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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 which can increase the risk of atherogenesis. We measured circulating proatherogenic inflammatory mediators in women with PCOS (8 lean - BMI, 18–25 kg/m$^2$, 8 obese -BMI, 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 (MMP-2), plasminogen activator inhibitor-1 (PAI-1), and activated nuclear factor κB (NFκB) in mononuclear cells. Truncal fat was determined by DEXA. Fasting MCP-1 levels were elevated in lean women with PCOS compared to lean controls (159.9±14.1 vs. 121.2±5.4 pg/ml, $p<0.02$). Hyperglycemia failed to suppress MMP-2 in lean women with PCOS compared to lean controls (1.7±1.2 vs. −4.8 ±1.6 pg/ml, $p<0.002$). Among women with PCOS, obese individuals exhibited higher fasting sICAM-1 (16.1±0.8 vs. 10.5±1.0 ng/ml, $p<0.03$) and PAI-1 (6.1±0.7 vs. 3.4±0.8 ng/ml, $p<0.03$) levels. Trend analysis revealed higher ($p<0.005$) IL-6, sICAM-1, CRP and PAI-1, systolic and diastolic blood pressures, triglycerides, fasting insulin and HOMA-IR in women with PCOS compared to weight-matched controls, and the highest levels in the obese regardless of PCOS status. Fasting MCP-1 levels correlated with activated NFκB during hyperglycemia ($p<0.05$) and androstendione ($p<0.004$). Truncal fat correlated with fasting IL-6 ($p<0.004$), sICAM-1 ($p<0.006$), CRP ($p<0.0009$) and PAI-1 ($p<0.02$). 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.

### Keywords

atherosclerosis; inflammation; hyperglycemia; abdominal adiposity



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## INTRODUCTION

The Polycystic Ovary Syndrome (PCOS) is one of the most common female endocrinopathies affecting between 4–10% 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.[4,5,6,7] 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 afflicted by obesity, which is another risk factor for developing atherosclerosis and hyperglycemia.[11,12]

PCOS 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.[13,14,15,16,17,18] 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 due to its ability to generate reactive oxygen species (ROS) from peripheral blood mononuclear cells (MNC). ROS-induced oxidative stress activates a transcription factor known as nuclear factor κB (NFκB), the cardinal signal of inflammation that promotes atherogenesis.[21,22,23] We have recently shown that in PCOS, ROS generation and NFκB activation are increased following oral glucose ingestion independent of obesity, and that both are related to circulating androgens.[24,25] NFκB regulates gene transcription of IL-6, a proinflammatory cytokine capable of inducing the endothelial expression of sICAM-1 and MCP-1. sICAM-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] IL-6 also stimulates CRP synthesis in the liver. CRP 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.[28,29,30] 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.

## MATERIALS AND METHODS

### Subjects

Sixteen women with PCOS (8 lean and 8 obese) between 20–33 years of age and 16 weight-matched control subjects (8 lean and 8 obese) between 20–40 years of age volunteered to participate in the study. Subjects in the present report represent part of a larger cohort that are 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 oligo-amenorrhea and hyperandrogenemia after excluding nonclassic congenital adrenal hyperplasia, Cushing's 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 prior to study participation. The metabolic syndrome was diagnosed by Adult Treatment Panel III (ATP 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.

## Study Design

All study subjects underwent an oral glucose tolerance test (OGTT) between days 5 and 8 following the onset of menses, and an overnight fast of ~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.

## Oral Glucose Tolerance Test (OGTT)

All subjects ingested a 75 gram 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 hours 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 WHO criteria with normal glucose tolerance defined as a 2 hour glucose stimulated value less than 140 mg/dl.[35] Insulin resistance was estimated by HOMA-IR using the following formula: fasting glucose (mM) × fasting insulin (μU/ml) / 22.5.[36]

## 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 absorptiometry (DEXA) to determine % total body fat and % truncal fat using the QDR 4500 Elite model scanner (Hologic Inc., Waltham, MA) as previously described.[24,38]

## Plasma Measurements

Plasma glucose concentrations were measured by the glucose oxidase method (YSI, Yellow Springs, OH) while plasma insulin concentrations were measured by a double antibody RIA (Linco Research, St. Charles, MO). Luteinizing hormone (LH), testosterone, androstenedione and dehydroepiandrosterone-sulfate (DHEA-S) levels were measured by RIA (Diagnostic Products Corporation, Los Angeles, CA). Plasma IL-6 concentrations were measured by 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.

### NFκB Electrophoretic Mobility Shift Assay

Nuclear extracts of DNA-binding protein were prepared from MNC using the method described by Andrews et al.[39] Total protein concentrations were determined using the BCA protein assay (Pierce Chemical Company, 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, Inc., Santa Cruz, CA) was radiolabeled with gamma-P32 (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ) using T4 kinase (Invitrogen Corporation, Carlsbad, CA). Nuclear extract (7.5 mcg) 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 1D Image Analysis software version 3.6 (Rochester, NY).

### Statistics

The StatView statistical package (SAS Institute, Cary, NC) was used for data analysis. Descriptive data, and primary dependent variables were compared using ANOVA for multiple group comparisons. Detection of significance by ANOVA was followed by a post hoc analysis using unpaired Student's 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 (ρ) 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 (ρ) correlation between the variable and a number between one and four assigned to each group, in the order of the observed pattern. All values are expressed as means ± SE. An α-level of 0.05 was used to determine statistical significance.

## RESULTS

### Age, Body Composition, Blood Pressure and Lipids

Obese women with PCOS were similar in age compared to obese controls whereas lean women with PCOS were slightly younger than lean controls (Table 1). Weight, body mass index (BMI), % total body fat, % truncal fat and waist circumference were significantly ($p<0.04$) greater in obese subjects compared to those who were lean whether or not they had PCOS, but were similar when women with PCOS were compared to weight-matched controls.

Systolic and diastolic blood pressures were significantly ($p<0.04$) higher in obese individuals whether or not they had PCOS, and in lean women with PCOS compared to 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 to lean controls, but not significantly. High density lipoprotein cholesterol (HDL) was significantly ($p<0.03$) lower in obese women with PCOS compared to lean individuals whether or not they had PCOS. There was a significant trend in systolic ($\rho=0.61$; $p<0.0008$) and diastolic ($\rho=0.60$; $p<0.0009$) blood pressures, and triglycerides ($\rho=0.48$; $p<0.008$), in which mean levels were higher in lean women with PCOS compared to lean controls, and in obese controls compared to lean women with PCOS, with the highest

levels evident in obese women with PCOS. HDL also exhibited a significant trend among groups, but in the opposite direction ($\rho$= -0.49; $p<0.0007$).

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

### Plasma Hormone Levels, Glycemic Status and Insulin Resistance

Circulating levels of LH, testosterone and androstenedione were significantly ($p<0.04$) elevated in women with PCOS compared to control subjects independent of body mass (Table 2). Circulating DHEA-S levels were significantly ($p<0.02$) elevated in lean and obese women with PCOS compared to obese controls, but were similar in lean women with PCOS compared to lean controls.

Glucose levels while fasting and 2 hours post glucose ingestion were similar in women with PCOS compared to 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 ($p<0.05$) in the obese whether or not they had PCOS compared to lean controls. HOMA-IR in lean women with PCOS was similar to obese controls, but was significantly ($p<0.02$) lower compared to obese women with PCOS. There was a significant increasing trend in fasting insulin ($\rho=0.65$; $p<0.0005$) and HOMA-IR ($\rho=0.65$; $p<0.0004$), in which mean levels exhibited the same pattern among groups observed for blood pressures and triglycerides.

### Plasma Inflammatory Mediator Levels and Intranuclear NFκB

Plasma IL-6 concentrations were significantly ($p<0.03$) higher in obese women with PCOS compared to lean subjects whether or not they had PCOS (Table 3). Obese women with PCOS exhibited significantly ($p<0.04$) higher plasma sICAM-1 levels compared to obese controls and both lean groups. Plasma sICAM levels were significantly higher ($p<0.05$) in obese controls compared to lean controls. Lean women with PCOS exhibited significantly ($p<0.03$) higher plasma MCP-1 levels compared to 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 ($p<0.05$) higher in the obese whether or not they had PCOS. CRP concentrations were also elevated in lean women with PCOS compared to lean controls, but not significantly.

Plasma MMP-2 levels were significantly ($p<0.05$) lower in the obese whether or not they had PCOS compared to lean controls, and similar in lean women with PCOS compared to either control group. Plasma PAI-1 concentrations were significantly ($p<0.03$) higher in obese women with PCOS compared to lean subjects whether or not they had PCOS. There was a significant trend in IL-6 ($\rho=0.74$; $p<0.0001$), sICAM-1 ($\rho=0.76$; $p<0.0001$), CRP ($\rho=0.76$; $p<0.0001$) and PAI-1 ($\rho=0.54$; $p<0.005$), in which mean levels were once again higher in lean women with PCOS compared to lean controls, and in obese controls compared to lean women with PCOS, with the highest levels evident in obese women with PCOS.

In response to the oral glucose load, the % change in MNC-derived intranuclear NFκB was similar ($p=0.73$) 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 % change in MNC-derived intranuclear NFκB was significantly ($p<0.04$) higher in both groups of women with PCOS compared to 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 ($p<0.05$) and obese controls ($p<0.009$) (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 MMP-2 was significantly different in lean women with PCOS compared to lean controls ($p<0.002$) and obese controls ($p<0.04$) but similar in obese women with PCOS compared to either control group. There was no change in any of the other plasma inflammatory mediators in response to glucose ingestion (data not shown).

### Correlation Analyses

HOMA-IR was positively correlated with BMI ($r=0.52$, $p<0.003$), % body fat ($r=0.41$, $p<0.03$), % truncal fat ($r=0.49$, $p<0.005$) and waist circumference ($r=0.61$, $p<0.0005$) 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. IL-6, sICAM-1, CRP and PAI-1 were directly correlated with BMI, % total body fat, % truncal fat and waist circumference. IL-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 was also a positive correlation between IL-6 and PAI-1, and a negative correlation between sICAM-1 and MMP-2. sICAM-1 and CRP were positively correlated with BMI, % total body fat, % truncal fat and waist circumference. IL-6 and PAI-1 were directly correlated with % total body fat and % truncal fat. IL-6 and CRP were also directly correlated with HOMA-IR.

Plasma MCP-1 was positively correlated with MNC-derived intranuclear NFκB ($\rho=0.41$, $p<0.05$) and plasma androstendione ($\rho=0.55$, $p<0.004$) for the combined groups. Plasma testosterone and androstenedione were positively correlated with the % change in MNC-derived intranuclear NFκB ($r=0.44$, $p<0.03$; $r=0.46$, $p<0.03$) for the combined groups (data not shown).

## 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 to lean controls, and fail to suppress MMP-2 levels under postprandial-like conditions. Obese women with PCOS exhibit elevated sICAM-1 levels compared to obese controls, and elevated PAI-1 levels compared to 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 to 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. MCP-1 is directly related to MNC-derived intranuclear NFκB 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 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 to 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 to lean controls. The lack of significant difference of these inflammatory mediators between these two 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 following 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 TNFα exhibits a similar response pattern when measured in cultured MNC from lean women with PCOS and young healthy men and women following 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. MNC 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 to 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 to 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 to 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 which 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 to 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 to lean women with PCOS or obese controls.

In PCOS, there may be a link between adiposity and plasma mediators of inflammation that promote atherosclerosis. While 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.[19,38,42,43,44] 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. Since 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 following 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 following ROS-induced oxidative stress from glucose-activated MNC to upregulate 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 to 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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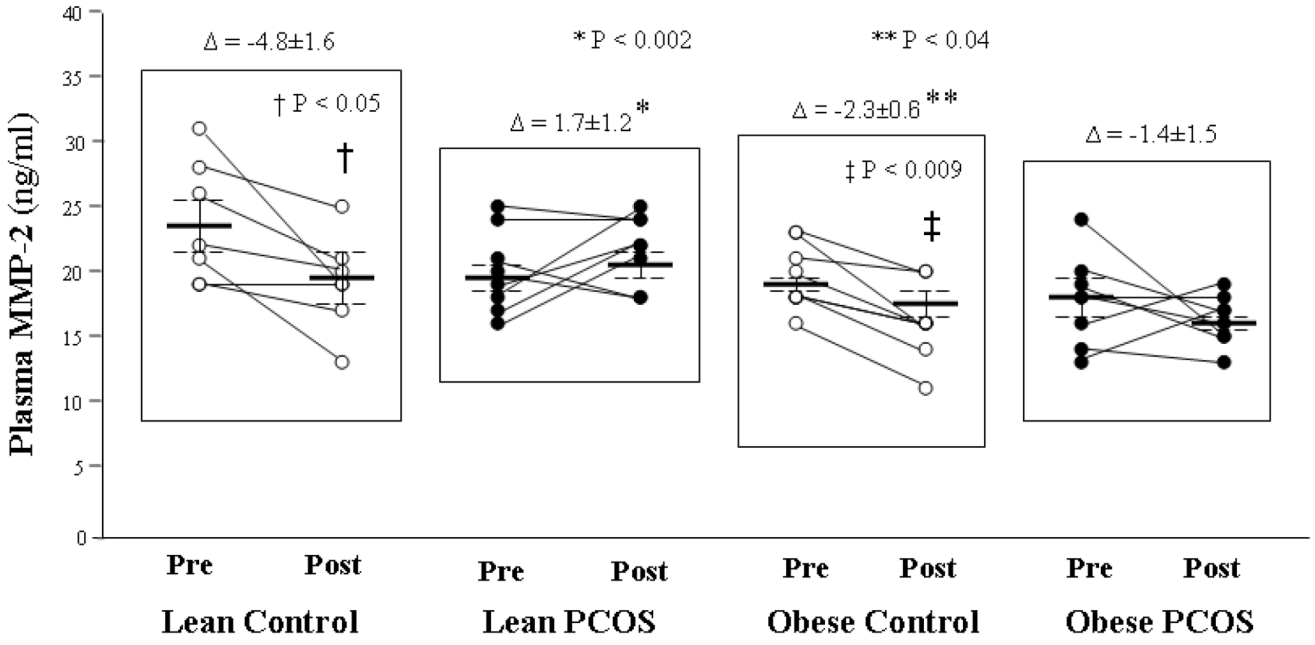


**Figure 1.**

Plasma matrix metalloprotease-2 (MMP-2) when fasting samples (pre) were compared to the samples collected 2 hours after glucose ingestion (post). Pre and post MMP-2 values were similar and superimposed for two 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, $P < 0.002$. **Incremental change (Δ) in MMP-2 during oral glucose challenge in lean women with PCOS was significantly different from that of obese controls, $P < 0.04$. †2 hours post glucose was significantly lower than fasting in lean controls, $P < 0.05$. ‡ 2 hours post glucose was significantly lower than fasting in obese controls, $P < 0.009$.

**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, yr | 27±2 [a] | 25±2 [b] | 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 [b,c] | 61.2±2.2 | 94.2±4.1 [d,e] |
| Body mass index, kg/m$^2$ | 23.0±0.9 | 36.1±0.6 [b,c] | 22.4±0.9 | 35.0±1.0 [d,e] |
| Total Body fat, % | 30.1±1.9 | 42.6±1.0 [b,c] | 29.3±2.0 | 425±1.0 [d,e] |
| Truncal Fat, % | 28.7±2.6 | 43.8±1.0 [b,c] | 26.3±2.7 | 42.3±0.9 [d,e] |
| Waist circumference, cm | 76.8±2.8 | 104.8±4.4 [b,c] | 74.9±3.0 | 1021±3.0 [d,e] |
| Systolic blood pressure, mmHg | 112±3 | 130±6 [b,c] | 104±3 | 118±5 [e] |
| Diastolic blood pressure, mmHg | 70±4 [a] | 78±5 [b] | 58±3 | 76±3 [e] |
| 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 [b,e] | 55±4 | 48±4 |
| LDL – cholesterol, mg/dl | 100±11 | 120±10 | 112±7 | 115±18 |

Values are expressed as means ± SE;

[a] Lean PCOS vs. Lean Control, P < 0.05

[b] Obese PCOS vs. Lean PCOS, P < 0.03

[c] Obese PCOS vs. Lean Control, P < 0.009

[d] Obese Control vs. Lean PCOS, P < 0.0001

[e] Obese Control vs. Lean Control, P < 0.04

**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 [a,b] | 8.8±1.5 [c,d,e] | 3.2±0.5 | 2.7±0.4 |
| Testosterone, ng/dl | 70.6±9.2 [a,b] | 87.4±10.9 [d,e] | 43.8±4.5 | 32.3±4.8 |
| Androstendione, ng/ml | 3.5±0.3 [a,b] | 3.4±0.2 [d,e] | 1.6±0.1 | 1.9±0.2 |
| DHEA-S, µg/dl | 318±42 [b] | 333±46 [d,e] | 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 Hour 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 [c,d] | 7.0±1.2 | 13.4±2.1 [f] |
| HOMA-IR, mM-µU/ml | 2.3±0.4 | 4.1±0.7 [c,e] | 1.5±0.2 | 2.9±0.5 [f] |

Values are expressed as means ± SE; Conversion factors to SI units: Testosterone ×3.467 (nmol/liter), Androstenedione ×3.492 (nmol/liter), DHEA-S ×0.002714 (umol/liter), Glucose ×0.0551 (mmol/liter), Insulin ×7.175 (pmol/liter).

[a] Lean PCOS vs. Lean Control, $P < 0.03$

[b] Lean PCOS vs. Obese Control, $P < 0.02$

[c] Obese PCOS vs. Lean PCOS, $P < 0.02$

[d] Obese PCOS vs. Obese Control, $P < 0.006$

[e] Obese PCOS vs. Lean Control, $P < 0.04$

[f] Obese Control vs. Lean Control, $P < 0.05$

**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 *a,b* | 0.6±0.1 | 2.1±0.5 |
| sICAM-1, ng/ml | 10.5±1.0 | 16.1±0.8 *a,b,c* | 8.2±1.0 | 13.0±1.3 *d* |
| MCP-1, pg/ml | 159.9±14.1 *e* | 145.0±12.8 | 121.2±5.4 | 123.3±9.8 *f* |
| CRP, **mg/l** | **1.2±0.4** | **5.7±1.7***a,b* | **0.3±0.1** | **6.5±1.1***d,f* |
| MMP-2, ng/ml | 20.0±1.1 | 17.6±1.3 *b* | 23.0±1.9 | 19.0±0.9 *d* |
| PAI-1, ng/ml | 3.4±0.8 *c* | 6.1±0.7 *b* | 3.5±0.9 | 5.5±0.7 |

Values are expressed as means ± SE; IL-6, Interleukin-6; sICAM-1, Soluble intracellular adhesion molecule-1; MCP-1, Monocyte chemotactic protein-1; CRP, C-reactive protein; MMP-2, Matrix metalloprotease-2; PAI-1, Plasminogen activator inhibitor-1.

*a* Obese PCOS vs. Lean PCOS, $P < 0.03$

*b* Obese PCOS vs. Lean Control, $P < 0.02$

*c* Obese PCOS vs. Obese Control, $P < 0.04$

*d* Obese Control vs. Lean Control, $P < 0.05$

*e* Lean PCOS vs. Lean Control, $P < 0.02$

*f* Obese Control vs. Lean PCOS, $P < 0.03$

**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$^2$)* | ρ | 0.580 | 0.640 | −0.031 | 0.690 | −0.210 | 0.493 |
| | P | 0.002* | 0.0004* | 0.866 | 0.0003* | 0.243 | 0.008* |
| Total body fat *(%)* | ρ | 0.574 | 0.740 | 0.032 | 0.782 | −0.264 | 0.577 |
| | P | 0.002* | 0.0001* | 0.860 | 0.0001* | 0.142 | 0.002* |
| Truncal fat *(%)* | ρ | 0.620 | 0.753 | 0.077 | 0.845 | −0.297 | 0.590 |
| | P | 0.0008* | 0.0001* | 0.675 | 0.0001* | 0.098 | 0.002* |
| Waist circum. *(cm)* | ρ | 0.564 | 0.601 | −0.078 | 0.588 | −0.236 | 0.467 |
| | P | 0.003* | 0.001* | 0.680 | 0.003* | 0.204 | 0.014* |
| HOMA-IR (mM-μU/ml) | ρ | 0.551 | 0.322 | 0.102 | 0.570 | −0.201 | 0.403 |
| | P | 0.003* | 0.071 | 0.575 | 0.002* | 0.263 | 0.030* |
| *IL-6 (pg/ml)* | ρ | ---------- | 0.442 | −0.003 | 0.685 | −0.487 | 0.366 |
| | P | ---------- | 0.017* | 0.989 | 0.0002* | 0.009* | 0.048* |
| *sICAM-1 (ng/ml)* | ρ | 0.442 | ---------- | 0.063 | 0.519 | −0.395 | 0.486 |
| | P | 0.017* | ---------- | 0.730 | 0.004* | 0.028* | 0.009* |
| *CRP (ng/ml)* | ρ | 0.685 | 0.519 | 0.165 | ---------- | 0.283 | 0.617 |
| | P | 0.0002* | 0.004* | 0.366 | ---------- | 0.116 | 0.0009* |

IL-6, Interleukin-6; sICAM-1, Soluble intracellular adhesion molecule-1; MCP-1, Monocyte chemotactic protein-1; CRP, C-reactive protein; MMP-2, Matrix metalloprotease-2; PAI-1, Plasminogen activator inhibitor-1

*P<0.05.

**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$^2$)* | ρ | 0.483 | 0.662 | −0.457 | 0.709 | −0.144 | 0.443 |
| | P | 0.071* | 0.010* | 0.087 | 0.006* | 0.577 | 0.098 |
| Total body fat *(%)* | ρ | 0.550 | 0.768 | −0.381 | 0.779 | −0.345 | 0.588 |
| | P | 0.040* | 0.003* | 0.153 | 0.003* | 0.182 | 0.028* |
| Truncal fat *(%)* | ρ | 0.589 | 0.723 | 0.326 | 0.870 | −0.302 | 0.654 |
| | P | 0.028* | 0.005* | 0.223 | 0.0008* | 0.242 | 0.014* |
| Waist circum. *(cm)* | ρ | 0.472 | 0.609 | −0.411 | 0.653 | −0.144 | 0.389 |
| | P | 0.077 | 0.018* | 0.124 | 0.011* | 0.577 | 0.145 |
| HOMA-IR (mM-μU/ml) | ρ | 0.479 | 0.176 | −0.129 | 0.694 | 0.300 | 0.325 |
| | P | 0.048* | 0.494 | 0.631 | 0.007* | 0.245 | 0.224 |
| *IL-6 (pg/ml)* | ρ | ---------- | 0.637 | −0.485 | 0.587 | −0.440 | 0.530 |
| | P | ---------- | 0.017* | 0.070 | 0.028* | 0.100 | 0.048* |
| *sICAM-1 (ng/ml)* | ρ | 0.637 | ---------- | −0.404 | 0.494 | −0.632 | 0.432 |
| | P | 0.017* | ---------- | 0.131 | 0.048* | 0.014* | 0.106 |
| *CRP (ng/ml)* | ρ | 0.587 | 0.494 | 0.071 | ---------- | −0.135 | 0.725 |
| | P | 0.028* | 0.048* | 0.789 | ---------- | 0.600 | 0.007* |

IL-6, Interleukin-6; sICAM-1, Soluble intracellular adhesion molecule-1; MCP-1, Monocyte chemotactic protein-1; CRP, C-reactive protein; MMP-2, Matrix metalloprotease-2; PAI-1, Plasminogen activator inhibitor-1

*P<0.05.