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ORIGINAL ARTICLE

Hyperandrogenism exerts an anti-inflammatory effect in obese women with polycystic ovary syndrome

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Abstract We determined the effect of chronic androgen suppression on inflammation in women with polycystic ovary syndrome (PCOS) compared to weight-matched controls. We performed a pilot project using samples from previous prospective, controlled studies. Nine women with PCOS (5 obese, 4 lean) and 9 ovulatory controls (5 obese, 4 lean) participated in the study. Androgens, C-reactive protein (CRP), interleukin-6 (IL-6), free fatty acids (FFA) and body weight were measured before and after 3 and 6 months of gonadotropin-releasing hormone (GnRH) agonist administration. GnRH agonist treatment decreased estradiol, testosterone and androstenedione to similar levels in all subjects. CRP and IL-6 increased in obese women with PCOS, was unaltered in lean women with PCOS and obese controls, and decreased in lean controls after 6 months of treatment. FFA decreased and body weight increased in obese women with PCOS, but did not change significantly in lean women with PCOS and in either control group after 6 months of treatment. The testosterone reduction was related to increases in weight and IL-6. The fall in FFA was related to the rise in CRP. The increases in weight and IL-6 were related to the rise in CRP. We propose that hyperandrogenism in PCOS may exert an anti-inflammatory effect when obesity is present, but may not promote inflammation in the disorder; and that circulating androgens have a pleiotropic effect on inflammation depending on the combination of PCOS and weight status in a given individual.

Keywords Androgens · Inflammation · Adiposity · Lypolysis

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Introduction

The polycystic ovary syndrome (PCOS) is one of the most common female endocrinopathies affecting between 8 and 12 % of reproductive-age women [1]. The disorder is characterized by hyperandrogenism, chronic oligo- or anovulation, and polycystic ovaries, with 2 out of these 3 findings required to diagnose PCOS [2]. As many as 70 % of women with PCOS exhibit insulin resistance, with the compensatory hyperinsulinemia considered to be a promoter of the hyperandrogenism [3, 4]. In addition, women with PCOS are often obese, which is strongly associated with insulin resistance and chronic low-grade inflammation [5–12].

We have previously shown that in PCOS, a dietary trigger such as glucose is capable of inciting an inflammatory response from peripheral blood mononuclear cells (MNC) independent of obesity [7–11]. Indeed, MNC of lean women with PCOS exhibit increased nuclear factor κB (NFκB) activation and altered tumor necrosis factor-α (TNF-α) release from MNC following oral glucose

ingestion [7–10]. It remains to be established whether inflamed adipose tissue is the principal contributor to the proinflammatory state in PCOS since there is increased prevalence of abdominal adiposity in PCOS across all weight classes [13]. Our previous reports have also demonstrated that circulating and molecular markers of inflammation are highly correlated with circulating androgens in the disorder [7–9]. These findings raise the possibility that in PCOS, either inflammation is a promoter of hyperandrogenism or conversely, hyperandrogenism is a promoter of inflammation. As a detractor to the latter concept, androgens such as testosterone promote lypolysis that limits the expansion of inflamed adipose tissue [14].

Circulating C-reactive protein (CRP) is an acute-phase reactant that has emerged as a major predictor of metabolic dysfunction in asymptomatic individuals, and also plays a functional role by promoting the uptake of lipids into MNC-derived foamy macrophages within atherosclerotic plaques [15, 16]. MNC-derived macrophages present in adipose tissue produce roughly half of adipose tissue interleukin-6 (IL-6), the endocrine cytokine that stimulates CRP synthesis in the liver [17]. Our recent meta-analysis revealed that CRP is the most reliable circulating marker of chronic low-grade inflammation in PCOS [18]. Nevertheless, CRP elevations in lean women with PCOS are rather modest compared with the obese whether or not they have PCOS [19, 20]. Equally as important is the fact that exacerbation or amelioration of inflammation in the clinical circumstance is reflected by a respective increase or decrease in CRP [21, 22].

We embarked on a pilot study using sera from previous studies [23, 24]. We examined the effect of chronic androgen suppression with a long-acting gonadotropin-releasing hormone (GnRH) agonist on circulating levels of CRP and IL-6 in women with PCOS compared with age- and weight-matched ovulatory controls. We also examined the relationship of the CRP and IL-6 responses to chronic androgen suppression with those of body weight, circulating free fatty acids (FFA), androgens and each other. We hypothesized that in response to chronic androgen suppression, CRP and IL-6 levels are altered in PCOS, and that these alterations are related to those of body mass, FFA and androgens.

Materials and methods

Participants

Sera were available from participants in our previous investigations of adrenal dysfunction in PCOS [23, 24]. The original cohort consisted of 12 women with PCOS (6 lean and 6 obese) and 9 control subjects (4 lean and 5 obese). Unfortunately, the sera of 3 women with PCOS (2 lean and 1 obese) had been discarded long before the current study was conceived. The women with PCOS were diagnosed on the basis of oligo-amenorrhea and hyperandrogenemia after excluding nonclassic congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, and thyroid disease. Polycystic ovaries were present on ultrasound in all subjects with PCOS. All control subjects were ovulatory as evidenced by regular menses, a biphasic basal body temperature curve, and a luteal range serum progesterone level (>5 ng/ml) after the temperature shift in the cycle before beginning the study. All control subjects exhibited normal circulating androgen levels and the absence of polycystic ovaries on ultrasound.

Diabetes, chronic inflammatory illnesses, and smoking were excluded in all subjects. None exhibited any minor inflammatory illnesses. None was taking medications that could affect carbohydrate metabolism or immune function for at least 6 weeks before and throughout study participation. None was involved in any regular exercise program for at least 6 months before the time of testing. All subjects provided written informed consent in accordance with Institutional Review Board guidelines for the protection of human subjects.

Study design

Subjects received a GnRH agonist (7.5 mg IM Lupron Depot; Abbott Laboratories, North Chicago, IL, USA) monthly at 8 AM for 6 months. The injections were begun without regard to the onset of menses in subjects with PCOS, and on day 3 of the menstrual cycle in ovulatory controls. All subjects underwent a fasting blood draw at 8 AM the day before beginning treatment as a baseline, and after 3 and 6 months of treatment.

All subjects were instructed to consume a healthy diet consisting of 50 % carbohydrate, 35 % fat, and 15 % protein, and maintained 72 h food records just before blood draw visits at 3 and 6 months that were reviewed by a dietician to monitor compliance. Height without shoes was measured to the nearest 1.0 cm at the beginning of the study. Body weight was measured to the nearest 0.1 kg at the beginning of the study as a baseline, and after 3 and 6 months of GnRH agonist treatment. Insulin resistance was estimated at baseline by HOMA-IR using the following formula: fasting glucose (mM) × fasting insulin/22.5 [25].

Serum measurements

Luteinizing hormone (LH), estradiol, testosterone, androstenedione, and DHEA-S were measured as previously described [26]. CRP was measured by a high-sensitivity

Tina-quant latex particle-enhanced immunoturbidimetric assay on a Roche Cobas c311 chemistry analyzer (Roche Diagnostics, Indianapolis, IN, USA). IL-6 was measured by high-sensitivity ELISA (eBiosience, Inc., San Diego, CA, USA). FFA was measured by an enzymatic colorimetric assay (NEFA-HR(2), Wako Diagnostics, Richmond, VA, USA). Glucose was measured using a hexokinase reagent on the same Roche Cobas c311 chemistry analyzer. Insulin was measured using a two-site immunoenzymatic assay on a DxI 800 automated system (Beckman Instruments, Chaska, MN, USA). The interassay and intraassay coefficients of variation for these assays were not greater than 6 and 12 %, respectively. The interassay and intra-assay coefficients of variation for the CRP assay in particular were less than 2.4 and 1.9 %, respectively.

Statistics

The StatView software package (SAS Institute, Cary, NC, USA) was used for statistical analysis. The primary endpoint was change from baseline of variables within group (0 vs. 3 months; 0 vs. 6 months). The secondary outcome was change from baseline among groups. Data are presented as mean ± SE. Descriptive data and differences in variables during treatment were compared between groups using unpaired Student's *t* test (PCOS vs. controls) or ANOVA for multiple group comparisons, followed by selective post hoc analysis. Change from baseline during treatment was analyzed using repeated measures ANOVA followed by selective post hoc paired Student's *t* tests. Treatment effects on CRP, the primary dependent variable, IL-6, FFA, androgens, and body weight were determined after calculating the incremental change (Δ) from baseline (3 months minus 0 months; 6 months minus 0 months) for each participant. The Spearman rank correlation coefficient was used to estimate the correlation between parameters. Results were considered significant at a two-tailed α-level of 0.05.

Results

Baseline body composition and endocrine status

Age and height were similar among groups (Table 1). Body weight and body mass index (BMI) were significantly ($P < 0.03$) greater in obese subjects compared with lean subjects whether or not they had PCOS, but were similar when women with PCOS were compared with weight-matched controls. Serum levels of LH, testosterone, androstenedione, and DHEA-S were significantly ($P < 0.05$) higher in women with PCOS compared with controls independent of body mass. Estradiol levels were significantly

Table 1 Baseline age, body composition, hormone, glucose and insulin levels, and HOMA-IR of subjects

	PCOS		Control	
	Lean ($n = 4$)	Obese ($n = 5$)	Lean ($n = 4$)	Obese ($n = 5$)
Age (year)	21 ± 2	25 ± 3	28 ± 3	29 ± 3
Height (cm)	163.4 ± 5.5	165.1 ± 2.5	163.8 ± 2.2	167.2 ± 2.6
Body weight (kg)	63.1 ± 5.3[a,b]	101.4 ± 4.7[c]	60.1 ± 1.3	100.1 ± 9.7[d]
Body mass index (kg/m^2)	23.5 ± 0.4[a,b]	37.4 ± 2.3[c]	22.4 ± 0.5	35.7 ± 3[d]
LH (mIU/ml)	20.2 ± 1.5[b,e]	14.4 ± 2.9[c,f]	5.1 ± 0.2	3.9 ± 0.6
Estradiol (pg/ml)	58.3 ± 1.2[b]	65.0 ± 5.6[f]	61.0 ± 3.1	40.1 ± 3.5[d]
Testosterone (ng/dl)	83.3 ± 19.0[b,e]	106.3 ± 14.0[c,f]	34.0 ± 7.4	38.5 ± 4.5
Androstenedione (ng/ml)	3.9 ± 0.7[e]	4.8 ± 0.5[c,f]	2.2 ± 0.2	2.8 ± 0.3
DHEA-S (μg/dl)	495 ± 82[b,e]	391 ± 45[c,f]	174 ± 36	166 ± 29
Fasting glucose (mg/dl)	75.0 ± 6.9	85.5 ± 2.8	76.5 ± 3.8	80.2 ± 4.7
Fasting insulin (μiU/ml)	20.6 ± 3.7[e]	21.9 ± 3.9[c,f]	7.5 ± 0.6	12.9 ± 0.7
HOMA-IR (mM-μU/ml)	3.7 ± 0.5[e]	4.7 ± 0.9[c,f]	1.4 ± 0.1	2.5 ± 0.1

Values are expressed as mean ± SE; conversion factors to SI units: testosterone ×3.467 (nmol/liter), androstenedione ×3.492 (nmol/liter), DHEA-S ×0.002714 (μmol/liter), Glucose ×0.0551 (mmol/liter), and insulin 7.175 (pmol/liter)

[a] Lean PCOS vs. obese PCOS, $P < 0.002$

[b] Lean PCOS vs. obese controls, $P < 0.03$

[c] Obese PCOS vs. lean controls, $P < 0.03$

[d] Obese controls vs. lean controls, $P < 0.002$

[e] Lean PCOS vs. lean controls, $P < 0.05$

[f] Obese PCOS vs. obese controls, $P < 0.04$

($P < 0.03$) higher in either PCOS group and in lean controls compared with obese controls.

Baseline metabolic and inflammation status

Fasting glucose levels were similar in women with PCOS compared with controls independent of body mass. Fasting insulin levels and HOMA-IR were significantly ($P < 0.05$) higher in women with PCOS compared with weight-matched controls, and in obese women with PCOS compared with lean controls (Table 1). Pretreatment FFA levels in obese subjects regardless of PCOS status were significantly ($P < 0.05$) higher compared with lean controls, and modestly higher ($P = 0.05$) compared with lean women with PCOS (Tables 2, 3, 4). Pretreatment FFA levels were similar in obese women with PCOS compared with obese controls and in lean women with PCOS compared with lean controls. Pretreatment IL-6 levels in obese subjects regardless of PCOS status were significantly ($P < 0.05$) higher compared with lean women with PCOS. Pretreatment fasting CRP levels were 2–3 times higher in obese subjects whether or not they had PCOS, but these differences were not statistically significant.

Treatment effect on estradiol and androgens

Serum levels of estradiol, testosterone, and androstenedione were significantly ($P < 0.04$) reduced to similar levels in women with PCOS and in control subjects after 3 months of GnRH agonist administration, and remained similar after 6 months of treatment (Fig. 1a–d). Serum estradiol levels in particular, remained <28 pg/ml in all subjects during treatment. In women with PCOS, serum DHEA-S levels exhibited a progressive 18 % decline by the end of 6 months of GnRH agonist treatment. This decline in DHEA-S approached significance ($P = 0.07$) compared with baseline but levels were not reduced to that of controls

Table 2 FFA levels at baseline (0 month), and after 3 and 6 months of GnRH agonist treatment in women with PCOS, and in control subjects

FFA (mmol/l)	GnRH agonist treatment (months)		
	0	3	6
PCOS			
Lean	0.47 ± 0.04	0.43 ± 0.04	0.51 ± 0.04
Obese	0.74 ± 0.10[a,c,d]	0.63 ± 0.09[b]	0.53 ± 0.06
Controls			
Lean	0.44 ± 0.72[e]	0.44 ± 0.07[e]	0.57 ± 0.02[e]
Obese	0.79 ± 0.12[f,g,h]	0.69 ± 0.9[h]	0.76 ± 0.12[h]

[a] Obese PCOS, 0 vs. 3 months, $P < 0.05$

[b] Obese PCOS, 3 vs. 6 months, $P < 0.04$

[c] 0 Months, obese PCOS vs. lean controls, $P < 0.05$

[d] 0 Months, obese PCOS vs. lean PCOS, $P = 0.09$

[e] 0, 3 and 6 months, lean PCOS vs. lean controls, $P = 0.95$

[f] 0 Months, obese controls vs. lean controls, $P < 0.03$

[g] 0 Months, obese controls vs. lean PCOS, $P = 0.05$

[h] 0, 3 and 6 months, obese PCOS vs. obese controls, $P = 0.95$

Table 3 IL-6 levels at baseline (0 month), and after 3 and 6 months of GnRH agonist treatment in women with PCOS, and in control subjects

IL-6 (pg/ml)	GnRH agonist treatment (months)		
	0	3	6
PCOS			
Lean	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.3
Obese	2.8 ± 0.3[a,d]	3.1 ± 0.3[f,g]	4.1 ± 0.7[h,i,j]
Controls			
Lean	1.8 ± 0.2[b]	1.1 ± 0.3[c]	0.9 ± 0.2[c]
Obese	2.6 ± 0.5[e]	2.4 ± 0.6	2.5 ± 0.5

[a] Obese PCOS, 0 vs. 6 months, $P < 0.05$

[b] Lean controls, 0 vs. 3 months, $P = 0.08$

[c] Lean controls, 0 vs. 6 months, $P = 0.08$

[d] 0 Months, obese PCOS vs. lean PCOS, $P < 0.03$

[e] 0 Months, obese controls vs. lean PCOS, $P < 0.05$

[f] 3 Months, obese PCOS vs. lean controls, $P < 0.008$

[g] 3 Months, obese PCOS vs. lean PCOS, $P < 0.02$

[h] 6 Months, obese PCOS vs. lean controls, $P < 0.007$

[i] 6 Months, obese PCOS vs. lean PCOS, $P < 0.006$

[j] 6 Months, obese PCOS vs. obese controls, $P < 0.05$

Table 4 CRP levels at baseline (0 month), and after 3 and 6 months of GnRH agonist treatment in women with PCOS, and in control subjects

CRP (mg/l)	GnRH agonist treatment (months)		
	0	3	6
PCOS			
Lean	2.0 ± 0.3	1.5 ± 0.6	2.3 ± 0.3
Obese	5.9 ± 3.0[a]	11.5 ± 4.3	15.0 ± 5.0[d,e]
Controls			
Lean	2.7 ± 0.2[b,c]	0.5 ± 0.2	0.4 ± 0.2
Obese	5.4 ± 3.0	6.2 ± 3.3	7.1 ± 3.1

Values are expressed as mean ± SE, *FFA* free fatty acids, *IL-6* interleukin-6, *CRP* C-reactive protein, *GnRH agonist* gonadotropin-releasing hormone agonist

Conversion factors to SI units: CRP ×9.524 (nmol/liter), IL-6 ×1.311 (pmol/liter)

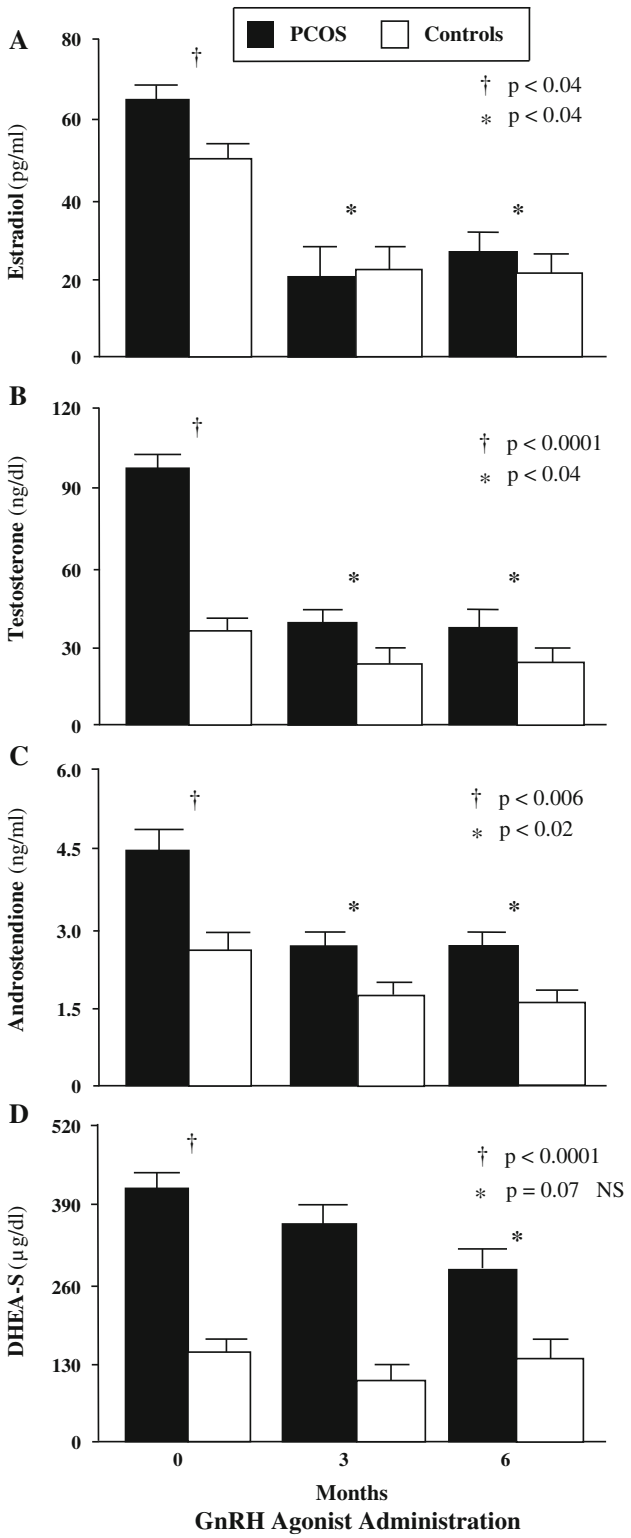
[a] Obese PCOS, 0 vs. 6 months, $P < 0.05$

[b] Lean controls, 0 vs. 3 months, $P < 0.02$

[c] Lean controls, 0 vs. 6 months, $P < 0.02$

[d] 6 Months, obese PCOS vs. lean controls, $P < 0.02$

[e] 6 Months, obese PCOS vs. lean PCOS, $P < 0.03$



◀**Fig. 1** Serum levels of **a** estradiol, **b** testosterone, **c** androstenedione, and **d** DHEA-S before and after 3 and 6 months of gonadotropin-releasing hormone (GnRH) agonist administration. Women with PCOS exhibited significantly higher baseline levels of estradiol (*, $P < 0.04$), testosterone (*, $P < 0.0001$), androstenedione (*, $P < 0.006$), and DHEA-S (*, $P < 0.0001$). GnRH agonist treatment significantly decreased estradiol (*, $P < 0.04$), testosterone (*, $P < 0.04$), and androstenedione (*, $P < 0.02$) to similar levels throughout treatment in women with PCOS and control subjects. DHEA-S progressively declined to approach significance (*, $P = 0.07$) in women with PCOS by the end of treatment, but was unaltered in control subjects

during this treatment interval. In contrast, serum DHEA-S levels in controls remained unaltered during treatment. The hormonal response to GnRH agonist treatment was similar whether or not women with PCOS and controls were lean or obese (data no shown).

Treatment effect on FFA and body weight

Obese women with PCOS exhibited a significant ($P < 0.05$) decline in FFA after 3 months of GnRH agonist treatment with a further decline ($P < 0.04$) in FFA after 6 months of treatment (Table 2). FFA remained unaltered in obese controls, and increased slightly in lean subjects regardless of PCOS status without achieving significance during GnRH agonist treatment. FFA remained similar in obese women with PCOS compared with obese controls and in lean women with PCOS compared with lean controls after 3 and 6 months of GnRH agonist treatment. The incremental Δ from baseline in FFA was significantly lower in obese women with PCOS compared with lean women with PCOS ($P < 0.03$) and lean controls ($P < 0.02$) after 6 months of GnRH agonist treatment (Fig. 2a).

All subjects were compliant with the recommended diet based on food record reviews after 3 and 6 months of treatment. Obese women with PCOS exhibited a significant weight increase after 3 months of GnRH agonist treatment compared to baseline (101.4 ± 4.7 vs. 103.8 ± 5.0 kg, $P < 0.009$), with a further weight increase by the end of 6 months of treatment (101.4 ± 4.7 vs. 109.3 ± 6.4 kg, $P < 0.03$). Body weight remained unaltered in lean women with PCOS, and declined slightly in control subjects regardless of weight class without achieving significance during GnRH agonist treatment (data not shown). The incremental Δ from baseline in body weight was significantly higher in obese women with PCOS compared with obese controls ($P < 0.02$) and lean controls ($P < 0.04$) after 3 and 6 months of GnRH agonist treatment (Fig. 2b).

Treatment effect on CRP and IL-6

Obese women with PCOS exhibited a progressive and significant ($P < 0.05$) increase in CRP by the end of 6 months of GnRH agonist treatment (Table 3). In contrast, lean controls exhibited a significant ($P < 0.02$) decline in CRP after 3 months of treatment that persisted after 6 months of treatment. CRP concentrations in lean women with PCOS and obese controls remained unaltered during GnRH agonist treatment.

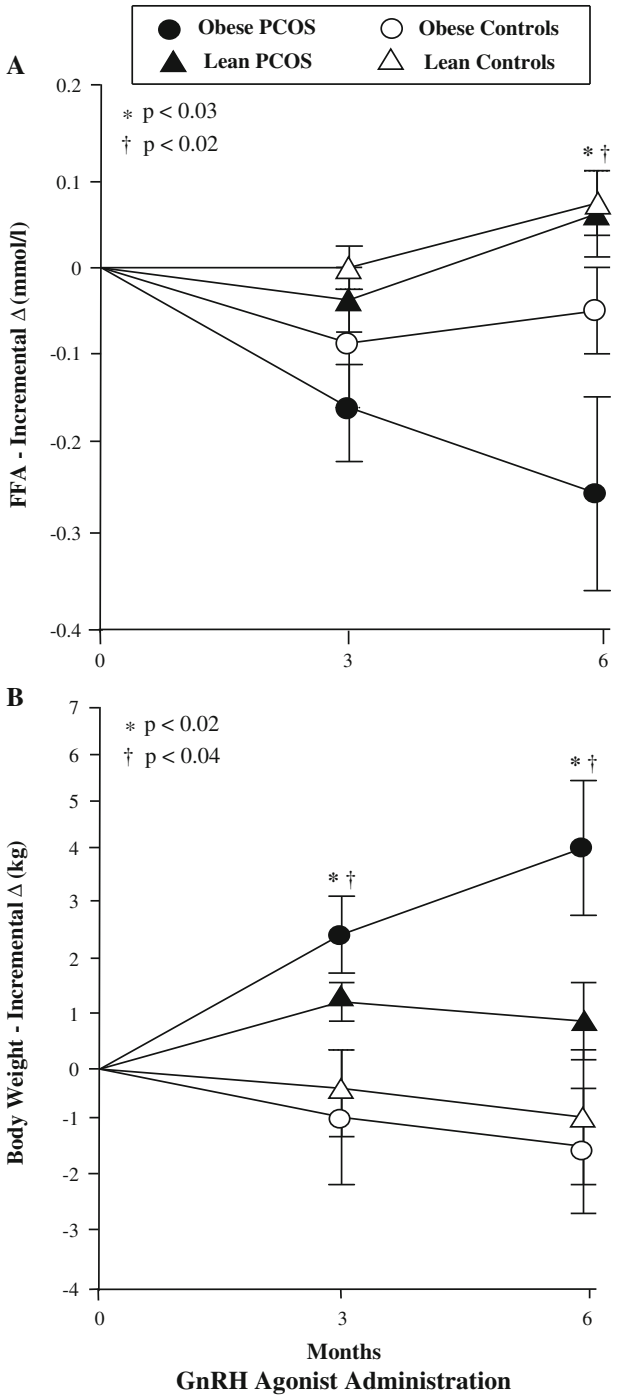


Fig. 2 The incremental change (Δ) from baseline in **a** serum free fatty acids (FFA) levels and **b** body weight after 3 and 6 months of gonadotropin-releasing hormone (GnRH) agonist administration. The incremental Δ in FFA was significantly lower in obese women with PCOS compared with lean women with PCOS (*, $P < 0.03$) and lean controls (†, $P < 0.02$) after 6 months of GnRH agonist treatment. The incremental Δ in body weight was significantly higher in obese women with PCOS compared to obese controls (*, $P < 0.02$) and lean controls (†, $P < 0.04$) after 3 and 6 months of GnRH agonist treatment

The incremental Δ from baseline in CRP was significantly ($P < 0.009$) higher in obese women with PCOS compared with lean controls after 3 and 6 months of GnRH agonist treatment (Fig. 3a). Obese women with PCOS also exhibited a significantly higher incremental Δ in CRP compared with obese controls ($P < 0.005$) and lean controls ($P < 0.007$) after 6 months of treatment.

Obese women with PCOS exhibited a progressive and significant ($P < 0.05$) increase in IL-6 by the end of 6 months of GnRH agonist treatment (Table 4). In contrast, lean controls exhibited a decline in IL-6 after 3 months of treatment that approached statistical significance ($P = 0.08$) and persisted after 6 months of treatment. IL-6 concentrations in lean women with PCOS and obese controls remained unaltered during GnRH agonist treatment.

The incremental Δ from baseline in IL-6 was significantly ($P < 0.03$) higher in obese women with PCOS compared with lean controls after 3 and 6 months of GnRH agonist treatment (Fig. 3b). Obese women with PCOS also exhibited a significantly higher incremental Δ in CRP compared with obese controls ($P < 0.04$) and lean controls ($P < 0.008$) after 6 months of treatment.

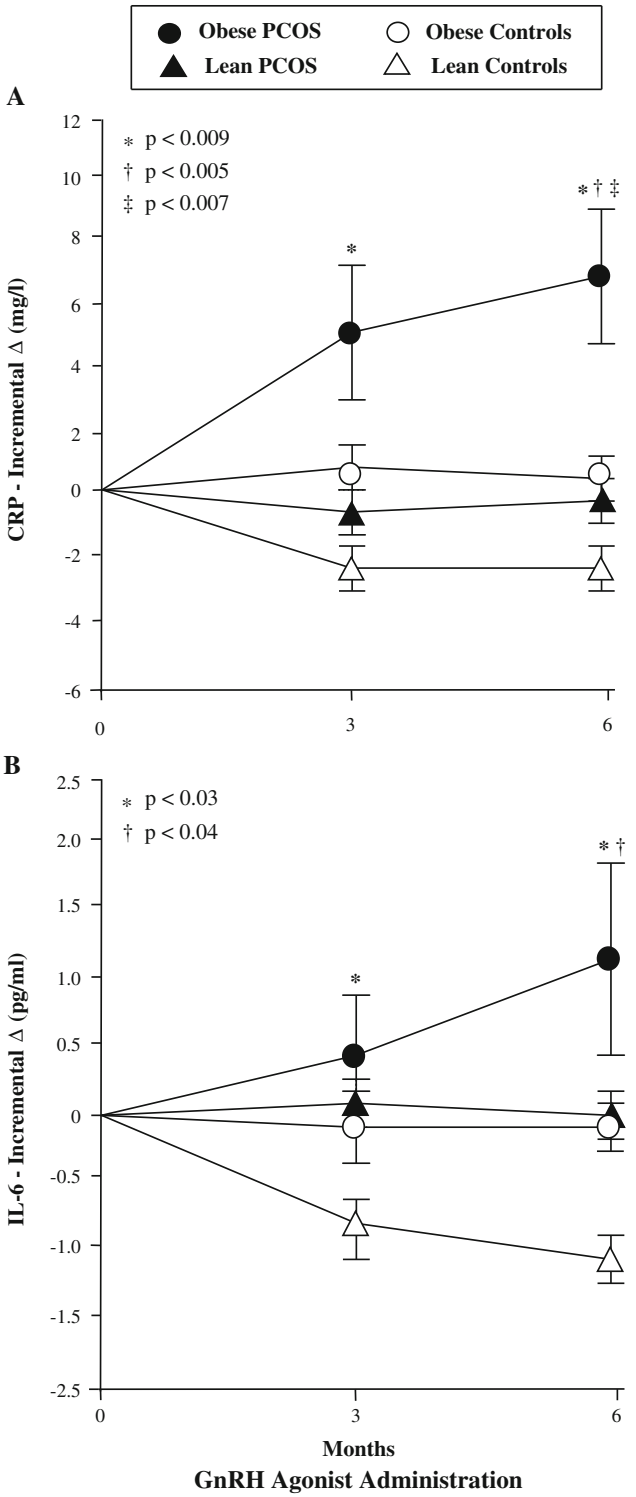
Correlations

At baseline, HOMA-IR was positively correlated with body weight, BMI and FFA levels for the combined groups (Table 5). Baseline FFA and IL-6 levels were positively correlated with baseline body weight and BMI. In addition, baseline IL-6 levels were positively correlated with baseline levels of FFA and CRP.

The 6-month incremental Δ in CRP and IL-6 was negatively correlated with the 6-month incremental Δ in testosterone for the combined groups (Table 6). The 6-month incremental Δ in CRP was also negatively correlated with the 6-month incremental Δ in FFA, and positively correlated with the 6-month incremental Δ in body weight, BMI and IL-6. The 6-month incremental Δ in body weight and BMI was negatively correlated with the 6-month incremental Δ in testosterone. Finally, the baseline and 6-month incremental Δ in androstenedione and DHEA-S did not correlate with any of the other parameters (data not shown).

Discussion

Our data suggest that hyperandrogenism may exert an anti-inflammatory effect in PCOS in the presence of obesity, and may not promote inflammation in the disorder. CRP and IL-6 increase in obese women with PCOS, and FFA



◀**Fig. 3** The incremental change (Δ) from baseline in **a** serum C-reactive protein (CRP) and **b** interleukin-6 (IL-6) levels after 3 and 6 months of gonadotropin-releasing hormone (GnRH) agonist administration. The incremental Δ in CRP was significantly higher in obese women with PCOS compared with lean controls (*, $P < 0.009$) after 3 and 6 months of GnRH agonist treatment; and compared to obese controls (†, $P < 0.005$) and lean controls (‡, $P < 0.007$) after 6 months of treatment. The incremental Δ in IL-6 was significantly higher in obese women with PCOS compared with lean controls (*, $P < 0.03$) after 3 and 6 months of GnRH agonist treatment; and compared with obese controls (†, $P < 0.04$) after 6 months of treatment

decrease in consort with weight gain in response to chronic androgen suppression following GnRH agonist administration. Furthermore, the rise in CRP is related to the increase in body weight, and the reduction in testosterone is related to rises in CRP, IL-6, and body weight during treatment. This suggests that hyperandrogenism in PCOS in combination with obesity exerts an anti-inflammatory effect by limiting expansion of inflamed adipose tissue. The lack of alteration in CRP, IL-6, FFA, and weight in obese controls and lean women with PCOS in response to GnRH agonist administration suggests that development of adiposity-related inflammation is not controlled by androgens in these individuals. On the other hand, the fall in CRP and IL-6 in healthy reproductive-age women who do not have PCOS or obesity in the face of unaltered FFA and weight in response to GnRH agonist administration suggests that in these individuals, androgen-induced alteration in inflammatory load is unrelated to factors affecting adiposity. Thus, androgens may impart a pleiotropic effect on inflammation depending on the combination of PCOS and weight status present in a given individual; and the anti-inflammatory effect of hyperandrogenism in PCOS may only occur after a threshold of body mass is superseded.

The ability of elevated circulating androgens to promote lipolysis may be responsible for the anti-inflammatory effect in obese women with PCOS [27]. Testosterone in particular, is known to stimulate catecholamine-induced hormone-sensitive lipase activity which in turn, limits adipose tissue expansion [14]. This is important because it is now clear that excess adipose tissue is a reservoir for MNC-derived macrophages that produce roughly half of adipose tissue IL-6, the endocrine cytokine that stimulates CRP synthesis in the liver [17, 28]. The fall in FFA levels during treatment may be the result of decreased lypolysis following androgen suppression to explain the progressive weight gain in obese women with PCOS that most likely represents expansion of the adipose tissue compartment over the 6 months of GnRH agonist administration. The rise in IL-6 levels during treatment may represent subsequent increased IL-6 production from inflamed adipose tissue which in turn stimulates the rise in CRP observed in these individuals. This concept is supported by the GnRH agonist-induced testosterone reduction that is related to increases in weight, BMI, and IL-6, the fall in FFA that is related to the rise in CRP, and the increases in weight, BMI and IL-6 that are related to the rise in CRP. The significant decline in FFA serves as a compelling surrogate for adipose accumulation in support of decreased lypolysis. This

Table 5 Spearman rank correlations of baseline (0 month) FFA, CRP, and IL-6 levels and HOMA-IR vs. baseline (0 month) body composition parameters, estradiol, testosterone, and each other for the combined groups

	Weight (kg)	BMI (kg/m^2)	Estradiol (pg/ml)	Testosterone (ng/dl)	HOMA-IR (mM-μU/ml)	IL-6 (pg/ml)	CRP (mg/l)
FFA (mmol/l)							
r	0.781	0.762	−0.254	0.033	0.497	0.601	0.364
P	0.001*	0.002*	0.295	0.892	0.040*	0.016*	0.135
CRP (mg/l)							
r	0.354	0.333	0.378	0.124	0.020	0.556	–
P	0.144	0.169	0.120	0.609	0.936	0.026*	–
IL-6 (pg/ml)							
r	0.767	0.740	0.057	0.202	0.392	–	–
P	0.002*	0.003*	0.822	0.418	0.117	–	–
HOMA-IR (mM-μU/ml)							
P	0.628	0.579	0.040	0.324	–	–	–
P	0.010*	0.017*	0.868	0.181	–	–	–

FFA free fatty acids, *CRP* C-reactive protein, *IL-6* lnterleukin-6, *HOMA-IR* homeostatic model assessment-insulin resistance, *BMI* body mass index

Conversion factors to SI units: CRP ×9.524 (nmol/liter), IL-6 ×1.311 (pmol/liter), estradiol ×3.671 (pmol/liter), and testosterone ×3.467 (nmol/liter)

* $P < 0.05$

Table 6 Spearman rank correlations of the incremental change from baseline (Δ) in FFA, CRP, IL-6, and testosterone and estradiol after 6 months of GnRH agonist treatment vs. the incremental change from baseline (Δ) in body composition parameters and each other after 6 months of GnRH agonist treatment for the combined groups

	Δ Weight (kg)	Δ BMI (kg/m^2)	Δ Estradiol (pg/ml)	Δ Testosterone (ng/dl)	Δ IL-6 (pg/ml)	Δ CRP (mg/l)
Δ FFA (mmol/l)						
r	−0.327	−0.320	−0.266	0.275	0.275	−0.613
P	0.234	0.247	0.338	0.322	0.341	0.027*
Δ CRP (mg/l)						
r	0.633	0.634	−0.007	−0.578	0.566	–
P	0.023*	0.022*	0.981	0.037*	0.049*	–
Δ IL-6 (pg/ml)						
r	0.467	0.471	0.022	−0.626	–	–
P	0.107	0.103	0.939	0.030*	–	–
Δ Testosterone (ng/dl)						
r	−0.822	−0.821	0.486	–	–	–
P	0.003*	0.003*	0.080	–	–	–
Δ Estradiol (pg/ml)						
r	−0.569	−0.568		–	–	–
P	0.039*	0.040*	–	–	–	–

FFA free fatty acids, *CRP* C-reactive protein, *IL-6* lnterleukin-6, *GnRH agonist* gonadotropin-releasing hormone agonist, *BMI* body mass index, and conversion factors to SI units: CRP ×9.524 (nmol/liter), IL-6 ×1.311 (pmol/liter), estradiol ×3.671 (pmol/liter), and testosterone ×3.467 (nmol/liter)

* $P < 0.05$

is in contrast to the elevations in FFA that result from dysregulated lypolysis associated with weight gain after excess caloric intake in simple obesity [29]. Further corroboration is provided by a previous report in which visceral fat accumulation was detected by serial single-slice CT scans in obese women with PCOS during 3 months of GnRH agonist treatment [30]. However, weight gain was not observed in this cohort. This may have been due to the shorter duration of treatment since most of the weight gain in our study occurred between 3 and 6 months of treatment.

Estrogen has also been shown to promote lypolysis [31], and the observed estrogen reduction is related to the

increases in weight and BMI during GnRH agonist treatment. However, the lack of association between the estrogen alteration and those of FFA, CRP, or IL-6 during treatment suggests that estrogen is less likely to contribute to the proposed anti-inflammatory effect. In addition, the decline in DHEA-S in women with PCOS during GnRH agonist treatment is most likely a separate phenomenon, and may reflect removal of an ovarian influence on adrenal androgen production as proposed in our previous studies [23, 24].

Circulating androgens have a limited effect on lipolysis in lean women with PCOS and obese reproductive-age women without PCOS. It is well documented that catecholamine resistance of subcutaneous adipose tissue precludes adequate induction of hormone-sensitive lipase activity in these individuals [32, 33]. This phenomenon can limit expansion of inflamed adipose tissue to explain the lack of alteration in FFA, CRP, IL-6, and body weight in both of these groups during GnRH agonist treatment. These findings support the concept that androgens do not control the factors responsible for obesity in reproductive-age women without PCOS. Most importantly, they suggest that hyperandrogenism does not promote the well-documented proinflammatory state in PCOS that is independent of obesity. Lypolysis is paradoxically increased in visceral adipose tissue of lean women with PCOS [34]. As demonstrated previously, however, decreased visceral adipose tissue lypolysis in response to GnRH agonist-induced suppression of hyperandrogenism may not manifest as overt weight gain [30]. This in turn, may not sufficiently increase the inflammatory load to raise CRP and IL-6.

Circulating androgens may raise the inflammatory load in lean healthy reproductive-age women which is a population that is not inflamed at baseline. CRP and IL-6 levels before GnRH agonist treatment are lower in the lean controls compared with obese individuals whether or not they have PCOS as corroborated by previous reports [19, 35]. Androgen suppression reduces CRP and IL-6 in lean controls as early as 3 months after GnRH agonist administration. In contrast, we have shown that 5 days of oral androgen administration to elevate circulating androgens to levels comparable to PCOS induces a proinflammatory response in lean healthy reproductive-age women, [36, 37]. This androgen treatment interval is too short to alter adiposity, and is consistent with the unaltered weight in lean controls of our current study in the face of the reduced inflammatory load following androgen suppression. Thus, the status of circulating androgens may serve as a barometer for inflammation in lean healthy reproductive-age women

The main limitation of the study is the modest sample size partially related to the loss of some samples since the original studies were performed. Nevertheless, the corroborative alteration pattern of testosterone, body weight, FFA, CRP, and IL-6 provides validity to our hypothesis. This report may serve as a basis for further hypothesis generation in the rapidly expanding field of chronic low-grade inflammation in PCOS.

In conclusion, circulating CRP and IL-6 and body weight increase in obese women with PCOS, but are unaltered in lean women with PCOS in response to chronic GnRH agonist-induced androgen suppression. Circulating CRP and IL-6 are also unaltered in obese controls, but decrease in lean controls without significant weight change in either group during similar treatment. We propose that hyperandrogenism in PCOS may exert an anti-inflammatory effect when obesity is present, and may not promote inflammation in the disorder. These unique observations suggest that androgens have a pleiotropic effect on inflammation depending on the combination of PCOS and weight status present in a given individual.

References

1. W.A. March, V.M. Moore, K.J. Willson, D.I.W. Phillips, R.J. Normal, M.J. Davies, The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum. Reprod. **25**, 544–551 (2010)
2. The Rotterdam ESHRE/ASRM-Sponsored PCOS Conference Workshop Group, Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Hum. Reprod. **19**, 41–47 (2004)
3. M.O. Goodarzi, S.G. Korenman, The importance of insulin resistance in polycystic ovary syndrome. Fertil. Steril. **77**, 255–258 (2002)
4. J.E. Nestler, D.J. Jakubowicz, A.F. de Vargas, C. Brik, N. Quintero, F. Medina, Insulin stimulates testosterone biosynthesis by human theca cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J. Clin. Endocrinol. Metab. **83**, 2001–2005 (1998)
5. O.G. Kolterman, J. Insel, M. Saekow, J.M. Olefsky, Mechanisms of insulin resistance in human obesity. Evidence for receptor and post receptor defects. J. Clin. Invest. **65**, 1272–1284 (1980)
6. T.P. Ciaraldi, O.G. Kolterman, J.M. Olefsky, Mechanism for the postreceptor defect in insulin action in human obesity. Decrease in glucose transport system activity. J. Clin. Invest. **68**, 875–878 (1981)
7. F. González, N.S. Rote, J. Minium, J.P. Kirwan, Hyperglycemia alters tumor necrosis factor-α release from mononuclear cells in women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. **90**, 5336–5342 (2005)
8. F. González, N.S. Rote, J. Minium, J.P. Kirwan, In vitro evidence that hyperglycemia stimulates tumor necrosis factor-α release in obese women with polycystic ovary syndrome. J. Endocrinol. **88**, 521–529 (2006)
9. F. González, N.S. Rote, J. Minium, J.P. Kirwan, Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J. Clin. Endocrinol. Metab. **91**, 336–340 (2006)
10. F. González, N.S. Rote, J. Minium, J.P. Kirwan, Increased activation of nuclear factor κB triggers inflammation and insulin

resistance in polycystic ovary syndrome. J. Clin. Endocrinol. Metab. **91**, 1508–1512 (2006)
11. F. González, N.S. Rote, J. Minium, J.P. Kirwan, Evidence of proatherogenic inflammation in polycystic ovary syndrome. Metabolism **58**, 954–962 (2009)
12. F. González, N.S. Rote, J. Minium, J.P. Kirwan, Elevated circulating levels of macrophage migration inhibitory factor in polycystic ovary syndrome. Cytokine **51**, 240–244 (2010)
13. E. Carmina, S. Bucchierri, A. Esposito, A. Del Puente, P. Mansueto, F. Orio, G. Di Fede, G. Rini, Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of it relation to insulin resistance. J. Clin. Endocrinol. Metab. **92**, 2500–2505 (2007)
14. X. Xu, G. De Pergola, P. Björntorp, The effects of androgens on the regulation of lipolysis in adipose precursor cells. Endocrinology **126**, 1229–1234 (1990)
15. P.M. Ridker, C.H. Hennekens, J.E. Buring, N. Rifai, C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N. Engl. J. Med. **342**, 836–843 (2000)
16. T.P. Zwaka, V. Hombach, J. Torzewski, C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. Circulation **103**, 1194–1197 (2001)
17. S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante Jr., Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. **112**, 1796–1808 (2003)
18. H.F. Escobar-Morreale, M. Luque-Ramírez, F. González, Serum inflammatory markers in polycystic ovary syndrome: a systematic review and meta-analysis. Fertil. Steril. **95**, 1048–1058 (2011)
19. N. Boulman, L.R. Leiba, S. Shachar, R. Linn, O. Zinder, Z. Blumenfeld, Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. J. Clin. Endocrinol. Metab. **89**, 2160–2165 (2004)
20. S.B. Kjøtrød, P. Romundstad, V. von Düring, A. Sunde, S.M. Carlsen, C-reactive protein levels are unaffected by metformin during pretreatment and an IVF cycle in women with polycystic ovary syndrome. Fertil. Steril. **89**, 635–641 (2008)
21. P.H. Dessein, B.I. Joffe, E. Stanwix, High sensitivity C-reactive protein as a disease activity marker in rheumatoid arthritis. J. Rheumatol. **31**, 1095–1097 (2004)
22. M.A. Rahmati, R.G. Craig, P. Homel, G.A. Kaysen, N.W. Levin, Serum markers of periodontal disease status and inflammation in hemodialysis patients. Am. J. Kidney Dis. **40**, 983–989 (2002)
23. F. González, L. Chang, T. Horab, R.A. Lobo, Evidence for heterogeneous etiologies of adrenal dysfunction in polycystic ovary syndrome. Fertil. Steril. **66**, 354–361 (1996)
24. F. González, L. Chang, T. Horab, F.Z. Stanczyk, K. Crickard, R.A. Lobo, Adrenal dynamic response to physiologic and pharmacologic ACTH stimulation before and after ovarian steroid modulation in polycystic ovary syndrome. Fertil. Steril. **71**, 439–444 (1999)
25. D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia **28**, 412–419 (1958)
26. F.Z. Stanczyk, C.C. Slater, D.E. Ramos, C. Azen, G. Cherala, C. Hakala, G. Abraham, S. Roy, Pharmacokinetics of dehydroepiandrosterone and its metabolites after long-term oral dehydroepiandrosterone treatment in postmenopausal women. Menopause **16**, 272–278 (2009)
27. H. Wahrenberg, I. Ek, S. Revnisdottir, K. Carlström, A. Bergqvist, P. Arner, Divergent effects of weight reduction and oral anticonception treatment on adrenergic lypolysis regulation in obese women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. **84**, 2182–2187 (1999)
28. T. Montalcini, V. Migliaccio, Y. Ferro, C. Gazzaruso, A. Pujia, Androgens for postmenopausal women's health? Endocrine. [Epub ahead of print] (12 May 2012). doi:10.1007/s12020-012-9692-1
29. M.D. Jensen, M.W. Haymond, R.A. Rizza, P.E. Cryer, J.M. Miles, Influence of body fat distribution on free fatty acid metabolism in obesity. J. Clin. Invest. **83**, 1168–1173 (1989)
30. D.A. Dumesic, D.H. Abbott, J.R. Eisner, R.R. Herrmann, J.E. Reed, T.J. Welch, M.D. Jensen, Pituitary desensitization to gonadotropin-releasing hormone increases abdominal adiposity in hyperandrogenic anovulatory women. Fertil. Steril. **70**, 94–101 (1998)
31. S. Pedersen, K. Kristensen, P.A. Hermann, J.A. Katzenellenbogen, R. Rochelsen, Estrogen controls lypolysis by up-regulating α2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor α. Implications for the female fat distribution. J. Clin. Endocrinol. Metab. **89**, 1869–1878 (2004)
32. D. Langin, A. Dicker, G. Tavernier, J. Hoffstedt, A. Mairal, M. Rydén, E. Arner, A. Sicard, C.M. Jenkins, N. Viguerie, V. van Harmelen, R.W. Gross, C. Holm, P. Arner, Adipocyte lipases and defect of lypolysis in human obesity. Diabetes **54**, 3190–3197 (2005)
33. I. Ek, P. Arner, A. Bergqvist, K. Carlström, H. Wahrenberg, Impaired adipocyte lypolysis in nonobese women with polycystic ovary syndrome: a possible link to insulin resistance? J. Clin. Endocrinol. Metab. **84**, 2182–2187 (1997)
34. I. Ek, P. Arner, M. Rydén, C. Holm, A. Thörn, J. Hoffstedt, H. Wahrenberg, A unique defect in the regulation of visceral fat cell lypolysis in the polycystic ovary syndrome as an early link to insulin resistance. Diabetes **51**, 484–492 (2002)
35. I. Tarkun, B.C. Arslan, Z. Cantürk, E. Türemen, T. Sahin, C. Duman, Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation. J. Clin. Endocrinol. Metab. **89**, 5592–5596 (2004)
36. F. González, K.S. Nair, J.K. Daniels, E. Basal, J.M. Schimke, Hyperandrogenism sensitizes mononuclear cells to promote glucose-induced inflammation in lean reproductive-age women. Am. J. Physiol. Endocrinol. Metab. **302**, E297–E306 (2012)
37. S.G. Witchel, S.E. Recabarren, F. González, E. Diamanti-Kandarakis, K.I. Cheang, A.J. Duleba, R.S. Legro, R. Homburg, R. Pasquali, R.A. Lobo, C.C. Zouboulis, F. Kelestimur, F. Fruzzetti, W. Futterweit, R.J. Norman, D.H. Abbott, Emerging concepts about prenatal genesis, aberrant metabolism and treatment paradigms in polycystic ovary syndrome. Endocrine. [Epub ahead of print] (4 June 2012). doi:10.1007/s12020-012-9701-4