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No difference in markers of adipose tissue inflammation between overweight women with polycystic ovary syndrome and weight-matched controls

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BACKGROUND: Previous studies have indicated that peripheral circulating markers of inflammation are elevated in women with polycystic ovary syndrome (PCOS), but thus far no studies concerning markers of inflammation in adipose tissue have been published. The aim of the study was to investigate whether patients with PCOS display increased expression of inflammatory markers in adipose tissue.

METHODS: Twenty overweight patients with PCOS, 10 lean patients with PCOS and 20 overweight controls had subcutaneous fat biopsies and blood samples taken. Adipose tissue levels of mRNA of inflammatory markers were determined by use of real-time PCR.

RESULTS: Overweight patients with PCOS had higher relative adipose tissue chemokine ligand 2 ($P < 0.01$), and its cognate receptor ($P < 0.05$), tumour necrosis factor-α ($P < 0.001$), interleukin (IL)-10 ($P < 0.001$) and IL-18 ($P < 0.001$) and the monocyte/macrophage markers CD14 ($P < 0.01$) and CD163 ($P < 0.01$) mRNA levels compared with lean women with PCOS. There were no differences between overweight patients with PCOS and overweight control subjects in this respect. Within the PCOS group, markers of adipose tissue inflammation correlated significantly with obesity-related metabolic disturbances, but when data were adjusted for age and BMI, most correlations were lost.

CONCLUSIONS: Overweight, rather than the PCOS diagnosis *per se*, appears to be the main explanatory variable for elevated adipose tissue inflammation in patients with PCOS.

Key words: polycystic ovary syndrome / adipose tissue / inflammatory markers / metabolic syndrome / overweight

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting 5–10% of women of child-bearing age (Knochenhauer *et al.*, 1998; Diamanti-Kandarakis *et al.*, 1999; Asuncion *et al.*, 2000; Azziz *et al.*, 2004; Lindholm *et al.*, 2008). Women with PCOS carry a number of risk markers for cardiovascular disease (CVD), such as obesity, type 2 diabetes, hypertension and dyslipidemia, and these risk factors remain, even after adjustment for age and BMI (Lo *et al.*, 2006).

Obesity is a common feature of PCOS, with prevalence rates varying between 38 and 88% (Barber *et al.*, 2006). The elevated metabolic risk profile associated with obesity is, to a large extent, dependent on body fat distribution. Central obesity is of importance in the definition of the metabolic syndrome, and especially visceral fat accumulation is associated with insulin resistance and CVD (Despres and Lemieux, 2006).

Obesity is characterized by a systemic low-grade inflammatory state that is thought to originate from the adipose tissue (Kershaw and Flier, 2004). The adipose tissue is an active endocrine organ that produces and releases hormones, pro- and anti-inflammatory cytokines and chemoattractant cytokines (chemokines). Pro-inflammatory molecules produced by the adipose tissue are proposed to be active participants in the development of insulin

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resistance and the increased risk of CVD associated with obesity (Mahadik *et al.*, 2008).

Recently, it was reported that adipose tissue in obesity is characterized by macrophage infiltration, and that macrophages are an important contributor to inflammation (Cancello and Clement, 2006). An increased macrophage infiltration could be caused by different chemokines thought to attract inflammatory cells to the adipose tissue. One such example is chemokine (C-C motif) ligand 2 (CCL2) (also known as monocyte chemoattractant protein-1, MCP-1) and its cognate receptor chemokine receptor 2 (CCR2), both of which contribute to systemic insulin resistance, macrophage infiltration and maintenance of macrophages in the adipose tissue (Kershaw and Flier, 2004; Cancello and Clement, 2006).

In addition to increased macrophage infiltration, the expression and release of pro-inflammatory cytokines, such as macrophage migration inhibitory factor (MIF), tumour necrosis factor-α (TNF-α) and interleukin-18 (IL-18), is increased in adipose tissue in the obese (Hotamisligil *et al.*, 1995; Bruun *et al.*, 2007; Skopkova *et al.*, 2007), and there is strong evidence that the expanded adipose tissue mass of the obese contributes, directly or indirectly, to the increased circulating levels of inflammatory markers found in obese patients (Hotamisligil, 2006).

Circulating markers of inflammation, including MCP-1, MIF and high-sensitive C-reactive protein (hs-CRP), are elevated in overweight women with PCOS in comparison with weight-matched controls (Diamanti-Kandarakis *et al.*, 2006; Glintborg *et al.*, 2009; Gonzalez *et al.*, 2009). However, thus far no studies concerning inflammatory markers in adipose tissue have been conducted in patients with PCOS.

Our hypothesis was that overweight/obesity would be associated with increased adipose tissue expression of inflammatory markers, irrespective of a PCOS diagnosis.

Materials and Methods

Subjects

Twenty overweight patients with PCOS, 10 lean patients with PCOS and 20 overweight control subjects were included at the Departments of Obstetrics and Gynaecology at Uppsala University Hospital, Umeå University Hospital and Sunderby Hospital, Sweden. All patients with PCOS were recruited from among patients seeking care for oligomenorrhoea and/or hirsutism at the out-patient wards of the participating clinics. The overweight control subjects were recruited by advertisement in local newspapers.

Overweight patients with PCOS and overweight control subjects had BMI >27.0 kg/m^2, whereas lean patients with PCOS had BMI <25.0 kg/m^2. The limit for BMI was chosen based on prior studies indicating an increased insulin resistance already at BMI 27 kg/m^2 in patients with PCOS (Gennarelli *et al.*, 2000).

PCOS was defined according to the Rotterdam criteria (Welt *et al.*, 2006). Two of the following three features had to be present for the PCOS diagnosis (i) oligomenorrhoea with eight or fewer menstruations in the previous 12 months or amenorrhoea (ii) clinical and/or biochemical signs of hyperandrogenism such as testosterone $>$ 2.7 nmol/l, elevated dehydroepiandrosterone sulphate ($>$9.2 μmol/l), free androgen index (FAI $\geq$5.0), or hirsutism ($>$7 on the Ferriman and Gallway scale) and (iii) polycystic ovaries on ultrasound examination [$>$12 follicles 2–9 mm diameter and/or increased ovarian volume ($>$10 ml)]. PCOS diagnosis also implied that thyroid disease, adrenocortical dysfunction and hyperprolactinemia were not present.

Control subjects had regular menstrual cycles (cycle length 25–31 days), normal androgen levels and no signs of polycystic ovaries on transvaginal ultrasound.

Inclusion criteria for the study were confirmed PCOS/control status and age 18–40 years. Exclusion criteria for the study (all groups) were obesity owing to organic disorder, presence of chronic illness and use of hormonal treatments 6 months prior to inclusion in the study (including combined oral contraceptives, progestagens, ovulatory stimulants, antidiabetics, cortisone, anti-inflammatory drugs, statins and anti-androgens).

The patients with PCOS and controls gave written informed consent and the Independent Ethical Review Board at Uppsala University, Sweden, approved the study.

Procedures before surgery

After overnight fasting, blood samples for glucose, insulin, triglycerides (TG), cholesterol, hs-CRP, IL-18, CCL2, testosterone and sex hormone-binding globulin (SHBG) were drawn between 08.00 and 08.30. The blood samples were stored at -20°C until analysed. FAI was calculated as testosterone (nmol/l)/SHBG (nmol/l) $\times$ 100, and homeostasis model assessment of insulin resistance (HOMA-IR) as fasting serum insulin (mU/l) $\times$ fasting serum glucose (mmol/l)/22.5 (Albareda *et al.*, 2000).

Height (to the nearest cm) and weight (to the nearest 200 g) was measured in stockinged feet and light clothes using standardized equipment. Waist circumference (to the nearest 0.5 cm) measurement was performed midway between the lower rib margin and iliac crest. Hip circumference (to the nearest 0.5 cm) was taken around the maximal girth of the hips. Blood pressure was measured (average of three readings) after 5 min rest, while seated.

Fat biopsies

Following local anaesthesia with prilocain (10 mg/ml) in the skin area to the right of the umbilicus, $\sim$1.5 cm^2 of superficial subcutaneous adipose tissue was excised through a 1.5 cm incision. The fat biopsies were placed into vials and snap frozen in liquid nitrogen and stored at -70°C until assayed.

Blood analyses

TG, hs-CRP and glucose were analysed on an Architect ci8200 (Abbott Laboratories, Abbott Park, IL, USA). The total imprecision (coefficient of variation, CV) of the instrument for the analytes were 1.1% at 0.9 mmol/l for TG, 0.8% at 8 mg/l for hs-CRP and 1.0% at 4.4 mmol/l for glucose. Insulin, SHBG and testosterone were analysed on a Modular E170 (Roche Diagnostics, Mannheim, Germany). The total CV of the instrument for the analytes was 1.6% at 7.0 mU/l for insulin, 1.5% at 43 nmol/l for SHBG and 6.8% at 3.9 nmol/l for testosterone. Serum levels of IL-18 (Human IL-18 ELISA kit, R&D Systems Abingdon, UK) and CCL2 (Quantikine Human MCP-1, R&D Systems) were measured using a commercially available immunoassay according to the instructions from the manufacturer (Lettesjo *et al.*, 2005). However, the wavelength correction for CCL2 was set to 620 nm instead of 540 nm in all experiments. The detection limit for IL-18 was $<$12.5 pg/ml and the minimum detectable concentration of CCL2 was $<$5.0 pg/ml.

RNA extraction, reverse transcription and real-time PCR

Total RNA was extracted from adipose biopsies using a RNeasyLipid Tissue Mini Kit (QIAGEN, Hilden, Germany). The yield and purity of RNA were determined by spectrophotometer (ND-1000 spectrophotometer, NanoDrop Technologies, Wilmington, DE9) and RNA integrity

was analysed by 1% agarose gel electrophoreses in the presence of ethidium bromide. One microgram of total RNA was reverse transcribed, using TaqMan RT reagents (High Capacity cDNA Reverse Transcription kit, Applied Biosystems, Foster City, CA, USA) and RNase inhibitor (Applied Biosystems) at a final concentration of 1.0 U/ml. Subsequently, specific mRNAs were run in duplicate on an ABI Prism 7900HT sequence detection system (Applied Biosystems) using Taqman Universal PCR Master Mix (Applied Biosystems). Ten inflammation-related genes were included in the analysis, including the chemokine CCL2 and its receptor CCR2; the pro-inflammatory cytokines MIF, TNF-α, IL-6 and IL-18; the anti-inflammatory cytokine IL-10; the monocyte/macrophage markers CD14 and CD163; and adiponectin. The following TaqMan gene expression assays (Applied Biosystems) were used; CCL2 (Hs00234140_ml), CCR2 (Hs00356601_m1), MIF (Hs00236988_g1), TNF-α (Hs00174128_m1), IL-6 (Hs00985639_m1), IL-10 (Hs00174086_m1), IL-18 (Hs999999040_ml), CD14 (Hs00169122_g1), CD163 (Hs01016657_m1), adiponectin (Hs00605917_m1), low density lipoprotein receptor-related protein 10 (LRP10: Hs00204094_ml), peptidylprolyl isomerase A (cyclophilin A)(PPIA: Hs999999904_ml), as well as acidic ribosomal phosphoprotein P0(RPLP0: Hs99999902_ml). The parameter cycle threshold (C_t) is defined as the cycle number at which the fluorescence intensity exceeds a fixed threshold. Relative amounts of mRNA for target genes were calculated using the comparative C_t method ($\Delta\Delta$t). Reference genes were evaluated by running LRP10, PPIA and RPLP0 on the full study cohort, and the NormFinder (Andersen et al., 2004) algorithm identified RPLP0 as the best gene to use in normalization. The expression of RPLP0 was thus used to normalize samples for the amount of cDNA used per reaction.

Statistics

The power analysis was based on the assumption that the difference between overweight patients with PCOS and overweight controls would be 25% of the difference between lean and obese women (Hotamisligil et al., 1995; Bruun et al., 2007). With this assumption, the study had >85% power to detect differences in adipose tissue mRNA expression between these two groups, given an α-error of 0.05. All variables were log-transformed prior to statistical analyses unless normally distributed. Comparisons between groups were made using one-way analysis of covariance (with age as covariate) with *post hoc* Tukey tests. Correlations were determined using Pearson correlation coefficient, followed by partial correlation controlling for age and BMI. For the correlation analyses the false discovery rate was used to protect against multiple testing. All values in the text and tables are displayed as mean $\pm$ SD, unless otherwise stated. The Statistical Package for the Social Sciences package was used for all analyses (SPPS, Inc., Chicago, IL, USA). A P-value < 0.05 was considered significant.

Results

Subject characteristics

All women with PCOS displayed oligomenorrhoea/amenorrhoea and polycystic ovaries, and the majority also had clinical or biochemical hyperandrogenism [16 (80.0%) among overweight patients with PCOS and 6 (60.0%) among lean patients with PCOS]. Baseline characteristics of the study population are shown in Table I. The overweight patients with PCOS were younger, had increased TG, FAI and lower systolic blood pressure than the overweight controls. Otherwise, there were no differences between overweight patients with PCOS and overweight control subjects. Because of the difference in age between groups, subsequent analyses were adjusted for age.

In comparison with lean PCOS patients, overweight patients with PCOS and overweight control subjects had increased serum concentrations of TG, insulin, HOMA-IR and hs-CRP. Likewise, overweight patients with PCOS and overweight control subjects had higher systolic and diastolic blood pressure than lean PCOS patients. There were no differences between the three groups in serum concentrations of IL-18 or CCL2, Table I.

Adipose tissue gene expression

Relative amounts of mRNA for inflammatory markers in adipose tissue from overweight and lean patients with PCOS as well as overweight controls are displayed in Fig. 1. Overweight patients with PCOS and overweight control subjects had increased relative amounts of CCL2, CCR2, TNF-α, IL-10, IL-18, CD14 and CD163, and decreased

Table I Clinical characteristics of women in a study of markers of adipose tissue inflammation in relation to PCOS and weight.

	Lean PCOS patients $n = 10$	Overweight PCOS patients $n = 20$	Overweight controls $n = 20$
Age, years	27.0 ± 4.8[b]	29.6 ± 5.1[b]	35.1 ± 5.7
BMI, kg/m^2	22.4 ± 2.0[a,b]	34.4 ± 4.8	34.5 ± 4.4
Waist circumference, cm	68 ± 26[a,b]	106 ± 13	112 ± 14
Waist-hip ratio	0.77 ± 0.77[a,b]	0.91 ± 0.06	0.93 ± 0.09
Glucose, mmol/l	4.5 ± 0.3	5.0 ± 0.5	5.2 ± 1.0
Insulin, mU/l	4.5 ± 1.51[a,b]	16.4 ± 12.1	12.6 ± 7.0
HOMA-IR	0.89 ± 0.30[a,b]	3.79 ± 3.20	3.08 ± 2.37
hs-CRP, mg/l	1.10 ± 0.99[a,b]	4.31 ± 3.16	3.48 ± 4.06
Serum CCL2, pg/ml	352 ± 96	371 ± 118	416 ± 138
Serum IL-18, pg/ml	215 ± 50	331 ± 164	283 ± 118
Serum TG, mmol/l	0.62 ± 0.16[a,b]	1.50 ± 0.70[b]	1.11 ± 0.38
Serum cholesterol, mmol/l	4.5 ± 0.90	4.8 ± 1.0	4.7 ± 0.7
Systolic blood pressure, mmHg	111 ± 10[b]	119 ± 11[b]	132 ± 10
Diastolic blood pressure, mmHg	72 ± 9[a,b]	80 ± 10	86 ± 9
Serum testosterone, nmol/l	1.7 ± 0.8	2.4 ± 1.0[b]	1.6 ± 0.8
Serum SHBG, nmol/l	49.8 ± 19.7[a]	33.4 ± 16.1	37.7 ± 17.7
FAI	5.46 ± 6.38[a]	9.12 ± 6.07[b]	4.84 ± 3.02

Data are mean ± SD.
CCL2, chemokine (C-C motif) ligand 2; CCR2, chemokine receptor 2; IL, interleukin; TG, triglycerides; hs-CRP, cholesterol high-sensitive C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; SHBG, sex hormone-binding globulin; FAI, free androgen index.
[a]Significantly different versus overweight patients with PCOS, $P < 0.025$–0.001, one-way analysis of covariance (ANCOVA), adjusted for age.
[b]Significantly different versus overweight control subjects, $P < 0.025$–0.001, ANCOVA, adjusted for age.

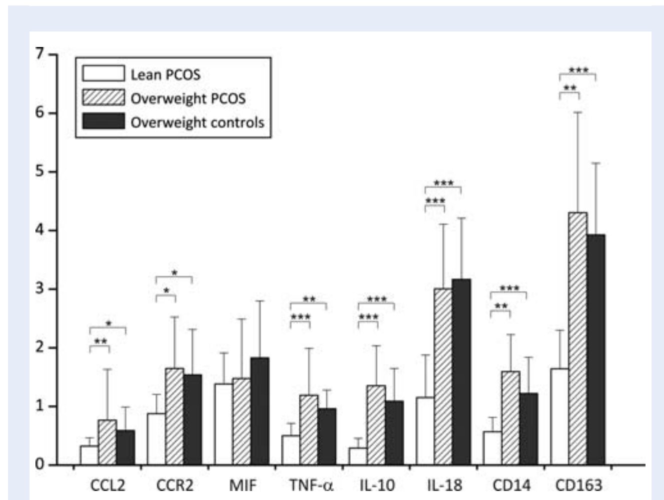

Figure 1 Levels of mRNA for inflammatory substances (mean ± SD) in abdominal superficial subcutaneous adipose tissue from lean patients with PCOS (white bars, $n = 10$), overweight patients with PCOS (dashed bars, $n = 20$) and overweight control subjects (black bars, $n = 20$). Levels of mRNA are relative to the RPLP0 gene, which was used to normalize data. CCL2, CCR2, macrophage MIF, TNF-α. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, one-way analysis of covariance, adjusted for age, with *post hoc* Tukey tests.

relative amounts of adiponectin adipose tissue mRNA, compared with lean patients with PCOS (data not shown in figure for adiponectin owing to high relative expression, i.e. 12.99 ± 1.80, 8.88 ± 1.97 and 9.56 ± 2.05 for lean and overweight patients with PCOS, and overweight control subjects, respectively, $P < 0.01$ for lean patients with PCOS versus overweight patients and overweight controls). There were no differences between overweight patients with PCOS and overweight controls in levels of adipose tissue mRNA for any of these inflammatory markers. MIF and IL-6 expression did not differ between groups (IL-6 data not shown owing to very low relative expression, i.e. 0.03 ± 0.02, 0.05 ± 0.03 and 0.04 ± 0.02 for lean and overweight patients with PCOS, and overweight controls, respectively).

When analyses were restricted to PCOS subjects fulfilling the hyperandrogenism criterion ($n = 16$) the results were unchanged (data not shown).

Adipose tissue mRNA levels and circulating levels of IL-18 and MCP-1 were positively correlated ($r = 0.44$, $P < 0.01$ and $r = 0.37$ $P < 0.05$, respectively).

Relationship between adipose tissue inflammation markers and obesity-related metabolic disturbances in patients with PCOS

Among patients with PCOS, the adipose tissue markers of inflammation, except for MIF, were correlated with most metabolic risk markers, including BMI, waist circumference, glucose, insulin, HOMA-IR, TG and systolic and diastolic blood pressures (Table II). CCR2, IL-10, IL-18, CD14 and CD163 mRNAs were negatively correlated with serum concentrations of SHBG, whereas adiponectin was positively correlated with SHBG. However, there were no correlations between FAI, testosterone or total cholesterol and the markers of adipose tissue inflammation (data not shown for testosterone and total cholesterol).

As depicted in Tables II and III, the main explanatory variable for the adipose tissue inflammatory markers in patients with PCOS was BMI. Following the adjustment for age and BMI (Table III), significant positive correlations only remained between adipose tissue TNF-α mRNA level and systolic blood pressure, and similarly IL-6 was positively correlated with insulin and HOMA-IR (data not shown for FAI). Adiponectin was negatively correlated with TG.

Discussion

This study demonstrates that the gene expression pattern of inflammation markers in abdominal superficial subcutaneous tissue is similar in overweight patients with PCOS and overweight control subjects. Furthermore, this finding remains when analyses are restricted to those patients with PCOS who displayed hyperandrogenism. In contrast, lean patients with PCOS display lower expression of adipose tissue inflammation markers compared with the two overweight groups. In concert with this finding, most of the associations between markers of adipose tissue inflammation and variables of the metabolic syndrome in patients with PCOS were lost when adjusted for age and BMI. Thus, overweight or obesity, rather than the PCOS diagnosis *per se* appears to be the main explanatory variable for increased subcutaneous adipose tissue inflammation in patients with PCOS.

Most phenotypes of PCOS—irrespective of degree of obesity—are hallmarked by a combination of androgen excess and insulin resistance. While the association with type 2 diabetes is well established, the precise CVD risk in PCOS women is still unclear, in part because there are no, or few, longitudinal studies examining cardiovascular events and/or mortality. However, biochemical hyperandrogenism is associated with CVD risk factors in young as well as middle-aged and post-menopausal healthy women (Sowers *et al.*, 2005; Sutton-Tyrrell *et al.*, 2005; Coviello *et al.*, 2006; Shaw *et al.*, 2008) and in post-menopausal women, hyperandrogenism has also been associated with CVD events (although not independently of BMI) (Rexrode *et al.*, 2003). Previous studies have indicated that serum concentrations of inflammatory markers, such as MCP-1 (i.e. CCL2) and CRP, are increased in overweight patients with PCOS compared with weight-matched controls (Gonzalez *et al.*, 2009), and that the inflammatory status is correlated with levels of hyperandrogenism (Glintborg *et al.*, 2009; Gonzalez *et al.*, 2009). Other studies have failed to display any differences between overweight controls and overweight patients with PCOS in terms of circulating TNF-α and IL-6 (Olszanecka-Glinianowicz *et al.*, 2007). In contrast with previous studies of inflammatory markers in PCOS patients, we were not able to confirm prior findings of increased circulating levels of hs-CRP, CCL2 and IL-18 in overweight PCOS patients, presumably because our study was powered primarily for the adipose tissue mRNA levels and not for the circulating levels of inflammatory markers. Similarly, we were unable to demonstrate a correlation between adipose tissue mRNA levels for inflammatory markers and FAI or testosterone. However, in line with previous studies SHBG, as an indirect measure of androgenicity and insulin resistance,

Table II Bivariate correlations between mRNA level for CCL2, CCR2, MIF, TNF-α, IL-6, IL-10, IL-18, CD14, CD163 (monocyte/macrophage markers) in adipose tissue and adiponectin and selected anthropometric/metabolic variables in all women with PCOS ($n = 30$).

	Age	BMI	Waist	Glucose	Insulin	HOMA-IR	TG	Systolic blood pressure	Diastolic blood pressure	FAI	SHBG
CCL2	0.01	0.52**	0.42*	0.55**	0.34	0.44*	0.33	0.52**	0.28	−0.08	−0.25
CCR2	0.08	0.52**	0.42	0.59**	0.50**	0.50**	0.50**	0.12	0.30	0.00	−0.50**
MIF	0.07	−0.08	−0.03	0.02	0.02	0.02	0.06	−0.07	−0.18	−0.10	0.09
TNF-α	0.40	0.55**	0.48*	0.58**	0.42*	0.41	0.46*	0.65**	0.52**	−0.18	−0.23
IL-6	0.01	0.40	0.55**	0.51**	0.63**	0.64**	0.54**	0.25	0.24	−0.16	−0.19
IL-10	0.22	0.78**	0.75**	0.69**	0.52**	0.52*	0.59**	0.56**	0.54**	0.13	−0.49*
IL-18	0.31	0.76**	0.73**	0.67**	0.54**	0.54**	0.57*	0.47*	0.55**	0.00	−0.38
CD14	0.12	0.78**	0.75**	0.67**	0.55**	0.55**	0.56**	0.53**	0.58**	0.04	−0.41
CD163	0.15	0.77**	0.68**	0.61**	0.51*	0.50*	0.54*	0.52*	0.55**	0.09	−0.45*
Adiponectin	−0.08	−0.61**	−0.57**	−0.50**	−0.58**	−0.57**	−0.65**	−0.22	−0.32	−0.16	0.54*

MIF, macrophage migration inhibitory factor; TNF-α, tumour necrosis factor-α .
*$P < 0.025$ and **$P < 0.005$, Pearson correlation coefficient.

Table III Partial correlations (controlling for age and BMI) between mRNA level for CCL2, CCR2, MIF, TNF-α, IL-6, IL-10, IL-18, CD14, CD163 in adipose tissue and adiponectin and selected anthropometric/metabolic variables in all patients with PCOS ($n = 30$).

	Waist	Glucose	Insulin	HOMA-IR	TG	Systolic blood pressure	Diastolic blood pressure	SHBG
CCL2	0.16	0.06	0.02	0.04	0.12	0.39	−0.13	0.19
CCR2	−0.02	0.41	0.22	0.23	0.26	−0.20	−0.06	−0.29
MIF	0.04	−0.06	0.19	0.17	0.15	0.04	−0.08	0.01
TNF-α	0.07	0.17	0.19	0.19	0.29	0.50*	0.13	0.15
IL-6	0.36	0.36	0.48*	0.51*	0.39	0.11	−0.04	0.02
IL-10	0.38	0.18	−0.12	−0.13	0.22	0.35	0.07	−0.14
IL-18	0.31	0.18	0.03	0.01	0.21	0.23	0.19	−0.06
CD14	0.33	0.15	−0.12	−0.11	0.09	0.34	0.20	0.12
CD163	0.18	0.08	−0.15	−0.13	−0.00	0.35	0.19	0.00
Adiponectin	−0.18	−0.06	−0.30	−0.25	−0.46*	0.01	0.01	0.36

*$P < 0.025$, partial correlation, controlling for age and BMI.

was negatively correlated with most of the inflammatory markers (Glintborg et al., 2009; Gonzalez et al., 2009).

Although most studies on PCOS report that obese women with PCOS are more insulin resistant than control obese women, there was no difference in our present study. The reason for this is unclear but may involve the fact that the overweight women with PCOS did not differ in waist circumference compared with controls. When women with PCOS are compared with control women matched for abdominal adiposity, the difference in insulin resistance between the groups is much less marked than if the two groups are matched for BMI (Holte et al., 1994). It is also possible that our relatively small comparative study was unable to reveal true differences in insulin sensitivity. The fact that, based upon waist circumference measurements and waist-hip ratio, obese women with PCOS and obese controls did not differ in this study goes against the notion that anovulatory women build up more truncal-abdominal fat. However, data from recent studies utilizing advanced imaging techniques are ambiguous. Using magnetic resonance imaging, Barber and colleges found that overweight PCOS cases and BMI/fat mass-matched controls were indistinguishable with respect to distribution of fat within visceral, abdominal subcutaneous and gluteofemoral subcutaneous fat depots (Barber et al., 2008). Furthermore, by use of computer tomography, it was recently demonstrated that obese women with PCOS only have a modest, but significant, increase in visceral fat compared with BMI/fat mass-matched non-PCOS women (Hutchison et al., 2011). Notably, these studies (Barber

et al., 2008; Hutchison *et al.,* 2011) included overweight and obese subjects, which may explain the discrepancy from previous studies where mainly lean subjects were included (Yildirim *et al.,* 2003). Concerning visceral adiposity and the close link to metabolic risk, it is unfortunate that we had only biopsies from the abdominal superficial subcutaneous adipose tissue depot. It has recently been reported that the different adipose tissue depots have different profiles of inflammatory markers (Harman-Boehm *et al.*, 2007; Huber *et al.*, 2008; Poulain-Godefroy *et al.*, 2008), and genomic and proteomic profiling studies of morbidly obese PCOS omental adipose tissue have revealed changes in components of several biological pathways (Corton *et al.,* 2007, 2008). Consequently, there might be differences in the inflammatory status of visceral adipose tissue in overweight PCOS versus weight-matched controls. However, it is not likely that the visceral tissue directly would account for the increased circulating concentrations of plasma cytokines and chemokines. Only a limited number of adipose-derived mediators are actually released into the circulation in amounts sufficient to result in the increased systemic levels which are associated with obesity (Dahlman *et al.,* 2005; Murdolo *et al.,* 2007). Indirectly, however, the portal drainage of visceral fat provides direct hepatic access to free fatty acids and adipokines that may activate hepatic immune mechanisms with production of inflammatory mediators, such as CRP (Despres and Lemieux, 2006). Further studies of adipose tissue depot-specific inflammation in relation to circulating levels of inflammatory markers in larger cohorts of women with PCOS are thus warranted.

Support for a possible causal relationship between obesity-associated low-grade inflammation and metabolic impairment is gained from epidemiological data indicating an association of markers of systemic inflammation (i.e. CRP, IL-6) with insulin resistance and future risk for development of diabetes type 2 (Pradhan *et al.*, 2001). In agreement with previous studies, we found a pronounced effect of increased body mass on adipose tissue expression of chemokines, pro-inflammatory cytokines and markers of inflammation (Hotamisligil, 2003; Bruun *et al.*, 2007; Huber *et al.*, 2008; Shakeri-Manesch *et al.*, 2009), whereas overweight patients with PCOS did not differ from overweight controls in this respect. We showed that MIF mRNA, however, was not influenced by fat mass in agreement with data from our laboratory where MIF expression in subcutaneous fat depots from white women was unaffected by fat mass (Evans *et al.*, 2011). Skurk *et al.* (2005) reported that secretion of MIF from isolated human subcutaneous adipocytes correlated with BMI, seemingly in conflict with our data. Importantly, the majority of MIF secreted from adipose tissue originates from non-fat cells in obese women (Fain *et al.*, 2006) and it is also likely that the adipocyte isolation and culturing procedure *per se* can influence the secretion pattern (Ruan *et al.*, 2003). Similar to MIF, we found no effect of fat mass on adipose tissue IL-6 mRNA. Although a minority of the body's circulating IL-6 is derived from adipose tissue (Mohamed-Ali *et al.*, 1997; Weisberg *et al.*, 2006), secretion from adipose tissue and circulating IL-6 levels are suggested to be a function of the degree of fat mass (Kern *et al.*, 2001). However, since we did not measure IL-6 secretion, this issue should be investigated further.

Several pro-inflammatory cytokines are elevated in parallel with many metabolic risk markers, whereas the anti-inflammatory and adipocyte-specific hormone adiponectin is consistently lower (Spranger *et al.*, 2003). In agreement with this, our lean patients with PCOS had higher levels of mRNA for adiponectin versus the overweight groups. Adiponectin exerts anti-inflammatory effects on adipocytes primarily via inhibition of the nuclear factor κB pathway (Ajuwon and Spurlock, 2005). As a result, release of a number of pro-inflammatory cytokines (e.g. MCP-1, IL-6) by adipocytes is suppressed (Ajuwon and Spurlock, 2005; Dietze-Schroeder *et al.*, 2005). Expression of the anti-inflammatory cytokine IL-10, on the other hand, was lower in the lean group which has been reported previously (Juge-Aubry *et al.*, 2005). The fact that anti-inflammatory cytokine IL-10 is upregulated in adipose tissue from obese humans suggests that adipose tissue may produce anti-inflammatory cytokines with the potential to limit the overall pro-inflammatory response commonly seen in obesity.

It is possible that adipokine secretion pattern might influence blood pressure parameters and in this respect, most focus has been on endocrine factors, such as leptin and adiponectin, which may activate and inhibit, respectively, the sympathetic nervous system (reviewed in Mathieu *et al.*, 2009). In this study, adipose tissue TNF-α (mainly autocrine/paracrine action) correlated with systolic blood pressure. Since TNF-α may act on immune cells and induce local and systemic inflammation, endothelial as well as vascular function might be altered. Indeed, systemic TNF-α has been suggested as an independent risk factor for elevated blood pressure in apparently healthy subjects (Bautista *et al.*, 2005). Notably, the contribution of adipose tissue TNF-α to the circulation has not been fully resolved and there is only a modest elevation of serum TNF-α concentrations in obesity (Hauner, 2005).

There are a number of limitations in this study. The study included a limited number of participants, which presumably is the reason why a difference in circulating levels of inflammatory markers was not detected. Furthermore, control subjects were significantly older than the patients with PCOS, but we adjusted for this age difference, and this appears not to have affected the primary outcome variables: as our study did not include a lean control group, we are unable to determine whether lean patients with PCOS differ in adipose tissue expression of inflammatory markers from weight-matched controls. Furthermore, the inclusion of a lean control group would have enhanced our understanding of how adipose tissue inflammation is influenced by hyperandrogenism and PCOS. Another limitation, discussed above, is that only adipose tissue biopsies from one anatomical location were available for study.

In conclusion, fat mass rather than a PCOS diagnosis *per se* seems to be the main determinant of increased inflammatory markers in subcutaneous adipose tissue. Further studies investigating the different clinical phenotypes of PCOS in terms of cardiovascular risk are warranted.

Authors' roles

Å.L., M.B., I.S.P. and J.B. designed the study and collected the data, C.B., I.D., T.H. and J.B. performed the data analysis. All authors have participated in the drafting and revising of the manuscript and have given their final approval of the version to be published.

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