PCOS Is Associated with Increased CD11c Expression and Crown-Like Structures in Adipose Tissue and Increased Central Abdominal Fat Depots Independent of Obesity

Zhi Hua Huang, Buvana Manickam, Victoria Ryvkin, Xiaohong Joe Zhou, Giamila Fantuzzi, Theodore Mazzone, and Susan Sam

Department of Medicine (Z.H.H., B.M., V.R., S.S.), Section of Endocrinology, Diabetes, and Metabolism; Department of Radiology and Center for Magnetic Resonance Research (X.J.Z.); and Department of Kinesiology and Nutrition (G.F.), University of Illinois, Chicago, Illinois 60612; and Department of Medicine (T.M.), NorthShore University Health System, Chicago, Illinois 60201

Context: Adipose tissue macrophage (ATM) infiltration is a major pathway for obesity-induced insulin resistance but has not been studied as a mechanism for insulin resistance in PCOS.

Objective: We tested whether polycystic ovary syndrome (PCOS) is associated with increased ATM infiltration, especially of inflammatory subtype identified by the CD11c marker.

Design and Setting: We conducted a case-control study at an academic medical center in the United States.

Participants and Interventions: Fourteen PCOS and 14 control women of similar age and body mass index (BMI) underwent a gluteal fat biopsy. Markers of ATM, integrins, TNF-α, and adiponectin, were analyzed by quantitative RT-PCR using a standard curve method. Crown-like structures (CLS) were identified by immunohistochemistry. Abdominal magnetic resonance imaging and frequently sampled iv glucose tolerance test were performed to assess abdominal fat and insulin sensitivity (SI).

Main Outcome: Women with PCOS were compared with control women of similar age and BMI for ATM markers, CLS density, adipose tissue expression of inflammatory cytokines and adiponectin, SI, and abdominal fat depots.

Results: Women with PCOS had an increase in CD11c expression ($P = 0.03$), CLS density ($P = 0.001$), α5 expression ($P = 0.009$), borderline increase in TNF-α expression ($P = 0.08$), and a decrease in adiponectin expression ($P = 0.02$) in gluteal adipose tissue. Visceral ($P = 0.009$) and sc abdominal fat ($P = 0.005$) were increased in PCOS. SI was lower in PCOS ($P = 0.008$).

Conclusions: PCOS is associated with an increase in CD11c expression and CLS density and a decrease in adiponectin expression in sc adipose tissue. Additionally, PCOS is associated with higher central abdominal fat depots independent of BMI. These alterations are present among mostly nonobese women and could represent mechanisms for insulin resistance. (***J Clin Endocrinol Metab*** **98: E17–E24, 2013)**

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in reproductive-age women and the leading cause of diabetes mellitus type 2 (DM2) and dyslipidemia in this population (1). This heightened risk for DM2 and metabolic disorders in PCOS is related to the presence of insulin resistance (2). It has been shown that

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2012–2697 Received July 6, 2012. Accepted October 2, 2012.
First Published Online November 8, 2012

Abbreviations: ATM, Adipose tissue macrophage; BMI, body mass index; CLS, crown-like structure; DI, disposition index; DM2, diabetes mellitus type 2; FSIGT, frequently sampled iv glucose tolerance test; MRI, magnetic resonance imaging; PCOS, polycystic ovary syndrome; SI, insulin sensitivity index.

nonobese women with PCOS have the same degree of insulin resistance as obese reproductively normal women (3, 4). Furthermore, obesity exacerbates the insulin resistance associated with PCOS so that obese women with PCOS are more insulin resistant compared with reproductively normal women of the same body mass index (BMI) (3, 4). The mechanisms for insulin resistance in PCOS are not well understood (2).

Recently, adipose tissue inflammation has been recognized as a major pathway for development of insulin resistance in obesity (5, 6). Obesity is characterized by adipose tissue macrophage (ATM) infiltration especially of inflammatory subtypes that are the source of inflammatory cytokines associated with insulin resistance (7–10). These inflammatory macrophages cluster around dead adipocytes in crown-like structures (CLSs), and CLS density correlates strongly with the degree of obesity and insulin resistance (11). In humans, weight loss after bariatric surgery has been associated with a 40% reduction in ATM infiltration accompanied by improvements in insulin sensitivity (12). The inflammatory macrophages in humans are characterized by CD11c expression, whereas CD206, CD14, and CD 163 are considered to be nonspecific macrophage markers (11). Not all obese humans have an increase in inflammatory ATM infiltration (13), and the factors that regulate ATM infiltration in obesity have not been well characterized. Furthermore, higher ATM density has been reported in obese men compared with obese women, raising the possibility that sex hormones may play a factor in ATM infiltration (11, 39). Because women with PCOS have higher androgen levels, we tested the hypothesis that PCOS is associated with increased inflammatory ATM infiltration characterized by an increase in adipose tissue CD11c expression. This potential novel mechanism for insulin resistance has never been explored in PCOS.

Additionally, we examined whether PCOS is associated with an increase in sc and visceral abdominal fat depots that are associated with increased risk for insulin resistance and metabolic disorders (14). Visceral fat is a strong surrogate for fat accumulation in ectopic locations such as liver, skeletal muscle, and pancreas (14). Ectopic fat accumulation accounts for the majority of harmful effects of obesity (13–15). Obese individuals who do not deposit their excess fat in such locations do not suffer the metabolic consequences usually attributed to obesity (15–17). To study the independent impact of PCOS from obesity, PCOS and control women were of similar BMI, and the majority of women included in both groups were nonobese.

Subjects and Methods

Subjects

Twenty-eight premenopausal women with (n = 14) and without (n = 14) PCOS were recruited for the study. Most women with PCOS (n = 11) and all of the control women were recruited from local advertisements. Three women with PCOS were recruited from the endocrinology clinic at University of Illinois. The diagnosis of PCOS was based on the National Institutes of Health criteria and defined by presence of oligomenorrhea (fewer than six menses per year) and biochemical hyperandrogenism based on elevated total or bioavailable testosterone levels (18). Thyroid hormone abnormalities, hyperprolactinemia, and nonclassical congenital hyperplasia due to 21-hydroxylase deficiency were excluded by appropriate laboratory testing in all women with PCOS. All control women had normal menstrual cycles, no evidence of androgen excess, and normal levels of dehydroepiandrosterone and total and bioavailable testosterone. Subjects were excluded if they were younger than 18 yr old or older than 40 yr old, were pregnant or lactating, or had any chronic disease including diabetes, hypertension, or psychiatric disorder. None of the subjects were receiving any cholesterol or antihypertension medications. None of the women have received any oral contraceptive or other form of hormonal contraception for at least 3 months before their participation. None of the women had received metformin or thiazolidinedione for at least 1 month before their participation in the study. The study was approved by the institutional review board at University of Illinois, and all subjects signed written informed consent before their participation in the study.

Data collection

All women were studied at the clinical research center at the University of Illinois and underwent a history and physical exam by a physician investigator that included detailed menstrual and medical history as well as assessment for hirsutism and other signs of hyperandrogenism and insulin resistance. Standardized forms were used to obtain medical history including information on exercise habits and alcohol and tobacco use. Height, weight, and waist measurements were determined on all subjects. A morning blood sample was obtained after an overnight fast from all subjects and included measurements of total and bioavailable testosterone, sex hormone binding globulin, glucose, insulin and adiponectin.

Determination of insulin sensitivity

A frequently sampled iv glucose tolerance test (FSIGT) was performed in the morning after 3 d of 300-g carbohydrate preparatory diet and a standard overnight fasting period of 10 h to study insulin sensitivity. All subjects had two iv catheters inserted, one in each arm, and were then allowed to rest for 30 min. At 0 min, glucose (0.3 g/kg) was injected over 1 min; at 20 min, insulin was injected at 0.02 U/kg over 20 sec. Blood samples were drawn at −15, −10, −5, −1, 0, 2, 3, 4, 5, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 90, and 100 min and every 20 min thereafter until 180 min. The insulin sensitivity index (SI) was calculated by application of the minimal model of glucose kinetics (MINMOD computer program Millennium version, copyright R. N. Bergman) to the dynamics of plasma glucose and insulin during the FSIGT (19, 20). The SI is a more sensitive measure of insulin sensitivity compared with fasting insulin lev-

els (19, 20). The acute insulin response to glucose was calculated as the increment area under curve from basal insulin values measured at 2–10 min and disposition index (DI) (β-cell compensation index) as the product of SI and acute insulin response to glucose (19, 20).

Determination of body composition and abdominal fat depots

Subjects underwent abdominal magnetic resonance imaging (MRI) scans in the supine position on a 3-T whole-body scanner (Signa HDxt; General Electric Healthcare, Milwaukee, WI) using a body coil with the protocol detailed previously (21). On each subject, an axial scan was performed using a gradient echo pulse sequence with water suppression to obtain a fat-only image, followed by two additional acquisitions at the same locations, one with lipid suppression to obtain a water-only image and the other without any suppression to serve as an anatomic reference. In all acquisitions, a total of five slices (slice thickness = 4 mm) were obtained with the central slice at the L3 level. The field of view was 36–40 cm^2, depending on the patient size. With a matrix size of 256 × 256, the in-plane resolution was between 1.40 and 1.56 mm. From the fat-only (I_f) and water-only (I_w) images, the fat signal intensity ratio, $I_f/(I_f + I_w)$, was calculated on a pixel-by-pixel basis, producing a map of fat signal intensity ratio. Using the calibration curve obtained from a previous phantom study, this ratio was converted to a quantitative fat percentage value (21). Fat volume in the visceral and sc regions was analyzed using AnalyzeDirect, Inc. (Stilwell, KS), and expressed as cubic centimeters.

In addition to MRI-based measurement, fat quantity was also determined by total-body dual-energy x-ray absorptiometry using Hologic QDR 4500 (Bedford, MA). Total fat quantity and quantity of fat in trunk area were measured.

Subcutaneous adipose tissue biopsy

The study was performed after an overnight fast and 3 d of 300-g carbohydrate diet. Adipose tissue was obtained from gluteal region through a small incision after using 1% lidocaine for local anesthesia (22). Adipose tissue was immediately placed in liquid nitrogen for later RNA extraction and stored at −80 C (22) or fixed in 10% neutral-buffered formalin for immunohistochemical staining.

Laboratory methods

Total testosterone was measured by turbulent flow liquid chromatography mass spectrometry that has an assay sensitivity of 1.0 ng/dl at Quest Diagnostics (Wood Dale, IL). Bioavailable testosterone was calculated based on constants for the binding of testosterone to SHBG and albumin. SHBG was measured by extraction, chromatography, and RIA, and albumin was measured by spectrophotometry. Plasma glucose was collected in a fluoride/oxalate tube and analyzed using spectrophotometry. Insulin was measured by a chemiluminescent sandwich immunoassay measuring down to 2 μU/ml. Serum adiponectin levels were measured using ELISA kits from Alpco (Salem, NH).

Expression of ATM, inflammatory markers, and adiponectin in sc adipose tissue

Macrophage infiltration and expression of inflammation markers and adiponectin in adipose tissues were measured by quantitative RT-PCR according to the previously published method (23). Briefly, total RNA was extracted using an RNeasy lipid tissue kit (QIAGEN, Valencia, CA) (24). First-strand cDNA is synthesized using random hexamer primers according to the manufacturer's instructions (Fermentas, Hanover, MD). Real-time PCR was performed on each sample in triplicates using the Mx3000p quantitative PCR system (Stratagene, La Jolla, CA). Reactions are carried out in a total volume of 25 μl using Brilliant II Sybr Green QPCR Master Mix (Stratagene). Primer sequences are shown in Table 1. Standard curves for this study were generated using pooled cDNA from two sc adipose tissue RNA samples (matched for age, gender, and BMI) (Zen Bio, Research Triangle Park, NC). Relative expression of each gene is normalized to three housekeeping gene (β-actin, *RPII*, and *HPRTI*).

Immunohistochemical staining in sc adipose tissues

Adipose tissues were fixed and embedded in paraffin, and 5-mm-thick consecutive sections were prepared. After antigen retrieval, adipose tissue sections were stained with anti-CD68 (1:400, ab955; Abcam, Cambridge, MA) and anti-CD11c (1:200, ab52632; Abcam) primary antibodies on contiguous slides followed by horseradish peroxidase-conjugated secondary antibody. Positive stained cells were visualized with 3,3′-diaminobenzidine and counterstained with hematoxylin. A CLS was identified as an adipocyte with more than 50% of its perimeter surrounded by CD68-positive cells (25). CLS density was defined as the number of CLSs per 500 adipocytes, with an average 800 ± 370 adipocytes (16 ± 7 high-power fields) counted from each subject in a blinded fashion. ATMs within CLSs were also positive for CD11c staining.

TABLE 1. PCR primer set

Genes of interest	Sense primer	Antisense primer
CD14	GCAGCCGAAGAGTTCACAAG	CAGCAGCAGCAACAAGCA
CD11c	AGCAGCCACGAACAATTCAC	GAGACCTCCACATCCATCCA
TNF-α	TTGTTCCTCAGCCTCTTCTCC	GGTTTGCTACAACATGGGCTAC
IL-6	AATAACCACCCCTGACCCAAC	AATCTGAGGTGCCCATGCTAC
CD206	TGGAGTGATGGTTCTCCTGTTT	CTGGTGTTGGCTCAGGTTTT
CD163	CAGGAAACCAGTCCCAAACA	AGCGACCTCCTCCATTTACC
Adiponectin	CCTGGTGAGAAGGGTGAGAAA	AAGTCTCCAATCCCACACTGAA
Integrin α5	GGAGTTCCAAGAGCAGCAAG	AGAGCCACGATCCATGAAGA
β-Actin	ACTCTTCCAGCCTTCCTTCCT	CAGTGATCTCCTTCTGCATCCT
HPRT I	CTGAGGATTTGGAAAGGGTGT	AATCCAGCAGGTCAGCAAAG
RPII	GCGCAAATTCACCAAGAGAG	GTGCTGTGGGTACGGATACAA

TABLE 2. Baseline characteristics, hormonal assessment, and measures of insulin sensitivity and secretion among PCOS and control women

Variable	PCOS (n = 14)	Control (n = 14)	*P*
Age (yr)	26 ± 5	27 ± 6	0.73
BMI (kg/m^2)	27.2 ± 4.1	25.5 ± 3.9	0.28
Waist (cm)	78 ± 9	77 ± 9	0.78
Bioavailable testosterone (ng/dl)	15 ± 6	6 ± 2	<0.001
SHBG (nmol/liter)	34 ± 15	45 ± 14	0.06
Fasting glucose (mg/dl)	79 ± 6	81 ± 5	0.37
Fasting insulin (μU/ml)	6 ± 4	5 ± 2	0.81
Adiponectin (μg/ml)	3.5 ± 1.3	4.6 ± 1.4	0.05
SI [$\times 10^{-4}$ min/(μU/ml)]	7 ± 4	15 ± 10	0.008
DI	1893 ± 1213	2491 ± 2081	0.62

Data are presented as mean ± SD. Variables were log transformed if not normally distributed and compared by unpaired *t* test. SI is the index of insulin sensitivity and DI is the index of β-cell function from FSIGT.

Statistical analyses

Mean, SD, medians, and quartiles were used to summarize data. Data were log transformed if not normally distributed. Continuous variables were compared by independent *t* test if normally distributed and by nonparametric Mann-Whitney *U* test if not normally distributed after log transformation. The distribution of BMI categories between the two groups were compared by χ^2 analysis. Correlations were performed using Spearman's correlation coefficients. Analyses were performed using the 18.0 PC package of SPSS statistical software (SPSS, Inc., Chicago, IL). $P \leq 0.05$ was considered significant.

Results

Baseline clinical and laboratory characteristics of women with PCOS and control women are summarized in Table 2. The mean age for women with PCOS was 26 ± 5 yr, which was similar to control women at 27 ± 6 yr (P = 0.73, Table 2). The mean BMI was 27.2 ± 4.1 kg/m^2 for women with PCOS and 25.5 ± 3.9 kg/m^2 for control women, differences that were not significant (P = 0.28, Table 2). The distribution of nonobese, overweight, and obese was similar between the two groups (P = 0.53): nonobese (BMI < 25 kg/m^2), eight control and five PCOS; overweight (25 kg/m^2 ≥ BMI < 30 kg/m^2), four control and six PCOS; and obese (BMI ≥ 30 kg/m^2), two control and three PCOS. The BMIs of the two obese women in the control group were 31.5 and 35.2 kg/m^2, and the three obese women with PCOS were 31.0, 33.6, and 36.3 kg/m^2. The mean waist circumference was 78 ± 9 cm for women with PCOS and 77 ± 9 cm for control women (P = 0.78, Table 2). There was no difference in distribution of race/ethnicity between the two groups (P = 0.70, data not shown). Only one woman smoked cigarettes in the control group, very few women in either group drank any alcohol, and the two groups did not differ in regards to these two factors or exercise habits (data not shown). As expected, women with PCOS had significantly higher bioavailable testosterone compared with control women (P < 0.001, Table 2) and borderline lower SHBG compared with control women (P = 0.06, Table 2). Fasting glucose (P = 0.37, Table 2) and insulin levels (P = 0.81, Table 2) were similar between PCOS and control women. Women with PCOS had borderline lower serum adiponectin levels compared with control women (P = 0.05, Table 2). The SI from FSIGT was significantly lower in women with PCOS compared with control women (P = 0.008, Table 2). However, DI, an index of β-cell function, did not differ between the two groups (P = 0.62, Table 2).

There was no difference in trunk fat between women with PCOS and control women (P = 0.28, Table 3) but trunk to total fat ratio was higher in PCOS compared with controls (P = 0.04, Table 3). Visceral (P = 0.009, Table 3) and sc abdominal (P = 0.005, Table 3) fat depots were significantly higher in women with PCOS compared with control women.

Women with PCOS had higher sc adipose tissue expression of the inflammatory ATMs identified by CD11c marker (P = 0.03, Fig. 1) as well as higher expression of $\alpha 5$ (P = 0.009, Fig. 1), an integrin that is expressed on ATMs (11, 26) and may be involved in stimulation of inflammatory signaling pathways (27). There were no differences between PCOS and control women in expression of CD14, CD163, and CD206. Women with PCOS also had borderline increased expression of TNF-α in sc fat compared with control women (P = 0.08, Fig. 1), but there was no difference in expression of IL-6 (Fig. 1). Adiponec-

TABLE 3. Comparison of body composition between PCOS and control women

Variable	PCOS (n = 14)	Control (n = 14)	*P*
Trunk fat	9878 (8254, 13, 085)	7472 (5826, 15, 647)	0.28
Trunk/total	0.44 (0.39, 0.45)	0.38 (0.35, 0.42)	0.04
Visceral (cm^3)	450 (312, 895)	230 (157, 337)	0.009
Subcutaneous (cm^3)	3020 (2609, 3218)	1772 (1589, 2229)	0.005

Data are presented as median (25th, 75th quartiles) and compared by Mann-Whitney *U* test.

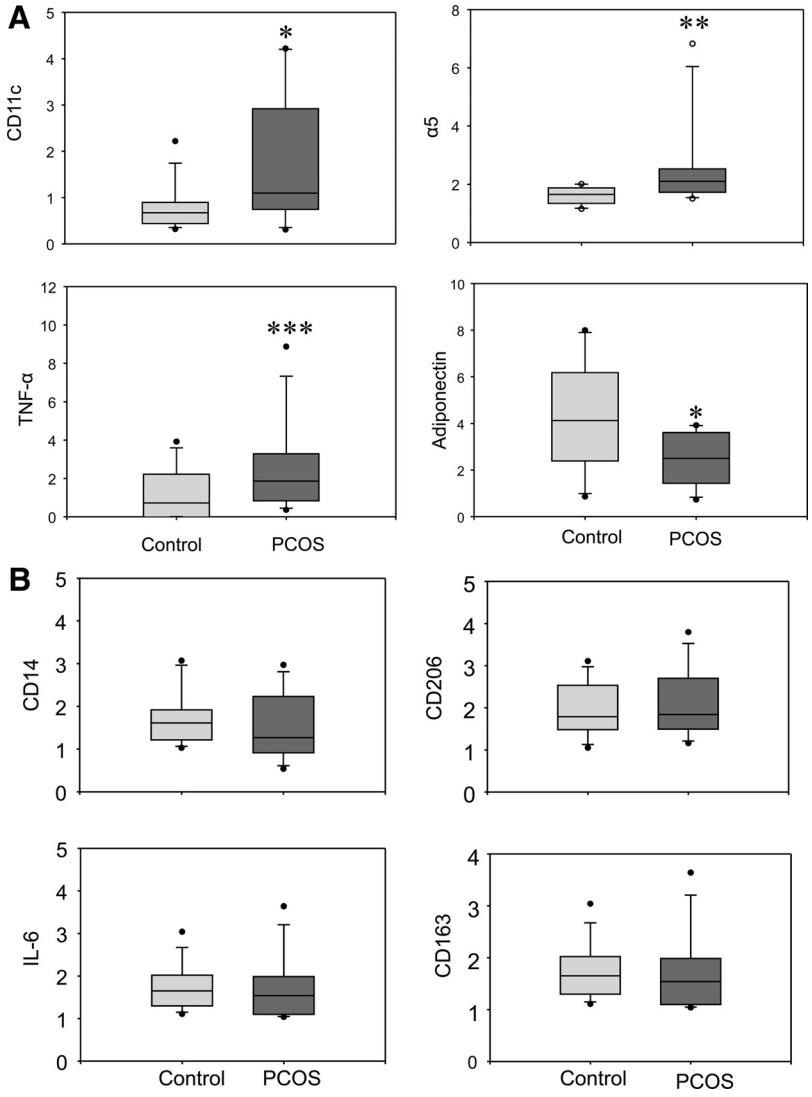

FIG. 1. A, Women with PCOS (n = 14) had significantly higher CD11c expression (*, $P = 0.03$), $\alpha 5$ expression (**, $P = 0.009$), and borderline higher TNF-α expression (***, $P = 0.08$). Women with PCOS had significantly lower adiponectin expression (*, $P = 0.02$) compared with control women. B, Expression of ATM markers (CD14, CD11c, CD206, and CD163), adiponectin, $\alpha 5$, IL-6, and TNF-α was analyzed by quantitative RT-PCR using a standard curve method. Relative expression of each gene is normalized to three housekeeping genes (β-actin, *RPII*, and *HPRTI*). Statistical comparisons were made by independent Mann-Whitney *U* test.

tin expression in sc fat was significantly reduced in women with PCOS compared with control women ($P = 0.02$, Fig. 1). Women with PCOS also had significant increase in CLS density identified by staining for both CD68 and CD11c on contiguous slides compared with control women ($P = 0.001$, Fig. 2).

CD11c expression was positively correlated to visceral ($P = 0.01$, Table 4) and sc abdominal fat depots ($P = 0.002$, Table 4) and borderline positively correlated to bioavailable testosterone levels ($P = 0.06$, Table 4). CLS density was positively correlated to bioavailable testosterone ($P = 0.01$, Table 4), visceral depot ($P = 0.009$, Table 4), sc abdominal fat depot ($P = 0.04$, Table 4), and BMI ($P = 0.04$, Table 4). The SI was negatively correlated to sc abdominal fat ($P = 0.04$, Table 4), borderline negatively correlated with trunk fat ($P = 0.06$, Table 4) and visceral fat ($P = 0.09$, Table 4), and positively correlated to adiponectin expression ($P = 0.009$, Table 4).

Discussion

This study demonstrates, for the first time, that PCOS is associated with an increase in CD11c expression and CLS density in adipose tissue. The accumulation of macrophages in adipose tissue is the primary feature of the chronic low-grade inflammatory state frequently observed in obesity (5, 6). The inflammatory ATMs cluster in CLSs around dead adipocytes and express the CD11c marker (10, 11). These macrophages are known to secrete numerous inflammatory cytokines such as TNF-α, IL-6, and monocyte chemotactic protein-1 that lead to insulin resistance in peripheral tissues (5, 6). Ablation of CD11c macrophages normalizes insulin sensitivity in obese insulin-resistant mice (28). Recently, there is evidence that dendritic cells rather than macrophages may be the source of increased CD11c expression in adipose tissue (29). Nonetheless, adipose tissue CD11c expression and CLS density are strongly and positively correlated with the degree of obesity, insulin resistance and metabolic syndrome in humans (11). Of note, the increase in CD11c adipose tissue expression and CLS density in PCOS in our study cannot be attributed to obesity because women with PCOS had similar BMI compared to control women, and additionally, the majority were not obese. Our findings indicate that increased CD11c expression and CLS density in adipose tissue is a potentially unrecognized mechanism for insulin resistance in PCOS. Furthermore, both findings may be related to hyperandrogenism because both CD11c expression and CLS density were positively correlated to bioavailable testosterone levels.

Consistent with an increased inflammatory state in adipose tissue of women with PCOS, we also observed an increase in expression of integrin $\alpha 5$ and a borderline in-

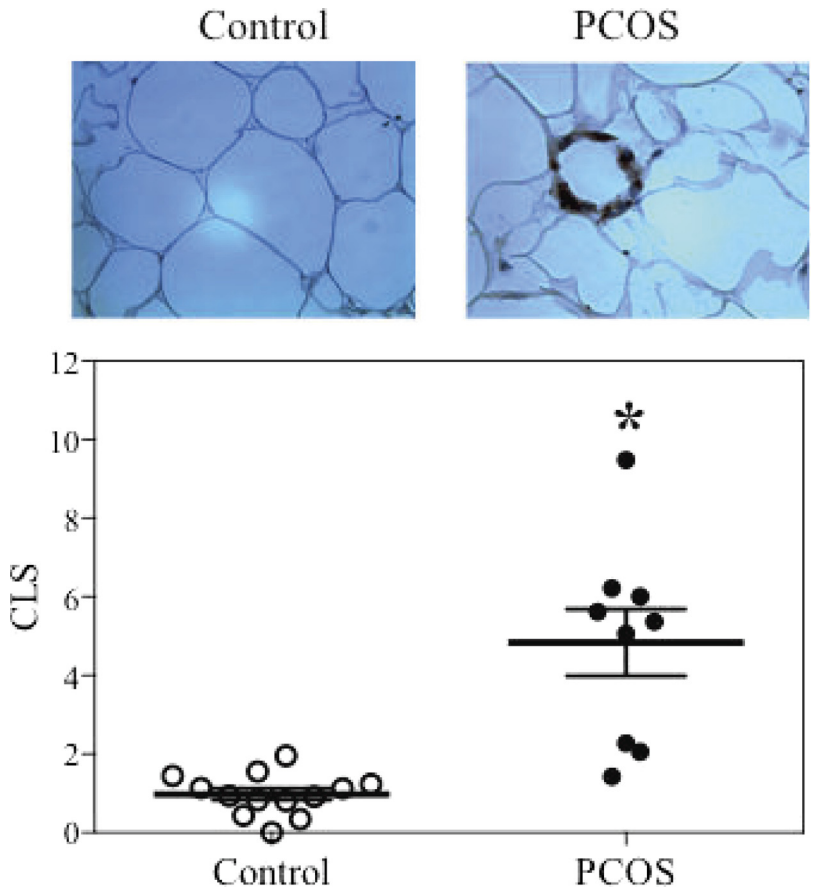

FIG. 2. *Top*, Photomicrograph representative of sc adipose tissue stained for CD68 showing a CLS in a PCOS subject. Magnification, ×40. Crown ATMs also stained positive for CD11c on contiguous slides (not shown). *Bottom*, Women with PCOS had significantly higher CLS density in sc adipose tissue compared with control women (*, $P = 0.001$).

crease in expression of TNF-α. The differences in TNF-α expression may have become significant with a larger sample size. TNF-α expression is increased in adipose tissue of obese mice (7) and humans (30) and has been shown to directly induce insulin resistance by inhibiting glucose disposal in peripheral tissues. CD11c macrophages in obese women have been shown to have high expression of integrins (11), and integrins such as α5 may be involved in activation of inflammatory signaling in ATMs and adipocytes (27). We did not detect an increase in the expression of CD14, CD206, and CD163 macrophages that are considered to have less proinflammatory phenotypes (11).

Expression of the insulin-sensitizing adipokine adiponectin was reduced in gluteal adipose tissue from women with PCOS. Consistent with this finding, we also detected lower circulating levels of serum adiponectin in women with PCOS compared with control women of similar BMI. Adiponectin is shown to down-regulate the expression of inflammatory cytokines and promote polarization of macrophages toward an antiinflammatory phenotype (31). Alternatively, cytokines such as TNF-α can reduce adiponectin expression leading to perpetuation of inflammation (32). In our study, adiponectin expression was reduced, whereas TNF-α expression was borderline increased in women with PCOS, indicating that the adipose tissue of these women has a more inflammatory milieu.

Additional findings of the study include increased amounts of visceral and sc abdominal fat in women with PCOS compared to control women of similar BMI. Our data are in contradiction to another study that reported no difference in visceral and sc abdominal fat in overweight women with PCOS compared with BMI-matched control women by MRI (33) but are consistent with previous reports that do show an increase in central fat in nonobese women with PCOS by dual-energy x-ray absorptiometry scan (34–36). The difference between the findings from these studies may be related to the degree of obesity. In our study, the majority of women were nonobese, similar to the study by Carmina *et al.* (36) who did detect an increase in central abdominal fat only in nonobese women with PCOS. Insulin sensitivity was lower in women with PCOS based on the SI, which is a more sensitive measure than fasting insulin levels, and this index was negatively correlated to both sc abdominal and visceral depots. Androgen excess has been associated with an increase in central fat depots in women. Increases in abdominal fat depots have been noted in postmenopausal women after receiving an anabolic steroid with weak an-

TABLE 4. Spearman's correlation coefficients between CD11c, CLS density, and SI and BMI, waist, trunk fat, bioavailable testosterone, visceral fat, sc abdominal fat, and adipose tissue adiponectin expression in women with PCOS (n = 14) and control women (n = 14)

Variable	CD11c	CLS	SI
BMI	0.32	0.44[a]	−0.19
Waist	0.24	0.14	−0.05
Trunk fat	0.21	0.38	−0.42[b]
Bioavailable testosterone	0.38[b]	0.51[a]	−0.28
Visceral fat	0.48[a]	0.63[a]	−0.43[c]
Subcutaneous abdominal fat	0.68[a]	0.52[a]	−0.51[a]
Adiponectin expression	0.06	−0.24	0.55[a]

[a] $P < 0.05$.

[b] $P = 0.06$.

[c] $P = 0.09$.

drogenic activity (37) as well as after administration of testosterone to female to male transsexuals (38).

The mechanism for the increase in adipose tissue CD11c expression and CLS density in women with PCOS remains to be determined. The CLSs were identified by contiguous staining for both CD11c and CD68, which is a specific macrophage marker, so the most likely source is macrophages, although dendritic cells cannot be ruled out (29). Obesity is an unlikely mediator because most women were nonobese and similar to control women for BMI. Sex differences in ATM infiltration have not been well studied, although in one report, men were found to have higher ATM density compared with BMI-matched women (39). In this study, we did observe a positive correlation between bioavailable testosterone, CD11c expression, and CLS density, raising the possibility that hyperandrogenism may be a contributing factor in this phenomenon. Additionally, we found positive correlations between both visceral and sc abdominal fat depots and CD11c expression and CLS density in gluteal adipose tissue. Adiponectin has been shown to promote macrophage polarization toward an antiinflammatory phenotype (31), and adiponectin expression is reduced with increasing visceral fat (32).

Our study has several limitations. Adipose tissue was obtained from the gluteal region and not central locations. Preferential infiltration of ATMs into omental fat has been well documented (40). However, a greater degree of CD11c infiltration in sc compared with visceral fat has also been reported in obese subjects with metabolic syndrome (11). Considering that we had planned to study a nonobese group of women, obtaining visceral fat would not have been possible. Furthermore, adiponectin expression is best studied in peripheral adipose tissue, so changes in gluteal expression may be more pertinent. The fact that ATM infiltration is increased in gluteal depots highly suggests that similar or greater differences would have been observed in central depots, which are considered to have greater metabolic risk. Additionally, the study may have lacked power to observe some differences in certain measures such as expression of adipose tissue TNF-α, fasting insulin levels, and waist circumference.

In summary, we report for the first time, that PCOS is associated with increased CD11c expression and CLS density in adipose tissue that appears to be independent of obesity. Adiponectin expression is also reduced in adipose tissue of women with PCOS and strongly correlates with the reduction in insulin sensitivity. Our findings also indicate that nonobese women with PCOS have an increase in abdominal fat depots that correlate with insulin sensitivity. The mechanisms for increased adipose tissue inflammation in PCOS are not well understood and should be subject to future studies.

Acknowledgments

We thank all the women for participating in this study. We also thank the nursing staff at University of Illinois Clinical Research Center for their invaluable help with all the studies.

Address all correspondence and requests for reprints to: Susan Sam, M.D., Section of Endocrinology, Diabetes, and Metabolism (MC 797), University of Illinois at Chicago, 1819 West Polk Street, Chicago, Illinois 60612. E-mail: susansam@uic.edu.

This project was supported by the following National Institutes of Health grants: K23 DK080988-01A1 to S.S., DK083328 to G.F., and UL1RR029879 to Clinical and Translational Science Award (CTSA) at University of Illinois.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Sam S, Dunaif A** 2003 Polycystic ovary syndrome: syndrome XX? Trends Endocrinol Metab 14:365–370
2. **Diamanti-Kandarakis E** 2006 Insulin resistance in PCOS. Endocrine 30:13–17
3. **Dunaif A, Segal KR, Futterweit W, Dobrjansky A** 1989 Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 38:1165–1174
4. **Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T** 1992 Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. Diabetes 41:1257–1266
5. **Olefsky JM, Glass CK** 2010 Macrophages, inflammation, and insulin resistance. Annu Rev Physiol 72:219–246
6. **Lumeng CN, Saltiel AR** 2011 Inflammatory links between obesity and metabolic disease. J Clin Invest 121:2111–2117
7. **Hotamisligil GS, Shargill NS, Spiegelman BM** 1993 Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 259:87–91
8. **Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H** 2003 Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112:1821–1830
9. **Lumeng CN, Bodzin JL, Saltiel AR** 2007 Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117:175–184
10. **Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS** 2010 Dynamic, M2-like remodeling phenotypes of $CD11c^+$ adipose tissue macrophages during high-fat diet-induced obesity in mice. Diabetes 59:1171–1181
11. **Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, Smyth GK, Wabitsch M, O'Brien PE, Harrison LC** 2010 Pro-inflammatory $CD11c^+CD206^+$ adipose tissue macrophages are associated with insulin resistance in human obesity. Diabetes 59:1648–1656
12. **Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, Coupaye M, Pelloux V, Hugol D, Bouillot JL, Bouloumié A, Barbatelli G, Cinti S, Svensson PA, Barsh GS, Zucker JD, Basdevant A, Langin D, Clément K** 2005 Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 54:2277–2286
13. **Klöting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, Stumvoll M, Blüher M** 2010 Insulin-sensitive obesity. Am J Physiol Endocrinol Metab 299:E506–E515
14. **Després JP, Lemieux I** 2006 Abdominal obesity and metabolic syndrome. Nature 444:881–887
15. **Fabbrini E, Magkos F, Mohammed BS, Pietka T, Abumrad NA, Patterson BW, Okunade A, Klein S** 2009 Intrahepatic fat, not vis-

ceral fat, is linked with metabolic complications of obesity. Proc Natl Acad Sci USA 106:15430–15435
16. **Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, Balletshofer B, Machicao F, Fritsche A, Häring HU** 2008 Identification and characterization of metabolically benign obesity in humans. Arch Intern Med 168:1609–1616
17. **Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, Sowers MR** 2008 The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999–2004). Arch Intern Med 168:1617–1624
18. **Zawadzki J, Dunaif A** 1992 Diagnostic criteria for polycystic ovary syndrome. Boston: Blackwell Scientific; 377–384
19. **Bergman RN, Phillips LS, Cobelli C** 1981 Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and β-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 68:1456–1467
20. **Bergman RN** 1989 Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38:1512–1527
21. **Cotler SJ, Guzman G, Layden-Almer J, Mazzone T, Layden TJ, Zhou XJ** 2007 Measurement of liver fat content using selective saturation at 3.0 T. J Magn Reson Imaging 25:743–748
22. **Yue L, Rasouli N, Ranganathan G, Kern PA, Mazzone T** 2004 Divergent effects of peroxisome proliferator-activated receptor γ agonists and tumor necrosis factor α on adipocyte ApoE expression. J Biol Chem 279:47626–47632
23. **Varma V, Yao-Borengasser A, Bodles AM, Rasouli N, Phanavanh B, Nolen GT, Kern EM, Nagarajan R, Spencer 3rd HJ, Lee MJ, Fried SK, McGehee Jr RE, Peterson CA, Kern PA** 2008 Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance. Diabetes 57:432–439
24. **Huang ZH, Reardon CA, Mazzone T** 2006 Endogenous ApoE expression modulates adipocyte triglyceride content and turnover. Diabetes 55:3394–3402
25. **Spencer M, Yao-Borengasser A, Unal R, Rasouli N, Gurley CM, Zhu B, Peterson CA, Kern PA** 2010 Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. Am J Physiol Endocrinol Metab 299:E1016–E1027
26. **Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Gyori G, Zlabinger GJ, Stulnig TM** 2007 Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. Int J Obes (Lond) 31: 1420–1428
27. **Zeyda M, Gollinger K, Todoric J, Kiefer FW, Keck M, Aszmann O, Prager G, Zlabinger GJ, Petzelbauer P, Stulnig TM** 2011 Osteopontin is an activator of human adipose tissue macrophages and directly affects adipocyte function. Endocrinology 152:2219–2227
28. **Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG** 2008 Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab 8:301–309
29. **Stefanovic-Racic M, Yang X, Turner MS, Mantell BS, Stolz DB, Sumpter TL, Sipula IJ, Dedousis N, Scott DK, Morel PA, Thomson AW, O'Doherty RM** 2012 Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in $CD11c^+$ cells in adipose tissue and liver. Diabetes 61:2330–2339
30. **Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA** 1996 The expression of TNFα by human muscle. Relationship to insulin resistance. J Clin Invest 97:1111–1116
31. **Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, Pedersen AA, Kalthoff C, Tullin S, Sams A, Summer R, Walsh K** 2010 Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. J Biol Chem 285:6153–6160
32. **Fantuzzi G** 2008 Adiponectin and inflammation: consensus and controversy. J Allergy Clin Immunol 121:326–330
33. **Barber TM, Golding SJ, Alvey C, Wass JA, Karpe F, Franks S, McCarthy MI** 2008 Global adiposity rather than abnormal regional fat distribution characterizes women with polycystic ovary syndrome. J Clin Endocrinol Metab 93:999–1004
34. **Kirchengast S, Huber J** 2001 Body composition characteristics and body fat distribution in lean women with polycystic ovary syndrome. Hum Reprod 16:1255–1260
35. **Rebuffé-Scrive M, Cullberg G, Lundberg PA, Lindstedt G, Björntorp P** 1989 Anthropometric variables and metabolism in polycystic ovarian disease. Horm Metab Res 21:391–397
36. **Carmina E, Bucchieri S, Esposito A, Del Puente A, Mansueto P, Orio F, Di Fede G, Rini G** 2007 Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. J Clin Endocrinol Metab 92:2500–2505
37. **Lovejoy JC, Bray GA, Bourgeois MO, Macchiavelli R, Rood JC, Greeson C, Partington C** 1996 Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women: a clinical research center study. J Clin Endocrinol Metab 81:2198–2203
38. **Elbers JM, Asscheman H, Seidell JC, Megens JA, Gooren LJ** 1997 Long-term testosterone administration increases visceral fat in female to male transsexuals. J Clin Endocrinol Metab 82:2044–2047
39. **Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, Coussieu C, Basdevant A, Bar Hen A, Bedossa P, Guerre-Millo M, Clément K** 2006 Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes 55:1554–1561
40. **Harman-Boehm I, Blüher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, Shai I, Klöting N, Stumvoll M, Bashan N, Rudich A** 2007 Macrophage infiltration into omental *versus* subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. J Clin Endocrinol Metab 92:2240–2247