# Elevated Serum Levels of Tumor Necrosis Factor Alpha in Normal-Weight Women With Polycystic Ovary Syndrome

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**Since an increase in tumor necrosis factor alpha (TNFα) expression has been associated with insulin resistance, this study was undertaken to determine the status of circulating TNFα and the relationship of TNFα with insulin levels, body weight, or both in women with polycystic ovary syndrome (PCOS). Fasting serum samples were analyzed in 34 subjects with PCOS, of whom 22 were obese (body mass index [BMI] >27 kg/m$^2$), and in 40 normal control women, of whom 20 were obese. Women with PCOS exhibited a significantly ($P < .02$) higher mean serum TNFα concentration compared with the controls. The serum TNFα level and BMI were directly correlated in women with PCOS ($r = .48$, $P < .005$) and highly correlated in controls ($r = .78$, $P < .001$). When subjects were classified by body weight, the mean serum TNFα concentration was significantly ($P < .001$) elevated in normal-weight women with PCOS compared with normal-weight controls. On the other hand, mean serum TNFα concentrations in obese women with PCOS and obese controls were similar and significantly ($P < .02$) higher than in normal-weight women with PCOS. A direct correlation between serum fasting insulin and TNFα was evident in controls ($r = .35$, $P < .03$), but not in women with PCOS. However, in the subgroup of obese women with PCOS, fasting insulin directly correlated ($r = .49$, $P < .03$) with TNFα and the median fasting serum insulin concentration was significantly ($P < .05$) higher compared with the level in normal-weight women with PCOS and all controls. Fasting insulin and TNFα were no longer correlated in controls as a group and in obese women with PCOS when controlling for body weight. Serum TNFα did not correlate with luteinizing hormone (LH), testosterone (T), or dehydroepiandrosterone sulfate (DHEAS) in women with PCOS. However, serum insulin was significantly correlated ($r = .49$, $P < .0004$) with T and the BMI exhibited a trend for correlation with serum T ($r = .33$, $P = .05$) in women with PCOS. Finally, the mean serum LH concentration was significantly ($P < .02$) higher in normal-weight women with PCOS versus obese women with PCOS, and serum LH levels exhibited a trend for an inverse correlation with the BMI ($r = .31$, $P = .09$) in women with PCOS. We conclude that (1) serum TNFα is increased in normal-weight women with PCOS and is even higher in obese individuals regardless of whether they have PCOS; (2) factors other than obesity are the cause of elevated serum TNFα in normal-weight women with PCOS; and (3) whereas increased circulating TNFα may mediate insulin resistance in obesity, which may in turn promote hyperandrogenism in obese women with PCOS, it remains to be demonstrated whether this is also the case in normal-weight women with PCOS.**


POLYCYSTIC OVARY SYNDROME (PCOS) is a disorder characterized by hyperandrogenism and chronic anovulation.[1,2] Women with PCOS are frequently insulin-resistant, with the resultant hyperinsulinemia considered to be the cause of hyperandrogenism in these individuals.[2,3] In addition, women with PCOS are often obese, which is well-documented to promote insulin resistance and non–insulin-dependent diabetes mellitus (NIDDM).[4,5]

In obesity-related diabetic syndromes, the molecular processes that mediate altered glucose homeostasis can involve tumor necrosis factor alpha (TNFα), a polypeptide cytokine produced by activated immune cells.[6] Several human and animal studies have documented overexpression of TNFα in adipose tissue when obesity or NIDDM is present.[7-9] We have recently demonstrated elevated plasma TNFα concentrations in human obesity that decrease with weight loss.[10] Chronic exposure to TNFα causes functional impairment or depletion of GLUT4, the insulin-sensitive glucose transport protein.[11] Since GLUT4 malfunction has been identified in PCOS,[12,13] it is possible that TNFα may contribute to the induction of this postreceptor defect.

Thus, this study was undertaken to determine the status of TNFα in PCOS. We compared serum TNFα concentrations in women with PCOS versus normal ovulatory women serving as controls and analyzed the relationship of TNFα with body weight, insulin levels, or both in these patients.

## SUBJECTS AND METHODS

### *Subjects*

Thirty-four women with PCOS were selected for study because they showed an elevation in at least one serum androgen (total testosterone [T] > 70 ng/dL, free T >0.8 ng/mL, or dehydroepiandrosterone sulfate [DHEAS] >300 μg/dL) and exhibited the classic features of oligomenorrhea and hirsutism. Women with PCOS had 3.3 ± 0.4 (mean ± SE) menses per year and a mean Ferriman-Gallwey hirsutism score of 10.8 ± 0.7. All had withdrawal bleeding after progestin administration. None had abnormal basal serum concentrations of prolactin, thyrotropin (TSH), or 17-hydroxyprogesterone.

Forty women with menstrual cyclicity and no evidence of hirsutism were selected as controls. The mean age of control subjects (25.7 ± 1.6 years) was similar to that of PCOS subjects (24.6 ± 1.0 years). The mean body mass index (BMI) of control subjects (28.8 ± 1.5 kg/m$^2$) was also similar to that of PCOS subjects (32.2 ± 1.3 kg/m$^2$). None of the subjects in either group used medication including oral contraceptive agents known to affect carbohydrate or sex hormone metabolism for at least 6 weeks before the study.

### *Protocol*

After provision of written informed consent, fasting blood samples were collected at 8 AM from all subjects during participation in several previous investigations[10,14,15] or during the course of routine clinical evaluation. Serum was separated and frozen at −70°C until used for

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assay. All blood sampling was performed without regard to the previous menstrual period in women with PCOS and during the early to midfollicular phase of the menstrual cycle in control subjects.

### *Assays*

The serum levels used to screen women with PCOS were measured from blood samples drawn within 6 months before the study. Screening levels of total T and DHEAS were measured with radioimmunoassay (RIA) kits from PANTEX (Santa Monica, CA), and 17-hydroxyprogesterone and prolactin levels were measured with RIA kits from Diagnostic Products Corp (Los Angeles, CA). Screening levels of TSH were measured with a RIA kit from Bio-Rad (Hercules, CA), and free T levels were measured by a method described previously.[16]

The actual study data were analyzed separately. The TNFα level was measured with an enzyme-linked immunosorbent assay kit (Quantikine) from R&D Systems (Minneapolis, MN), which has a sensitivity of 0.4 pg/mL to allow for measurement of TNFα in the normal range in human serum. The insulin level was measured with a RIA kit from Linco Laboratories (St Louis, MO). Luteinizing hormone (LH), T, and DHEAS levels were measured with RIA kits from Diagnostic Products Corp. The intraassay and interassay coefficients of variation did not exceed 7% and 12%, respectively, for all assays.

### *Statistical Analysis*

Student's unpaired $t$ test for normal data or the Mann-Whitney rank-sum test for data lacking normality were used, plus ANOVA for multiple group comparisons. In the latter instance, the Kruskal-Wallis method was used when data lacked normality. Correlation analyses were initially performed by linear regression using the least-squares method. This was followed by a multivariate regression analysis in which body weight was used as a continuous variable to determine if any significant correlations of fasting insulin with TNFα or T were independent of body weight. Differences were considered significant at a $P$ level less than .05.

## RESULTS

Women with PCOS exhibited a significantly ($P < .02$) higher mean serum TNFα concentration compared with the control subjects (PCOS, 3.83 ± 0.17 pg/mL; control, 2.78 ± 0.35 pg/mL). The serum TNFα concentration and BMI were directly correlated in women with PCOS ($r = .48$, $P < .005$) and highly correlated in control subjects ($r = .78$, $P < .001$; Fig 1). Figure 2 shows the individual TNFα levels and Table 1 lists the mean BMI and serum TNFα concentration for women with PCOS and control subjects classified as normal-weight (BMI ≤ 27 kg/m$^2$) or obese (BMI > 27 kg/m$^2$). Whereas obese women with PCOS were weight-matched to obese controls, the mean BMI of normal-weight women with PCOS was slightly greater than that of normal-weight controls, achieving statistical significance ($P < .01$). The mean TNFα concentration was similar in obese women with PCOS and obese control subjects. On the other hand, the mean TNFα concentration was significantly higher in obese women with PCOS and obese controls ($P < .02$) compared with normal-weight women with PCOS and significantly higher ($P < .001$) in normal-weight women with PCOS compared with normal-weight controls. These findings were unchanged when only normal-weight women with PCOS who were weight-matched to normal-weight controls were considered for the comparison of TNFα levels among groups. A correlation between the serum TNFα level and BMI was lacking when women with PCOS or control subjects were classified by body weight as normal-weight or obese.

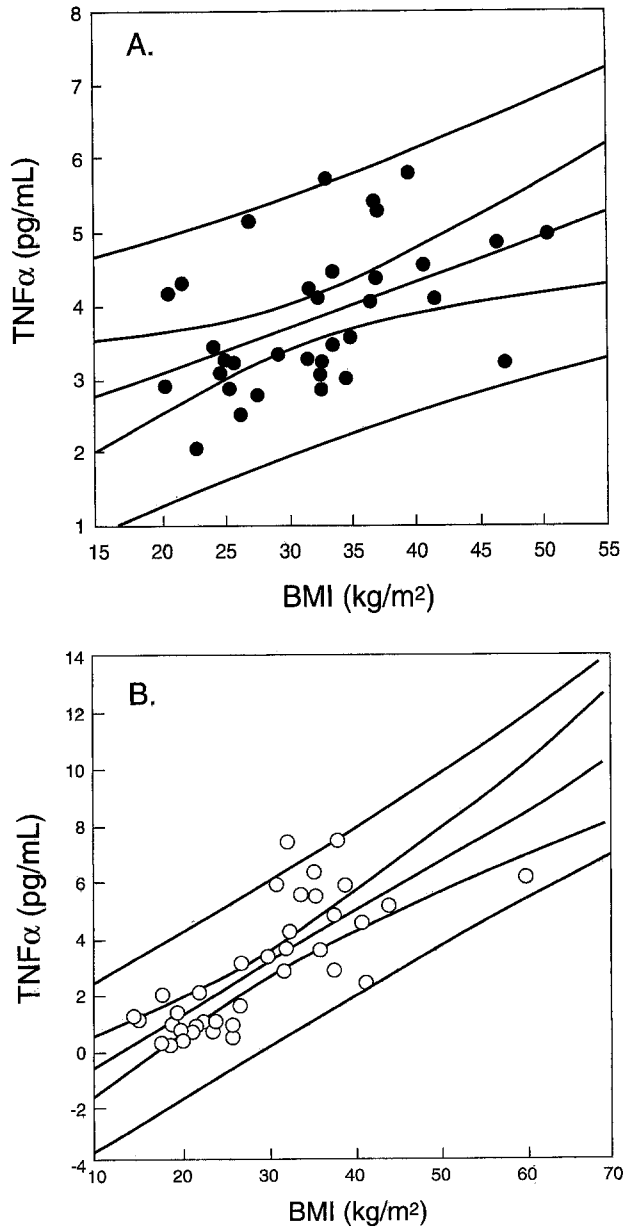


**Fig 1. Linear correlation between serum TNFα and BMI in (A) women with PCOS ($r = .48$, $P < .005$) and (B) control subjects ($r = .78$, $P < .001$).**

Women with PCOS exhibited a significantly ($P < .003$) higher mean serum insulin concentration compared with control subjects (PCOS, 36.8 ± 8.5 μU/mL; control, 14.2 ± 3.1 μU/mL). A direct correlation between TNFα and insulin levels was evident in control subjects ($r = .35$, $P < .03$; data not shown), but not in women with PCOS. When dividing women with PCOS and controls by body weight, the median serum insulin concentration in obese women with PCOS (37.3 μU/mL) was significantly ($P < .05$) higher than that in normal-weight women with PCOS (10.2 μU/mL) or in control subjects whether obese (12.2 μU/mL) or normal-weight (9.7 μU/mL; Fig 3A). In fact, a

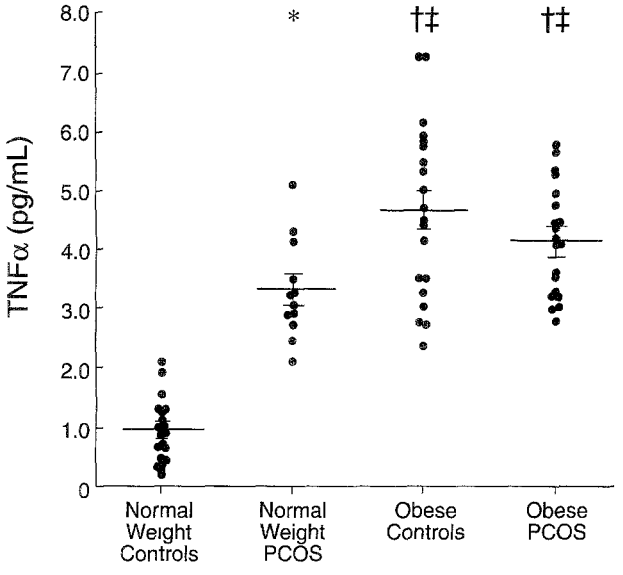


**Fig 2. Serum TNFα concentrations in women with PCOS and controls classified by body weight. *Significant ($P < .01$) difference between normal-weight women with PCOS and normal-weight controls. †Significant ($P < .0001$) difference between obese women with PCOS or obese controls and normal-weight controls. ‡Significant ($P < .02$) difference between obese women with PCOS or obese controls and normal-weight women with PCOS.**

direct correlation between serum insulin and TNFα was only evident in this subgroup of women with PCOS ($r = .49$, $P < .03$; Fig 3B), not in normal-weight women with PCOS or normal-weight or obese control subjects. However, after controlling for body weight, a correlation between serum insulin and TNFα was not observed in controls or women with PCOS before or after division by body weight.

Figure 4 depicts a direct correlation between serum insulin and T in women with PCOS ($r = .49$, $P < .0004$). When controlling for body weight, the correlation between insulin and T persisted in women with PCOS as a group ($P < .007$) and in those of normal weight ($P < .002$), but not in obese women with PCOS. In addition, a comparison between the serum T level and BMI in women with PCOS showed a trend for correlation that approached significance ($r = .32$, $P = .05$; data not shown). The mean serum hormone concentrations of women with PCOS are listed in Table 2. Although the mean serum levels of T and DHEAS were similarly elevated in accordance with the entry criteria in both normal-weight and obese women with PCOS, the mean serum LH concentration was significantly ($P < .02$) higher in normal-weight women with PCOS versus obese women with PCOS. In addition, a comparison between the serum LH concentration and BMI in women with PCOS showed a trend for an inverse correlation that approached significance ($r = .31$, $P = .09$; data not shown).

There was no correlation between serum concentrations of TNFα and LH, T, or DHEAS in women with PCOS. There also was no correlation between the serum concentration of TNFα and age in women with PCOS or control subjects.

**Table 1. BMI and Serum TNFα Concentrations in Women With PCOS and Controls Classified by Body Weight (mean ± SE)**

| Group | BMI (kg/m²) | TNFα (pg/mL) |
|---|---|---|
| Obese PCOS (n = 22) | 36.6 ± 1.2* | 4.11 ± 0.19‡§ |
| Obese control (n = 20) | 36.7 ± 1.6* | 4.65 ± 0.33‡§ |
| Normal-weight PCOS (n = 12) | 24.2 ± 0.7† | 3.31 ± 0.25‖ |
| Weight-matched normal-weight PCOS (n = 6) | 22.3 ± 0.7 | 3.32 ± 0.54‖ |
| Normal-weight control (n = 20) | 20.8 ± 0.8 | 0.91 ± 0.12 |

*$P < .001$, obese (PCOS or control) *v* normal-weight (PCOS or control).

†$P < .01$, normal-weight PCOS *v* normal-weight control.

‡$P < .02$, obese PCOS or obese control *v* weight-matched and all normal-weight PCOS.

§$P < .0001$, obese PCOS or obese control *v* normal-weight control.

‖$P < .001$, weight-matched or all normal-weight PCOS *v* normal-weight control.

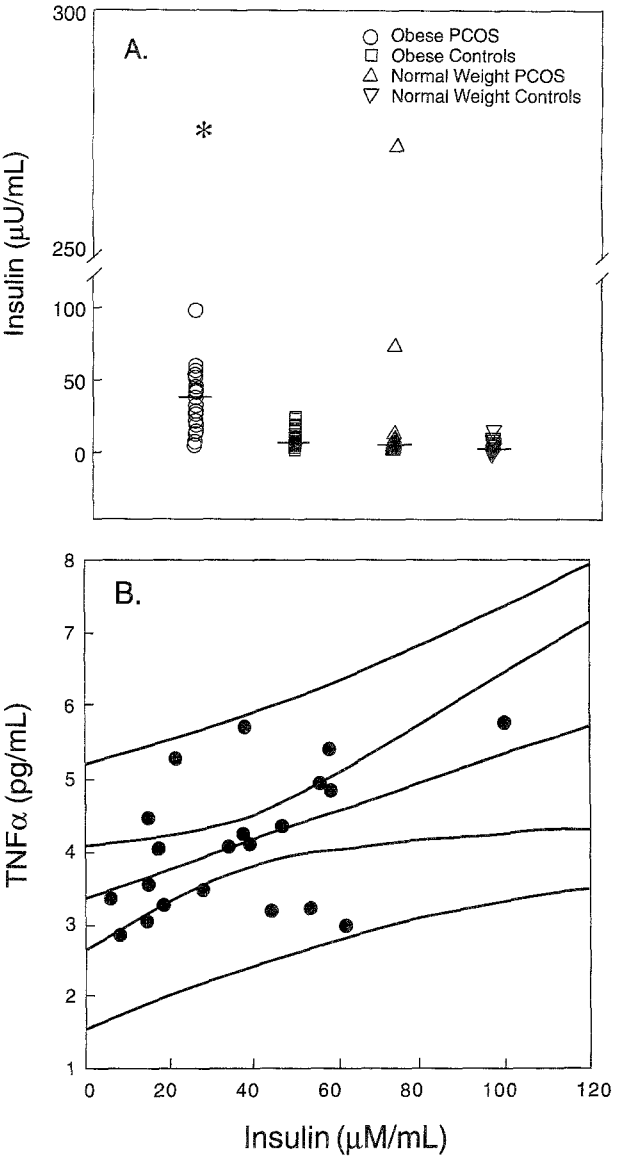


**Fig 3. (A) Serum insulin concentrations in obese women with PCOS, obese controls, normal-weight women with PCOS, and normal-weight controls. *Significant ($P < .05$) difference between the median serum insulin concentration in obese women with PCOS *v* the other 3 subgroups. (B) Linear correlation between serum TNFα and insulin in obese women with PCOS ($r = .49$, $P < .03$).**

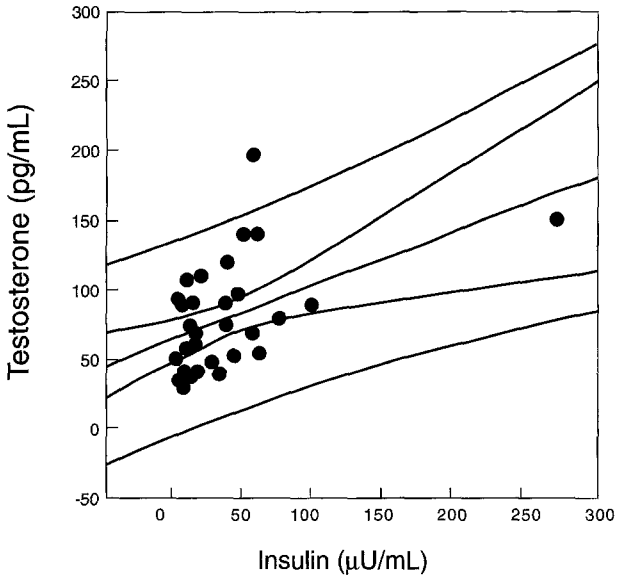


**Fig 4. Linear correlation between serum insulin and T in women with PCOS ($r = .49$, $P < .0004$).**

## DISCUSSION

Our data clearly show that plasma TNFα concentrations are elevated in women with PCOS. While obesity may be the major promoter of increased serum TNFα concentrations in control subjects, factors other than obesity may be involved in promoting elevated serum TNFα levels in women with PCOS. This is suggested by the correlation between serum TNFα and body weight observed in both groups, which was better in control subjects than in women with PCOS. In addition, stratification according to body weight revealed significantly higher TNFα levels in normal-weight women with PCOS compared with normal-weight control subjects. However, in the presence of obesity, this difference was no longer observed with even higher TNFα levels detected in obese individuals regardless of whether they had PCOS. Thus, our data are consistent with our previous demonstration that in the obese, plasma TNFα concentrations are elevated and decrease with weight loss,[10] but in women with PCOS, TNFα concentrations can be elevated independently of obesity. Whereas the source of excess circulating TNFα in PCOS remains to be elucidated in the absence of obesity, it is likely that adipose tissue is the predominant source of the even-higher serum TNFα concentrations observed when obesity is present.

The issue of insulin resistance is cardinal to the pathogenesis of hyperandrogenism in PCOS and therefore needs to be addressed. TNFα expression and serum concentrations have been shown to be elevated in animals with obesity, and the increased insulin resistance in these animals is reversed by infusion of agents that bind/neutralize TNFα.[8] Our data also suggest that insulin resistance may be mediated by obesity-related increases in circulating TNFα. While the fasting insulin level directly correlated with the TNFα level in control subjects, we were unable to demonstrate a similar correlation in women with PCOS as a group. This latter observation may represent a "washout" phenomenon caused by the impact of factors other than obesity involved in promoting excess circulating TNFα in women with PCOS. On the other hand, a significant correlation between insulin and TNFα was only evident in obese women with PCOS. This subgroup probably had the greatest insulin resistance, as suggested by their significantly higher fasting insulin compared with the other subgroups. However, the impact of obesity on the relationship between insulin and TNFα is apparent by the absence of a correlation between these two substances in control subjects as a group and in obese women with PCOS when controlling for body weight. Thus, excess circulating TNFα arising from obesity may be a mediator of insulin resistance independently of PCOS.

**Table 2. Fasting Serum Hormone Levels in Subjects With PCOS (mean ± SE)**

| Hormone | Normal-Weight PCOS | Obese PCOS |
|---|---|---|
| LH (mIU/mL) | 20.6 ± 2.4 | 12.5 ± 1.9* |
| T (ng/dL) | 72.7 ± 10.0 | 82.4 ± 8.6 |
| DHEAS (μg/dL) | 413 ± 38 | 407 ± 22 |

*$P < .02$.

Our data are insufficient to determine if the mediation of insulin resistance by elevated serum TNFα in PCOS can be independent of obesity. We were unable to demonstrate elevated fasting insulin concentrations or a correlation between insulin and TNFα in normal-weight women with PCOS, but similar findings were also evident in obese controls. Morales et al,[17] on the other hand, have recently demonstrated that insulin resistance is greater in normal-weight women with PCOS versus normal-weight control women and that in obese women with PCOS it is the greatest, using the Bergman minimal model.[18] This latter method of assessing insulin resistance is more sensitive than the fasting insulin concentration used by our group, and could be an additional reason that no correlation was observed between serum insulin and TNFα in women with PCOS as a group, as well as those of normal weight. However, the observations reported by Morales et al[17] parallel our current data on TNFα to some extent. Thus, it would be of interest in the future to correlate serum TNFα with insulin sensitivity as obtained by the Bergman method in women with PCOS, with a special focus on those of normal weight.

TNFα originating from adipose tissue may be involved in the classic relationship between hyperinsulinemia and hyperandrogenism in PCOS. Although the serum concentrations of TNFα and T did not correlate, insulin and T were highly correlated in women with PCOS, as described previously.[2,3,17] However, this latter correlation was no longer apparent in obese women with PCOS when controlling for body weight, and there was a trend for a correlation between T and body weight in women with PCOS as a group. Therefore, TNFα may indirectly promote hyperandrogenism in obese women with PCOS through its ability to mediate insulin resistance in obesity. Nevertheless, the possibility that TNFα can directly induce androgen excess in women with PCOS cannot be definitively excluded by the basal T level used herein as the sole assessment of androgen production. Moreover, it would be of interest to correlate serum

TNFα with a dynamic measure of androgen production such as serum T after gonadotropin-releasing hormone agonist stimulation.

LH is a known promoter of ovarian hyperandrogenism[17,19,20] and is likely to elicit this effect in normal-weight women with PCOS. This is suggested by the significantly higher mean serum LH concentration in these patients compared with obese women with PCOS as described previously,[21,22] and by the trend for an inverse correlation between serum LH and body weight in normal-weight women with PCOS. However, any possible role of the increased circulating TNFα concentrations in this subgroup of women with PCOS either to induce excess LH secretion or to potentiate the effect of LH on ovarian androgen secretion is beyond the scope of this study.

In conclusion, serum TNFα is increased in women with PCOS independently of obesity. However, this increase is even greater in obese individuals regardless of whether they have PCOS. Factors other than obesity may be responsible for elevated serum TNFα levels in normal-weight women with PCOS. Increased circulating TNFα may mediate insulin resistance in obesity, which may in turn promote hyperandrogenism in obese women with PCOS. However, it remains to be determined whether this is also the case in normal-weight women with PCOS.

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