

C-Reactive Protein before and after Weight Loss in Overweight Women with and without Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is associated with reproductive and metabolic abnormalities. It is unknown whether overweight women with and without PCOS achieve similar benefits from weight loss for cardiovascular risk factors.

Method: Overweight body mass index-matched women with ($n = 15$) and without ($n = 17$) PCOS (weight, 95.3 ± 17.6 kg; body mass index, 35.6 ± 5.3 kg/m², mean \pm SD) followed an 8-wk weight loss regime.

Results: All subjects had similar reductions in weight (3.9 ± 3.6 kg, 3.8%, vs. 4.5 ± 4.1 kg, 4.7%, respectively, for PCOS and non-PCOS), waist circumference, fat mass, triglycerides, free testosterone, and fasting and postprandial insulin. At baseline, C-reactive protein (CRP) between groups was not significantly different (5.5 ± 3.1 mg/liter for PCOS vs. 4.9 ± 3.0 mg/liter for non-PCOS). There was a significant interaction between PCOS status and CRP ($P = 0.016$)

such that CRP decreased with weight loss for non-PCOS women (-1.2 ± 1.8 mg/liter; $P = 0.025$) but not for PCOS women. For all women, the change in CRP correlated with the change in weight ($r = 0.560$; $P = 0.003$), fat mass ($r = 0.477$; $P = 0.016$), and postprandial insulin ($r = 0.402$; $P = 0.046$). Adiponectin, IL-6, and TNF- α were not significantly different between groups before or after weight loss. Only subjects with baseline CRP levels below the median (4.52 mg/liter) showed increases in adiponectin (0.98 ± 1.3 μ g/liter) ($P = 0.015$) and greater reductions in triglycerides ($P = 0.001$) with weight loss.

Conclusion: A 4–5% weight loss improved lipid, glucose, and insulin profiles in women with and without PCOS. This degree of weight loss was not effective in lowering CRP concentrations in PCOS women, suggesting that greater weight loss is required in this group to achieve equivalent cardiovascular benefit to non-PCOS women. (*J Clin Endocrinol Metab* 92: 2944–2951, 2007)

POLYCYSTIC OVARY SYNDROME (PCOS) is a common endocrine condition affecting 4–8% of women of reproductive age and is associated with menstrual dysfunction, infertility, hyperandrogenism, and an increased prevalence of generalized and abdominal obesity (1). Peripheral insulin resistance (IR) is present in 50–80% of women with PCOS and is further worsened by the presence of obesity (2). IR is implicated in the etiology of PCOS through insulin stimulating ovarian androgen production and reducing hepatic synthesis of SHBG (3). Metabolic complications associated with IR are also increased in PCOS independent of obesity, including an adverse cardiovascular risk profile (impaired fibrinolysis, endothelial dysfunction, dyslipidemia, and subclinical atherosclerosis) and increased prevalence of the metabolic syndrome, impaired glucose tolerance, and type 2 diabetes mellitus (4).

High-sensitivity C-reactive protein (CRP) is an acute-phase reactant hepatically synthesized in response to IL-6

and TNF- α (5). There is evidence that it both is a marker for low-grade chronic inflammation and plays an active role in atherosclerosis (6). CRP independently predicts cardiovascular events and coronary heart disease (7), and elevated levels are linked to visceral fat accumulation, IR, the metabolic syndrome, and type 2 diabetes mellitus (8). The adipose tissue secretes a variety of bioactive active substances (adipocytokines) that additionally contribute to inflammation and metabolic disease including leptin, TNF- α , IL-6, IL-18, plasminogen activator inhibitor type 1, and adiponectin. Adiponectin has proposed insulin-sensitizing (9), antiatherogenic, and antiinflammatory properties (10), and an inverse association exists between adiponectin and CRP (11). There is emerging evidence that novel cardiovascular risk factors are also dysregulated in PCOS, and elevated CRP (12, 13), IL-6 (14, 15), and TNF- α (16) and reduced adiponectin (17–19) have been documented in obese and nonobese women with PCOS.

Although there is no conclusive evidence that long-term cardiovascular morbidity and mortality are elevated (20), prudent treatment should target metabolic risk factors at the earliest stage because weight loss normalizes cardiovascular and diabetic risk factors and reduces CRP in women with (21) and without PCOS (22–24). In the general population, adiponectin, IL-6, and TNF- α either increase or not change after weight loss (22, 25, 26). The effect of weight loss on adiponectin, IL-6, and TNF- α in PCOS has not yet been studied.

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Abbreviations: BMI, Body mass index; CRP, C-reactive protein; FAI, free androgen index; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; MTT, meal tolerance test; PCOS, polycystic ovary syndrome; TFFM, total fat-free mass; TFM, total fat mass.

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The aim of this study was to examine the relationship between CRP, adiponectin, IL-6, and TNF- α before and after weight loss in overweight women with and without PCOS.

Subjects and Methods

Subjects and recruitment

Overweight premenopausal women (European Caucasian) with ($n = 18$) and without ($n = 19$) PCOS were recruited through public advertisement. The study was approved by the Human Ethics committees of the Commonwealth Scientific and Industrial Research Organisation Division of Health Sciences and Nutrition, The Royal Adelaide Hospital, and the Women's and Children's Hospital of South Australia, and all subjects gave informed written consent. PCOS was diagnosed according to Rotterdam consensus group criteria as previously described (21, 27). Exclusion criteria were pregnancy, breastfeeding, body mass index (BMI) less than 25 kg/m², and use of oral contraceptives, endocrine hormonal treatment, or insulin-sensitizing agents (subjects were required to cease oral contraceptives 4 wk and hormonal treatment/insulin-sensitizing agents 2 wk before commencement of the study). The PCOS and non-PCOS groups were matched for BMI and smoking status. Consort criteria are documented in Fig. 1.

Study design and dietary treatment

The study was conducted on an outpatient basis over 8 wk. Subjects followed an energy-restricted diet whereby two meals daily were replaced with commercially available meal replacements (Slimfast; Unilever Australasia, Epping, New South Wales, Australia) (28). Nutritional intake was assessed from fortnightly 3-d consecutive dietary food records (one weekday and two weekend days) and daily dietary checklists. Dietary compliance was determined by subject adherence to the Slimfast regime and calculated with Diet 4/Nutrient Calculation Software (Xyris Software, Highgate Hill, Australia).

Subjects attended the clinic fortnightly and were weighed in light clothes with no shoes (Mettler scales, model AMZ14; A&D Mercury, Kinomoto, Japan). At wk 0 and 8, waist circumference, total fat mass (TFM) and total fat-free mass (TFFM) (by bioelectrical impedance) were measured as previously described (21); overnight fasting venous blood samples were taken for assessment of glucose, insulin, lipids, CRP, adiponectin, IL-6, TNF- α , testosterone, and SHBG; and exercise was assessed by a 7-d 24-h physical activity record (29). Subjects documented their menstrual cycles for the study duration and 6 months before study commencement. During the intervention, ovulation status was determined from twice-weekly first morning urine samples assessed for total urinary pregnanediol-3-glucuronide (30). For the fortnight before study commencement, subjects weighed themselves daily to ensure weight stability, defined as a change of no more than 2% of initial body weight.

At wk 0 and 8, subjects performed a meal tolerance test (MTT). On the evening before the MTT, subjects consumed the same meal (3820 kJ, 20% of energy from protein, 17% fat, and 62% carbohydrate) and refrained from alcohol for 24 h. A cannula was inserted into a lower arm vein, and a fasting blood sample was taken for assessment of glucose and insulin. Subjects consumed a liquid preload of Slimfast (936 kJ, 12 g protein, 2 g fat, and 35 g carbohydrate) within 5 min, and subsequent blood samples were taken at 15, 30, 45, 60, 90, 120, and 180 min.

Biochemical measurements

SHBG, total testosterone (bound and unbound) (31), low-density lipoprotein cholesterol (LDL-C) (32), insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, plasma glucose, and CRP (21) and urinary pregnanediol-3-glucuronide (32) were measured as previously described. The analytical sensitivity for the CRP assay was 0.03 mg/liter, and the functional sensitivity was 0.11 mg/liter. IL-6 and TNF- α were measured in serum using the Lincoplex high-sensitivity human cytokine kit (Linco, St. Charles, MO), according to manufacturer's instructions. The mean intra- and interassay coefficient of variance were 4 and 7% for TNF α and were both 4% for IL-6. Adiponectin was measured in duplicate using a commercial enzyme immunoassay kit (R&D Systems, Minneapolis, MN). The homeostatic model assessment (HOMA) was used as a surrogate measure of insulin

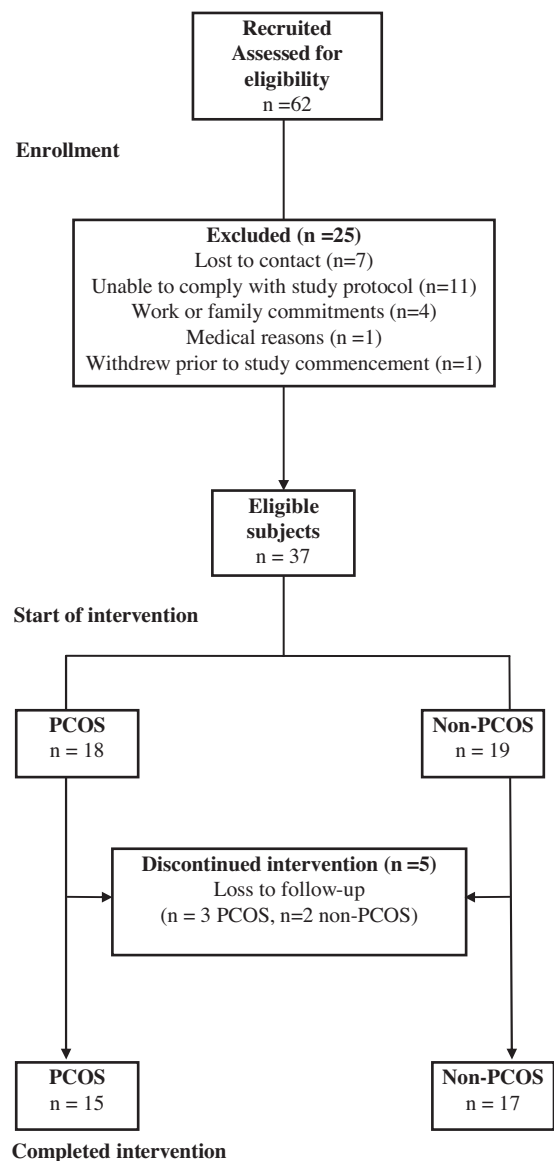


FIG. 1. Flow diagram of enrollment, commencement, and completion of the 8-wk weight loss protocol incorporating subjects with ($n = 15$) and without ($n = 17$) PCOS.

sensitivity, calculated as [fasting insulin (mU/liter) \times fasting glucose (mmol/liter)/22.5] (33). A HOMA score of more than 2.61 or a fasting insulin of more than 12.1 mU/liter was defined as IR (34). The free androgen index (FAI) (testosterone/SHBG \times 100) and equilibrium binding equations for determination of free testosterone (35) were used as surrogate estimates of free testosterone. Biochemical assays were performed in a single assay at the completion of the study, and all samples for individuals were analyzed in the same assay.

Statistics

Data were presented as means \pm SD except where indicated and log transformed where nonnormally distributed. Results are presented for 32 subjects ($n = 15$ PCOS; $n = 17$ non-PCOS) except for MTT insulin and glucose ($n = 15$ PCOS; $n = 15$ non-PCOS), CRP ($n = 15$ PCOS; $n = 15$ non-PCOS), and IL-6, TNF- α , dietary analysis, TFM, TFFM, waist circumference, and menstrual cyclicity ($n = 14$ PCOS; $n = 17$ non-PCOS) due to incomplete data. Subjects with an isolated CRP of more than 10 mg/liter ($n = 3$ PCOS; $n = 2$ non-PCOS) were excluded from the CRP analysis. Two-tailed statistical analysis was performed using SPSS for

Windows 14.0 software (SPSS Inc., Chicago, IL) with statistical significance set at an α -level of $P \leq 0.05$. Baseline data were assessed using a one-way ANOVA. Comparisons between time points were assessed using repeated-measures ANOVA with PCOS as between-subject factor. In specific analyses, weight, BMI, age, and weight loss were included as covariates. In the event of an interaction, *post hoc* pairwise comparisons were performed. Relationships between variables were examined using bivariate and partial correlations. Subjects with baseline CRP above and below the median (4.53 mg/liter) were assessed separately with baseline CRP status as the between-subject factor. This study had 65% power to detect a difference of 1.6 mg/liter between subjects with and without PCOS for CRP to statistical significance of $P < 0.05$. To confirm the observed differences between subjects with and without PCOS of CRP to statistical significance of $P < 0.05$ and 80% power, 19 subjects for each group would be needed. For changes in adiponectin with weight loss, 124 subjects would be needed in each group to detect a difference of 633.4 ng/ml between the subjects with and without PCOS to statistical significance of $P < 0.05$ and 80% power.

Results

Subjects, physical activity, and dietary intake

Thirty-two subjects completed the intervention (15 PCOS, 17 non-PCOS) with an overall dropout rate of 13.5% and similar dropouts between subjects with and without PCOS (Fig. 1). Baseline characteristics are shown in Table 1. Activity levels were comparable for all subjects at wk 0 and did not change throughout the study. The dietary intervention was well tolerated by all subjects with no adverse events documented. There were no differences between subjects with and without PCOS for energy (5217.0 ± 722.1 kJ), fat (34.4 ± 10.6 g, $24.3 \pm 6.0\%$), carbohydrate (158.5 ± 28.2 g, $51.6 \pm 5.6\%$), and protein (72.6 ± 9.3 g, $22.4 \pm 2.4\%$) intake.

Weight loss and body composition

There was no significant difference in weight loss between subjects with and without PCOS (3.9 ± 3.6 , or 4%, *vs.* 4.5 ± 4.1 kg, or 4.7%). A mean weight loss of 4.2 ± 3.8 kg (4.3%) occurring for combined subjects. There were no differences in the proportion of subjects with and without PCOS who lost

greater than 5% of their initial body weight (40 *vs.* 47.1%). Reductions in waist circumference, TFM, and TFFM occurred for all subjects with no differential effect of PCOS status (Table 2).

Fasting lipids, adiponectin, IL-6, TNF- α , and CRP

There was no effect of PCOS status on changes in lipids and no significant change in total cholesterol, LDL-C, and HDL-C over the study. Decreases in triglycerides occurred equivalently for subjects with and without PCOS (Table 2). There were no differences between subjects with and without PCOS at wk 0 or 8 for adiponectin ($P = 0.641$; $P = 0.259$), IL-6 ($P = 0.121$; $P = 0.060$), or TNF- α ($P = 0.751$; $P = 0.706$). There was no change in adiponectin, IL-6, or TNF- α with weight loss or effect of PCOS status on change in adiponectin or IL-6 after weight loss (Table 2). There was a trend for a time-by-PCOS status effect with TNF- α ($P = 0.073$) such that TNF- α increased with weight loss for the subjects with PCOS ($P = 0.028$) and did not change for the non-PCOS subjects ($P = 0.574$) (Table 2).

At wk 0, there was no difference in CRP between subjects with and without PCOS (5.5 ± 3.1 *vs.* 4.9 ± 3.0 mg/liter, $P = 0.603$). There was a significant interaction between PCOS status and CRP change (time-by-PCOS status $P = 0.016$) such that CRP decreased with weight loss for non-PCOS women (-1.2 ± 1.8 mg/liter, $P = 0.025$) but did not change for PCOS women ($P = 0.418$). At wk 8, there was a trend for subjects with PCOS to have higher CRP (5.9 ± 3.3 *vs.* 3.7 ± 2.7 mg/liter, $P = 0.066$) (Fig. 2, A and B). When subjects with baseline CRP above and below the median (4.52 mg/liter) were assessed separately (7.4 ± 2.2 *vs.* 2.5 ± 1.2 mg/liter), there was a time-by-baseline CRP-status effect for changes in adiponectin ($P = 0.029$) and triglycerides ($P = 0.015$) with weight loss such that only subjects with baseline CRP below the median showed increases in adiponectin (0.98 ± 1.3 μ g/

TABLE 1. Subject baseline characteristics

| | PCOS (n = 15) | Non-PCOS (n = 17) |
|---|---------------------|---------------------|
| Age (yr) ^a | 31.7 \pm 6.2 | 37.1 \pm 4.7 |
| Weight (kg) | 95.1 \pm 19.3 | 95.5 \pm 16.5 |
| BMI (kg/m ²) | 35.7 \pm 5.8 | 35.5 \pm 5.1 |
| Waist circumference (cm) | 114.4 \pm 3.7 | 112.6 \pm 3.4 |
| Fat mass (kg) | 35.0 \pm 2.2 | 35.0 \pm 1.9 |
| Fat free mass (kg) | 60.1 \pm 3.0 | 61.1 \pm 2.3 |
| Glucose (mmol/liter) | 5.3 \pm 0.8 | 5.2 \pm 0.5 |
| Insulin (mU/liter) ^b | 21.5 \pm 14.2 | 12.2 \pm 6.4 |
| HOMA ^c | 5.3 \pm 3.7 | 2.8 \pm 1.6 |
| Adiponectin (μ g/liter) | 7034.5 \pm 4257.7 | 7063.7 \pm 3111.2 |
| IL-6 (pg/ml) | 16.0 \pm 9.8 | 11.2 \pm 8.4 |
| TNF- α (pg/ml) | 6.0 \pm 5.1 | 5.8 \pm 3.5 |
| Testosterone (nmol/liter) ^d | 3.3 \pm 1.0 | 2.0 \pm 0.5 |
| Free testosterone (pmol/liter) ^d | 82.2 \pm 35.9 | 43.6 \pm 13.7 |
| SHBG (nmol/liter) | 20.8 \pm 10.7 | 24.7 \pm 7.7 |
| FAI ^d | 21.6 \pm 18.2 | 9.0 \pm 4.3 |

Data are presented as mean \pm SD. For conversion from mmol/liter to mg/dl for glucose, multiply by 18; for conversion from mU/liter to pmol/liter for insulin, multiply by 6.95. Measurements were made at the wk 0 visit and were assessed using one-way ANOVA with PCOS status as the fixed factor.

^a $P = 0.009$ for PCOS *vs.* non-PCOS.

^b $P = 0.039$ for PCOS *vs.* non-PCOS.

^c $P = 0.053$ for PCOS *vs.* non-PCOS.

^d $P < 0.001$ for PCOS *vs.* non-PCOS.

TABLE 2. Change in weight, body composition, lipids, HOMA, adiponectin, and reproductive hormones before and after 8 wk of energy restriction on one dietary pattern (meal replacements) for overweight women with and without PCOS

| | PCOS | Non-PCOS | <i>P</i> , effect of weight loss | <i>P</i> , PCOS <i>vs.</i> non-PCOS |
|---|--------------|-------------|----------------------------------|-------------------------------------|
| Weight (kg) ^a | -3.9 ± 3.6 | -4.5 ± 4.1 | <0.001 | 0.642 |
| Waist circumference (cm) ^a | -6.1 ± 6.0 | -7.2 ± 4.8 | <0.001 | 0.595 |
| TFFM (kg) ^a | -1.2 ± 1.6 | -1.9 ± 2.4 | <0.001 | 0.330 |
| TFM (kg) ^a | -2.7 ± 2.5 | -3.2 ± 2.6 | <0.001 | 0.566 |
| Total cholesterol (mmol/liter) | -0.2 ± 0.7 | -0.2 ± 0.8 | 0.167 | 0.770 |
| LDL-C (mmol/liter) | -0.03 ± 0.7 | -0.07 ± 0.5 | 0.598 | 0.851 |
| HDL-C (mmol/liter) | -0.05 ± 0.2 | 0.00 ± 0.2 | 0.550 | 0.506 |
| Triglycerides (mmol/liter) ^a | -0.3 ± 1.5 | -0.2 ± 0.8 | 0.021 | 0.955 |
| Adiponectin (μg/liter) | -0.2 ± 1.9 | 0.4 ± 1.8 | 0.412 | 0.166 |
| IL-6 (pg/ml) | 0.9 ± 3.8 | 0.03 ± 1.3 | 0.654 | 0.126 |
| TNF-α (pg/ml) | 0.9 ± 1.7 | -0.1 ± 1.1 | 0.179 | 0.073 |
| Insulin (mU/liter) ^a | -3.9 ± 10.8 | -3.0 ± 4.7 | 0.001 | 0.397 |
| HOMA ^a | -1.1 ± 2.8 | -0.7 ± 1.3 | 0.004 | 0.435 |
| Testosterone (nmol/liter) ^a | -0.5 ± 0.7 | -0.3 ± 0.4 | 0.001 | 0.200 |
| SHBG (nmol/liter) ^a | 0.9 ± 3.6 | 3.8 ± 5.8 | 0.009 | 0.102 |
| FAI ^a | -4.2 ± 4.7 | -2.0 ± 2.1 | <0.001 | 0.754 |
| Free testosterone (pmol/liter) ^a | -15.6 ± 19.2 | -8.6 ± 9.6 | <0.001 | 0.911 |

Data are presented as mean ± SD. Data were assessed using repeated-measures ANOVA with time as within-subject factor and PCOS status as between-subject factors. PCOS n = 15, and non-PCOS n = 17 except for weight, waist circumference, TFFM, and TFM (PCOS n = 14; non-PCOS n = 17). For conversion from mmol/liter to mg/dl for glucose, multiply by 18; for conversion from mU/liter to pmol/liter for insulin, multiply by 6.95; for conversion from mmol/liter to mg/dl for total cholesterol, LDL-C, and HDL-C, multiply by 38.67; and for conversion from mmol/liter to mg/dl for triglycerides, multiply by 88.57.

^a *P* < 0.05 for effect of time (wk 0–8).

liter, *P* = 0.015) and decreases in triglycerides (0.4 ± 0.5 mmol/liter, *P* = 0.001) with weight loss.

CRP correlated with SHBG (wk 0, *r* = -0.60 and *P* = 0.001; wk 8, *r* = -0.44 and *P* = 0.021), insulin (wk 0, *r* = 0.36 and *P* = 0.064; wk 8, *r* = 0.53 and *P* = 0.005), HOMA (wk 0, *r* = 0.38 and *P* = 0.052; wk 8, *r* = 0.53 and *P* = 0.004), and FAI (wk 0, *r* = 0.43 and *P* = 0.025). These relationships remained after adjusting for weight or BMI at each time point. Adiponectin correlated with insulin (wk 0, *r* = -0.53 and *P* = 0.002; wk 8, *r* = -0.53 and *P* = 0.002), HOMA (wk 0, *r* = -0.54 and *P* = 0.001; wk 8, *r* = -0.54 and *P* = 0.001), HDL-C (wk 0, *r* = 0.53 and *P* = 0.002; wk 8, *r* = 0.42 and *P* = 0.016), triglycerides (wk 0, *r* = -0.65 and *P* < 0.001), and MTT glucose (wk 8, *r* = -0.505 and *P* = 0.005). The correlation between adiponectin and MTT glucose was removed after adjusting for weight or BMI. TNF-α correlated with IL-6 (wk 8, *r* = 0.388 and *P* = 0.031), area under the curve for insulin (wk 8, *r* = 0.49 and *P* = 0.007), insulin (wk 0, *r* = 0.39 and *P* = 0.031; wk 8, *r* = 0.605 and *P* < 0.001), and HOMA (wk 0, *r* = 0.36 and *P* = 0.049; wk 8, *r* = 0.60 and *P* < 0.001). The change in CRP with weight loss correlated with the change in weight (*r* = 0.55; *P* = 0.003), FFM (*r* = 0.55; *P* = 0.005), FM (*r* = 0.48; *P* = 0.016), and MTT insulin (*r* = 0.46; *P* = 0.025). The correlation between the change in CRP and MTT insulin was removed after adjusting for the effect of weight loss.

Fasting and postprandial glucose and insulin

There was no change in fasting glucose with weight loss and no effect of PCOS status on glucose or changes in glucose with weight loss. There was an effect of PCOS status on MTT glucose (*P* = 0.026) such that it decreased with weight loss only for subjects with PCOS (*P* = 0.029) (Fig. 3, A and B). At wk 0 and 8, subjects with PCOS had significantly higher fasting insulin and HOMA than subjects without PCOS (Table 2 and Fig. 3, C and D). At wk 0, there was no difference in the proportion of subjects with IR with or without PCOS

(10 of 15 *vs.* eight of 17, *P* = 0.265), whereas at wk 8, significantly more subjects with PCOS had IR than subjects without PCOS (nine of 15 *vs.* four of 17, *P* = 0.026). After weight loss, similar decreases in HOMA and fasting insulin (Table 2 and Fig. 3) occurred for all subjects. After weight loss, MTT insulin significantly decreased similarly for subjects with and without PCOS (*P* < 0.001), although subjects without PCOS had a lower insulin response at both time points (*P* = 0.050 for between-subject effect) (Fig. 3).

Reproductive hormones and menstrual cyclicity

There was no effect of PCOS status on changes in SHBG, FAI, and free or total testosterone. After weight loss, testosterone, FAI, and free testosterone decreased and SHBG increased equivalently for all subjects (Table 2). The subjects without PCOS had double the number of ovulations over the study compared with subjects with PCOS (1.9 *vs.* 1.0 ovulation, *P* < 0.001). For the subjects with PCOS, 11 of 15 ovulated at least once and four of 15 were previously amenorrheic and ovulated or recommenced menstruation during the study.

Discussion

We report for the first time that moderate weight loss (4%) does not reduce CRP in overweight women with PCOS compared with weight-matched controls undergoing a similar amount of weight loss (4.7%). Although the effect of weight loss on reducing CRP is well documented (22–24), there are limited data on the effects of weight loss on reducing CRP in women with PCOS. In previous work by our group in overweight women with PCOS (n = 34), a 5.6-kg weight loss in association with a 2.8-mU/liter reduction in fasting insulin decreased CRP (3.3–2.8 mg/liter) (21). Changes in CRP with weight loss have been previously related to changes in weight (24), waist circumference (26), and IR (36). IL-6 and

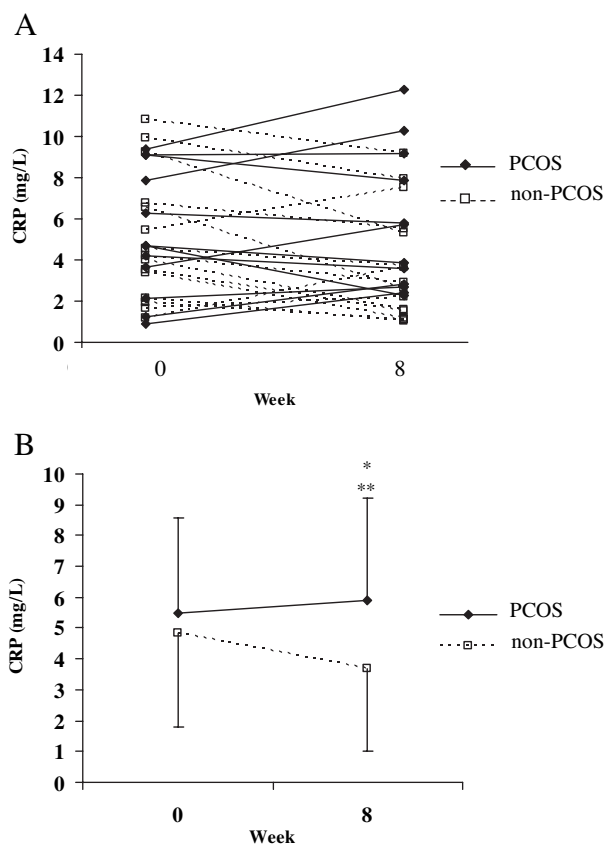


FIG. 2. CRP before and after 8 wk of energy restriction on one dietary pattern (meal replacements) in overweight women with ($n = 12$) or without ($n = 15$) PCOS for individual values (A) and combined mean values (B). In A, data are presented as individual subject values. In B, data are presented as mean \pm SD. Data were assessed using repeated-measures ANOVA with time as within-subject factor and PCOS status as between-subject factor. For conversion from mg/dl to mg/liter for CRP, multiply by 10. *, Time-by-PCOS status interaction ($P = 0.016$); **, trend for a difference at wk 8 ($P = 0.066$).

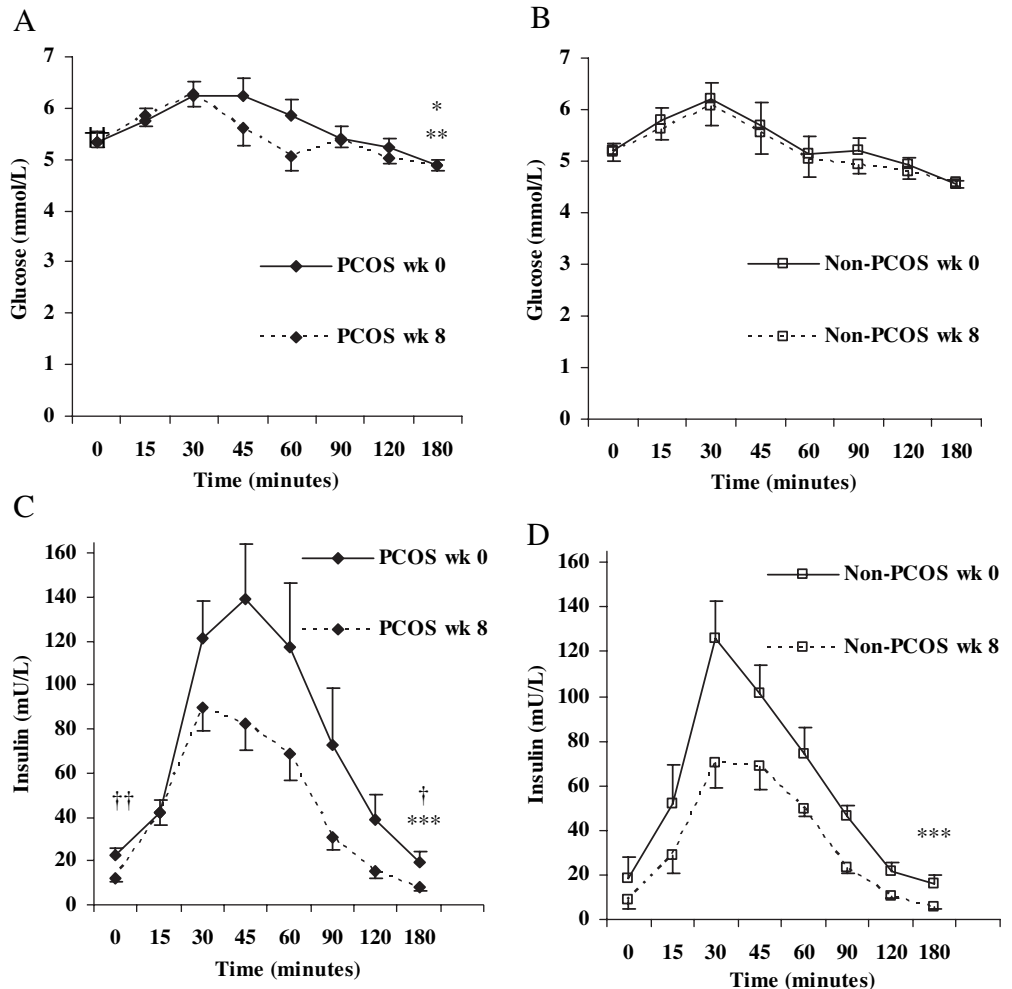
TNF- α stimulate CRP production, and our observed differences in CRP could potentially be explained by the tendency of the PCOS women to display a more inflammatory state after weight loss as evidenced by trends for higher IL-6 and increases in TNF- α with weight loss. In this study, although we observed equivalent reductions in weight and fasting insulin, significantly more women with PCOS than without PCOS were classified with IR after weight loss. The metabolic benefits conferred by weight loss, specifically reductions in IR, may therefore be contingent on reduction on a key level of abdominal or visceral abdominal fat (37). For participants with relatively less IR at baseline, a lesser degree of weight loss may be sufficient to observe metabolic improvements. In support of this, after obesity surgery, maximal decreases in CRP occurred for participants who were relatively less IR at baseline (36). Conversely, equivalent weight losses in subjects with and without IR (8.7 vs. 8.4 kg) induced decreases in CRP only for subjects with IR (3.9–1.2 mg/dl) (38), although this may be accounted for by the relatively low CRP levels before weight loss (1.2 mg/liter) for the insulin-sensitive subjects, unlike our controls. Alternatively, the insignificantly greater degree of weight loss observed in this study

for the women without PCOS (0.6 kg, 0.7%) may have been crucial in achieving a key reduction in IR with resultant positive effects on CRP because no reductions in CRP were observed for subjects who achieved a 3% compared with a 15% weight loss in a 2-yr dietary and exercise intervention study (23).

We report for the first time that modest weight loss does not increase adiponectin or decrease IL-6 or TNF- α in overweight women with PCOS. In contrast to previous findings (22, 23, 25), we observed no increase in adiponectin or decrease in IL-6 and TNF- α after weight loss for women without PCOS. Although the similar response for subjects with and without PCOS is to be expected given the comparable reductions in weight and waist circumference, the lack of a reduction for all subjects after weight loss is surprising. A number of studies have observed no changes in adiponectin, IL-6, or TNF- α after significant reductions in weight (5–9 kg) and insulin in dietary and exercise interventions (26, 39). In this study, the modest weight reduction (<5%) was potentially too small to confer metabolic benefits to all subjects, as evidenced by the lack of a reduction in adiponectin, total cholesterol, HDL-C, and LDL-C. This is supported by observed increases in adiponectin, reductions in IL-6, and improvements in lipid profiles after a 15% weight loss over a 2-yr dietary and exercise intervention compared with no changes in adiponectin or IL-6 for controls who 3% of their initial body weight (23). However, in a *post hoc* analysis, we observed increases in adiponectin and reductions in triglycerides after weight loss for both women with and without PCOS with significantly lower baseline CRP. This suggests that subjects with an adverse inflammatory profile may demonstrate less favorable metabolic improvements after weight loss. The evidence to support this is unclear (40). Despite the significantly higher fasting and postprandial insulin for women with PCOS in this study, all subjects pre-weight loss displayed an equivalently moderately increased metabolic risk profile including elevated CRP (>3 mg/liter), BMI, waist circumference, triglycerides, and LDL-C and reduced HDL-C. It is possible that the participants in this study are not representative of the general population where differences in cardiovascular risk profiles are commonly observed between women with and without PCOS (41). Where women with PCOS display an elevated cardiovascular risk profile in association with elevated inflammatory markers, a greater degree of weight loss may be required to achieve similar metabolic benefits to subjects without PCOS.

We and other investigators have reported similar CRP, adiponectin, IL-6, and TNF- α in overweight women with and without PCOS (42, 43), in contrast to alternative reports of elevated CRP (12, 13), IL-6 (14, 15), and TNF- α (16) and reduced adiponectin (17–19) in lean and overweight women with PCOS compared with weight-matched controls. The latter studies often observed these differences in conjunction with significantly elevated surrogate measures of IR for the women with PCOS, supporting the proposed association between cytokines, adipokines, IR, and inflammation (44). Although less likely, it is possible that alterations in adiposity rather than IR are primarily responsible for mediating changes in cytokines and adipocytokines with weight loss. In a number of studies, weight status was more strongly asso-

FIG. 3. Fasting and post-meal values of plasma glucose and insulin at wk 0 and 8 for overweight women with ($n = 15$) (A, glucose; C, insulin) or without ($n = 15$) (B, glucose; D, insulin) PCOS. Data are presented as mean \pm SEM. Minute zero data were compared by one-way ANOVA with PCOS status as fixed factor. Week zero and 8 data were compared by repeated-measures ANOVA with week and blood sampling time as within-subject factors and PCOS status as between-subject factors. For conversion from mmol/liter to mg/dl for glucose, multiply by 18; for conversion from mU/liter to pmol/liter for insulin, multiply by 6.95. *, Significant week-by-minute-by-PCOS status interaction ($P = 0.026$) (repeated-measures ANOVA); **, significant decrease in postprandial glucose with weight loss for subjects with PCOS ($P = 0.029$) (repeated-measures ANOVA); ***, significant decrease in fasting ($P < 0.002$) and postprandial insulin ($P < 0.001$) with weight loss for subjects with and without PCOS (repeated-measures ANOVA); †, significant between-subject effect of PCOS status for postprandial insulin at wk 0 and 8 ($P = 0.05$) (repeated-measures ANOVA); ††, significant difference at 0 min between subjects with and without PCOS at wk 0 ($P = 0.039$) and wk 8 ($P = 0.011$) (one-way ANOVA).



ciated with CRP, IL-6, and TNF- α than surrogate measures of IR (42, 43), and no association was reported between adiponectin and insulin sensitivity as assessed by the euglycemic hyperinsulinemic clamp after adjustment for BMI (45). The similar CRP, adiponectin, IL-6, and TNF- α levels may be a function of the similar weight and waist circumference between the women with and without PCOS.

There is some suggestion that the ratio of the high to low molecular weight form of adiponectin influences hepatic insulin sensitivity and that this may be differently modulated by weight loss than total adiponectin (46). We measured only total adiponectin and cannot comment on any differential effect of weight loss on adiponectin molecular forms in women with and without PCOS. It is proposed that obesity-associated increases in TNF- α and IL-6 reduce adiponectin expression and thus insulin sensitivity through inhibiting adiponectin promoter activity and reducing adiponectin mRNA expression and secretion (47). In PCOS, IR is predominantly associated with post-receptor defects in insulin signaling (3). Obesity-associated IR is thus metabolically distinct from PCOS-associated IR (3), and it is possible that adiponectin, IL-6, and TNF- α are not involved in the mediation of IR in PCOS. However, there is a proposed role for androgens in inhibiting adiponectin expression and secre-

tion (48) and IL-6 and TNF- α in altering follicular function or gonadotropin secretion (15). The implications of this with regard to their circulating levels in PCOS are unclear.

Despite good associations by a variety of investigators of waist circumference with visceral adiposity (49) and fasting measures of insulin sensitivity with the euglycemic hyperinsulinemic clamp (34), more precise measures may have elucidated subtle differences between the women with and without PCOS. Waist circumference does not distinguish between the relative proportions of sc and visceral abdominal adipose tissue and was not associated with insulin sensitivity assessed by euglycemic hyperinsulinemic clamp in the European Group for the Study of IR (50). We were not able to perform a detailed assessment of metabolic risk to aid in interpretation of results, and it is possible that despite the similar waist circumferences, differences in visceral abdominal fat existed between subjects with and without PCOS. This could account for the differences in fasting insulin and HOMA and the differential effect of weight loss on CRP in PCOS in this study. Furthermore, aging is associated with an increase in IR, central and visceral adiposity, and CRP (51–53) and reductions in adiponectin (54), and variations in inflammatory markers, IR, and glucose homeostasis have also been reported over the course of the menstrual cycle (55,

56). The lack of controlling for age and menstrual cycle stage is an additional weakness of this study. Furthermore, although this study had 65% power to detect the observed changes in CRP with weight loss, we lacked sufficient power to detect changes in adiponectin with weight loss between subjects with and without PCOS.

Although modest weight loss improves reproductive features in PCOS, effects on the metabolic profile were more varied. Despite improvements in triglycerides, insulin, and glucose homeostasis for all subjects, modest weight loss had no effect on CRP, adiponectin, IL-6, and TNF- α in overweight women with PCOS. Clinically, this suggests overweight women with PCOS may require a greater weight loss target (>5%) to achieve reductions in inflammatory markers such as CRP. This may be related to the elevated IR commonly observed in PCOS, which either may not be improved by weight loss in all women with PCOS or may require a greater reduction in weight, abdominal or visceral adiposity, and androgens to be ameliorated.

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