Soluble CD36 and Risk Markers of Insulin Resistance and Atherosclerosis Are Elevated in Polycystic Ovary Syndrome and Significantly Reduced During Pioglitazone Treatment

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OBJECTIVE — We investigated the relation between soluble CD36 (sCD36), risk markers of atherosclerosis and body composition, and glucose and lipid metabolism in polycystic ovary syndrome (PCOS).

RESEARCH DESIGN AND METHODS — Thirty PCOS patients were randomized to 30 mg/day pioglitazone or placebo for 16 weeks. Fourteen weight-matched healthy female subjects were included as control subjects. sCD36, oxidized LDL (oxLDL), high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6, euglycemic-hyperinsulinemic clamps, and whole-body dual-energy X-ray absorptiometry scans were performed.

RESULTS — sCD36 (2.87 relative units [0.88–9.36] vs. 1.67 relative units [0.72–3.89]), oxLDL (44.9 units/l [26.9–75.1] vs. 36.1 units/l [23.4–55.5]), and hsCRP (0.26 mg/dl [0.03–2.41] vs. 0.12 mg/dl [0.02–0.81]) were significantly increased in PCOS patients versus control subjects (geometric mean ± 2 SD). In PCOS, positive correlations were found between central fat mass and sCD36 ($r = 0.43$), hsCRP ($r = 0.43$), and IL-6 ($r = 0.42$) (all $P < 0.05$). After adjusting for fat mass, sCD36 and oxLDL correlated inversely with measures of insulin-stimulated glucose metabolism and positively with lipid oxidation during insulin stimulation in PCOS patients and control subjects ($n = 44$). sCD36 and oxLDL were significant independent predictors of glucose and lipid metabolism, whereas hsCRP and IL-6 showed no significant contribution.

Following pioglitazone treatment, insulin sensitivity increased, whereas sCD36 (3.21 relative units [0.76–13.6] vs. 2.33 relative units [0.84–6.46]) and hsCRP decreased ($P < 0.05$). No significant changes were measured in body composition.

CONCLUSIONS — sCD36 and oxLDL correlated with measures of insulin sensitivity independent of central fat mass. Pioglitazone treatment reduced sCD36 while improving insulin-stimulated glucose metabolism, further supporting the association between sCD36 and insulin resistance in PCOS.

Polycystic ovary syndrome (PCOS) is characterized by anovulation, hyperandrogenemia, and/or polycystic ovaries. About 50% of PCOS patients fulfill the criteria of the metabolic syndrome. A five- to eightfold increased risk for type 2 diabetes in PCOS patients compared with weight-matched female control subjects makes the syndrome of high socioeconomic importance (1). Previous studies found significantly increased risk of hypertension and coronary heart disease in women with irregular cycles, suggesting a 4- to 11-fold increased risk for coronary heart disease (2). Increased risk factors for coronary heart disease in PCOS patients were documented in several previous studies, including atherogenic lipid profiles, increased coronary artery calcification, and echocardiographic abnormalities (3).

Abdominal obesity is seen in overweight and in most normal-weight PCOS patients. Abdominal obesity, especially visceral adiposity, is associated with insulin resistance and increased levels of inflammatory markers including high-sensitive C-reactive protein (hsCRP) and cytokines. Previous studies (4,5) reported higher levels of inflammatory markers in PCOS patients compared with weight-matched control subjects.

CD36 is expressed on the surface of monoytes and macrophages (6). CD36 may initiate atherosclerotic lesions and be an important risk factor of cardiovascular disease (6). The foam-cell formation process is initiated and enhanced by the binding of oxidized LDL (oxLDL) to CD36 receptors (6). In mice lacking the CD36 receptor, foam-cell formation and vascular lesion development are interrupted (7).

In ob/ob mice, increased CD36 expression on macrophages was caused by defective insulin signaling, which was reverted during peroxisome proliferator–activated receptor-γ agonist treatment (8). These results suggested important relations between decreased insulin sensi-

tivity, foam-cell formation, and, possibly, future atherosclerosis

Based on a hypothesis that CD36 could be released into circulation as part of a low-grade inflammatory state with increased macrophage activation in patients with type 2 diabetes, Handberg et al. (9) developed an assay for the measurement of soluble CD36 (sCD36) in plasma. Handberg et al. found a significant inverse correlation between sCD36 and insulin-stimulated glucose disposal. sCD36 correlated positively with insulin and BMI, but no measures of central fat mass were performed. BMI and fasting plasma glucose were independent predictors of sCD36 levels, thus suggesting a correlation between obesity, poor glycemic control, and accelerated atherosclerosis risk in patients with type 2 diabetes (9).

Increased insulin sensitivity and decreased insulin levels are followed by improved ovulatory function in PCOS. Few studies exist evaluating the effects of peroxisome proliferator–activated receptor-γ treatment on inflammatory markers in PCOS (10,11). In the present study, we tested the hypothesis that PCOS patients had significantly higher sCD36 levels than control subjects and that sCD36 correlated with fat mass and measures of glucose metabolism. oxLDL, hsCRP, and interleukin (IL)-6 levels were furthermore included to evaluate possible mechanisms for increased inflammation in PCOS and how this is affected by peroxisome proliferator–activated receptor-γ treatment.

RESEARCH DESIGN AND METHODS

— The study subjects have previously been described in two articles on insulin sensitivity, IGF-1, and growth hormone levels during pioglitazone treatment in PCOS patients (12,13). In brief, 30 reproductive-age Caucasian women with PCOS were included. Criteria for PCOS were irregular periods with cycle length >35 days and testosterone above reference interval and/or hirsutism. Included patients had elevated fasting insulin and/or were obese. Fourteen healthy, Caucasian, premenopausal women matched to PCOS patients for BMI and age were studied as control subjects. All control subjects had regular menses (period lengths 28–34 days) and did not suffer from hyperandrogenemia or hirsutism. The study was approved by the local ethics committee and by the Danish Medicines Agency, and all subjects gave written informed consent.

After initial examination (see description below), patients were randomized to 30 mg/day pioglitazone (Actos; Takeda, Lilly) or placebo in a double-blind fashion (treatment modality blinded for patients and doctors). After a treatment period of 16 weeks, patients were admitted for repeated examinations similar to the initial evaluation program. Two patients were excluded from the study. One patient in the placebo group became pregnant and one patient on pioglitazone treatment experienced side effects (dizziness, ankle edema, and anxiety) and was excluded after 1 week of treatment.

Examinations

Examinations were performed during the follicular phase in patients with oligomenorrhea. Patients with amenorrhea (period length >3 months) were examined randomly. The evaluation program of included subjects was performed as previously described (12,13).

Clinical examination. Included subjects underwent a clinical examination including establishment of Ferriman-Gallwey score, BMI, waist-to-hip ratio, height, and weight.

Whole-body dual-energy X-ray absorptiometry scan. Dual-energy X-ray absorptiometry in whole-body array mode (Hologic QDR-4500) was used to measure whole-body fat mass and to differentiate between extremital and central fat mass. The coefficient of variation (CV) for replicate scans of the same individual was 0.8% for whole-body fat mass.

Euglycemic-hyperinsulinemic clamp. The clamp protocol has previously been described (13). In brief, after a 120-min basal tracer equilibration period, insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at a rate of 40 mU/m^2 per min for 180 min, and plasma glucose levels were clamped at ~5 mmol/l using a variable infusion rate of 20% glucose. 3-^{3}H glucose was added to the glucose infusate to maintain constant levels of plasma-specific activity during the clamp period. Indirect calorimetry was performed during the last 40 min of the basal and insulin infusion periods using a ventilated hood system (Deltatrac 2; SensorMedics, Yorba Linda, CA), and average gas exchanges were used to calculate glucose and lipid oxidation rates and total energy expenditure.

Calculations

Steele's non–steady state formulas were used to calculate rates of total glucose appearance (R_a) and glucose disposal (R_d), assuming a glucose distribution volume of 200 ml/kg body wt and a pool fraction of 0.65. Nonoxidative glucose metabolism was calculated as the difference between R_d and glucose oxidation determined by indirect calorimetry. Glucose infusion rate during the last 20 min of the insulin infusion period was used for calculation of whole-body insulin sensitivity (S_i). S_i was calculated as the glucose infusion rate divided by the incremental increase in insulin concentration from the basal to the insulin-stimulated period during the clamp and divided by the mean glucose concentration during the last 40 min of the insulin clamp.

Assays

Sex hormones

Serum total testosterone was analyzed using a specific radioimmunoassay after extraction as previously described (14). This method has a close correlation with the determination of testosterone levels using mass spectrometry. Sex hormone–binding globulin was analyzed by a time-resolved immunoassay using a AutoDELFIA commercial kit (Wallac Oy, Turku, Finland). The intra-assay CV for total testosterone was 8.2% and for sex hormone–binding globulin 5.2%. The interassay CV for total testosterone was 13.8% and for sex hormone–binding globulin 7.5%.

Plasma free fatty acids, sCD36, oxLDL, hsCRP, and IL-6

Plasma free fatty acids (FFAs) were analyzed by enzymatic colonimetric reactions (Modular P; Roche). sCD36 was measured using an in-house enzyme-linked immunosorbent assay (ELISA) (9). A pool of EDTA plasma, aliquoted and stored in the −80°C freezer, was analyzed in seven dilutions in each run and used as a standard concentration curve. Two dilutions of another EDTA pool were used as internal controls, and each control was run in quadruplicates on each ELISA plate. Patient EDTA plasma samples were analyzed in duplicates. Total CV was 11.9–17.3%.

oxLDL was measured in plasma by a competitive ELISA (Mercodia, Uppsala, Sweden) with a total CV of 6.5–9.6%. hsCRP was measured by a solid-phase, chemiluminescent immunometric assay

Table 1—Fasting and insulin-stimulated metabolic characteristics in control subjects and PCOS patients

	PCOS subjects	Control subjects	Pioglitazone		Placebo	
			Pretreatment	Posttreatment	Pretreatment	Posttreatment
n	30	14	15	14	15	14
BMI (kg/m²)	33.1 (25.2–43.9)	33.2 (23.8–46.4)	33.3 (27.2–40.5)	33.8 (26.2–43.5)	33.1 (23.2–47.0)	34.6 (24.4–47.8)
Total fat mass (kg)	36.4 (21.5–61.4)	34.6 (22.4–52.3)	33.9 (24.5–47.7)	35.3 (23.8–50.5)	34.9 (21.2–57.6)	35.5 (20.9–60.3)
Central fat mass (kg)	18.2 (10.5–31.6)	18.2 (10.8–31.6)	17.8 (12.9–25.4)	18.3 (11.8–28.2)	18.9 (9.5–37.5)	18.6 (10.9–32.1)
Free testosterone (nmol/l)	0.045 (0.018–0.112)*	0.026 (0.015–0.082)	0.048 (0.017–0.190)	0.046 (0.020–0.107)	0.041 (0.018–0.093)	0.040 (0.015–0.109)
sCD36 (arbitrary units)	2.87 (0.88–9.36)†	1.67 (0.72–3.89)	3.21 (0.76–13.6)	2.33 (0.84–6.46)*	2.54 (1.14–5.67)	3.22 (0.90–11.5)
oxLDL (units/l)	44.9 (26.9–75.1)†	36.1 (23.4–55.5)	46.2 (25.0–85.2)	41.8 (24.6–70.9)	43.6 (29.4–64.7)	40.8 (28.3–58.7)
hsCRP (mg/dl)	0.26 (0.03–2.41)†	0.12 (0.02–0.81)	0.36 (0.06–2.20)	0.24 (0.04–1.58)‡	0.19 (0.02–2.22)	0.25 (0.01–1.33)
IL-6 (pg/ml)	1.35 (0.43–4.21)	0.97 (0.28–3.44)	1.47 (0.44–4.97)	1.64 (0.60–4.40)	1.23 (0.42–3.57)	1.32 (0.50–3.51)
Insulin stimulated						
S_i (during clamp) (10^{-2} mg/min per m²)	8.1 (2.9–23.0)*	14.7 (7.7–28.0)	6.9 (2.1–22.0)§	9.7 (4.2–22.6)‡	9.6 (4.5–20.1)	9.3 (4.2–20.7)
Total glucose disposal (R_d) (mg/m² per min)	144 (84–244)*	286 (166–492)	134 (76–237)	173 (89–338)‡	155 (98–244)	155 (82–305)
Nonoxidative glucose metabolism (mg/m² per min)	57 (20–163)*	136 (45–418)	50 (16–154)	76 (31–183)‡	64 (26–156)	67 (21–208)
Glucose oxidation (mg/m² per min)	82 (50–136)*	133 (72–456)	78 (44–137)	95 (51–179)‡	87 (57–135)	86 (46–159)
Lipid oxidation (mg/m² per min)	22 (11–46)*	8 (2–31)	24 (12–49)	17 (5–57)‡	20 (9–43)	20 (8–52)
FFAs (mmol/l)	0.059 (0.024–0.144)*	0.026 (0.015–0.046)	0.067 (0.030–0.145)	0.042 (0.013–0.134)‡	0.053 (0.020–0.140)	0.053 (0.016–0.174)

Data are geometric mean (−2 SD to +2 SD). *P < 0.001 pioglitazone vs. control subjects. †P < 0.05 pretreatment values of PCOS vs. control subjects. ‡P < 0.05 pioglitazone vs. pretreatment pioglitazone. §Pretreatment pioglitazone vs. pretreatment placebo.

(Immulite 2000) with a within-run CV of 2.8–8.7% and a total CV of 3.1–8.7%. IL-6 was determined by a high-sensitivity solid-phase ELISA from R&D (Abingdon, U.K.). Intra-assay CV was 6.9–7.8%, and interassay CV was 6.5–9.6%, according to the manufacturer.

Statistical analysis

Fasting and insulin-stimulated hormonal and metabolic variables and sCD36, ox-LDL, hsCRP, and IL-6 levels were approximately log-Gaussian distributed. Data were backtransformed and presented a geometric mean (±2 SD), as previously described by Altman (15). Pretreatment differences between PCOS patients in the pioglitazone and placebo group and control subjects were tested using unpaired t tests on log-transformed values.

Basal differences between patients randomized to pioglitazone and placebo were taken into account by comparing Δ values of hormonal and metabolic variables between the placebo and pioglitazone groups using Mann-Whitney U tests.

The relationship between pretreatment levels of sCD36, oxLDL, hsCRP, IL-6, and metabolic and body composition variables were evaluated by Spearman's nonparametric correlation tests. Body composition parameters were entered in the analyses (BMI, waist-to-hip ratio, and total and central fat mass). Entered measures of metabolic parameters were S_i, R_d, insulin-stimulated oxidative and nonoxidative glucose metabolism, lipid oxidation, and FFA levels during insulin stimulation.

Correlations between the individual markers sCD36, oxLDL, hsCRP, or IL-6 and glucose and lipid metabolism were corrected for BMI or central fat mass using multiple regression analyses. In each analysis, the logarithm of one of the parameters S_i, R_d, insulin-stimulated oxidative and nonoxidative glucose metabolism, lipid oxidation, or FFA levels during insulin stimulation was entered as the dependent variable. As independent variables, BMI or central fat mass and one of the markers sCD36, oxLDL, hsCRP, or IL-6 were entered in each analysis. In this way, two independent variables (one measure of fat mass and one marker of inflammation/insulin resistance) were entered in each analysis.

The contribution of sCD36, oxLDL, hsCRP, and IL-6 to measures of glucose and lipid metabolism were furthermore evaluated using multiple regression. The

same dependent variables were entered as described above (S_i, R_d, insulin-stimulated oxidative and nonoxidative glucose metabolism, lipid oxidation, or FFA levels during insulin stimulation), whereas all the measures sCD36, oxLDL, hsCRP, and IL-6 were entered at the same time as independent variables. All statistics were performed using SPSS 13.0 (SPSS, Chicago, IL) for calculations, and P values <0.05 were considered significant.

RESULTS

Glucose metabolism, body composition, and hormonal parameters in PCOS patients and control subjects

PCOS patients had higher levels of free testosterone than control subjects (Table 1). During the insulin-stimulated state, PCOS patients had higher FFA levels and rates of lipid oxidation and lower S_i, R_d, and oxidative and nonoxidative glucose metabolism than control subjects (all $P < 0.001$).

Pioglitazone treatment improved S_i, insulin-stimulated R_d, oxidative and non-oxidative glucose metabolism, and the ability of insulin to suppress FFA and lipid oxidation when compared with changes during placebo. No significant changes were observed in testosterone levels and body composition.

sCD36, oxLDL, hsCRP, and IL-6 levels in PCOS patients versus control subjects: effect of pioglitazone and placebo treatment

Pretreatment levels of sCD36, oxLDL, and hsCRP were increased in PCOS patients versus control subjects, whereas no significant differences were found in IL-6 levels. sCD36 and hsCRP decreased during pioglitazone treatment when compared with changes during placebo treatment.

sCD36, oxLDL, hsCRP, and IL-6 levels and body composition and metabolic parameters

Representative scatter plots with correlations between sCD36 and metabolic variables in control subjects and PCOS patients are shown in Fig. 1.

sCD36

In PCOS patients ($n = 30$), sCD36 significantly correlated with central fat mass ($r = 0.43$, $P = 0.02$), insulin-stimulated oxidative glucose metabolism ($r = -0.57$, $P = 0.001$), and lipid oxidation
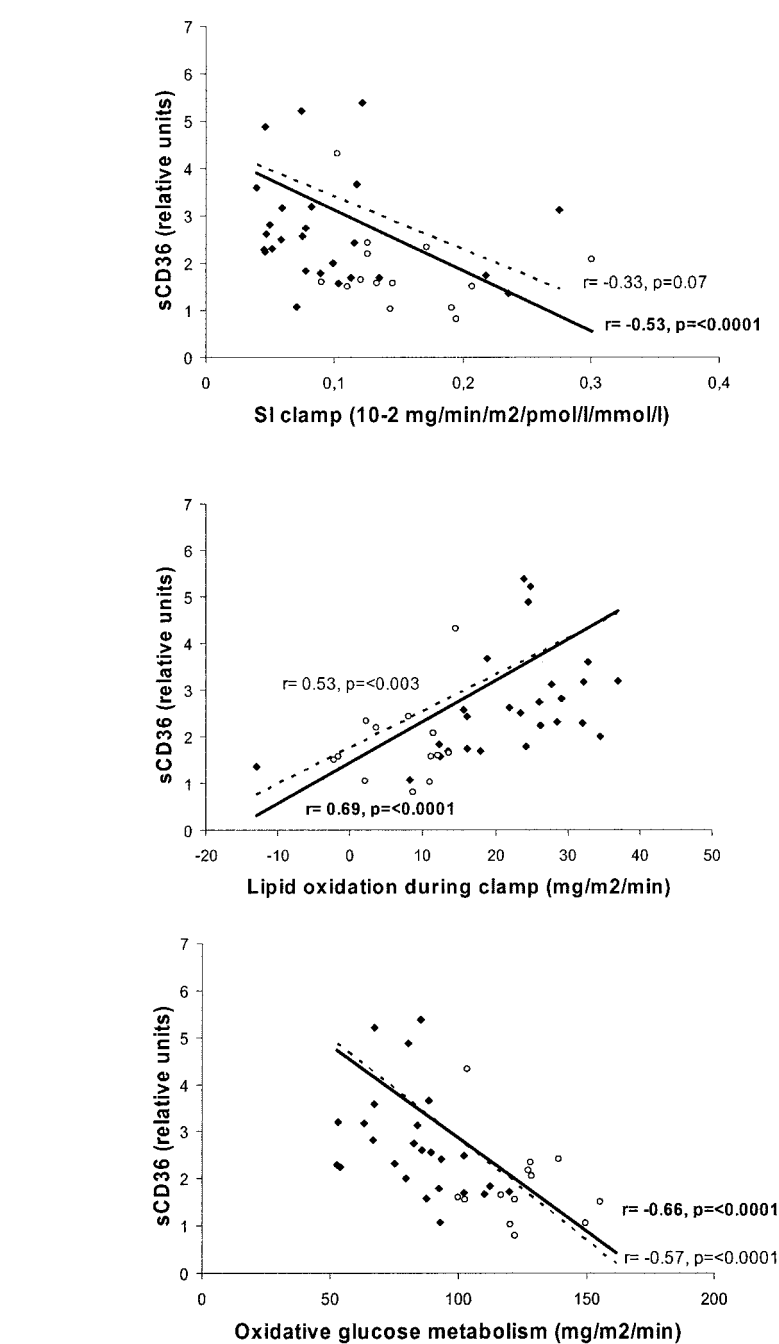

Figure 1—*Correlations between sCD36 and metabolic variables in PCOS patients and control subjects. Bivariate correlation analyses between sCD36 and measures of glucose and lipid metabolism. R values and P levels are presented for Spearman's nonparametric correlation tests in PCOS patients and PCOS plus control subjects. ■, PCOS; □, control subjects. Regression lines: fat lines: PCOS plus control subjects; dotted lines: PCOS.*

during insulin stimulation ($r = 0.53$, $P = 0.003$). When pooling patients and control subjects ($n = 44$), significant inverse correlations were found between sCD36 and S_i ($r = -0.53$), insulin-stimulated R_d ($r = -0.55$), insulin-stimulated glucose oxidation ($r = -0.66$) (all $P < 0.001$), and insulin-stimulated nonoxidative glucose metabolism ($r = -0.36$, $P < 0.01$).

Positive correlations were found between sCD36 and FFA levels ($r = 0.54$) and lipid oxidation ($r = 0.69$) during insulin stimulation (both $P < 0.001$).

oxLDL

The only significant correlation in PCOS patients was between oxLDL and insulin-stimulated nonoxidative glucose metabo-

lism ($r = -0.39$, $P = 0.04$). All other P values for correlations between oxLDL and metabolic parameters were >0.15, and for correlations with fat mass measures P values were >0.5.

In PCOS and control subjects ($n = 44$), inverse correlations were found between oxLDL and measures of S_i and insulin-stimulated glucose metabolism ($r = -0.32$ to -0.51, all $P < 0.03$), and positive correlations were found with FFA levels during insulin stimulation ($r = 0.52$, $P < 0.001$).

hsCRP

In PCOS patients, hsCRP correlated with all measures of body composition ($r = 0.43$–0.59, all $P < 0.01$), whereas no significant correlations were found with entered metabolic parameters (all $P > 0.3$). In pooled PCOS and control subjects, correlations between hsCRP and measures of body composition remained significant ($r = 0.42$–0.60, all $P < 0.005$). Inverse correlations were found between hsCRP and insulin-stimulated oxidative and nonoxidative glucose metabolism ($r = -0.29$ and -0.27, $P = 0.05$), and positive correlations were found with FFA levels during insulin stimulation ($r = 0.39$, $P = 0.009$).

IL-6

Positive correlations were found between IL-6 and all measures of body composition in PCOS patients as well as in PCOS and control subjects ($r = 0.37$–0.52, all $P < 0.05$), whereas no significant correlations were found with metabolic parameters (all $P > 0.4$).

Bivariate correlations between sCD36, oxLDL, hsCRP, and IL-6

IL-6 showed a significant positive correlation with hsCRP in both patients ($r = 0.60$, $P < 0.001$) and in patients and control subjects ($r = 0.60$, $P < 0.001$). Otherwise, no significant correlations were found between inflammatory markers ($P > 0.3$).

Inflammatory markers in relation to total and free testosterone levels

In PCOS patients, a positive correlation was found between oxLDL and free testosterone ($r = 0.36$, $P = 0.05$), which remained significant in pooled patient and control subject data. No significant correlations were found between sCD36, hsCRP, or IL-6 and testosterone levels ($P > 0.3$).

Table 2—*BMI-corrected correlations between inflammatory markers and measures of insulin sensitivity and glucose and lipid metabolism in PCOS patients and control subjects (*n = 44*)*

	R^2 model (P values)	BMI-adjusted β coefficient (P values)
sCD36		
S_i	0.16 (0.03)*	−0.38 (0.01)*
R_d	0.16 (0.03)*	−0.027 (0.01)*
Insulin-stimulated oxidative glucose	0.21 (0.009)*	−0.0028 (0.004)*
Insulin-stimulated FFAs	0.15 (0.04)*	0.03 (0.05)*
Insulin-stim lipid oxidation	0.13 (0.05)*	0.04 (0.02)*
oxLDL		
S_i	0.18 (0.01)*	−0.008 (0.006)*
R_d	0.28 (<0.001)†	−0.008 (0.001)†
Insulin-stimulated oxidative glucose	0.13 (0.05)*	−0.004 (0.03)*
Insulin-stimulated nonoxidative glucose	0.21 (0.009)*	−0.01 (0.003)*
Insulin-stimulated FFAs	0.27 (0.001)†	0.009 (0.002)*

Entered dependent variables: logarithm of S_i, insulin-stimulated R_d, insulin-stimulated oxidative and non-oxidative glucose metabolism, and lipid oxidation and FFA levels during insulin stimulation. Entered independent variables: BMI and one of the markers: sCD36, oxLDL, hsCRP, or IL-6. Only data from models with significant R^2 values are presented. *$P < 0.05$; †$P < 0.001$.

BMI-adjusted correlations between sCD36, oxLDL, hsCRP, or IL-6 and glucose and lipid metabolism

sCD36 showed BMI-independent significant inverse associations with S_i, R_d, and insulin-stimulated oxidative glucose metabolism, whereas positive correlations were found with FFAs and lipid oxidation during insulin stimulation (Table 2). After adjusting for BMI, oxLDL was inversely correlated with S_i, R_d, and insulin-stimulated oxidative and nonoxidative glucose metabolism, whereas a positive BMI-independent correlation was found with FFAs during insulin stimulation. In all cases, similar correlation coefficients and levels of significance were found when entering central fat mass in regression analyses (data not shown). No significant associations were found when entering hsCRP and IL-6 in multiple regression analyses.

Contribution of sCD36, oxLDL, hsCRP, and IL-6 to S_i, glucose, and lipid metabolism

sCD36 and oxLDL were significant independent predictors of all measures of S_i, glucose, and lipid metabolism, whereas hsCRP and IL-6 showed no significant contribution. The proportion of the variation in glucose and lipid metabolism explained by the inflammatory markers varied from 25% for lipid oxidation to 43% for R_d.

CONCLUSIONS — In the present study, we applied the measurement of

sCD36 in PCOS at baseline and during pioglitazone treatment. sCD36 showed fat mass–independent correlations with clamp-established measures of glucose and lipid metabolism, and increased sCD36 levels were significantly reversed during pioglitazone-induced increased insulin sensitivity. These results supported that sCD36 is an independent marker of insulin resistance in PCOS.

Our findings of significant correlations between sCD36 and measures of glucose metabolism and FFAs in PCOS patients and control subjects are in agreement with the results of Handberg et al. (9) in patients with type 2 diabetes. sCD36 levels were significantly decreased, whereas no significant changes were observed in central fat mass after pioglitazone treatment. Multiple regression analyses further supported the fat mass–independent correlation between sCD36 and insulin sensitivity. However, no direct measures of intra-abdominal and subcutaneous fat mass were available in the present study, and computed tomography or magnetic resonance scans are needed to determine the relation between sCD36 levels and intra-abdominal versus subcutaneous fat distribution in PCOS patients.

Recent studies suggested that LDL must be oxidized to be taken up by macrophages, making oxLDL the atherogenic form of LDL (16). Increased levels of ox-LDL in PCOS patients compared with weight-matched control subjects were documented in a recent study (17) in

overweight and normal-weight sub-groups of PCOS patients. In this study, Macut et al. (17) examined 179 PCOS patients (79 overweight and 100 normal weight) and 56 (20 overweight and 36 normal weight) control subjects. PCOS was defined according to the Rotterdam criteria (18). Macut et al. found comparable oxLDL levels in normal-weight and overweight PCOS patients. These findings suggested a minor association between body weight and oxLDL levels in PCOS and are in agreement with the BMI-independent correlation between oxLDL and glucose and lipid metabolism in the present study.

We found significant associations, similar to those with sCD36, between oxLDL and measures of fat mass and glucose and lipid metabolism. During multiple regression analyses, sCD36 and oxLDL were significant independent predictors of glucose and lipid metabolism, whereas hsCRP and IL-6 showed no significant contribution. The lack of a direct relationship between oxLDL and sCD36 indicates that the underlying mechanism of elevated sCD36 may be related to insulin resistance rather than to increased oxLDL levels per se.

In the present study, oxLDL showed a significant positive correlation with free testosterone, but this correlation became insignificant after correcting for BMI. These findings are supported by previous studies in PCOS and suggest that the increase in levels of oxLDL in PCOS more closely relates to insulin resistance and abdominal obesity than to hyperandrogenemia (17,19).

hsCRP is secreted in response to cytokines including IL-6. Previous studies showed that a high hsCRP level was the strongest univariate predictor for the risk of cardiovascular events (rev. in 20). hsCRP may not only be a marker of inflammatory disease but may also amplify the inflammation process by further activation of monocytes and endothelial cells (20). In the present study, significantly higher hsCRP levels in PCOS patients than in control subjects supported the increased risk of atherosclerosis in PCOS. Furthermore, significant positive correlations between hsCRP and IL-6 and fat mass measures in both patients and control subjects support a relationship between abdominal and overall fat mass and hsCRP and IL-6. hsCRP and IL-6 were increased in PCOS patients compared with control subjects of matched body composition and therefore suggested pos-

sible associations between hsCRP and IL-6 and insulin sensitivity or hyperandrogenemia. We found no significant correlations between hsCRP and testosterone levels. Pioglitazone-mediated improved insulin sensitivity was accompanied by decreased hsCRP levels, whereas no significant changes were measured in body composition or testosterone levels. Overall, these findings suggest parallel improvements of insulin sensitivity and hsCRP levels during pioglitazone treatment. Supporting the relation between hsCRP and IL-6, we found significant positive correlations between these markers, although no significant improvement of IL-6 levels was observed during pioglitazone treatment.

Some previous studies (21) have found significantly increased hsCRP levels in PCOS patients compared with weight-matched control subjects, although this could not be reproduced in other studies (22,23). In agreement with our study, two previous studies (22,23) measured IL-6 levels in PCOS and found no significant differences between PCOS patients and control subjects. In one previous study by Puder et al. (21), the authors included hsCRP, and a dual-energy X-ray absorptiometry scan established measures of fat mass in PCOS patients and control subjects and found significant correlations between hsCRP and fat mass measures. As in the present study, most previous studies were performed in small study populations, and this may have affected results.

In conclusion, hsCRP and IL-6 levels were correlated with central and total fat mass, while sCD36 and oxLDL correlated with measures of insulin sensitivity independent of BMI and central fat mass. Pioglitazone treatment reduced sCD36 while improving insulin-stimulated glucose metabolism, further supporting the association between insulin sensitivity and sCD36 in PCOS.

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