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# Low Grade Chronic Inflammation in Women with Polycystic Ovarian Syndrome

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**ABSTRACT**

Low grade chronic inflammation as reflected by increased C-reactive protein (CRP) concentrations independently predicts those at risk for coronary heart disease (CHD) and type 2 diabetes. Women with polycystic ovarian syndrome (PCOS) are insulin resistant and have increased risk for CHD and type 2 diabetes, but currently there are no data on markers of inflammation in women with PCOS. Seventeen women with PCOS (defined on the basis of elevated testosterone and oligomenorrhea) and 15 healthy women matched as a group for body mass index were recruited. Measurement of CRP concentrations was made using a highly sensitive assay. Insulin resistance was assessed using the hyperinsulinemic euglycemic clamp technique. The women with PCOS had significantly elevated CRP concentrations relative to controls (geometric means, 2.12 and 0.67 mg/L, respectively; $P = 0.016$). Log CRP correlated with body mass index in both PCOS and controls ($r = 0.58$; $P < 0.05$ and $r = 0.78$; $P < 0.01$, respectively) and inversely with insulin sensitivity ($r = -0.57$; $P < 0.05$ and $r = -0.69$; $P < 0.01$). Total testosterone did not correlate with log CRP in either group. On adjustment for body mass index and age, there remained a significant difference in log CRP between PCOS and controls ($t = 2.13$; $P < 0.05$). On further adjustment for insulin sensitivity, log CRP was no longer significantly different between groups ($t = 1.51$; $P = 0.14$). We conclude that women with PCOS have significantly increased CRP concentrations relative to women with normal menstrual rhythm and normal androgen levels. We propose low grade chronic inflammation as a novel mechanism contributing to increased risk of CHD and type 2 diabetes in these women. (*J Clin Endocrinol Metab* **86:** 2453–2455, 2001)

THE POLYCYSTIC ovarian syndrome (PCOS) affects between 5–10% of premenopausal women (1). It is recognized to have a metabolic component consisting of hyperinsulinemic insulin resistance. Women with PCOS exhibit a decrease in insulin sensitivity of between 30–40%, a deficit similar to that seen in subjects with type 2 diabetes mellitus (2). PCOS also carries with it an increased lifetime risk of both diabetes and cardiovascular disease. For example, postmenopausal women with a past history of PCOS have a 3-fold increased prevalence (3) of hypertension, whereas up to 40% of premenopausal women with PCOS have either impaired glucose tolerance or type 2 diabetes (4, 5). The evidence for an association between PCOS and cardiovascular disease is less robust and comes largely from cross-sectional studies showing an increased prevalence and severity of coronary artery disease in PCOS (6–8).

Low grade chronic inflammation independently predicts coronary heart disease (CHD) (9, 10) and more recently has been linked to the insulin resistance syndrome (11, 12). Furthermore, two large prospective studies have suggested that markers of low grade chronic inflammation independently predict those at high risk for type 2 diabetes (13, 14). Currently there are no data on markers of inflammation in women with PCOS. In light of the above observations, in this study we investigated the hypothesis that C-reactive protein (CRP) concentrations are elevated in women with PCOS with respect to healthy controls.

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## Subjects and Methods

### *Subjects*

The study was approved by the Western Hospitals National Health Service Trust ethics committee, and all subjects gave written informed consent. PCOS was defined as androgen excess (total testosterone >3.6 nmol/L or a free androgen index ≥9) with ovulatory dysfunction (less than six menstrual cycles per yr) once specific disorders, such as adult-onset congenital adrenal hyperplasia, hyperprolactinemia, and androgen-secreting neoplasia, had been excluded. The patients were recruited from the endocrine clinics of the North Glasgow University Trusts and were not taking any medication at the time of the study. Control patients, recruited from the Glasgow area, were volunteers who had regular menstrual cyclex and normal androgen levels and were taking no medication. For each woman with PCOS we attempted to recruit a control within 2 body mass index (BMI) units. This was achieved for all but two patients. All subjects were studied in the early follicular phase if they were regularly cycling or at least 8 weeks after their last menstrual period if they were oligomenorrheic. None of the patients had clinical evidence (history or examination) of recent or ongoing infection.

### *Whole body insulin sensitivity*

Subjects were studied after an overnight fast. Baseline blood pressure and heart rate were recorded after 20 min of supine rest. An indwelling cannula was then inserted into the left antecubital vein for administration of glucose and insulin and retrogradely into the right dorsal hand vein for blood sampling. Insulin sensitivity was assessed using a modified version of the hyperinsulinemic euglycemic clamp described by DeFronzo *et al.* (15). In brief, a primed, constant rate infusion of soluble insulin (1.5 mU/kg·min) was administered for 180 min, and a variable rate infusion of 20% glucose was administered to maintain euglycemia (5.2 mmol/L). Insulin was prepared in 45 mL 0.9% NaCl; 5 mL of the patient's own blood were added to prevent adsorption of insulin to

plastic surfaces. The infusion was administered using a Braun perfusor pump (Braun, Melsungen, Germany). Glucose (20%) was infused from 4–180 min using an IVAC iv infusion system (Basingstoke, UK); the glucose infusion rate was adjusted manually according to glucose concentrations (Beckman Coulter, Inc., Hialeah, FL), measured at 5-min intervals in 2-mL arterialized blood samples collected from a dorsal right hand vein surrounded by a heated box (60 C; Department of Physiology, University of Nottingham, Nottkingham, UK). Under steady state conditions, M (insulin sensitivity) was calculated from the glucose infusion rate and the serum glucose concentration (15). Samples were also collected for the measurement of serum insulin concentrations at 60-, 120-, 150-, and 180-min intervals. M/I (insulin sensitivity index) was calculated using the steady state insulin concentration. Blood pressure and heart rate were recorded every 30 min using a semiautomatic sphygmomanometer (Dinamap, Bracknell, UK).

### *Clinical measures*

Ovarian morphology was assessed (in each case by the same operator) using either transabdominal or transvaginal ultrasound. Anthropometric measurements were made by the same trained observer using standard techniques (according to WHO). Body weight was measured using analog scales (Seca, Germany) to within 500 g in light clothing; height was measured barefoot using a stadiometer to within 0.5 cm. BMI was calculated as follows: weight (kilograms)/height (meters)$^2$.

### *Biochemistry*

Routine biochemical analysis for all subjects was carried out using an Olympus Corp. AU5200 autoanalyzer (New Hyde Park, NY). Baseline hormonal profiles (testosterone, progesterone, PRL, 17-hydroxyprogesterone, and FSH/LH) were measured using a Bayer Corp. Immuno 1 autoanalyzer (Tarrytown, NY), and sex hormone-binding globulin (SHBG) was measured using the Immulite 2000 (Diagnostic Products, Los Angeles, CA). The free androgen index was calculated by total testosterone/SHBG × 100. CRP was measured using a sensitive double antibody sandwich enzyme-linked immunosorbent assay with rabbit antihuman CRP and peroxidase-conjugated rabbit antihuman CRP. Standard curves were linear up to 5 mg/L and were logarithmic thereafter. The inter- and intraassay coefficients of variation were less than 10% across the range of measured results (16).

### *Statistics*

CRP concentrations were skewed and were transformed on to a logarithmic scale before analysis. Results are shown as the mean and SD. Data from the two groups were compared using standard statistical tests. Simple linear correlation was applied to the data from the PCOS cohort and the control group separately, with log CRP taken as the response variable. Multivariate regression analyses were used to correct for the effects of age, BMI, and insulin sensitivity (M/I) on the difference in log CRP between the cases and controls.

## Results

Patient characteristics are shown in Table 1. There was no difference in BMI between groups, although the women with PCOS had a lower mean age. The proportion of smokers in each group was similar. As expected, the women with PCOS had significantly ($P < 0.001$) elevated mean testosterone and lower mean SHBG concentrations. Sixteen of the 17 women with PCOS, but none of the controls, had evidence of polycystic ovaries on ultrasound ($P < 0.001$). Insulin sensitivity was lower in women with PCOS, although this did not reach statistical significance ($P = 0.069$).

**TABLE 1.** Characteristics of women with PCOS and healthy controls

| | PCOS (n = 17) | Control (n = 15) | *P* value |
|---|---|---|---|
| Body mass index (kg/m$^2$) | 31.5 (5.7) | 30.3 (7.8) | NS |
| Age (yr) | 26.50 (5.6) | 33.1 (6.2) | 0.004 |
| Current smokers (n) | 8 | 5 | NS |
| Testosterone (nmol/L) | 3.2 (0.8) | 1.5 (0.6) | <0.001 |
| SHBG (nmol/L) | 27.0 (15.4) | 50.9 (15.0) | <0.001 |
| M/I | 4.2 (2.5) | 6.1 (3.1) | 0.069 |
| Glucose (mmol/L) | 4.7 (0.6) | 4.5 (0.4) | NS |

Figure 1 shows CRP concentrations in both groups. The geometric means for the women with PCOS and the control group were 2.12 and 0.67 mg/L, respectively ($P = 0.016$). Log CRP correlated with BMI in both PCOS and controls (r = 0.58; $P < 0.05$ and r = 0.78; $P < 0.01$, respectively) and inversely with M/I (r = −0.57; $P < 0.05$ and r = −0.69; $P < 0.01$). Total testosterone did not correlate with log CRP in either group.

On adjustment for BMI and age, there remained a significant difference in log CRP between cases and controls ($t = 2.13$; $P < 0.05$). On further adjustment for insulin sensitivity, log CRP was no longer significantly different between groups ($t = 1.51$; $P = 0.14$).

## Discussion

There is increasing evidence to suggest a role for inflammation in the pathogenesis of cardiovascular disease. Low grade chronic inflammation, demonstrated by persistent moderately elevated CRP concentrations within the normal range, has been shown to be independently predictive of both myocardial infarction and ischemic stroke in prospective studies (9, 10, 17). The same phenomenon is observed whether individuals studied are apparently healthy (with or without classical cardiovascular risk factors) or have established vascular disease (18, 19). Indeed, in a recently reported prospective nested case-control study among 28,263 apparently healthy postmenopausal women followed for 3 yr, CRP was the strongest univariate predictor of the risk of cardiovascular events among a panel of 12 baseline measures, including lipids and homocysteine (9).

This is the first study to examine low grade chronic inflammation in women with PCOS. We have shown that CRP concentrations measured using a highly sensitive assay are

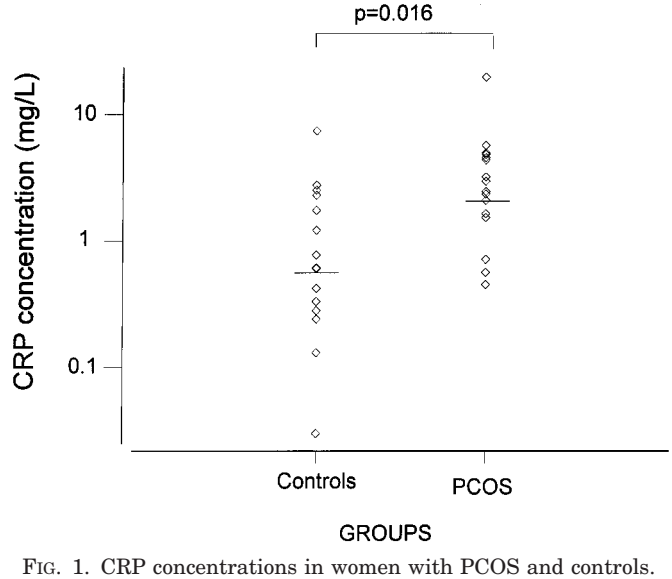


FIG. 1. CRP concentrations in women with PCOS and controls.

significantly increased in women with PCOS relative to those in healthy women with normal menstrual rhythm and normal androgen levels after correction for BMI. In keeping with previous data, we also noted that CRP concentrations in both PCOS and controls correlated with the degree of obesity and inversely with insulin sensitivity (11, 12), although not with total testosterone concentrations.

Interestingly, although BMI was strongly correlated with increased CRP concentrations in both the women with PCOS and the controls, other workers have recently reported that visceral adiposity correlates with CRP concentrations independently of total adiposity in a cross-sectional study of 168 men (20). These data are consistent with the idea that adipose tissue-derived cytokine expression (tumor necrosis factor-$\alpha$ and interleukin-6) may be an important contributor to low grade chronic inflammation. In other words, accumulation of visceral adipose tissue may be a key factor underpinning features of the metabolic syndrome and of low grade chronic inflammation. These combined observations would also explain the correlation of insulin sensitivity to CRP, as recently noted by Festa and colleagues (12).

Although not measured in this study, women with PCOS are likely to have increased visceral fat mass relative to BMI-matched controls, as the waist to hip ratio is often higher in such women (21). It would be interesting to examine in future studies whether visceral fat area predicts CRP concentrations independently of total adiposity in women with PCOS. The lack of a correlation between CRP concentrations and total testosterone in either the cases or controls suggests that endogenous hyperandrogenemia is not linked to low grade chronic inflammation. This observation is consistent with recent data from our group demonstrating a lack of association between total testosterone and lipid and lipoprotein subfraction concentrations in women with PCOS (22). These combined observations accord with a recent prospective case-referent study, which, by employing measurement of urinary sex hormone metabolites, showed that high androgen output is not related to an increased CHD risk in women (23).

Our finding of increased CRP in PCOS is also relevant because recent data suggest that CRP, rather than being simply a marker of low grade chronic inflammation, may be directly involved in the atherogenic process by promoting endothelial dysfunction and complement activation (24, 25). Currently, evidence is conflicting with respect to the presence or absence of endothelial dysfunction in women with PCOS (26, 27).

In conclusion, we have shown for the first time that women with PCOS have significantly increased CRP concentrations relative to those in healthy women with normal menstrual rhythm and normal androgens. We propose low grade chronic inflammation as a novel mechanism contributing to the increased risk of CHD and type 2 diabetes in these women.

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