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Plasma interleukin 6 levels are elevated in polycystic ovary syndrome independently of obesity or sleep apnea

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Abstract

Premenopausal women with polycystic ovary syndrome (PCOS) are at a much higher risk for excessive daytime sleepiness, fatigue, and insulin resistance than control women. Elevated levels of interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) are presumably part of the pathogenesis of these clinical manifestations. Forty-two obese women with PCOS, 17 body mass index–comparable obese controls, and 15 normal-weight controls free from apnea participated in the study that included one 8-hour nighttime polysomnography, single morning cytokine plasma concentrations, and insulin resistance indices. Women with PCOS exhibited higher plasma concentrations of IL-6 than obese controls, who had intermediate values, or normal-weight controls, who had the lowest values (4.75 ± 0.5 vs 3.65 ± 0.4 vs 1.84 ± 0.3 pg/mL, $P < .01$). Tumor necrosis factor α values were higher in PCOS and obese controls compared with normal-weight controls, but the difference was not statistically significant (4.05 ± 0.3 vs 3.79 ± 0.2 vs 3.14 ± 0.2 pg/mL, $P = .103$). Based on backward regression analysis, IL-6 levels had a stronger association with the PCOS group than with the obese group, and the sleep or hypoxia variables did not make a significant contribution to either IL-6 or TNF-α. Both IL-6 and TNF-α correlated positively with body mass index ($P < .01$) in obese controls but not in women with PCOS. Furthermore, within the PCOS group, IL-6 and TNF-α correlated more strongly with indices of insulin resistance than obesity. We conclude that IL-6 levels are elevated in obese women with PCOS independently of obesity or sleep apnea and may represent a pathophysiologic link to insulin resistance.

1. Introduction

Polycystic ovary syndrome (PCOS), the most common endocrine disorder of premenopausal women, is characterized by chronic gonadotropin-dependent hyperandrogenism, oligoanovulation, oligoamenorrhea, and insulin resistance [1]. We and others recently reported that sleep apnea and excessive daytime sleepiness (EDS) were markedly and significantly more frequent in women with PCOS than in premenopausal controls, even when controlling for obesity [2-5], prompting leading experts in the field to suggest that sleep apnea and sleepiness should be added to the clinical manifestations of this disorder [6]. Furthermore, in our study, the strongest predictor of sleep apnea/sleepiness was insulin resistance.

In 1997, we reported that the pro-inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) are elevated in sleep apnea and disorders of EDS and that these cytokines may be mediators of EDS and fatigue in humans [7]. Further studies demonstrated that hypercytokinemia, visceral fat, and insulin resistance are independently and strongly associated with sleep apnea and EDS [8-10].

Few studies have assessed the peripheral levels of IL-6 and TNF-α in women with PCOS, and the results are inconsistent [11-14]. All of them have focused on normal-weight or mildly overweight women with PCOS, whereas a recent study suggested that obesity and not PCOS per se

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was the major determinant of serum inflammatory markers in these premenopausal women [15].

The purpose of our study was to assess whether IL-6 and TNF-α are elevated in obese, premenopausal, women with PCOS while controlling for obesity and sleep apnea. To accomplish this objective, we included 3 groups in the study: obese women with PCOS; body mass index (BMI)–matched, obese controls; and age-matched, normal-weight controls. All 3 groups were free from sleep apnea and were monitored in the sleep laboratory for one night and were assessed for single cytokine plasma concentrations and insulin resistance indices.

2. Subjects and methods

2.1. Subjects

Forty-two female patients with PCOS, 17 obese controls, and 15 normal-weight, female controls participated in the study. The subjects were recruited from the PCOS clinic, the obesity clinic, or through advertisement in the local community. Women with PCOS and obese controls were weight-matched, and, in terms of age, women with PCOS and normal-weight controls were age-matched, whereas obese controls were slightly older than those in the other 2 groups. For women with PCOS, obese controls, and normal-weight controls, their mean ± SE ages were 29.6 ± 0.9, 35.7 ± 1.0, and 31.9 ± 1.5 years, respectively ($P < .05$ between women with PCOS and obese controls) and their BMIs were 38.7 ± 1.4, 36.9 ± 1.0, and 23.3 ± 0.5 kg/m^2, respectively ($P < .05$ between women with PCOS or obese controls and normal-weight controls). The ethnic backgrounds of the groups were as follows: PCOS—41 whites and 1 African American; obese controls—15 whites, 1 Hispanic, and 1 other; and thin controls—13 whites and 2 Hispanics.

Women with PCOS, to qualify for the study, had to have a diagnosis made by the presence of chronic anovulation (6 or fewer menstrual periods per year) in association with elevated circulating androgen levels (total testosterone more than 201.1 nmol/L and/or free and weakly bound testosterone more than 55.5 nmol/L) [2,16]. Nonclassic adrenal 21-hydroxylase deficiency, hyperprolactinemia, and androgen-secreting tumors were excluded by appropriate tests before the diagnosis of PCOS was made. All the women with PCOS had oligoamenorrhea and polycystic ovaries, by ultrasound examination. All 3 groups were premenopausal, whereas obese and normal-weight controls had no history of irregular menstrual cycles or fertility problems. Potential participants in the study were screened in the sleep laboratory for one night for possible primary sleep disorders; subjects with sleep apnea or nocturnal myoclonus were excluded from the study. Both PCOS and control subjects with a diagnosis of diabetes mellitus or who were receiving treatment with psychotropics, steroids, sympathomimetics, or sympatholytics, including β-blockers, were excluded from the study. All patients and controls were asked to abstain from nonsteroidal anti-inflammatory medication for 1 week before the study.

The study was approved by the institutional review board of Hershey Medical Center, Penn State University, and all subjects gave written informed consent.

2.2. Procedures

A thorough medical assessment, including physical examination, routine laboratory tests (including complete blood cell count, urinalysis, thyroid function tests, and electrocardiography), and sleep history, was completed for each patient and control subject.

All subjects were evaluated for one night in the sleep laboratory in sound-attenuated, light- and temperature-controlled rooms. During this evaluation, they were continuously monitored for 8 hours (Model 15 Neurodata Amplifier System, Grass-Telefactor Instruments, West Warwick, RI). The sleep records were subsequently scored independently, according to standardized criteria [17].

Respiration was monitored throughout the night by use of thermocouples at the nose and mouth (model no. 1450, Sleepmate Technologies, Midlothian, VA) and thoracic strain gauges. All-night recordings of hemoglobin oxygen saturation (SaO_2) were obtained with an oximeter (model 8800, Noonin Medical, Plymouth, MN) attached to the finger. An apnea was considered present if a breath cessation exceeded 10 seconds. Each apnea was categorized in terms of obstructive (chest wall movement present) or central (chest wall movement absent). In addition, hypopneas were considered present when a reduction in airflow of approximately 50% was indicated at the nose or mouth and was associated with a reduction of 4% of SaO_2.

Blood pressure was measured in the evening during the physical examination using a pneumoelectric microprocessor-controlled instrument. The recorded blood pressure was the average of 3 consecutive readings during a 5-minute period after 10 minutes of rest in the supine position.

2.3. Assays

Single blood samples for measurement of plasma IL-6 and TNF-α were drawn from the 3 groups in the morning between 06:00 and 07:00 AM after completion of the nocturnal sleep laboratory recording. In addition, in the group of women with PCOS, single blood samples for measurement of testosterone, non–sex hormone-binding globulin-bound testosterone, fasting blood glucose, and insulin were obtained within a year from the sleep study. Plasma was stored at −70°C until assay. All samples were processed in the same manner. Plasma IL-6 and TNF-α were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) [7,10]. The intra- and interassay coefficients of variation (CVs) ranged from 3.2% to 8.5% and 3.5% to 8.7%, respectively, for IL-6 and from 5.6% to 6.1% and 7.5% to 10.4%, respectively, for TNF-α.

The lower detection limits for IL-6 and TNF-α were 0.18 and 0.094 pg/mL, respectively. Samples were run in duplicate, and standards were run in triplicate. Assays for testosterone were performed using Diagnostic Products (Los Angeles, CA) Coat-A-Count kits; the interassay CVs were 8% and 5%, respectively [18]. Free and weakly bound testosterone was measured by a modification of the procedure of Tremblay and Dube [19], which precipitates out sex hormone–binding globulin and measures what remains; the interassay CV was 7%. Insulin was determined with a double-antibody method using reagents obtained from Linco Research (St Charles, MO). The sensitivity of this assay is 2 μU/mL, with 0.2% cross-reactivity with proinsulin. The intra- and interassay CVs are less than 10%. Plasma glucose levels were determined by the glucose oxidase technique [18].

2.4. Statistical analyses

For comparisons among the 3 groups in Table 1, the linear model that allowed for heterogeneous variances for the 3 groups using the maximum likelihood methods was used [20] or the Kruskal-Wallis test when the variable that displayed high level of skewness was used. If the overall test was significant, pairwise comparisons among the 3 groups were further conducted using the Student *t* test or the Wilcoxon rank sum test. For analysis of cytokine data, we performed logarithm transformation of IL-6 and TNF-α to satisfy the normality assumption for each of the 3 groups. Furthermore, we tested linear contrasts to assess whether there is a trend between the measured cytokine levels among the groups. The trend test is appropriate when the primary interest is to test whether there exists a dose-response relationship between the cytokine levels and the 3 study groups. However, because it is expected that the 3 groups might not be equally spaced in terms of cytokine levels, we tried a variety of coefficients for the contrasts, with the ratios of the distances between PCOS to obese controls vs obese controls to normal controls ranging from 1 to 2.5. A backward non-stepwise regression analysis was performed with the significance level to stay of .1 to assess the relative contribution of PCOS group, obesity group, percentage of sleep time, slow wave sleep, and minimum SaO_2 in the elevation of plasma IL-6 and TNF-α levels. The initial model included PCOS group, obese group, percentage of sleep time, slow wave sleep, and minimum SaO_2. Relations among plasma cytokines and glucose, insulin, glucose-to-insulin ratio, the homeostatic model assessment (HOMA), and testosterone were assessed individually using Pearson correlation and the linear bivariate regression analysis that also included BMI. The demographic, sleep, and respiratory data are expressed as the mean ± SE in Table 1 for the purpose of cross-comparisons between

Table 1
Demographic, sleep, and respiratory data in women with PCOS, obese controls, and normal-weight controls

	PCOS (n = 42)	Obese controls (n = 17)	Normal-weight controls (n = 15)	Overall test
Age (y)	29.6 ± 0.9[a]	35.7 ± 1.0[b]	31.9 ± 1.5	**[c]
BMI	38.7 ± 1.4[d]	36.9 ± 1.0[b]	23.3 ± 0.5	**[c]
Heart rate	79.5 ± 2.6[d]	78.6 ± 3.2[b]	71.5 ± 1.7	*[c]
Respiratory rate	16.7 ± 0.5	17.8 ± 1.0	15.6 ± 0.5	NS
Systolic blood pressure	124.1 ± 2.0	127.9 ± 3.2	122.5 ± 5.8	NS
Diastolic blood pressure	79.5 ± 1.7	77.9 ± 3.1	76.3 ± 3.4	NS
Sleep latency (min)	31.8 ± 4.8	26.4 ± 4.9	20.4 ± 4.6	NS
Wake time after sleep onset (min)	60.3 ± 6.6	56.3 ± 8.4	46.0 ± 9.3	NS
Total wake time (min)	92.1 ± 9.9	82.7 ± 12.1	66.4 ± 10.2	NS
Total sleep time (min)	376.9 ± 12.4	394.9 ± 11.8	412.9 ± 10.1	#[c]
% Sleep time	79.8 ± 2.3	82.7 ± 2.5	86.1 ± 2.1	NS
% Stage 1	5.7 ± 0.9	6.0 ± 0.9	4.9 ± 0.8	NS
% Stage 2	64.8 ± 1.5[a]	70.3 ± 2.0[b]	63.5 ± 2.5	#[c]
% SWS	12.6 ± 1.1[a]	5.7 ± 1.6	10.3 ± 2.0	**[e]
% REM	16.9 ± 1.1[d]	18.0 ± 2.0	21.3 ± 1.5	#[c]
REM latency (min)	140.1 ± 10.6[d]	140.3 ± 18.2[b]	103.2 ± 14.9	#[e]
Apnea/hypopnea index	1.2 ± 0.6	0.9 ± 0.4	0.0 ± 0.0	NS
Minimum oxygen saturation	94.8 ± 0.7	93.2 ± 0.9[b]	95.8 ± 0.4	*[c]
Glucose (μg/dL)	96.9 ± 4.0			
Insulin (μg/mL)	25.1 ± 2.5			
Glucose-to-insulin ratio	5.0 ± 0.4			
HOMA	6.2 ± 0.9			

Values are expressed as mean ± SE except for age and BMI, which were expressed as mean ± SD. SWS indicates slow wave sleep. NS indicates not significant for overall F test or Kruskal-Wallis test. *$P < .05$; **$P < .01$; #$P < .10$.

[a] $P < .05$; PCOS vs obese controls.

[b] $P < .05$; obese controls vs normal-weight controls.

[c] F test.

[d] $P < .05$; PCOS vs normal-weight controls.

[e] Kruskall-Wallis test.

groups. The statistical significance level selected for all analyses was .05.

3. Results

3.1. Sleep, respiratory, and blood pressure data

Although most findings were not statistically significant, women with PCOS tended to sleep not as well as the other 2 groups as suggested by a decrease in total sleep time ($P < .1$), increases of sleep latency, wake time after sleep onset, or total wake time compared with normal-weight controls (Table 1). Women with PCOS demonstrated a significantly lower percentage of stage 2 sleep ($P < .05$) and a significantly higher percentage of slow wave sleep ($P < .05$) than obese controls. In addition, percentages of rapid eye movement (REM) sleep and REM latency were lower and longer, respectively, in women with PCOS compared with normal-weight controls ($P < .05$).

Indices of apnea/hypopnea index were not different among the 3 groups. Minimum Sa_{O_2} was not different between women with PCOS and obese controls, whereas it was slightly lower in obese controls compared with normal-weight controls ($P < .05$).

Heart rate in normal-weight controls was significantly lower than that in women with PCOS ($P < .05$). In terms of respiratory rate, and systolic and diastolic blood pressures, no statistically significant differences were observed among the 3 groups ($P = .212$ for respiratory rate, $P = .564$ for systolic blood pressure, and $P = .671$ for diastolic blood pressure).

3.2. Plasma cytokines

Plasma TNF-α morning values were higher in the group of women with PCOS (4.05 ± 0.3 pg/mL) and in the obese controls (3.79 ± 0.2 pg/mL), compared with the normal-weight controls (3.14 ± 0.2 pg/mL), but the difference was not statistically significant ($P = .103$) (Table 2). Plasma IL-6 values were highest in the group of women with PCOS (4.72 ± 0.5 pg/mL), intermediate in the obese controls (3.65 ± 0.4 pg/mL), and lowest in the normal-weight controls (1.84 ± 0.3 pg/mL), with the difference between women with PCOS, obese controls, and normal-weight controls being significant ($P < .01$) (Table 2). The trend tests based on linear contrast for IL-6 showed a significant dose-response effect for all different ratios of the distances between PCOS to obese controls vs obese controls to normal controls ranged from 1 to 2.5 ($P < .001$). The results remain the same after adjusting for age using analysis of covariance. Both log IL-6 and log TNF-α values correlated positively with BMI in the obese group, whereas there was no significance in the PCOS group (Fig. 1). The P values for testing whether the PCOS and obese groups have the same regression slopes of BMI are 0.025 for log IL-6 and 0.214 for log TNF-α, respectively.

Table 2
Plasma IL-6 and TNF-α in women with PCOS, obese controls, and normal-weight controls

	PCOS (n = 42)	Obese controls (n = 17)	Normal-weight controls (n = 15)	Overall[a] F test (P)
IL-6 (pg/mL)	4.72 ± 0.5*	3.65 ± 0.4†	1.84 ± 0.3	<.001
TNF-α (pg/mL)	4.05 ± 0.3	3.79 ± 0.2	3.14 ± 0.2	.103

Values are expressed as mean ± SE. Data are not age-adjusted.
[a] Based on log-transformed data.
* $P < .05$; PCOS vs normal-weight controls.
† $P < .05$; obese controls vs normal-weight controls.

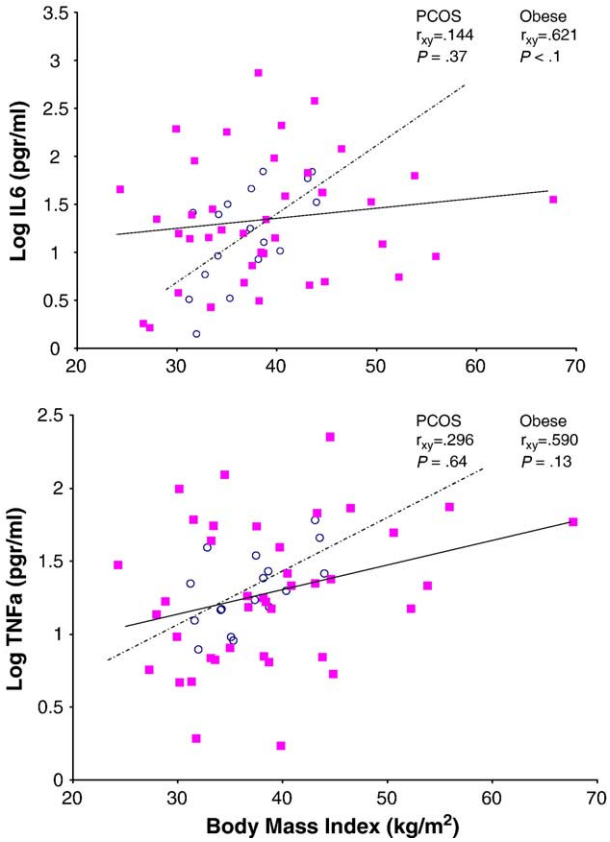

Fig. 1. Plasma IL-6 (top) and TNF-α (bottom) are positively correlated with BMI in obese controls, but not in obese women with PCOS. ■ indicates women with PCOS; ○, obese controls.

Based on a backward regression analysis for log IL-6, the percentage of sleep time was first eliminated (partial R^2 = 0.3%), followed by minimum Sa_{O_2} (1.3%,) and slow wave sleep (2.5%) (all P values > .1). Furthermore, the PCOS group was more significantly associated with log IL-6 levels than the obese group ($P < .0001$ vs $P = .005$; partial R^2 = 16.2% vs 9.7%). For log TNF-α, the PCOS and obese groups, and the sleep or hypoxia variables did not make a significant contribution (all $P > .1$; partial R^2 all <2.5%).

3.3. Association between plasma cytokine concentrations, insulin resistance indices, and testosterone in PCOS

The associations between cytokines and glucose, insulin, glucose-to-insulin ratio, HOMA, and testosterone were

Table 3
Pearson correlation analyses between cytokine plasma levels and indices of insulin resistance in the PCOS group

	Glucose		Insulin		Glucose-to-insulin ratio		Log (HOMA)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Log (TNF-α)	−0.01	.963	0.35	.030	−0.36	.026	0.33	.046
Log (IL-6)	0.38	.017	0.15	.370	−0.05	.758	0.31	.060

assessed only within the PCOS group because this was the only group that we had complete cytokine, glucose, insulin, and testosterone data.

Plasma log TNF-α correlated with fasting plasma insulin levels ($r = 0.35$; $P < .030$) and glucose-to-insulin ratio ($r = -0.36$; $P < .026$) (Table 3). In addition, plasma log IL-6 correlated with fasting plasma glucose levels ($r = 0.38$; $P < .02$), but not with insulin levels. Both log IL-6 and log TNF-α correlated with log HOMA ($r = 0.31$ for log IL-6, $P = .06$ and $r = 0.33$ for log TNF-α, $P = .046$). Finally, free testosterone correlated with insulin levels ($r = 0.53$; $P < .0007$) and glucose-to-insulin ratio ($r = 0.30$; $P = .06$). In a bivariate regression analysis, glucose-to-insulin ratio, which was treated either as a dichotomous variable (≤4.5 indicates insulin resistance) or as a log-transformed continuous variable, was a marginally significant predictor ($P = .08$ and .07, respectively) of log TNF-α levels, whereas BMI was not ($P = .30$). In addition, in a bivariate regression analysis, when HOMA was used as an index of insulin sensitivity, HOMA appeared to have a stronger association with IL-6 and TNF-α levels compared with BMI. Specifically, among subjects with PCOS, HOMA was associated with a 22.1% increase in TNF-α (95% confidence interval [CI], −5.3% to 57.6%, $P = .14$) per doubling of HOMA and 1.0% increase in TNF-α (95% CI, −1.0% to 3.0%, $P = .26$) per doubling of BMI. Furthermore, HOMA was associated with a 33.6% increase in IL-6 (95% CI, −4.2% to 86.4%, $P = .10$) per doubling of HOMA and 0.4% increase in TNF-α (95% CI, −1.9% to 2.8%, $P = .76$) per doubling of BMI. There was also no correlation between free testosterone with either IL-6 or TNF-α.

4. Discussion

Our study provides evidence that IL-6 is elevated in premenopausal obese women with PCOS independently of obesity or sleep apnea. Tumor necrosis factor α may be elevated in obese women with PCOS compared with normal-weight controls. These results suggest that hypercytokinemia associated with PCOS may contribute to the behavioral and physical signs and symptoms associated with this common endocrine disorder, including those related to sleep.

Daytime sleepiness is reported frequently by women with PCOS even after ruling out sleep apnea [2,21]. In our previous study, which included a large control group (N = 453) from a general randomized sample, women with PCOS reported more frequent daytime sleepiness than the controls (80% vs 27%, respectively) even when controlling for obesity [2]. We previously proposed that IL-6 and TNF-α may be mediators of sleepiness associated with sleep apnea and obesity [7,8,10]. We speculate that the same cytokines may be mediators of sleepiness and fatigue reported by obese women with PCOS when controlling for obesity or sleep apnea.

In this study, in a dose-response manner, obese women with PCOS had the highest IL-6 levels, obese intermediate, and lean controls the lowest. Furthermore, in a regression analysis, as well as the analysis of variance, the primary determinant (in terms of contribution to the total variation and/or statistical significance) of IL-6 levels was PCOS, followed by obesity, suggesting that PCOS is the principal factor leading to elevated plasma IL-6 levels. Given the limitations of the small sample size of this study, studies with a larger group of obese controls are needed. In addition, because of the disparity in sample sizes among the 3 groups, a future study where the sample sizes are more similar would be desirable to confirm these findings.

What factors other than obesity or sleep apnea may be associated with the elevated IL-6 levels observed in obese women with PCOS? Polycystic ovary syndrome is frequently associated with insulin resistance and is considered a human model of this condition [1,6]. In our study, TNF-α and IL-6 were positively associated with indices of insulin resistance even after controlling for BMI. These findings are in fact confirmatory of our earlier study in males, in which we found that visceral fat values obtained by computed tomography scan—an excellent surrogate for degree of insulin resistance—were better determinants of sleep apnea than obesity evaluated by BMI [8]. The correlation between pro-inflammatory cytokines and insulin resistance indices observed in our study may indicate a pathophysiologic link of hypercytokinemia and insulin resistance in obese women with PCOS, which in our patients with PCOS overpowers the effect of obesity. Whether these associations are also applicable in normal-weight women with PCOS should be the focus of future studies.

Our results expand and are consistent with previous studies that reported increased IL-6 and TNF-α levels in normal-weight women with PCOS [11-13]. Two previous studies that failed to show an independent effect in mildly overweight obese women with PCOS on plasma inflammatory cytokines might have been due to the effect of using a heterogeneous control group, which included both obese women referred for treatment of their obesity and lean women [15], or that women with PCOS and controls were not matched for BMI [22]. Furthermore, we should note that in our study, the cytokine differences between PCOS and obese controls might have been attenuated because obese women were older than patients with PCOS.

Similar to previous findings in men [7,10], obese women had higher levels of IL-6 and TNF-α than lean women, and these 2 cytokines were positively related to BMI. These data

suggest that fat tissue is a major source of IL-6 and TNF-α in women too. That the correlation of BMI with cytokines was weaker in the women with PCOS compared with obese controls suggests that other factors such as insulin resistance and/or hormonal differences affected their association in the PCOS group. In addition, that IL-6 levels were surprisingly low in the morbidly obese women with PCOS might be the result of an increased inhibitory effect of estradiol and estrone on IL-6 secretion in these patients [23]. The latter 2 hormones are the respective aromatization products of testosterone and δ-4-androstenedione, both of which are hypersecreted by the ovaries and adrenal cortices of patients with PCOS [24]. The weaker association of IL-6 with BMI in women compared with men may reflect that IL-6 secretion might be influenced by visceral fat [25], which is the predominant fat in obese men. Similar to our previous study [2], the nighttime sleep patterns (with the exception of slow wave sleep that was similar between PCOS and lean controls) of the PCOS group were slightly impaired compared with normal-weight and obese controls. Whether this is related to the neurohormonal abnormalities of PCOS or the presumably higher anxiety and stress experienced by these women remains to be determined. However, this sleep abnormality did not contribute significantly to IL-6 and TNF-α levels.

An important question is whether the reported elevations of plasma cytokines are of any clinical or biologic relevance. Although there is no direct answer to this question from our observational or experimental studies, it should be noted that the increases in plasma levels of IL-6 and TNF-α that we reported in this study are similar with the increases associated with conditions such as obesity [8] or aging [26], which are associated with increased morbidity and mortality. Moreover, elevations or reductions of IL-6 and its surrogate marker C-reactive protein similar to those reported in our studies have been associated either with an increased risk for cardiovascular morbidity and mortality [27-31] or with a cardioprotective function, respectively [32]. Finally, our recent study, in which neutralizing TNF-α peripherally and reducing IL-6 levels in patients with obstructive sleep apnea in a range similar to those observed in our studies was associated with a significant reduction of EDS, provides further support of the potential significance of the reported elevations of peripheral cytokine concentrations [33].

In conclusion, PCOS in obese women is associated with elevation of IL-6 levels independently of obesity or sleep apnea. The associations of IL-6 and TNF-α with insulin resistance/HOMA within the PCOS group suggest that the pro-inflammatory cytokines may be one of the pathways leading to the insulin resistance and cardiovascular disease associated with PCOS. Finally, the results of this study add to the evidence from observational [7,8] and recent interventional studies [33] that the pro-inflammatory cytokines IL-6 and TNF-α are important mechanisms involved in the multifaceted association of sleepiness/fatigue, insulin resistance, and obesity. Thus, the use of neutralizing antibodies of these cytokines may represent a novel treatment of these ailments that are associated with many disorders, including PCOS, and plague an increasing number of people in modern societies.

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