Adipose Tissue Has Aberrant Morphology and Function in PCOS: Enlarged Adipocytes and Low Serum Adiponectin, But Not Circulating Sex Steroids, Are Strongly Associated with Insulin Resistance

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Context: Comprehensive characterization of the adipose tissue in women with polycystic ovary syndrome (PCOS), over a wide range of body mass indices (BMIs), is lacking. Mechanisms behind insulin resistance in PCOS are unclear.

Objective: To characterize the adipose tissue of women with PCOS and controls matched pair-wise for age and BMI, and to identify factors, among adipose tissue characteristics and serum sex steroids, that are associated with insulin sensitivity in PCOS.

Design/Outcome Measures: Seventy-four PCOS women and 31 controls were included. BMI was 18–47 (PCOS) and 19–41 kg/m^2 (controls). Anthropometric variables, volumes of subcutaneous/visceral adipose tissue (magnetic resonance imaging; MRI), and insulin sensitivity (clamp) were investigated. Adipose tissue biopsies were obtained to determine adipocyte size, lipoprotein lipase (LPL) activity, and macrophage density. Circulating testosterone, free testosterone, free 17β-estradiol, SHBG, glycerol, adiponectin, and serum amyloid A were measured/calculated.

Results: Comparison of 31 pairs revealed lower insulin sensitivity, hyperandrogenemia, and higher free 17β-estradiol in PCOS. Abdominal adipose tissue volumes/distribution did not differ in the groups, but PCOS women had higher waist-to-hip ratio, enlarged adipocytes, reduced adiponectin, and lower LPL activity. In regression analysis, adipocyte size, adiponectin, and waist circumference were the factors most strongly associated with insulin sensitivity in PCOS (R^2=0.681, $P < 0.001$).

Conclusions: In PCOS, adipose tissue has aberrant morphology/function. Increased waist-to-hip ratio indicates abdominal/visceral fat accumulation, but this is not supported by MRI. Enlarged adipocytes and reduced serum adiponectin, together with a large waistline, rather than androgen excess, may be central factors in the pathogenesis/maintenance of insulin resistance in PCOS. (***J Clin Endocrinol Metab*** **96: E304–E311, 2011)**

Polycystic ovary syndrome (PCOS) affects 5–10% of women of reproductive age (1). The three main characteristics are hyperandrogenism, ovulatory dysfunction, and polycystic ovarian (PCO) morphology; according to the 2003 Rotterdam criteria, at least two are required for diagnosis (2). PCOS is associated with obesity and insulin resistance, which increase the risk for type 2 diabetes and possibly cardiovascular disease (1). The pathogenesis of PCOS is unknown but may involve a genetic predisposition influenced by gestational environment, lifestyle factors, or both (1).

About 50% of women with PCOS are overweight or obese, though the figure varies somewhat between countries and ethnic groups (1). Most women with PCOS are thought to have an abdominal body fat distribution, regardless of body mass index (BMI) (3). However, mag-

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Abbreviations: BMI, Body mass index; CMIA, chemiluminescent microparticle immunoassay; GDR, glucose disposal rate; HOMA, homeostasis model assessment; hs-CRP, high-sensitivity C-reactive protein; LPL, lipoprotein lipase; MRI, magnetic resonance imaging; PCO, polycystic ovarian; PCOS, polycystic ovary syndrome; SAA, serum amyloid A.

netic resonance imaging (MRI) of PCOS women and controls matched for BMI and fat mass showed no difference in body fat distribution (4). Both groups had a global distribution, despite significant differences in insulin resistance, but were not matched for age, which may influence abdominal adiposity and insulin sensitivity (4).

Other features of adipose tissue in women with PCOS may be aberrant. In a small group of young nonobese women with PCOS, the lipolytic effect of catecholamines was decreased in sc adipocytes and increased in visceral adipocytes, favoring release of fatty acids from visceral depots (5). Subcutaneous fat cells were enlarged by about 25%, possibly reflecting the lipolytic catecholamine resistance (5). In other small studies, adipocytes were enlarged (6) or of normal size (7). Adipose tissue lipoprotein lipase (LPL) activity, reflecting delivery of fatty acids or monoacylglycerol, is reported to be unchanged or reduced, respectively, in women with PCOS (8, 9). A meta-analysis suggested that serum adiponectin levels are lower in women with PCOS than controls after controlling for BMI (10), indicating disturbed release of certain adipokines from adipose tissue. Low-grade inflammation in PCOS has also been hypothesized (11), but macrophage infiltration of adipose tissue has not been investigated in women with PCOS. Thus, analysis of adipose tissue features in women with PCOS has yielded inconsistent results, and comprehensive studies over a wide range of BMIs are lacking.

Insulin resistance with compensatory hyperinsulinemia is the major metabolic abnormality in PCOS, but the underlying mechanisms are unclear (12). A key candidate is hyperandrogenemia. Exposure of females to androgens causes insulin resistance in animal models (13), and antiandrogen treatment increases insulin sensitivity in women with PCOS (14). Obesity and adipose tissue characteristics are other candidates, although women with PCOS display insulin resistance regardless of BMI (15). An intrinsic defect in insulin action has also been suggested (12).

In this study, we characterized adipose tissue in women with PCOS and controls, matched pair-wise for age and BMI, in terms of distribution, adipocyte size, *in vivo* lipolytic activity (serum glycerol), LPL activity, circulating levels of the adipokines adiponectin and serum amyloid A (SAA), and macrophage density. We also sought to identify, among these characteristics as well as circulating sex steroids and SHBG, factors associated with insulin sensitivity in PCOS.

Materials and Methods

Subjects

The study was conducted at Sahlgrenska Academy, University of Gothenburg. Women with PCOS and controls were recruited by advertising in local newspapers and in frequently visited places in the community. Potential participants were asked to describe their medical history and underwent a gynecological examination and two-dimensional transvaginal ultrasonography (HDI 5000, ATL, Bothell, WA) to investigate ovarian morphology.

Inclusion criteria for women with PCOS were PCO morphology (12 or more 2- to 9-mm follicles or >10 ml in volume, in at least one ovary) and clinical signs of hyperandrogenism (hirsutism, acne) and/or oligo/amenorrhea (2). Hirsutism was defined as a Ferriman Gallwey score ≥8 (16). Acne was determined by an affirmative answer to the question *Do you have excessive acne?* Oligomenorrhea was defined as an intermenstrual interval >35 days and <8 menstrual bleedings in the past year. Amenorrhea was defined as absent menstrual bleeding or none in the past 90 days.

Exclusion criteria for controls were evidence of PCO, excessive acne or hirsutism, or menstrual irregularities (cycles >35 days). Exclusion criteria for all women were age <18 or >37 years, pharmacological treatment within 12 weeks (including hormonal contraceptives, naturopathic preparations, and homeopathic substances), breast feeding within 24 weeks, cardiovascular disease, diabetes mellitus, or other endocrine disorders (*e.g.*, congenital adrenal hyperplasia, Cushing's syndrome, or androgen-secreting tumors). Seventy-four women with PCOS and 31 controls were included. The controls were matched pairwise to 31 women with PCOS by age (±5 yr) and BMI (±2 kg/m^2). The majority of the pairs were matched continuously during enrollment. Six pairs were matched after inclusion of all participants to meet the matching criteria.

All participants gave oral and written consent. The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Ethical Review Board, University of Gothenburg.

Study procedure

Participants were examined, including blood and adipose tissue sampling, in the morning after an overnight fast. Controls were examined during the early follicular phase (d 1–7 of the menstrual cycle) with the exception of MRI, which was performed during d 11–17 of the menstrual cycle. Because most women with PCOS had oligo/amenorrhea, the examination day was chosen independently of cycle day.

Anthropometry

Body height, body weight, waist and hip circumferences, sagittal diameter, BMI, and waist-to-hip ratio were measured/calculated by standard protocols.

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Abdominal adipose tissue volumes

MRI was performed with a 1.5-Tesla scanner (Intera; Philips Medical Systems, Best, The Netherlands). For abdominal adipose tissue imaging, an axial multi-slice T1-weighted gradient echo acquisition, consisting of 16 10-mm sections without gaps centered at the L4–L5 intervertebral disk level, was performed during breath hold. The repetition time was 129 ms, echo time 4.6 ms, flip angle 80 degree, and total acquisition time 16.1 s. The whole-body coil was used for signal transmission and reception. MRI data were transferred to a workstation to quantify total, visceral, and sc adipose tissues as described (17).

Insulin sensitivity

The euglycemic hyperinsulinemic clamp and homeostasis model assessment (HOMA) were used to assess insulin sensitivity. The clamp examination was performed as described (18). Insulin (Actrapid, 100 IU/ml; Novo Nordisk, Bagsvaerd, Denmark) was infused at 500 mU/ml in isotonic saline containing 2 ml of plasma from the subject to prevent insulin loss. A 10-min primed insulin infusion was followed by a constant infusion (0.12 U/kg body weight/min) for 120 min. Blood glucose levels were determined before infusion, every 10 min during the first 90 min of infusion, and every 5 min during the last 30 min. Euglycemia (5.5 mmol/liter) was maintained by infusing 20% glucose (1.1 mol/liter); the rate was adjusted to maintain a glucose level of 5.5 mmol/liter. The glucose infusion rate during the last 30 min (steady-state), normalized to body weight, was used to determine the glucose disposal rate (GDR, mg/kg $\times$ min). HOMA was calculated as fasting insulin (mU/liter) $\times$ fasting glucose (mmol/liter)/22.5 (19). For logistical reasons, 32 of 74 women with PCOS (including four in matched pairs) and one control did not undergo clamp evaluation. Simple regression analysis revealed that logHOMA was strongly associated with *clamp*-derived GDR ($R = 0.71$, $P = 7.6 \times 10^{-20}$) and GDR could therefore be predicted for these participants.

Adipose tissue biopsy

A needle biopsy of sc abdominal adipose tissue was obtained under local anesthesia two-thirds of the distance from the iliac crest to the umbilicus. The specimen was prepared as described below.

Adipocyte size

Mean adipocyte diameter was determined by computerized image analysis (20). The cell suspension was placed between a siliconized glass slide and a cover slip and transferred to the microscope stage (Axioplan 2 imaging, Carl Zeiss, Oberkochen, Germany; $\times 5$ objective). Nine random fields were photographed with a CCD camera (Axiocam, Carl Zeiss). Images were analyzed with Leica software (QWin V3, Leica Microsystems, Wetzlar, Germany). Validation of the Leica analysis macro revealed that the correlation coefficients for results obtained with the previously used Zeiss macro and the currently used Leica macro were 0.99589–0.99990. Microspheres 98.00 μm in diameter (Dynal, Invitrogen, Oslo, Norway) served as a reference. Mean adipocyte volume was calculated with the Goldrick formula (21).

LPL activity

Adipose tissue LPL activity was measured as described (22). Samples from matched pairs were analyzed in the same assay to avoid interassay variation. One mU of lipase activity represents 1 nmol of fatty acids released per minute.

Immunohistochemistry

Adipose tissue specimens were fixed with neutral-buffered formalin, embedded in paraffin, and sectioned at 5 μm. After heat-induced antigen retrieval, sections were incubated with monoclonal antibody against human CD68 (clone PG-M1, DakoCytomation, Denmark) to detect macrophages. HRP-Envision (DakoCytomation) was used as the secondary antibody and diaminobenzidine/H_2O_2 for detection. Sections were counterstained with hematoxylin, mounted, scanned (Mirax Desk Digital Slide Scanner, Zeiss, Göttingen, Germany), and analyzed (Mirax Viewer, Zeiss). Macrophages (immunoreactive CD68-positive cells) and crown-like structures (adipocyte surrounded by at least three macrophages) were counted by a single observer (blinded to the source of the section) and normalized for analyzed section area.

Biochemical assays

Serum SHBG was measured with a chemiluminescent microparticle immunoassay (CMIA) (Architect SHBG reagent kit; Abbott Laboratories, Diagnostic Division, Chicago, IL); adiponectin with a human adiponectin immunoassay (R&D Systems, Minneapolis, MN); SAA with a human SAA enzyme-linked immunosorbent assay (Biosource International, Nivelles, Belgium); glycerol with a Randox radiometric glycerol *kit* (GY105; Crumlin, UK) and a Konelab 30 autoanalyzer (Thermo Clinical Labsystems, Vantaa, Finland); high-sensitivity C-reactive protein (hs-CRP) with a particle-enhanced immunoturbidimetric assay (Tina-quant C-reactive protein (latex) high-sensitive assay; Roche Diagnostics); and insulin with an immunometric two-step sandwich method and chemiluminescence technology (Advia Centaur Insulin ReadyPack; Bayer HealthCare, Tarrytown, NY). Plasma glucose was measured at 37 C with an enzymatic photometric method (Roche Diagnostics, Mannheim, Germany). Gas chromatography/mass spectroscopy (GC-MS) was used to analyze serum testosterone (limit of detection, 0.02 ng/ml) and serum 17β-estradiol (limit of detection, 1.00 pg/ml). Free testosterone and free estradiol were calculated as described by Vermeulen *et al.* (23) and Van den Beld *et al.* (24). These assays, and corresponding results, for circulating levels of testosterone, 17β-estradiol, and SHBG, and calculated free testosterone in the participants of the present study have been described (25).

Statistical analyses

Thirty-one controls were matched pair-wise to 31 women with PCOS. The matching resulted in a nonnegative correlation between the value of the women with PCOS, on any variable, and the corresponding value of her control. Thus, the difference between women with PCOS and controls could be analyzed with a nonpaired instead of a paired test. A nonpaired test provides higher statistical power if the matching variables are of small importance (resulting in a low correlation between PCOS women and controls) and if the number of individuals missing her match is large enough. However, analysis of confidence intervals for the two options revealed that the paired test was more powerful in almost all cases. Thus, for pair-wise comparisons between the BMI- and age-matched cases and controls, paired *t* tests were used.

To identify the relative independent determinants of insulin sensitivity in women with PCOS (n = 74), linear regression analyses were performed with GDR as the dependent variable and

MRI-estimated abdominal adipose tissue volumes, anthropometric variables, and other variables related to adipose tissue and sex steroids as covariates. All variables were skewed, except age, height, and adipocyte volume, and transformed before analysis. To transform continuous variables to normally distributed variables, we used $\Phi^{-1}[F(x)]$, where Φ^{-1} is the inverse of the standardized normal distribution function and F is the empirical distribution function. That transformation yielded an almost perfect normal distribution, which is not the case for the logarithmic transformation. $P < 0.05$ was considered significant. All statistical analyses were conducted in SPSS (v17.0 for Windows, SPSS, Chicago, IL).

Results

PCOS phenotypes and matching

All 74 women with PCOS had PCO morphology; 17 (11 with a matched control) had signs of hyperandrogenism, seven (three with a matched control) had oligo/amenorrhea, and 50 (17 with a matched control) had both hyperandrogenism and oligo/amenorrhea.

The BMI was 18.2–47.4 kg/m^2 in women with PCOS and 18.2–40.4 kg/m^2 in those with a matched control. All 31 pairs met the BMI criterion (±2 kg/m^2). The BMI difference (PCOS *vs.* control) ranged from −1.86 to 1.75 kg/m^2 (mean, 0.11 ± 1.04 kg/m^2). The proportion of overweight/obesity (BMI ≥25 kg/m^2) was 51% in women with PCOS and 39% in those with a matched control. The age range was 21–37 years in women with PCOS, also in those with matched controls. All 31 pairs met the age criterion (±5 years). The age difference (PCOS *vs.* control) ranged from −5 to 5 years (mean, 0.65 ± 2.60 years).

Characteristics of women with PCOS and matched controls

Age, anthropometric variables, metabolic and hormonal status, and adipose tissue characteristics of women with PCOS and matched controls are shown in Table 1. Women with PCOS had markedly higher levels of serum testosterone, free testosterone, and free 17β-estradiol, lower levels of serum SHBG, and lower insulin sensitivity determined by the clamp technique. The waist-to-hip ratio was higher in women with PCOS, but there were no other differences in anthropometric variables or adipose tissue volumes/distribution between the groups (Table 1). Because of logistic difficulties, one woman with PCOS did not undergo MRI examination and was excluded from analysis of adipose tissue volumes.

TABLE 1. Characteristics of women with PCOS and controls pair-wise matched for age and body mass index

Variable	PCOS (n = 31)	Controls (n = 31)	No. of pairs with missing data	P[a]
Age, y	28.5 ± 3.6	27.8 ± 3.5	0	0.178
Body mass index, kg/m^2	24.8 ± 4.8	24.7 ± 4.9	0	0.093
Weight, kg	69.8 ± 13.4	70.9 ± 16.9	0	0.997
Height, cm	167.9 ± 6.4	169.0 ± 6.5	0	0.295
Waist circumference, cm	83.5 ± 11.4	82.8 ± 11.3	0	0.682
Hip circumference, cm	102.7 ± 8.4	105.3 ± 10.2	0	0.128
Waist-to-hip ratio	0.81 ± 0.06	0.78 ± 0.06	0	0.034
Sagittal diameter, cm	18.2 ± 3.0	17.9 ± 2.8	0	0.222
MRI-determined adipose tissue volumes and ratios				
Total abdominal, liter	4.66 ± 2.09	4.64 ± 2.50	1	0.375
Subcutaneous abdominal, liter	3.73 ± 1.76	3.77 ± 2.02	1	0.757
Visceral, liter	0.92 ± 0.48	0.85 ± 0.59	1	0.099
Visceral/total abdominal	0.20 ± 0.06	0.18 ± 0.07	1	0.199
Visceral/subcutaneous abdominal	0.26 ± 0.10	0.23 ± 0.12	1	0.191
Serum glycerol, μmol/liter	73 ± 29	64 ± 22	0	0.200
Adipose tissue lipoprotein lipase activity, mU/g tissue	22.3 ± 15.2	33.0 ± 18.9	2	0.031
Adipocyte volume, picoliter	590 ± 187	536 ± 166	4	0.036
Adipose tissue macrophage density, no./mm^2[b]	8.22 ± 4.42	7.68 ± 4.47	12	0.535
Serum adipokines				
Adiponectin, μg/ml[b]	9.55 ± 5.32	13.32 ± 7.66	3	0.016
Serum amyloid A, μg/ml[b]	19.9 ± 14.8	20.4 ± 13.8	3	0.449
Glucose disposal rate, mg/kg × min	11.0 ± 3.0	13.0 ± 4.1	0	0.009
Serum sex steroids and binding protein				
Testosterone, ng/ml	0.43 ± 0.19	0.21 ± 0.08	0	<0.001
Sex hormone binding globulin, nmol/liter	49.0 ± 25.1	69.4 ± 30.4	0	0.001
Free testosterone, pmol/liter	23.3 ± 12.9	8.7 ± 5.2	0	<0.001
Free 17β-estradiol, nmol/liter	4.69 ± 2.43	1.81 ± 0.82	0	<0.001

Plus–minus values are means ± SD.

[a] Paired sample t test (performed with transformed data, except for age, height, and adipocyte volume).

[b] Three pairs including women with serum hs-CRP >10 mg/liter were excluded.

Because previous reports are inconsistent or lacking, adipose tissue characteristics were further evaluated. The mean volume of abdominal sc adipocytes was greater in women with PCOS than in controls (Table 1). Regarding the distribution of adipocyte diameter, there was a significant difference in the mean between the groups ($P = 0.029$) but not in SD ($P = 0.763$), skewness ($P = 0.696$), or kurtosis ($P = 0.271$). Thus, the adipocyte size distribution for a woman with PCOS was translated to the right compared with that of her control, but there were no differences in the distribution shape. The mean diameter of reference microspheres was 98.3 ± 0.26 μm (range 97.9–98.8 μm; n = 50). In each cell population, 1076–3407 cells (mean 2217 ± 590) were analyzed. Owing to limited amounts of adipose tissue and technical reasons, four pairs were not included in the analysis of adipocyte size.

The circulating concentration of glycerol, an indirect marker of lipolytic activity, was similar in the groups. Women with PCOS had lower adipose tissue LPL activity (Table 1). In two pairs, limited amounts of adipose tissue precluded analysis of LPL activity.

Serum adiponectin was lower in women with PCOS, while serum SAA was similar in the groups (Table 1). Macrophage density (number of CD68-positive cells normalized for section area), was also similar in the groups (Table 1, Fig. 1). Intact adipose tissue sections were obtained from the majority of the biopsies. Crown-like structures were observed in three of 19 women with PCOS (range, one to five crown-like structures/section) and in two of 19 controls (range, one to two crown-like structures). In cases of poor section quality, macrophage density was not analyzed (nine pairs excluded). Two women with PCOS and one control among the pairs had serum *hs-CRP* >10 mg/liter, indicating acute phase, and were excluded from analyses of adiponectin, SAA, and macrophage density (three pairs excluded). Macrophage density was analyzed in 19 pairs. There was no difference in circulating levels of hs-CRP between women with PCOS and controls (1.18 ± 1.25 *vs.* 0.85 ± 0.71 mg/liter, $P = 0.336$, n = 28).

Factors involved in insulin resistance

A linear regression analysis was performed to identify factors associated with insulin sensitivity (GDR) in women with PCOS. Anthropometric variables, abdominal adipose tissue volumes assessed by MRI, all other adipose tissue characteristics, and sex steroid-related variables were initially included. Adipose tissue volumes and macrophage density did not contribute to the models and were excluded from the final analysis to allow inclusion of more women with PCOS. Exclusion of these variables did not change the outcome, and β-standardized coefficients and determination coefficients (R^2) were only marginally influenced. A complete data set from 60 women with PCOS was processed in the final analysis (missing information on adipocyte size in 10 women, owing to limited amounts of adipose tissue and technical/logistic reasons, and hs-CRP >10 mg/liter in four women) (Table 2). The best regression model included adipocyte volume, serum adiponectin, and waist circumference; adipocyte volume and waist circumference were negatively, and serum adiponectin was positively, associated with insulin sensitivity (Table 2). Adipocyte volume was the variable most strongly associated with insulin sensitivity (GDR) in women with PCOS (Table 2, Fig. 2).

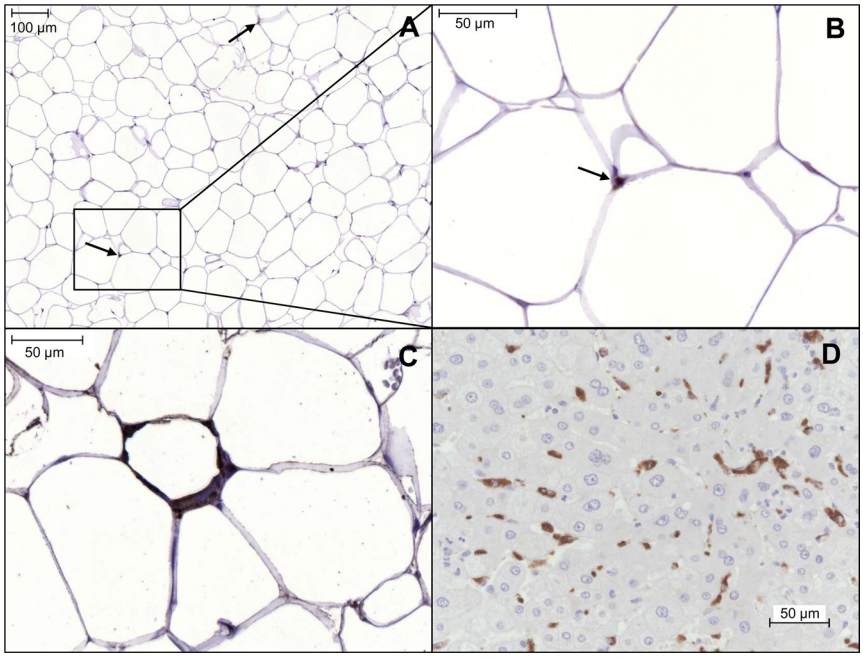

FIG. 1. CD68 immunoreactivity of human adipose tissue and liver (positive control). Sections were counterstained with hematoxylin. Positive signal appears brown. A, Adipose tissue section. *Arrows* indicate scattered macrophages. B, High-power image of macrophage (*arrow*) residing between adipocytes. C, High-power image of adipocyte surrounded by macrophages, forming a crown-like structure. D, Kupffer cells of human liver.

Discussion

This study shows that the adipose tissue of women with PCOS displays aberrant morphology and function compared with controls matched pair-wise for age and BMI. Women with PCOS had larger adipocytes, lower serum adiponectin levels, and lower adipose tissue LPL activity. Women with PCOS also had an increased waist-to-hip ratio

TABLE 2. Factors most strongly associated with insulin sensitivity in women with PCOS

Model and variables	β	R^2
Model I		
Adipocyte volume	−0.740[a]	0.548[a]
Model II		
Adipocyte volume	−0.658[a]	0.637[a]
Adiponectin	0.310[a]	
Model III		
Adipocyte volume	−0.439[a]	0.681[a]
Adiponectin	0.281[a]	
Waist circumference	−0.311[b]	

Multiple linear regression (stepwise) including age, height, weight, BMI, waist, hip, waist-to-hip ratio, sagittal diameter, testosterone, SHBG, free testosterone, free 17β-estradiol, glycerol, LPL activity, adiponectin, SAA, and adipocyte volume as independent variables. The inclusion criterion for the model was an F probability of 0.05; the exclusion criterion was an F probability of 0.1. β, β standardized coefficient; R^2, determination coefficient.

[a] $P < 0.001$; [b] $P < 0.01$, $n = 60$.

but no other differences in anthropometric variables or abdominal adipose tissue volumes and distribution were observed. Adipocyte size, circulating adiponectin, and waist circumference were the variables most strongly associated with insulin sensitivity in women with PCOS. Thus, the adipose tissue related factors enlarged fat cells, reduced serum adiponectin, and a large waistline may be more important than hyperandrogenemia in development or maintenance of insulin resistance in PCOS.

An increased waist-to-hip ratio, as reported in women with PCOS in the present study, indicates an abdominal/visceral body fat distribution. However, this indication was not supported by MRI; abdominal adipose tissue volumes were similar in the groups. Gluteofemoral adipose tissue volumes were not assessed in the present study why it cannot be excluded that women with the syndrome store less fat in this region. Fat accumulation on the trunk and in visceral depots has been reported in women with PCOS regardless of BMI, possibly in part explaining their insulin resistance (26–28); in those studies, adiposity and fat distribution were assessed with a lipometer (26), by ultrasonography (27), or by dual energy x-ray absorptiometry (28). In one study, investigation of fat distribution with MRI in women with PCOS and controls, matched for BMI and fat mass, revealed no difference in fat accumulation in visceral, abdominal sc, and gluteofemoral depots, despite significant differences in insulin resistance (4), consistent with our findings. We used a multi-slice protocol, instead of the commonly used single-slice approach (4), to determine adipose tissue volumes, which allowed collection of data from the major part of the abdomen. The MRI technique allows area and volume determinations of specific adipose tissue compartments with high precision and reproducibility. Clearly, the view of an increased abdominal and visceral fat accumulation in PCOS should be reevaluated.

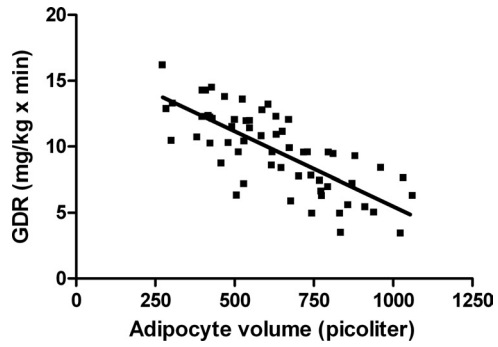

FIG. 2. Correlation between GDR and adipocyte size in women with PCOS. R = 0.753, $P = 3.9 \times 10^{-12}$, n = 60.

Adipocyte size predicts incidence of type 2 diabetes in women (29). Using a computerized technique that allows rapid analysis of 10-fold more cells than conventional methods, we observed an increased mean sc adipocyte size in women with PCOS and revealed that this is attributable to a rightward shift of the adipocyte size distribution curve. These results suggest that adipose tissue in PCOS is characterized by aberrant cellularity, which probably alters adipose tissue function and most likely contributes to metabolic disturbances and the increased risk of type 2 diabetes in women with the syndrome.

Adipocyte size reflects the balance between triglyceride storage and mobilization. LPL controls the delivery of fatty acids, derived from circulating triglycerides in plasma lipoproteins, to adipose tissue (30). In women with PCOS, adipose tissue LPL activity was lower than in controls, consistent with an inhibitory effect of androgens (31). A contributing factor to the lower LPL activity could be that women with PCOS have fewer adipocytes per volume of tissue. In patients with type 2 diabetes, reduced adipose tissue LPL activity, resulting in postprandial dyslipidemia, has been reported (32), as well as a tendency toward lower LPL activity in adipose tissue homogenates and post-heparin plasma (33). The circulating glycerol level, an indirect marker of *in vivo* lipolytic activity, was reported to be one-third lower in nonobese, otherwise healthy women with PCOS than in controls (5), possibly reflecting catecholamine resistance in sc fat cells (5). Circulating glycerol levels were similar in the groups we studied, possibly as a result of insulin resistance in women with PCOS giving a net effect of unchanged glycerol levels.

Adiponectin is secreted from adipocytes and has antidiabetic, antiinflammatory, and antiatherogenic properties. Further, circulating adiponectin levels are inversely associated with body weight and insulin resistance (34). Conflicting results regarding adiponectin levels in women with PCOS have been reported (35). However, in a recent meta-analysis, and in line with our findings, adiponectin

levels were lower in women with PCOS after controlling for BMI (10). It is interesting to note that high-molecular-weight adiponectin, the multimer most closely associated with insulin sensitivity, has been reported to be selectively reduced in women with PCOS independent of BMI and insulin resistance (36). Further, testosterone has been demonstrated to impede the secretion of high-molecular-weight adiponectin from *in vitro* differentiated rat adipocytes (37).

SAA is an acute-phase protein produced by the liver in response to inflammatory stimuli (38). However, in the nonacute phase, adipose tissue may be the major source of SAA (39), and SAA mRNA expression is markedly higher in large than in small adipocytes (40). SAA has been implicated in inflammation, insulin resistance, and atherosclerosis (41) and could therefore be a direct link between obesity and its comorbidities. In our study, circulating levels of SAA and hs-CRP were similar in the groups. Thus, except for low adiponectin levels, we found no signs of inflammation in the circulation of women with PCOS.

The hypothesis that an increased abundance of macrophages in adipose tissue contributes to insulin resistance and low-grade inflammation is primarily based on studies in rodents (42). However, correlations between macrophage density in human adipose tissue and BMI and adipocyte size have been reported (42). The macrophage density in adipose tissue was similar in the PCOS and control groups, consistent with the absence of an inflammatory status in the PCOS group.

As confirmed in the present investigation euglycemic clamp studies demonstrated insulin resistance in women with PCOS regardless of BMI (15). Hyperandrogenemia is a potential key cause, but other mechanisms are likely involved (12). In a regression analysis to identify factors associated with insulin sensitivity in women with PCOS, adipocyte size, circulating adiponectin, and waist circumference constituted the best model. Interestingly, the sex steroids and SHBG were excluded in this multivariate analysis. Thus, adipose tissue features, particularly hypertrophic adipocytes, low secretion of adiponectin, and a large waistline, may have a strong negative impact on insulin sensitivity in women with PCOS.

The inclusion of women with untreated PCOS, over a wide range of BMIs, the pair-wise matching by age and BMI, and the rigorous inclusion/exclusion criteria are strengths of the present study. The fact that several metabolic and endocrine features were investigated in the same group of women with PCOS is another advantage, as is the use of several gold standard techniques. Matching the pairs for body fat would have further strengthened our findings. Because women receiving hormonal or other treatments were excluded, the observations cannot be generalized to those who are on treatment.

In summary, the adipose tissue of women with PCOS displays aberrant morphology and function compared with age/BMI-matched controls; women with PCOS have enlarged adipocytes, reduced serum adiponectin, and lower adipose tissue LPL activity. An increased waist-to-hip ratio indicates abdominal/visceral fat accumulation in women with the syndrome but this is not supported by MRI. Adipose tissue abnormalities in PCOS— enlarged fat cells and reduced serum adiponectin—together with a large waistline, rather than androgen excess, may be central factors in the pathogenesis or maintenance of insulin resistance in women with PCOS.

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