Obese PCOS vs lean PCOS; $P$ less than .02.
§ Obese PCOS vs obese control; $P$ less than .006.
‖ Obese PCOS vs lean control; $P$ less than .04.
¶ Obese control vs lean control; $P$ less than .05.

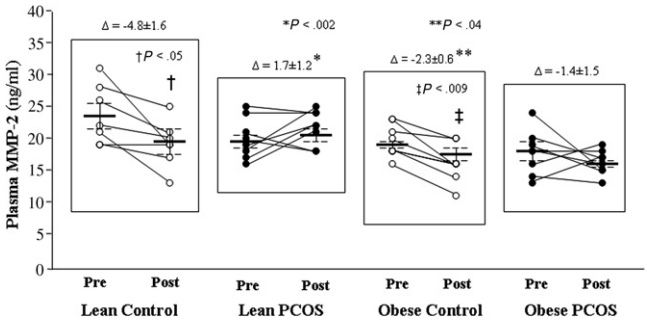


Fig. 1. Plasma MMP-2 when fasting samples (pre) were compared with the samples collected 2 hours after glucose ingestion (post). Pre and post MMP-2 values were similar and superimposed for 2 study subjects in each of the control groups. *Incremental change (Δ) in MMP-2 during oral glucose challenge in lean women with PCOS was significantly different from that of lean controls; *P* less than .002. **Incremental change (Δ) in MMP-2 during oral glucose challenge in lean women with PCOS was significantly different from that of obese controls; *P* less than .04. †2-Hour postglucose level was significantly lower than fasting level in lean controls; *P* less than .05. ‡2-Hour postglucose level was significantly lower than fasting level in obese controls; *P* less than .009.

insulin ($\rho$ = 0.65, $P < .0005$) and HOMA-IR ($\rho$ = 0.65, $P < .0004$), in which mean levels exhibited the same pattern among groups observed for blood pressures and triglycerides.

### 3.3. Plasma inflammatory mediator levels and intranuclear NFκB

Plasma IL-6 concentrations were significantly ($P < .03$) higher in obese women with PCOS compared with lean subjects whether or not they had PCOS (Table 3). Obese women with PCOS exhibited significantly ($P < .04$) higher plasma sICAM-1 levels compared with obese controls and both lean groups. Plasma sICAM levels were significantly higher ($P < .05$) in obese controls compared with lean controls. Lean women with PCOS exhibited significantly ($P < .03$) higher plasma MCP-1 levels compared with either control group. Plasma MCP-1 levels were similar in both groups of women with PCOS and in both control groups. Plasma CRP concentrations were significantly ($P < .05$) higher in the obese whether or not they had PCOS. C-reactive protein concentrations were also elevated in lean women with PCOS compared with lean controls, but not significantly.

Plasma MMP-2 levels were significantly ($P < .05$) lower in the obese whether or not they had PCOS compared with lean controls and were similar in lean women with PCOS compared with either control group. Plasma PAI-1 concentrations were significantly ($P < .03$) higher in obese women with PCOS compared with lean subjects whether or not they had PCOS. There was a significant trend in IL-6 ($\rho$ = 0.74, $P < .0001$), sICAM-1 ($\rho$ = 0.76, $P < .0001$), CRP ($\rho$ = 0.76, $P < .0001$), and PAI-1 ($\rho$ = 0.54, $P < .005$), in which mean levels were once again higher in lean women with PCOS compared with lean controls and in obese controls compared with lean women with PCOS, with the highest levels evident in obese women with PCOS.

In response to the oral glucose load, the percentage change in MNC-derived intranuclear NFκB was similar ($P$ = .73) in lean women with PCOS (25.5% ± 21.2%), obese women with PCOS (33.2% ± 16.0%), and obese controls (9.2% ± 6.2%). However, the percentage change in MNC-derived intranuclear NFκB was significantly ($P < .04$) higher in both groups of women with PCOS compared with lean controls (−21.2% ± 13.3%), which was suppressed. In response to the oral glucose load, plasma MMP-2 was significantly suppressed in lean controls ($P < .05$) and obese controls ($P < .009$) (Fig. 1). Plasma MMP-2 was also suppressed in obese women with PCOS, but not significantly. In contrast, plasma MMP-2 failed to suppress in lean women with PCOS. The maximum incremental change in

Table 4
Spearman rank correlations for the combined groups

| | | IL-6, pg/mL | sICAM-1, ng/mL | MCP-1, pg/mL | CRP, ng/mL | MMP-2, ng/mL | PAI-1, ng/mL |
|---|---|---|---|---|---|---|---|
| BMI, kg/m$^2$ | $\rho$ | 0.580 | 0.640 | −0.031 | 0.690 | −0.210 | 0.493 |
| | $P$ | .002* | .0004* | .866 | .0003* | .243 | .008* |
| Total body fat, % | $\rho$ | 0.574 | 0.740 | 0.032 | 0.782 | −0.264 | 0.577 |
| | $P$ | .002* | .0001* | .860 | .0001* | .142 | .002* |
| Truncal fat, % | $\rho$ | 0.620 | 0.753 | 0.077 | 0.845 | −0.297 | 0.590 |
| | $P$ | .0008* | .0001* | .675 | .0001* | .098 | .002* |
| Waist circumference, cm | $\rho$ | 0.564 | 0.601 | −0.078 | 0.588 | −0.236 | 0.467 |
| | $P$ | .003* | .001* | .680 | .003* | .204 | .014* |
| HOMA-IR, mmol/L-μU/mL | $\rho$ | 0.551 | 0.322 | 0.102 | 0.570 | −0.201 | 0.403 |
| | $P$ | .003* | .071 | .575 | .002* | .263 | .030* |
| IL-6, pg/mL | $\rho$ | – | 0.442 | −0.003 | 0.685 | −0.487 | 0.366 |
| | $P$ | – | .017* | .989 | .0002* | .009* | .048* |
| sICAM-1, ng/mL | $\rho$ | 0.442 | – | 0.063 | 0.519 | −0.395 | 0.486 |
| | $P$ | .017* | – | .730 | .004* | .028* | .009* |
| CRP, ng/mL | $\rho$ | 0.685 | 0.519 | 0.165 | – | 0.283 | 0.617 |
| | $P$ | .0002* | .004* | .366 | – | .116 | .0009* |

* $P$ less than .05.

MMP-2 was significantly different in lean women with PCOS compared with lean controls ($P < .002$) and obese controls ($P < .04$) but similar in obese women with PCOS compared with either control group. There was no change in any of the other plasma inflammatory mediators in response to glucose ingestion (data not shown).

### 3.4. Correlation analyses

The HOMA-IR was positively correlated with BMI ($r = 0.52$, $P < .003$), percentage body fat ($r = 0.41$, $P < .03$), percentage truncal fat (r = 0.49, $P < .005$), and waist circumference ($r = 0.61$, $P < .0005$) for the combined groups (data not shown).

For the combined groups, there was a positive correlation between IL-6 and sICAM-1 (Table 4). Each of these levels was directly correlated with CRP and PAI-1. There was also a positive correlation between CRP and PAI-1. In contrast, IL-6 and sICAM-1 were negatively correlated with MMP-2. Interleukin-6, sICAM-1, CRP, and PAI-1 were directly correlated with BMI, percentage total body fat, percentage truncal fat, and waist circumference. Interleukin-6, CRP, and PAI-1 were directly correlated with HOMA-IR.

In women with PCOS, there was a positive correlation between IL-6 and sICAM-1 (Table 5). Each of these levels was directly correlated with CRP. There were also a positive correlation between IL-6 and PAI-1, and a negative correlation between sICAM-1 and MMP-2. Soluble intercellular adhesion molecule–1 and CRP were positively correlated with BMI, percentage total body fat, percentage truncal fat, and waist circumference. Interleukin-6 and PAI-1 were directly correlated with percentage total body fat and percentage truncal fat. Interleukin-6 and CRP were also directly correlated with HOMA-IR.

Plasma MCP-1 was positively correlated with MNC-derived intranuclear NF$\kappa$B ($\rho = 0.41$, $P < .05$) and plasma androstenedione ($\rho = 0.55$, $P < .004$) for the combined groups. Plasma testosterone and androstenedione were positively correlated with the percentage change in MNC-derived intranuclear NF$\kappa$B ($r = 0.44$, $P < .03$; $r = 0.46$, $P < .03$) for the combined groups (data not shown).

## 4. Discussion

Our data clearly show that both PCOS and obesity make significant contributions to elevations in plasma inflammatory mediators collectively involved in atherogenesis. Lean women with PCOS exhibit elevated MCP-1 levels compared with lean controls and fail to suppress MMP-2 levels under postprandial-like conditions. Obese women with PCOS exhibit elevated sICAM-1 levels compared with obese controls and elevated PAI-1 levels compared with lean subjects whether or not they have PCOS. There is also an increasing trend among groups in plasma IL-6, sICAM-1, CRP, and PAI-1, with higher levels evident in women with PCOS compared with weight-matched controls and the highest levels evident in the obese whether or not they have PCOS. Systolic and diastolic blood pressures, triglycerides, fasting insulin, and HOMA-IR also exhibit an increasing trend of similar pattern among groups, along with a decreasing trend in HDL. These additional findings provide support that both PCOS and obesity also contribute to elevated blood pressure, lipid abnormalities, and insulin resistance in the development of atherosclerosis. Monocyte chemotactic protein–1 is directly related to MNC-derived intranuclear NF$\kappa$B and circulating androstenedione, suggesting that proatherogenic inflammation may be promoted by hyperandrogenism in PCOS. Furthermore, the independent associations between multiple plasma inflammatory mediators and abdominal adiposity suggest that the accumulation of abdominal fat is an important contributing factor

Table 5
Spearman rank correlations in women with PCOS

| | | IL-6, pg/mL | sICAM-1, ng/mL | MCP-1, pg/mL | CRP, ng/mL | MMP-2, ng/mL | PAI-1, ng/mL |
|---|---|---|---|---|---|---|---|
| BMI, kg/m$^2$ | $\rho$ | 0.483 | 0.662 | −0.457 | 0.709 | −0.144 | 0.443 |
| | $P$ | .071* | .010* | .087 | .006* | .577 | .098 |
| Total body fat, % | $\rho$ | 0.550 | 0.768 | −0.381 | 0.779 | −0.345 | 0.588 |
| | $P$ | .040* | .003* | .153 | .003* | .182 | .028* |
| Truncal fat, % | $\rho$ | 0.589 | 0.723 | 0.326 | 0.870 | −0.302 | 0.654 |
| | $P$ | .028* | .005* | .223 | .0008* | .242 | .014* |
| Waist circumference, cm | $\rho$ | 0.472 | 0.609 | −0.411 | 0.653 | −0.144 | 0.389 |
| | $P$ | .077 | .018* | .124 | .011* | .577 | .145 |
| HOMA-IR, mmol/L-$\mu$U/mL | $\rho$ | 0.479 | 0.176 | −0.129 | 0.694 | 0.300 | 0.325 |
| | $P$ | .048* | .494 | .631 | .007* | .245 | .224 |
| IL-6, pg/mL | $\rho$ | – | 0.637 | −0.485 | 0.587 | −0.440 | 0.530 |
| | $P$ | – | .017* | .070 | .028* | .100 | .048* |
| sICAM-1, ng/mL | $\rho$ | 0.637 | – | −0.404 | 0.494 | −0.632 | 0.432 |
| | $P$ | .017* | – | .131 | .048* | .014* | .106 |
| CRP, ng/mL | $\rho$ | 0.587 | 0.494 | 0.071 | – | −0.135 | 0.725 |
| | $P$ | .028* | .048* | .789 | – | .600 | .007* |

* $P$ less than .05.

in promoting atherogenesis and subsequent cardiovascular events in obese women with PCOS.

Lean women with PCOS may be at increased risk for accelerated atherogenesis and subsequent atherosclerotic plaque rupture. The increase in intranuclear NFκB from MNC in response to oral glucose ingestion in lean women with PCOS compared with lean controls is consistent with our previous report [24]. The resultant inflammatory signal may be responsible for the elevated plasma MCP-1 levels, and this may facilitate migration of MNC into the vascular interstitium. This scenario is further corroborated by the direct relationship between MCP-1 and intranuclear NFκB. Plasma IL-6, sICAM-1, CRP, and PAI-1 were modestly higher in lean women with PCOS compared with lean controls. The lack of significant difference of these inflammatory mediators between these 2 groups may be due to the high variability in the small sample size and is thus a limitation of the study. Lean women with PCOS also fail to suppress plasma MMP-2 after oral glucose ingestion, and this may promote atherosclerotic plaque rupture. In contrast, control subjects regardless of body mass exhibit plasma MMP-2 suppression, suggesting that this is the normal in vivo response to physiologic hyperglycemia for preservation of blood vessel integrity. We have previously reported that the proinflammatory cytokine tumor necrosis factor–α exhibits a similar response pattern when measured in cultured MNC from lean women with PCOS and young healthy men and women after oral glucose ingestion [33,40,41]. Thus, women with PCOS demonstrate a unique proinflammatory, proatherogenic risk profile that is independent of obesity and is exacerbated by physiologic hyperglycemia.

Obese women with PCOS may also be at increased risk for accelerated atherogenesis and thrombosis. Mononuclear cells obtained from obese women with PCOS exhibit increases in intranuclear NFκB in response to oral glucose ingestion. Increased NFκB activation may contribute to the elevated plasma sICAM-1 levels in obese women with PCOS compared with obese controls and lean women with PCOS. This phenomenon may in turn promote the attachment of MNC to vascular endothelium in obese women with PCOS. The elevated plasma PAI-1 levels in obese women with PCOS compared with lean subjects regardless of whether they have PCOS may promote thrombosis. The elevations in plasma IL-6 and CRP appear to be more a function of obesity than PCOS per se because they are markedly elevated in obese subjects compared with those who are lean regardless of PCOS status. Elevated IL-6 levels may further perpetuate increases in circulating sICAM-1 and stimulate increases in circulating CRP that may in turn promote lipid uptake by MNC-derived foamy macrophages within atherosclerotic plaques. The latter scenario is further supported by the direct relationship between IL-6 and CRP. Furthermore, obese women with PCOS exhibit evidence of insulin resistance based on an increase in HOMA-IR, a feature highly associated with atherosclerosis [8]. This is confirmed in the present study by the direct relationship of HOMA-IR with plasma IL-6 and CRP levels. It is unclear why plasma MMP-2 levels are lower in the obese whether or not they have PCOS compared with lean controls. This finding is in contrast to a previous study reporting elevated MMP-2 levels in obese women with PCOS [17]. Nevertheless, the presence of PCOS in combination with obesity may result in greater risk of inflammation-related atherogenesis compared with lean women with PCOS or obese controls.

In PCOS, there may be a link between adiposity and plasma mediators of inflammation that promote atherosclerosis. Although not evident in the present study, our group and other investigators have previously shown that, aside from obese women with PCOS, abdominal adiposity can be increased in lean women with the disorder [19,38,42-44]. Moreover, plasma levels of IL-6, sICAM-1, CRP, and PAI-1 are directly related to measures of adiposity, particularly abdominal adiposity for the combined groups and in women with PCOS. Because activated MNC-derived macrophages produce roughly half of the IL-6 in the expanded adipose mass of obese individuals, it is possible that the inflamed adipose tissue of obese women with PCOS, especially in the abdominal region, is a perpetuator of these elevated inflammatory mediators in plasma [45,46]. Thus, these data are striking because they suggest that adiposity-related inflammation may initiate a proatherogenic milieu in women with PCOS at an early age.

In PCOS, hyperandrogenism may be capable of promoting inflammation that can lead to atherosclerosis. The direct correlation of the plasma levels of testosterone and androstenedione with intranuclear NFκB is consistent with our previous reports [24,33,47]. The direct relationship between the plasma levels of androstenedione and MCP-1 provides further corroboration. In vitro studies have shown that adhesion of MNC to vascular endothelium and oxidation of LDL by MNC-derived macrophages are increased after androgen exposure [48,49]. Furthermore, experimentally induced hyperandrogenism favors the development of atherosclerosis in cholesterol-fed female cynomolgus monkeys [50]. We have previously shown that, in PCOS, hyperglycemia causes an increase in ROS generation from MNC [33]. Thus, hyperandrogenism in PCOS may perpetuate NFκB activation after ROS-induced oxidative stress from glucose-activated MNC to up-regulate the transcription of inflammatory mediators that are involved in atherogenesis.

In conclusion, women with PCOS are in a proinflammatory state that places them at an increased risk of developing atherosclerosis. Lean women with PCOS exhibit elevations in plasma MCP-1 and failed suppression of plasma MMP-2 during physiologic hyperglycemia. Obese women with PCOS exhibit elevations in plasma sICAM and PAI-1 independent of obesity. There is also a clear trend among groups in plasma IL-6, sICAM-1, CRP, and PAI-1; systolic and diastolic blood pressures; triglycerides; fasting insulin;

and HOMA-IR, with higher levels evident in women with PCOS compared with weight-matched controls and the highest levels evident in the obese regardless of PCOS status. Thus, both PCOS and obesity significantly contribute to elevations in proatherogenic inflammatory mediators and blood pressure, lipid abnormalities, and insulin resistance. Furthermore, the association of plasma inflammatory mediators with abdominal fat and circulating androgens suggests that, in PCOS, increased abdominal adiposity and hyperandrogenism can contribute significantly to the promotion of atherogenesis.

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## References

[1] Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997;18:774-800.

[2] Knochenhauer ES, Key TJ, Kahser-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern Unites States: a prospective study. J Clin Endocrinol Metab 1998;83:3078-82.

[3] The Rotterdam ESHRE/ASRM-Sponsored PCOS Conference Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19-25.

[4] The Rotterdam ESHRE/ASRM-Sponsored PCOS Conference Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Hum Reprod 2004;19:41-7.

[5] Goodarzi MO, Korenman SG. The importance of insulin resistance in polycystic ovary syndrome. Fertil Steril 2002;77:255-8.

[6] Burghen GA, Givens JR, Kitabachi AE. Correlation of hyperandrogenism with hyperinsulinemia in polycystic ovarian disease. J Clin Endocrinol Metab 1980;50:113-6.

[7] Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F. Insulin stimulates testosterone biosynthesis by human theca cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab 1998;83:2001-5.

[8] Nigro J, Osman N, Dart AM, Little PJ. Insulin resistance and atherosclerosis. Endocr Rev 2006;27:242-59.

[9] Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy II PF, Fitzpatrick LA. Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2003;88:2562-8.

[10] Talbott EO, Zborowski JV, Rager JR, Boudreaux MY, Edmundowicz DA, Guzick DS. Evidence for an association between metabolic cardiovascular syndrome and coronary and aortic calcification among women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004;89:5454-61.

[11] NIH, NHLBI. Clinical guidelines on the identification, evaluation and treatment of overweight and obesity in adults—the evidence report. Obesity Res 1998;6(Suppl 2):51S-209S.

[12] Colditz GA, Willett WC, Rotnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes mellitus in women. Ann Intern Med 1995;122:481-6.

[13] Vgontzas AN, Trakada G, Bixler EO, Lin HM, Pejovic S, Zoumakis E, et al. Plasma interleukin 6 levels are elevated in polycystic ovary syndrome independently of obesity or sleep apnea. Metabolism 2006;55:1076-82.

[14] Diamanti-Kandarakis E, Alexandraki K, Piperi C, Protogerou A, Katsikis T, Paterakis T, et al. Inflammatory and endothelial markers in women with polycystic ovary syndrome. Eur J Clin Invest 2006;36:691-7.

[15] Hu WH, Qiao J, Zhao SY, Zhang XW, Li MZ. Monocyte chemoattractant protein–1 and its correlation with lipoprotein in polycystic ovary syndrome. Beijing Da Xue Xue Bao 2006; 38:487-91.

[16] Boulman N, Leiba LR, Shachar S, Linn R, Zinder O, Blumenfeld Z. Increased C-reactive protean levels in the polycystic ovary syndrome: a marker of cardiovascular disease. J Clin Endocrinol Metab 2004;89:2160-5.

[17] Lewandowski KC, Komorowski J, O'Callaghan CJ, Tan BK, Chen J, Prelevic GM, et al. Increased circulating levels of matrix metalloproteinase–2 and –9 in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:1173-7.

[18] Orio Jr F, Palomba S, Cascella T, Tauchmanova L, Nardo LG, Di Biase S, et al. Is plasminogen activator inhibitor–1 a cardiovascular risk factor in young women with polycystic ovary syndrome? Reprod Biomed Online 2004;9:505-10.

[19] Ibanez L, de Zegher F. Ethinylestradiol-drospirenone, flutamide-metformin, or both for adolescents and women with hyperinsulinemic hyperandrogenism: opposite effects on adipocytokines and body adiposity. J Clin Endocrinol Metab 2004;89:1592-7.

[20] Morin-Papunen L, Bautio K, Ruokonen A, Hedberg P. Metformin reduces serum C-reactive protein levels in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2003;88:4649-54.

[21] Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002;23:599-622.

[22] Mohanty P, Hamouda W, Garg R, Aljada A, Ghanim H, Dandona P. Glucose challenge stimulates reactive oxygen species generation by leucocytes. J Clin Endocrinol Metab 2000;85:2970-3.

[23] Barnes PJ, Karin M. Nuclear factor–κB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997;336:1066-71.

[24] González F, Rote NS, Minium J, Kirwan JP. Reactive oxygen species–induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:336-40.

[25] González F, Rote NS, Minium J, Kirwan JP. Increased activation of nuclear factor κB triggers inflammation and insulin resistance in polycystic ovary syndrome. J Clin Endocrinol Metab 2006; 91:1508-12.

[26] Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, et al. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity 1997;6:315-25.

[27] Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-74.

[28] Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1991;265:621-36.

[29] Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.

[30] Zwaka TP, Hombach V, Torzewski J. C-reactive protein–mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. Circulation 2001;103:1194-7.

[31] Vaughan DE. PAI-1 and atherothrombosis. J Thromb Haemost 1883;3:1879-83.

[32] Newby AC. Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Res 2005;85:1-31.

[33] González F, Minium J, Rote NS, Kirwan JP. Hyperglycemia alters tumor necrosis factor–α release from mononuclear cells in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005;90: 5336-5342.

[34] Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.

[35] Modan M, Harris MI, Halkin H. Evaluation of WHO and NDDG criteria for impaired glucose tolerance. Results from two national samples. Diabetes 1989;38:1630-5.

[36] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1885;28:412-9.

[37] Kohrt WM, Kirwan JP, King DS, Staten MA, Holloszy JO. Insulin resistance of aging is related to abdominal obesity. Diabetes 1993;42:273-81.

[38] Taylor RW, Keil D, Gold EJ, Williams SM, Goulding A. Body mass index, waist girth, and waist to hip ratio as indexes of total and regional adiposity in women: evaluation using receiver operating characteristic curves. Am J Clin Nutr 1998;67:44-9.

[39] Andrews NC, Faller DV. A rapid micropreparation technique for extraction of DNA-binding proteins from limited numbers of mammalian cells. Nucleic Acids Res 1991;19:2499.

[40] González F, Minium J, Rote NS, Kirwan JP. Altered tumor necrosis factor–α release from mononuclear cells of obese reproductive-age women during hyperglycemia. Metabolism 2006;55:271-7.

[41] Kirwan JP, Krishnan RK, Weaver JA, Del Aguila LF, Evans WJ. Human aging is associated with altered TNF-α production during hyperglycemia and hyperinsulinemia. Am J Physiol Endocrinol Metab 2001;281:E1137-E1143.

[42] Kirchengast S, Huber J. Body composition characteristics and body fat distribution in lean women with polycystic ovary syndrome. Hum Reprod 2001;16:1255-60.

[43] Ibanez L, de Zegher F. Flutamide-metformin plus an oral contraceptive (OC) for young women with polycystic ovary syndrome: switch from third- to fourth-generation OC reduces body adiposity. Hum Reprod 2004;19:1725-7.

[44] Yildirim B, Sabir N, Kaleli B. Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome. Fertil Steril 2003;79:1358-64.

[45] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796-808.

[46] Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adiponectin by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinol 2004;145:2273-82.

[47] González F, Rote NS, Minium J, Kirwan JP. In vitro evidence that hyperglycemia stimulates tumor necrosis factor–α release in obese women with polycystic ovary syndrome. J Endocrinol 2006;188: 521-9.

[48] McCrohon JA, Jessup W, Handelsman DJ, Celermajer DS. Androgen exposure increases human monocyte adhesion to vascular endothelium and endothelial cell expression of vascular adhesion molecule–1. Circulation 1999;99:2317-22.

[49] Zhu XD, Bonet B, Knopp RH. 17β-Estradiol, progesterone and testosterone inversely modulate low-density lipoprotein oxidation and cytotoxicity in cultured placental trophoblast and macrophages. Am J Obstet Gynecol 1997;177:196-209.

[50] Adams MR, Williams JK, Kaplan JR. Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. Arterioscler Thromb Vasc Biol 1995;15:562-70.