CLINICAL STUDY

Body fat and insulin resistance independently predict increased serum C-reactive protein in hyperandrogenic women with polycystic ovary syndrome

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Abstract

Objective: Increased serum C-reactive protein (CRP), an independent predictor of coronary heart disease, was reported in women with polycystic ovary syndrome (PCOS). It remains unclear whether this finding is due to the association between PCOS and either insulin resistance, obesity, or androgen excess, which are all common features of this condition. The aims of this study were to assess whether increased serum CRP is a specific feature of PCOS and to investigate the mechanisms underlying this association.

Design and methods: Serum high-sensitivity CRP (hs-CRP) was measured in 86 hyperandrogenic women (age 21.6 ± 4.2 years, body mass index (BMI) 23.6 ± 3.5 kg/m²), 50 with PCOS and 36 with idiopathic hyperandrogenism (HA). Thirty-five BMI-matched healthy women were also studied as controls. In these subjects, endocrine and metabolic profiles were assessed. In all hyperandrogenic subjects and 14 controls, insulin sensitivity was measured by the glucose clamp technique. Body fat was measured by bioelectrical impedance.

Results: Hs-CRP concentrations were higher in PCOS women (3.43 ± 2.01 mg/l) than in HA subjects and healthy women (2.43 ± 1.04 , $P < 0.005$; and 2.75 ± 0.86 mg/l, $P < 0.05$ respectively versus PCOS). In multiple regression analyses, increased serum hs-CRP was independently predicted by higher body fat and lower insulin sensitivity. However, in lean women, serum-free testosterone was an additional, negative, predictive variable.

Conclusions: PCOS is accompanied by a low-grade chronic inflammation. Body fat appears the main determining factor of this finding, which is only partly explained by insulin resistance. At least in lean women, androgen excess *per se* seems to play an additional, possibly protective, role in this association.

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Introduction

A number of studies indicate that serum high-sensitivity C-reactive protein (hs-CRP) is a novel independent cardiovascular risk factor (1). This protein, synthesized by the liver, is a sensitive and stable marker of low-grade chronic inflammation, a key process in the pathogenesis of atherosclerosis (2). Hs-CRP serum concentration, even within the normal range, independently predicted myocardial infarction and ischemic stroke in subjects of the general population – either with or without classical cardiovascular risk factors – as well as in patients with cardiovascular disease (3–5). More recently, hs-CRP levels have also been linked to insulin resistance and the associated features (6, 7). In the general population, hs-CRP levels increase as a function of the number of metabolic alterations and are associated with the metabolic syndrome, a cluster of metabolic disorders that increases the risk of atherosclerosis (8). In addition,

large prospective studies showed that hs-CRP independently predicted risk for type 2 diabetes (9, 10).

Some previous studies assessed serum hs-CRP levels in young women with polycystic ovary syndrome (PCOS), a clinical disorder characterized by hyperandrogenism and ovarian dysfunction. Many of these women show a cluster of cardiovascular risk factors, such as obesity, insulin resistance, lipid abnormalities, hypertension, and abnormal glucose tolerance (11), suggesting that they may have an increased cardiovascular risk later in life. Several of these studies showed higher levels of serum hs-CRP in PCOS women, as compared with controls (12–20). However, this finding was not confirmed by other studies (21–25). The reasons for these discrepancies are not readily understood.

Most studies carried out in PCOS women examined obese subjects and reported a relationship between hs-CRP levels and body weight (13, 16, 22, 26). As hs-CRP production in the liver is modulated by some

adipokines, such as interleukin-6 and tumour necrosis factor α , some authors have hypothesized that increased body fat may be the mechanism underlying the association between increased serum hs-CRP and PCOS. However, some studies have reported increased serum hs-CRP also in nonobese PCOS subjects (12, 15), or after adjusting for body fat (19). It still remains unclear whether insulin resistance, androgen excess, or other undefined aspects linked to the PCOS status may affect this feature.

As hyperandrogenism is often diagnosed in young subjects, before the onset of a full-blown metabolic syndrome and possibly of atherosclerosis (27), hyperandrogenic women offer a unique model to study the relationships between insulin resistance and markers of inflammation in an early phase of their natural history, also allowing us to evaluate the potential role of androgens in this association.

There were two aims in this study: i) to assess whether increased serum hs-CRP is a specific feature of PCOS; and ii) to investigate which factors predict low-grade inflammation in hyperandrogenic subjects.

Materials and methods

Subjects

Eighty-six hyperandrogenic women (age 21.6 ± 4.2 years, body mass index (BMI) 23.6 ± 3.5 kg/m²), referred to the Division of Endocrinology and Metabolism of Verona Hospital, Italy – a tertiary care academic medical center – for hirsutism (modified Ferriman–Gallwey score ≥ 8) and/or hyperandrogenic oligoamenorrhea (< 8 menses per year) were included in the study. Androgen-secreting tumors, congenital adrenal hyperplasia, thyroid dysfunction, hyperprolactinemia, Cushing's syndrome, and diabetes mellitus were ruled out in all subjects. According to both the Rotterdam criteria (28) and the NIH criteria (29), 50 of these women had PCOS. The other 36 patients, not fulfilling criteria for PCOS diagnosis and classified as idiopathic hyperandrogenism (HA), had hirsutism with normal or increased serum androgens, ovulatory menses, and normal ultrasonographic morphology of the ovaries. In this subgroup of women, 18 were hyperandrogenemic and 18 were normoandrogenemic.

All women were studied in the early follicular phase of the menstrual cycle, or after at least 3 months of amenorrhea. None of the women were suffering from any other disease, including recent infectious diseases, or had been taking metformin, oral contraceptives, or any other medications (including statins or acetylsalicylic acid) potentially interfering with the study in the previous 6 months.

Thirty-five BMI-matched healthy nonhirsute women, without polycystic ovaries and with regular ovulatory cycles, served as controls. Matching was based on BMI

ranges versus the entire cohort of hyperandrogenic women. The control group included lean volunteers and women referred to the outpatient clinic of the same medical center for simple obesity.

All subjects gave their informed consent to the study that was conducted in accordance with the Helsinki Declaration and approved by our local Ethical Committee.

Protocol

A complete physical examination, including measurement of hirsutism score (30), height, weight, systolic, and diastolic blood pressure, was carried out in all subjects.

Blood samples for endocrine and metabolic parameters and hs-CRP assay were collected at ~ 0800 h, after overnight fasting. The endocrine profile comprised assessment of total and free testosterone, DHEAS, gonadotropins, and 17-hydroxyprogesterone. In addition, response to a GnRH agonist challenge (31) was carried out by measuring gonadotropins and 17-hydroxyprogesterone on blood drawn 24 h after s.c. injection of 100 μ g buserelin (Suprefact, Hoechst Roussel, Milan, Italy).

The metabolic profile comprised the measurement of serum lipids (total, low density lipoprotein and high density lipoprotein cholesterol, triglycerides) and plasma glucose, and insulin measurement at 0, 30, 60, and 120 min during an oral glucose tolerance test, performed according to the WHO procedures (32).

Insulin sensitivity was measured by the hyperinsulinemic euglycemic clamp technique, as previously described (33). Briefly, after overnight fasting, a continuous insulin infusion (Humulin R, Lilly, Indianapolis, IN, USA) was started and maintained at a constant rate of 80 mU/kg $\times$ min for 120 min. Euglycemia was maintained throughout the test by a variable infusion of 20% dextrose, adjusted by monitoring plasma glucose levels in arterialized venous blood every 5–10 min. As in nondiabetic hyperandrogenic women and controls, endogenous glucose production is negligible at this insulin infusion rate (33), the amount of glucose infused into each subject may be considered equivalent to the whole body insulin-mediated glucose uptake. Because muscle is responsible for most insulin-induced glucose metabolism (34), glucose disposal data were expressed per fat-free mass, as assessed by bioelectrical impedance (BIA 103, Akern, Florence, Italy) (35). Glucose and lipid oxidation during the clamp studies, at baseline and during hyperinsulinemia, was measured by indirect calorimetry (36), as previously described (33).

Assays

Serum steroids, gonadotropins, insulin, lipids, and plasma glucose were assayed, as previously described (33).

Serum hs-CRP was determined by a highly sensitive method (Dimension RxL, Dade, Behring, Italy). The intra- and interassay coefficients of variation for this assay were 3.0 and 4.3% respectively.

Statistical analysis

ANOVA, followed by the Scheffé test for multiple comparisons, was used to compare groups of hyperandrogenic women and controls. Analysis of covariance was carried out to compare between groups CRP values and insulin sensitivity adjusted for age and BMI. Univariate and multiple regression analyses were used to correlate CRP levels with relevant hormonal and metabolic variables. In multiple regression analyses, age, fat mass, insulin sensitivity, and serum androgens (either free or total testosterone) or the PCOS status were included as independent variables. Additional independent variables were also included in some analyses, as indicated. Serum hs-CRP and other skewed variables were log-transformed before analysis to ensure normality of data and then back-transformed to their natural units for presentation in tables and figures.

Results are shown as the mean $\pm$ s.d. *P* values ≤ 0.05 were considered statistically significant. Analyses were performed using Statview 5.0.1 software (SAS Institute, Cary, NC, USA).

Results

Physical and biochemical characteristics of the subjects categorized into PCOS, HA, and controls are shown

in Table 1. Age was significantly different between groups. BMI, fat mass, and fat-free mass were not statistically different in the two groups of hyperandrogenic subjects and controls.

Serum total and free testosterone were significantly increased in both groups of hyperandrogenic women as compared with controls. HA women had significantly higher DHEAS than controls. Women with PCOS, but not the HA women, had significantly higher serum 17-hydroxyprogesterone, at baseline and after GnRH agonist stimulation, than controls.

Systolic and diastolic blood pressure and triglycerides were higher in PCOS women than in controls. Fasting plasma glucose and insulin were not significantly different between groups. However, plasma glucose and insulin 2 h after oral glucose load were increased in both groups of hyperandrogenic women as compared with controls, though these differences were of borderline statistical significance in the HA group. Consistently, insulin-mediated glucose uptake in the glucose clamp was significantly lower in both groups of hyperandrogenic women (PCOS 10.3 ± 2.8 , HA 11.8 ± 3.0 mg/kg fat-free mass $\times$ min) than in controls (13.9 ± 2.8 mg/kg fat-free mass $\times$ min, $P < 0.01$ versus PCOS, $P < 0.05$ versus HA). A statistically significant difference was also found between PCOS and HA subjects ($P < 0.05$). Differences in insulin sensitivity between groups were maintained after adjusting for age and BMI. No differences in serum hs-CRP, as well as in the anthropometric and metabolic features, were found when hyperandrogenic and normoandrogenic HA women were compared (see Table 1 of the Supplementary Materials section, which can be viewed online at <http://www.eje-online.org/supplemental/>).

Table 1 Main characteristics of subjects under study (mean $\pm$ s.d.).

	PCOS (n=50)	HA (n=36)	Controls (n=35)	P by ANOVA
Age (year)	22.4 $\pm$ 4.1 ^{†, §}	20.3 $\pm$ 3.9 [†]	25.4 $\pm$ 4.6	0.001
BMI (kg/m ²)	24.0 $\pm$ 4.0	23.0 $\pm$ 2.6	23.4 $\pm$ 5.2	NS
Fat mass (kg)	20.5 $\pm$ 7.0	18.8 $\pm$ 3.7	18.4 $\pm$ 9.6	NS
Fat-free mass (kg)	42.9 $\pm$ 6.8	42.4 $\pm$ 4.1	42.1 $\pm$ 8.2	NS
Fasting glucose (mmol/l)	4.7 $\pm$ 0.4	4.7 $\pm$ 0.5	4.7 $\pm$ 0.5	NS
Fasting insulin (pmol/l)	80.4 $\pm$ 34.4	82.5 $\pm$ 41.6	79.6 $\pm$ 43.0	NS
2 h-glucose OGTT (mmol/l)	5.5 $\pm$ 1.0 [†]	5.2 $\pm$ 1.1	4.6 $\pm$ 0.9	0.005
2 h-insulin OGTT (pmol/l)	561.8 $\pm$ 378.8 [†]	500.1 $\pm$ 473.5	299.9 $\pm$ 135.6	0.035
Insulinogenic index (mU/mmol)	48.6 $\pm$ 35.4	56.9 $\pm$ 48.5	37.4 $\pm$ 27.6	NS
Insulin-mediated glucose uptake (mg/kg $\times$ min)	7.1 $\pm$ 2.1 ^{†, §}	8.3 $\pm$ 2.1 [*]	9.8 $\pm$ 2.1	0.001
Insulin-mediated glucose uptake (mg/kg FFM $\times$ min)	10.3 $\pm$ 2.8 ^{†, ‡}	11.8 $\pm$ 3.0 [*]	13.9 $\pm$ 2.0	0.001
Hs-CRP (mg/l)	3.43 $\pm$ 2.01 ^{*, §}	2.43 $\pm$ 1.04	2.75 $\pm$ 0.86	0.008
Total cholesterol (mmol/l)	4.85 $\pm$ 0.75	4.45 $\pm$ 0.94	4.57 $\pm$ 0.59	NS
HDL cholesterol (mmol/l)	1.46 $\pm$ 0.30	1.50 $\pm$ 0.32	1.66 $\pm$ 0.38	0.059
Triglycerides (mmol/l)	1.15 $\pm$ 0.70 [*]	0.84 $\pm$ 0.27	0.84 $\pm$ 0.30	0.038
Systolic blood pressure (mmHg)	128 $\pm$ 14 ^{†, ‡}	121 $\pm$ 8	120 $\pm$ 12	0.012
Diastolic blood pressure (mmHg)	77 $\pm$ 9 ^{†, §}	69 $\pm$ 7	72 $\pm$ 8	0.001
Total testosterone (nmol/l)	3.11 $\pm$ 1.45 [*]	3.63 $\pm$ 1.72 [†]	2.09 $\pm$ 0.99	0.010
Free testosterone (pmol/l)	11.9 $\pm$ 5.1 [†]	11.2 $\pm$ 6.8 [*]	6.9 $\pm$ 3.8	0.024
DHEAS (μ mol/l)	6.07 $\pm$ 1.74	7.20 $\pm$ 2.46 [†]	5.23 $\pm$ 2.62	0.023
17OH-progesterone (nmol/l)	4.58 $\pm$ 2.19 ^{†, §}	3.49 $\pm$ 1.49	2.97 $\pm$ 1.14	0.001
17OH-progesterone after buserelin (nmol/l)	12.08 $\pm$ 4.78 ^{†, §}	7.18 $\pm$ 3.31	5.45 $\pm$ 1.33	0.001

* $P < 0.05$ and [†] $P < 0.01$ versus controls; [‡] $P < 0.05$ and [§] $P < 0.01$ versus HA. NS, not significant.

In the whole population of hyperandrogenic women (PCOS+HA), body fat was negatively associated with the glucose clamp derived measure of insulin sensitivity ($r = -0.21$, $P < 0.05$). However, a significant interaction was found between this relationship and the PCOS status ($P < 0.05$). When this relationship was assessed separately in the two groups of hyperandrogenic women, a similar finding was found in the PCOS group ($r = -0.39$, $P < 0.01$), but not in HA women ($r = 0.18$, $P = 0.31$).

Serum hs-CRP was significantly higher in PCOS (3.43 ± 2.01 mg/l) than in HA women (2.43 ± 1.04 mg/l, $P < 0.005$) and controls (2.75 ± 0.86 mg/l, $P < 0.05$). However, no statistically significant difference was found between HA and controls ($P = 0.37$). Similar results were found after adjusting these data for age and BMI.

In the whole population under study, simple regression analyses showed several relationships between serum hs-CRP and metabolic and endocrine features (Table 2). Similar results were found when the whole sample of hyperandrogenic women (PCOS+HA) or PCOS women only were included in the analysis. Statistically significant relationships in these subgroups are reported in the Supplementary Materials section (Table 2, which can be viewed online at <http://www.eje-online.org/supplemental/>). Conversely, no relationships between serum hs-CRP and these variables were found when either HA women or healthy controls were separately analyzed. However, the sample size of these subgroups was smaller.

In the whole population of women, serum hs-CRP was also negatively correlated with insulin sensitivity, as measured by the whole body insulin-stimulated

Figure 1 Relationship between serum hs-CRP and the glucose clamp derived measure of insulin sensitivity ($n = 100$).

glucose uptake in the glucose clamp (Fig. 1). Moreover, serum hs-CRP was correlated with insulin-induced changes in the substrate oxidation during the clamp studies: the higher the serum hs-CRP, the lower the increase in glucose oxidation and the lower the suppression of lipid oxidation observed during hyperinsulinemia (Fig. 2).

In multivariate analyses, when the main factors potentially influencing the inflammatory status – insulin sensitivity, body fat, hyperandrogenism, and age – were included as independent variables, an activation of the inflammatory system was independently predicted, in the whole sample (controls+PCOS+HA), by body fat, which was the main predictor of serum hs-CRP, clamp-derived measures of insulin sensitivity and age (Table 3). Body fat and insulin sensitivity, but not age, were also the independent predictors of serum hs-CRP in the sample of hyperandrogenic women (PCOS+HA; Table 4). As a whole, 35–40% of total variance of serum hs-CRP was explained by this model. Similar results were obtained by substituting in the model body fat with BMI or free testosterone with either total testosterone or 17-hydroxyprogesterone after GnRH agonist stimulation (data not shown). Similarly, also substituting serum-free testosterone with the PCOS status did not substantially improve the model, and the PCOS status *per se* was not an independent predictor (Table 4). In the subgroup of PCOS women alone, again fat mass and insulin sensitivity were independent predictors of hs-CRP (Table 3, Supplementary Materials section, which can be viewed online at <http://www.eje-online.org/supplemental/>). On the other hand, in HA women alone, free testosterone was the only statistically significant independent predictor of serum hs-CRP, whereas fat mass was of borderline significance. However, this analysis was constrained by the small sample size, and the variance explained by the model in this subgroup was only 21%.

Table 2 Univariate correlations between serum high-sensitivity C-reactive protein and metabolic and hormonal parameters in the whole population of women (controls+polycystic ovary syndrome+idiopathic hyperandrogenism, $n = 121$).

	<i>r</i>	<i>P</i>
BMI	0.26	0.037
Fat mass	0.39	0.001
Fat-free mass	0.21	0.034
Fasting glucose	0.07	0.430
Fasting insulin	0.13	0.212
Insulinogenic index	0.12	0.264
Insulin-mediated glucose uptake ^a	−0.40	0.001
Total cholesterol	−0.05	0.576
HDL cholesterol	−0.20	0.073
Triglycerides	0.13	0.166
Systolic blood pressure	0.22	0.030
Diastolic blood pressure	0.32	0.001
LH/FSH	0.28	0.007
Free testosterone	−0.08	0.469
Total testosterone	−0.29	0.024
DHEAS	0.01	0.992
17OH-progesterone	0.04	0.733
17OH-progesterone after buserelin	0.21	0.039

Statistically significant correlations are in bold type.

^aAvailable in all the 86 hyperandrogenic women and in 14 controls.

Figure 2 Relationships between serum hs-CRP and insulin-induced changes in glucose oxidation (panel A) and lipid oxidation (panel B) during the clamp studies ($n=100$).

We also assessed the effect of inclusion of LH/FSH ratio and diastolic blood pressure, which showed statistically significant relationships with serum hs-CRP in univariate analyses, as additional independent variables. After inclusion of these variables in the model together with insulin sensitivity, fat mass, free testosterone and age, insulin sensitivity was no longer a predictor of serum hs-CRP (Table 4, Supplementary Materials section, which can be viewed online at <http://www.eje-online.org/supplemental/>). However, in addition to fat mass, LH/FSH ratio and free testosterone were independent predictors of inflammation both in the whole population and in the sample of hyperandrogenic subjects (PCOS+HA), whereas only fat mass remained independently associated with serum hs-CRP in the PCOS subgroup. Similar results were obtained by

substituting in the model the diastolic with the systolic blood pressure (data not shown).

To further assess the effect of body fat on the inflammatory status, we analyzed separately our population according to BMI (≤ 25 or > 25 kg/m²). When multivariate analyses were carried out in lean subjects ($n=75$), the independent effect of body fat was lower, and free testosterone was an additional independent predictive variable (Table 5). In this model, increased serum-free testosterone was associated with lower serum hs-CRP levels. A similar, but not statistically significant, trend was obtained substituting serum-free testosterone with total testosterone (standard coefficient -0.25 , $P=0.086$) in the model, whereas serum DHEAS did not show any association with serum CRP (standard coefficient -0.01 , $P=0.93$). On the other hand, in overweight-obese women ($n=25$), there was evidence of a prevalent effect of body fat (standardized coefficient $=0.424$) and insulin sensitivity (standardized coefficient $= -0.369$). However, the limited sample size did not allow a reliable multivariate analysis in this subgroup.

Discussion

In the present study, we measured serum hs-CRP in both PCOS and HA women, and we found that only PCOS women had higher levels of this protein, when compared with BMI-matched controls. In this population of young hyperandrogenic women, a close relationship between serum hs-CRP and several classic cardiovascular risk factors was found. In particular, a positive correlation with body fat mass and blood pressure and a negative correlation with insulin-mediated glucose uptake during the glucose clamp studies were observed. Some endocrine features typical of PCOS women, such as the LH/FSH ratio and the serum 17-hydroxyprogesterone response to GnRH agonist, were also associated with serum hs-CRP levels. In multiple regression analysis, when the main factors potentially influencing the inflammatory status (insulin sensitivity, body fat, serum androgens, and age) were included as independent variables, both fat mass and insulin resistance were independent predictors of the

Table 3 Predictors of serum high-sensitivity C-reactive protein in the multivariate analysis in the whole population under study (controls+polycystic ovary syndrome+idiopathic hyperandrogenism, $n=121$).

Independent variables	β coefficient	S.E.M.	Standardized coefficient	P value
Insulin-mediated glucose uptake	-0.166	0.041	-0.348	0.001
Fat mass	0.098	0.018	0.434	0.001
Hyperandrogenic status	0.230	0.377	0.054	0.544
Age	0.069	0.029	0.202	0.021

Table 4 Predictors of serum high-sensitivity C-reactive protein in multivariate analysis models in hyperandrogenic women (polycystic ovary syndrome + idiopathic hyperandrogenism, $n=86$).

Independent variables	β coefficient	S.E.M.	Standardized coefficient	P value
Model A				
Insulin-mediated glucose uptake	-0.117	0.043	-0.276	0.009
Fat mass	0.102	0.022	0.481	0.001
Free testosterone	-0.130	0.077	-0.176	0.097
Age	0.028	0.030	0.094	0.367
Model B				
Insulin-mediated glucose uptake	-0.134	0.050	-0.253	0.009
Fat mass	0.128	0.024	0.489	0.001
PCOS status	0.260	0.291	0.084	0.376
Age	0.064	0.034	0.174	0.065

hs-CRP variance, whether considering the whole population of hyperandrogenic women or the PCOS subgroup alone. Age was an additional variable in the entire cohort (controls + PCOS + HA).

Increased adiposity is a common finding in PCOS women. Although the molecular mechanisms responsible for this association remain unclear, there is evidence that increased body fat has a negative impact on several clinical features of these women (37). Consistently with previous data, our results support the hypothesis that, in these women, body fat has a major role also in stoking up a low-grade chronic inflammation, with the associated potential risk of atherosclerosis.

In our sample, PCOS status was not independently associated with serum hs-CRP, when insulin resistance – as measured by the glucose clamp, the gold standard method – was included in the multivariate analysis. A number of previous studies detected significant correlation between serum hs-CRP and either insulin levels or other surrogate indices of insulin resistance (12, 13, 15, 21, 22). However, although these surrogate estimates correlate with glucose clamp derived measures of insulin sensitivity and are widely used in epidemiological studies, they are poor substitutes for direct measurements of insulin resistance (38).

To the best of our knowledge, only two previous studies measured insulin sensitivity by the glucose clamp technique, in these analyses. Kelly *et al.* compared 17 obese women with PCOS – all with androgen excess and menstrual dysfunction – and 15 BMI-matched nonhyperandrogenic controls, reporting that differences in serum hs-CRP between groups were no longer significant after controlling for BMI and insulin sensitivity (12); in this study, serum androgens were not included in the analysis. Morin-Papunen *et al.* studied 52 PCOS women – 32 obese and 20 nonobese, all with ultrasonographic evidence of polycystic ovaries and either menstrual dysfunction

or hyperandrogenism (26). In this study, in a multivariate analysis, serum hs-CRP was associated with BMI and waist-to-hip ratio (WHR) but not with insulin sensitivity. Moreover, change in WHR after metformin treatment was the only feature associated with the reduction in serum hs-CRP reported after treatment. This finding differs from the results reported by Moran *et al.* in a similar setting (39). These authors reported that a moderate weight loss did not affect hs-CRP levels in obese PCOS women despite a significant reduction in waist circumference, in contrast with findings in nonhyperandrogenic women. Again, these discrepancies are not easily explained. However, these data suggest that other mechanisms, besides body fat and fat distribution, may explain the increase in serum hs-CRP reported in women with PCOS.

Most women with PCOS, either lean or obese, have insulin resistance and hyperinsulinemia. As insulin has a physiologic inhibitory effect on acute-phase protein synthesis in the liver (40), hepatic insulin resistance is expected to lead to increased synthesis of hs-CRP.

The characteristics of hyperandrogenic women we studied and the methods we used allowed us to assess the role of PCOS status independently of the effect of body fat excess and hyperandrogenemia and to accurately assess the effect of insulin resistance on hs-CRP levels. We found that increased serum hs-CRP is a specific feature of PCOS women, and that both body fat and insulin resistance independently predicted this finding. In our study, hyperandrogenic women not fulfilling PCOS diagnosis did not show differences from controls in terms of serum hs-CRP. These data agree with those by Carmina *et al.* in the only previous study that measured serum hs-CRP levels in HA women (18).

As body fat was similar in the two groups of hyperandrogenic women and fat mass was the main predictor of serum hs-CRP, these findings suggest that obesity may have a distinctive adverse effect in PCOS subjects. In actual fact, consistent with data from Holte *et al.* (41), we found a significant interaction between the PCOS or non-PCOS status, and the relationship between body fat and insulin sensitivity. In other words, fat excess showed a greater impact on insulin resistance in PCOS subjects than in women with a similar degree of androgen excess but without the typical characteristics of PCOS.

Table 5 Predictors of serum high-sensitivity C-reactive protein in the multivariate analysis in the subgroup of lean (body mass index ≤ 25 kg/m²) women ($n=75$).

Independent variables	β coefficient	S.E.M.	Standardized coefficient	P value
Insulin-mediated glucose uptake	-0.108	0.047	-0.297	0.024
Fat mass	0.103	0.036	0.356	0.006
Free testosterone	-0.174	0.081	-0.270	0.037
Age	0.044	0.033	0.110	0.198

The mechanisms enhancing in PCOS women the adverse effect of body fat on the inflammatory status remain elusive. Some recent studies reported a striking increase in the prevalence of nonalcoholic fatty liver disease (NAFLD) in PCOS women (42). Interestingly, there is now evidence suggesting that NAFLD *per se* may aggravate insulin resistance through the release of proinflammatory mediators, including CRP and inflammatory cytokines, and intrahepatic fat *per se* (43). Moreover, we recently found that PCOS subjects with increased serum alanine aminotransferase concentrations, a surrogate measure of NAFLD, have an impaired insulin sensitivity, independent of the effect of age and adiposity, as compared with subjects without abnormalities in serum alanine aminotransferase (44). We could thus speculate that NAFLD could be the link between body fat, insulin resistance, and inflammation in PCOS women. However, further research is needed to verify this hypothesis. Interestingly, in our study, serum hs-CRP was correlated with the impairment in the ability of insulin to stimulate glucose oxidation and to suppress fatty acid oxidation. This finding is consistent with the notion that an increased availability and utilization of fatty acids as a preferential fuel in the liver and in other tissues may favor inflammation, insulin resistance, and NAFLD (45).

In the whole sample of hyperandrogenic women, we failed to observe any independent effect of serum androgens in explaining serum hs-CRP variance. Interestingly, hs-CRP was negatively correlated with serum testosterone but positively correlated with some endocrine features typical of ovarian hyperandrogenism, such as the GnRH-stimulated 17-hydroxyprogesterone levels and the LH/FSH ratio. Furthermore, when the analysis was carried out only in lean women, testosterone was an additional independent variable associated with serum hs-CRP. To the best of our knowledge, no previous studies assessed in multivariate analyses the independent effect of serum androgens on hs-CRP levels, although in simple correlation analyses different results were reported (12, 15, 20). These findings as well are not easily explained. Interestingly, studies carried out in animal models reported a protective effect of androgens on oxidative stress and chronic inflammation (46, 47). Based on these findings, we could hypothesize that androgens have a direct anti-inflammatory effect, but that this action is masked in PCOS women by the prevalent effect of fat mass and insulin resistance. This speculation could explain why their role arises only in lean women, when the effect of adiposity is blunted. Consistently, previous studies reported that serum androstenedione and testosterone were inversely associated with intima-media thickness in pre- and postmenopausal women (48, 49). We could also speculate that the protective effect of androgens on inflammation might contain the increase in the risk of cardiovascular events that is expected in PCOS women, considering the many metabolic abnormalities they

have. In this respect, there are limited and controversial data on the actual risk of cardiovascular disease in these subjects (50). However, this speculation does not exclude that the net effect of androgen excess on cardiovascular risk may be deleterious in these subjects. On the contrary, the increased serum hs-CRP levels we found in our PCOS women, despite the negative association between androgens and this feature, suggest that the overall effect of PCOS on the inflammatory status was adverse. Interestingly, in a recent paper by Shaw *et al.* (51) carried out in postmenopausal women evaluated for suspected ischemia, women with clinical features of PCOS, defined by a premenopausal history of irregular menses and current biochemical evidence of hyperandrogenemia, showed an increased 5-year risk of cardiovascular events. Remarkably, in PCOS women with high hs-CRP levels, this risk was as much as 12.2-fold higher than in non-PCOS subjects with normal hs-CRP.

After inclusion of LH/FSH ratio and blood pressure as independent variables in the multivariate analyses, insulin sensitivity was no longer an independent predictor of inflammation. On the other hand, in the whole population and in the hyperandrogenic sample, LH/FSH ratio and free testosterone were associated with serum hs-CRP. The meaning of this finding is not readily explained. Interestingly, recent data showed that insulin sensitivity might modulate the regulatory effect of androgens on gonadotropin-releasing hormone pulse generator activity in women (52), suggesting an interaction between insulin action, androgen levels, and gonadotropins secretion. However, further research is needed to confirm this finding and to establish the direction of relationships in this complex biological network.

The strengths of the study rely on the large sample of hyperandrogenic women, on the quantitative assessment of body fat, and, more importantly, on the direct measurement of insulin resistance by the gold standard method. However, there are also some limitations to our study. First, the measurements of serum androgens, obtained by commercial methods, were not accurate. Therefore, conclusions about the role of androgens cannot be definitive. Second, since PCOS subjects without ovulatory dysfunction and without hyperandrogenism were not included in the study, we cannot establish whether subjects with these phenotypes behave as the classical and more common PCOS phenotype in terms of inflammatory status. Furthermore, age was different between groups. However, these differences were small and data were adjusted for these differences. Another limitation of the study is in the differences in sample size between groups, which could have limited our ability to recognize in HA women and healthy control subgroups some correlations between serum hs-CRP and other variables.

In conclusion, low-grade chronic inflammation is a specific feature of young PCOS women, suggesting an

increased cardiovascular risk in these subjects. Body fat appears to be the main factor responsible for the association between PCOS and serum hs-CRP, with the independent contribution of insulin resistance. At least in lean women, androgen excess *per se* seems to play an additional, possibly protective, role in this association. However, this does not exclude that androgens might have an overall adverse effect on the cardiovascular risk of these subjects.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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References

- Ridker PM, Hennekens CH, Buring JE & Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine* 2000 **342** 836–843.
- Pepys MB & Hirschfield GM. C-reactive protein: a critical update. *Journal of Clinical Investigation* 2003 **111** 1805–1812.
- Haverkate F, Thompson SG, Pyke SDM, Gallimore JR & Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet* 1997 **349** 462–466.
- DeBeer FC, Hind CR, Fox KM, Allan RM, Maseri A & Pepys MB. Measurement of serum C-reactive protein concentration in myocardial ischaemia and infarction. *British Heart Journal* 1982 **47** 239–243.
- Koenig W, Sund M, Frohlich M, Fischer H-G, Lowel H, Doring A, Hutchinson WL & Pepys MB. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (monitoring trends and determinants in cardiovascular disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 1999 **99** 237–242.
- Yudkin JS, Stehouwer CD, Emeis JJ & Coppack SW. C-reactive protein in healthy subjects. Associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arteriosclerosis, Thrombosis, and Vascular Biology* 1999 **19** 972–978.
- Festa A, D'Agostino R Jr, Howard G, Mykkanen L, Tracy RP & Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 2000 **102** 42–47.
- Ridker PM, Buring JE, Cook NR & Rafai N. C-reactive protein, the metabolic syndrome and the risk of incident cardiovascular events: an 8-year follow up of 14719 initially healthy American women. *Circulation* 2003 **107** 391–397.
- Pradhan AD, Manson JE, Rifai N, Buring JE & Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American Medical Association* 2001 **286** 327–334.
- Festa A, D'Agostino R Jr, Tracy RP & Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes mellitus: the Insulin Resistance Atherosclerosis Study (IRAS). *Diabetes* 2002 **51** 1131–1137.
- Apridonidze T, Essah PA, Iuorno MJ & Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 1929–1935.
- Kelly CCJ, Lyall H, Petrie JR, Gould GW, Connell JMC & Sattar N. Low-grade chronic inflammation in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 2453–2455.
- Fenkci V, Fenkci S, Yilmazer M & Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. *Fertility and Sterility* 2003 **80** 123–127.
- Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Zinder O & Blumenfeld Z. Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2160–2165.
- Tarkun I, Arslan BC, Canturk Z, Turemen E, Sahin T & Duman C. Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 5592–5596.
- Talbott EO, Zborowski JV, Boudreaux MY, McHugh-Pemu KP, Sutton-Tyrrell K & Guzick DS. The relationship between C-reactive protein and carotid intima-media wall thickness in the middle-aged women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 6061–6067.
- Orio F, Palomba S, Cascella T, Di Biase S, Manguso F, Tauchmanova L, Nardo LG, Labella D, Savastano S, Russo T, Zullo F, Colao A & Lombardi G. The increase of leukocytes as a new putative marker of low-grade chronic inflammation and early cardiovascular risk in polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 2–5.
- Carmina E, Chu MC, Longo RA, Rini GB & Lobo RA. Phenotypic variation in hyperandrogenic women influences the findings of abnormal metabolic and cardiovascular risk parameters. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 2545–2549.
- Puder JJ, Varga S, Kraenzlin M, De Geyter C, Keller U & Muller B. Central fat excess in polycystic ovary syndrome: relation to low-grade inflammation and insulin resistance. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6014–6021.
- Diamanti-Kandarakis E, Paterakis T, Alexandraki K, Piperi C, Aessopos A, Katsikis I, Katsilambros N, Kretsas G & Panidis D. Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. *Human Reproduction* 2006 **21** 1426–1431.
- Escobar-Morreale HF, Villuendas G, Botella-Carretero JJ, Sancho J & San Millan JL. Obesity, and not insulin resistance, is the major determinant of serum inflammatory cardiovascular risk markers in pre-menopausal women. *Diabetologia* 2003 **46** 625–633.
- Mohlig M, Spranger J, Osterhoff M, Ristow M, Pfeiffer AF, Schill T, Schlosser HW, Brabant G & Schofl C. The polycystic ovary syndrome *per se* is not associated with increased chronic inflammation. *European Journal of Endocrinology* 2004 **150** 525–532.
- Bickerton AS, Clark N, Meeking D, Shaw KM, Crook M, Lumb P, Turner C & Cummings MH. Cardiovascular risk in women with polycystic ovarian syndrome (PCOS). *Journal of Clinical Pathology* 2005 **58** 151–154.
- Meyer C, McGrath BP & Teede HJ. Overweight women with polycystic ovary syndrome have evidence of subclinical cardiovascular disease. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 5711–5716.
- Topcu S, Caliskan M, Ozcimen EE, Tok D, Uckuyu A, Erdogan D, Gullu H, Yildirim A, Zeyneloglu H & Muderrisoglu H. Do young women with polycystic ovary syndrome show early evidence of preclinical coronary artery disease? *Human Reproduction* 2006 **21** 930–935.
- Morin-Papunen L, Rautio K, Ruokonen A, Hedberg P, Puukka M & Tapanainen JS. Metformin reduces serum C-reactive protein levels in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 4649–4654.

- 27 Dahlgren E, Janson PO, Johansson S, Lapidus S & Oden A. Polycystic ovary syndrome and risk for myocardial infarction. Evaluated from a risk factor model based on a prospective population study of women. *Acta Obstetrica et Gynecologica Scandinavica* 1992 **71** 599–604.
- 28 The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction* 2004 **19** 41–47.
- 29 Zawadzki JK & Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In *Polycystic Ovary Syndrome*, pp 59–69. Eds A Dunaif, JR Givens, FP Haseltine & GR Merriam, Oxford, UK: Blackwell, 1992.
- 30 Hatch R, Rosenfield RL, Kim MH & Tredway D. Hirsutism: implications, etiology, and management. *American Journal of Obstetrics and Gynecology* 1981 **140** 815–830.
- 31 Rosenfield RL, Ehrmann DA, Barnes RB & Sheikh Z. Gonadotropin-releasing hormone agonist as a probe for the pathogenesis and diagnosis of ovarian hyperandrogenism. *Annals of the New York Academy of Sciences* 1993 **687** 162–181.
- 32 World Health Organization Study Group. Diabetes mellitus. *World Health Organization Technical Report Series* 1985 **727** 1–113.
- 33 Moghetti P, Tosi F, Castello R, Magnani CM, Negri C, Brun E, Furlani L, Caputo M & Muggeo M. The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 952–960.
- 34 DeFronzo RA. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988 **37** 667–687.
- 35 Gray DS, Bray GA, Gemayel N & Kaplan K. Effect of obesity on bioelectrical impedance. *American Journal of Clinical Nutrition* 1989 **50** 255–260.
- 36 Ferrannini E. The theoretical bases of indirect calorimetry: a review. *Metabolism* 1988 **37** 287–301.
- 37 Diamanti-Kandarakis E. Role of obesity and adiposity in polycystic ovary syndrome. *International Journal of Obesity* 2007 **31** S8–S13.
- 38 Diamanti-Kandarakis E, Kouli C, Alexandraki K & Spina G. Failure of mathematical indices to accurately assess insulin resistance in lean, overweight, or obese women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1273–1276.
- 39 Moran LJ, Noakes M, Clifton PM, Wittert GA, Belobrajdic DP & Norman RJ. C-reactive protein before and after weight loss in overweight women with and without polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 2944–2951.
- 40 Campos SP & Baumann H. Insulin is a prominent modulator of the cytokine-stimulated expression of acute-phase plasma protein. *Molecular and Cellular Biology* 1992 **12** 1789–1797.
- 41 Holte J, Berg T, Berne C, Berglund L & Lithell H. Enhanced insulin response to glucose in relation to insulin resistance in women with polycystic ovary syndrome and normal glucose tolerance. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 1052–1058.
- 42 Gambarin-Gelwan M, Kinkhabwala SV, Schiano TD, Bodian C, Yeh HC & Futterweit W. Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *Clinical Gastroenterology and Hepatology* 2007 **5** 496–501.
- 43 Kotronen A & Yki-Järvinen H. Fatty liver: a novel component of the metabolic syndrome. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2008 **28** 27–38.
- 44 Targher G, Solagna E, Tosi F, Castello R, Spiazzi G, Zoppini G, Muggeo M, Day CP & Moghetti P. Abnormal serum alanine aminotransferase levels are associated with impaired insulin sensitivity in young women with polycystic ovary syndrome. *Journal of Endocrinological Investigation*, 2009 In Press.
- 45 Boden G. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. *Current Diabetes Reports* 2006 **6** 177–181.
- 46 Lee SH, Hoe JS, Lee MY & Han HJ. Effect of dihydrotestosterone on hydrogen peroxide-induced apoptosis of mouse embryonic stem cell. *Journal of Cellular Physiology* 2008 **216** 269–275.
- 47 Li S, Li X & Li Y. Regulation of atherosclerotic plaque growth and stability by testosterone and its receptor via influence of inflammatory reaction. *Vascular Pharmacology* 2008 **49** 14–18.
- 48 Bernini GP, Sgrò M, Moretti A, Argenio GF, Barlascini CO, Cristofani R & Salvetti A. Endogenous androgens and carotid intimal-medial thickness in women. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2008–2012.
- 49 Bernini GP, Moretti A, Sgrò M, Argenio GF, Barlascini CO, Cristofani R & Salvetti A. Influence of endogenous androgens on carotid wall in postmenopausal women. *Menopause* 2001 **1** 43–50.
- 50 Legro RS. Polycystic ovary syndrome and cardiovascular disease: a premature association? *Endocrine Reviews* 2003 **24** 302–312.
- 51 Shaw LJ, Bairey Merz CN, Azziz R, Stanczyk FZ, Sopko G, Braunstein GD, Kelsey SE, Kip KE, Cooper-Dehoff RM, Johnson BD, Vaccarino V, Reis SE, Bittner V, Hodgson TK, Rogers W & Pepine CJ. Postmenopausal women with a history of irregular menses and elevated androgen measurements at high risk for worsening cardiovascular event-free survival: results from the National Institutes of Health – National Heart, Lung, and Blood Institute sponsored Women's Ischemia Syndrome Evaluation. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 1276–1284.
- 52 Blank SK, McCartney CR, Chhabra S, Helm KD, Eagleson CA, Chang RJ & Marshall JC. Modulation of gonadotropin-releasing hormone pulse generator sensitivity to progesterone inhibition in hyperandrogenic adolescent girls – implications for regulation of pubertal maturation. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 2360–2366.

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