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Differential effects of insulin sensitivity on androgens in obese women with polycystic ovary syndrome or normal ovulation

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Abstract

The differential effects of insulin sensitivity and adiposity on androgen concentrations in women with polycystic ovary syndrome (PCOS) are unclear. To address this issue, we divided 43 overweight women into 4 groups based on both their clinical classification (PCOS or normal) and whether they were insulin resistant (IR) or insulin sensitive (IS) by their steady-state plasma glucose concentrations. Total testosterone concentrations were significantly increased as a function of both clinical classification (PCOS vs normal, $P < .0001$) and steady-state plasma glucose concentration (IR vs IS, $P = .002$). Mean testosterone concentrations were higher in PCOS-IR compared with PCOS-IS, normal-IR, or normal-IS women ($P < .005$). In addition, there was a statistically significant interaction ($P = .03$) between clinical classification (PCOS vs normal) and insulin sensitivity (IR vs IS) for testosterone concentrations. In contrast, androstenedione concentrations were higher in women with PCOS ($P = .001$), irrespective of whether they were IR or IS ($P = .31$); and no interaction between clinical classification and insulin sensitivity was discerned ($P = .34$). These results indicate that both PCOS and insulin resistance independently contributed to increased total testosterone concentrations within a group of overweight/obese women. These findings are consistent with the hypothesis that the ovaries of women with PCOS are hypersensitive to the ability of insulin to increase testosterone production and that the more insulin resistant the patient, the higher the testosterone concentration. In contrast, androstenedione concentrations seem to be independent of differences in insulin resistance. Our findings emphasize the need to increase understanding of the factors that modulate ovarian androgen secretion.

1. Introduction

Women with polycystic ovary syndrome (PCOS) are characterized by increased prevalence of insulin resistance [1-5] as well as obesity [6]. Although excess adiposity increases the likelihood of an individual becoming insulin resistant [7-10], not all obese women are insulin resistant [7-10]; nor do all insulin-resistant women develop PCOS [11]. The complex relationship among obesity, insulin resistance, and the hyperandrogenic state in PCOS is still unclear. For example, differences in the ability of compensatory hyperinsulinemia in insulin-resistant women to produce a hyperandrogenic state have been suggested to play a central role in determining who will or will not develop PCOS [12]. Alternatively, others have emphasized that obesity is responsible for both insulin resistance and hyperandrogenism [13].

The goal of this study was to test that neither insulin resistance nor obesity, by itself, leads to the development of PCOS. To address this issue, we recruited subjects who were similar for body mass index (BMI) in both healthy and PCOS women; and we measured insulin sensitivity directly by insulin-mediated glucose disposal (IMGU) rather than using surrogate measurements of insulin sensitivity such as homeostasis model assessment of insulin resistance or quantitative insulin sensitivity check index.

We created 4 groups that were similar for BMI but with different insulin sensitivity and different disease status. Average plasma concentrations of total testosterone, free testosterone, and androstenedione were compared among these 4 groups. This sampling design helped us to distinguish any associations of clinical diagnosis of PCOS or insulin resistance with androgen metabolism while minimizing the confounding effect of obesity.

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2. Materials and methods

2.1. Subjects

The study sample consisted of 43 women selected from a larger number of individuals who had volunteered in response to local newspaper advertisements describing 2 clinical research studies: one aimed at defining the relationship between obesity, insulin resistance, and cardiovascular disease, whereas the other was focused on the metabolic impact of an insulin-sensitizing drug on insulin-resistant women with PCOS. The women with a normal menstrual history were selected from a group of 57 obese individuals who had volunteered for a weight loss study, whereas the women with PCOS were selected from 42 subjects volunteering for our study evaluating the impact of rosiglitazone in women with PCOS. In both instances, Stanford University Medical Center's Institutional Review Board approved the experimental protocol; and informed consent was obtained before subjects were screened at the Medical Center's General Clinical Research Center. To qualify for enrollment, volunteers had to be in otherwise good health, with a BMI of at least 25.0 kg/m^2, a fasting plasma glucose concentration less than 126 mg/dL, and normal laboratory results on a routine blood and chemical screening panel. *Polycystic ovary syndrome* was defined according to the 1998 National Institute of Child Health and Human Development consensus criteria [14] as having oligomenorrhea or amenorrhea (8 or fewer menses per year, or 45 mean days between bleeding episodes), clinical (Ferryman-Gallway score $\geq$8) and biochemical evidence of hyperandrogenism, and normal serum thyroid-stimulating hormone and prolactin concentrations as determined in the Stanford Hospital Clinical Laboratory.

2.2. Study procedures

Volunteers meeting the above general entry criteria were then admitted to the General Clinical Research Center, where IMGU was quantified by a modification [15] of the insulin suppression test (IST) as described and validated by our research group [16,17]. Briefly, after an overnight fast and before infusion, baseline blood samples were taken; and then subjects were infused for 180 minutes with octreotide (0.27 μg/[m^2·min]), insulin (32 mIU/[m^2·min]), and glucose (267 mg/[m^2·min]). Blood was drawn at 10-minute intervals during the final 30 minutes of the infusion to measure plasma glucose and insulin concentrations, and the mean of these repeated measurements was taken as an estimate of average steady-state plasma glucose (SSPG) concentrations within each individual; a higher SSPG concentration indicated a more insulin-resistant individual.

The result of the IST was used to define an individual as being either insulin resistant (IR; SSPG concentration $\geq$180 mg/dL) or insulin sensitive (IS; SSPG concentration $\leq$100 mg/dL). These values represent the upper and lower tertiles of SSPG concentrations that were measured in 490 healthy volunteers in a previous study [18]. Furthermore, results from 2 prospective studies have shown that dividing a group of apparently healthy individuals into the third with the highest SSPG concentrations (IR) and lowest SSPG concentrations (IS) defines individuals as being either at increased (IR) or decreased (IS) risk to develop adverse consequences related to a loss of insulin sensitivity [19,20].

Volunteers who met the criteria for being either IR or IS were then considered eligible to be included in this analysis; and plasma obtained from the overnight fasting sample from the IST was used to measure concentrations of glucose, insulin, and androgenic hormones . Based upon the results of the IST, we were able to enroll 23 (IR = 11 and IS = 12) women with PCOS and 20 women (IR = 12 and IS = 8) with a normal menstrual history (normal-IS, normal-IR). The ages of the study population ranged from 24 to 40 years, and their BMI values were from 25.5 to 33.7 kg/m^2.

Plasma glucose and insulin concentrations were quantified as described previously [18-20]; and determinations of testosterone (coefficient of variation [CV] = 2.8%-6.8%), free testosterone (CV = 0.22%-7.5%), and androstenedione (CV = 6.6%-10.3%) were made using enzyme-linked immunosorbent assay kits (Diagnostic Systems Laboratories, Webster, TX).

2.3. Statistical methods

Data on each experimental variable are summarized as the mean ± 1 SD. Group means for baseline characteristics (Table 1) were compared using *t* tests. These comparisons were made without any adjustment for multiple comparisons to conserve power for assessing how well the case-control design was achieved. In contrast, a more conservative approach was taken in regard to type I error for analysis of the androgen concentrations to minimize the possibility of false-positive findings on study outcomes. Specifically, overall tests of differences in means among groups were evaluated using 2-way analysis of variance (ANOVA). The 2-way ANOVA model consisted of main effects for disease (PCOS vs normal) and insulin sensitivity

Table 1
Demographic and clinical characteristics (mean ± SD)

	PCOS		Normal	
	IR (n = 11)	IS (n = 12)	IR (n = 12)	IS (n = 8)
SSPG (mg/dL)	232 ± 25^{a}	80 ± 18^{b}	224 ± 33^{a}	76 ± 21^{b}
Age (y)	32 ± 5^{ab}	29 ± 6^{a}	35 ± 3^{c}	34 ± 5^{bc}
BMI (kg/m^2)	28.7 ± 2.3^{abc}	28.4 ± 2.3^{ad}	30.3 ± 2.3^{bde}	30.8 ± 1.8^{ce}
Fasting glucose (mg/dL)	98 ± 8^{ab}	92 ± 8^{ac}	99 ± 10^{bc}	85 ± 3^{d}
Fasting insulin (mIU/L)	18 ± 9^{a}	7 ± 5^{b}	16 ± 7^{a}	7 ± 3^{b}

Within each row of this table, means with different superscripts differ significantly at $P < .05$, whereas means with the same symbol do not differ.

(IR vs IS) as well as their interaction. Where a significant interaction was detected, we tested "simple effects" [21,22] by comparing means between each pair of groups; and we maintained an overall type I error rate of 5% across the multiple simple effect comparisons within each outcome using sequential Bonferroni adjustment [23,24]. Data for androgen concentrations met assumptions for ANOVA, being approximately normally distributed and homoscedastic. Statistical analyses were performed in Statview version 5 (SAS Institute, Cary, NC).

3. Results

3.1. Demographic and clinical characteristics

By selection, SSPG concentrations were approximately 3-fold higher in the PCOS-IR and normal-IR as compared with the PCOS-IS and normal-IS groups (Table 1). The SSPG concentrations were higher to the same degree in the PCOS-IR and normal-IR groups, and SSPG concentrations in the PCOS-IS and normal-IS groups were also identical. The PCOS-IS subjects were younger, and the BMI were lower in both PCOS groups. Finally, consistent with the differences in SSPG concentrations, fasting plasma glucose and insulin concentration were significantly higher in the IR groups.

3.2. Androgen concentrations

The data in Fig. 1A demonstrate that total testosterone concentrations were significantly increased (2.06 ± 1.3 vs 083 ± 0.42 ng/mL, $P > .001$) when the values in the 2 PCOS groups (PCOS-IR + PCOS-IS) were compared with those in the 2 normal groups (normal IR + normal IS). Total testosterone concentrations were also higher in the IR individuals, irrespective of clinical diagnosis ($P < .002$). In addition, the interaction between disease state (PCOS vs normal) and insulin sensitivity (IR vs IS) was statistically significant ($P = .03$). This interaction arose because the difference between IR and IS was much stronger in PCOS (difference in means = 1.46 ng/mL, standard error = 0.35 ng/mL) than in normal (difference in means = 0.28 ng/mL, standard error = 0.39 ng/mL). Analysis of simple effects reveals that mean total testosterone concentrations were higher in PCOS-IR than in any other group: PCOS-IR vs normal-IR (difference in means = 1.88 ng/mL, standard error = 0.35 ng/mL, $P = .0004$), PCOS-IR vs PCOS-IS (difference in means = 1.46 ng/mL, standard error = 0.35 ng/mL, $P = .0042$), and PCOS-IR vs normal-IS (difference in means = 2.16 ng/mL, standard error = 0.39 ng/mL, $P = .0007$). In addition, mean testosterone concentrations between normal groups were not significantly different.

The results in Fig. 1B indicate that free testosterone concentrations were also significantly higher ($P < .005$) in the 2 groups with PCOS as compared with the combined IR-normal and IS-normal groups (4.3 ± 1.5 vs 2.8 ± 1.0). Total testosterone and free testosterone concentrations were also increased as a function of being IR ($P < .05$). However, in contrast to total testosterone measurements, there was no statistically significant interaction between disease state and insulin sensitivity ($P = .56$).

In contrast to the changes in total testosterone and free testosterone concentrations, the results in Fig. 1C demonstrate that androstenedione concentrations were modulated only by disease status. Thus, androstenedione concentrations were significantly higher in those with PCOS ($P = .001$), irrespective of whether they were IR or IS ($P = .31$); and no interaction between disease state and insulin sensitivity was discerned ($P = .34$).

4. Discussion

The most important pathophysiologic finding in this study was the identification of significant interaction between disease state (PCOS vs normal) and insulin sensitivity (IR vs IS) in the modulation of total plasma testosterone concentration. This finding is consistent with the fact that the highest total testosterone concentrations were seen in the PCOS-IR group and the lowest levels were seen in the normal-IS individuals. The significant interaction between the presence of PCOS and insulin resistance is consistent with the notion of ovarian hypersensitivity to insulin stimulation of testosterone production in women with PCOS as recently proposed by Baillargeon and Nestler [23]. Their suggested formulation provides an explanation for the fact that total and free testosterone concentrations were increased in the PCOS-IS patients in this study, as well as the demonstration by Baillargeon and Carpentier [24] that androgen concentrations fall when lean, normoinsulinemic, insulin-sensitive individuals with PCOS are given diazoxide, an agent that decreases insulin secretion. Implicit in these notions is that insulin stimulates ovarian androgen secretion and that this can be shown to occur in vitro using ovarian androgen tissue from women with PCOS [25-28]. Our findings clearly do not provide additional information regarding direct ovarian responses to insulin or androgen signaling, but the notion of ovarian hypersensitivity to insulin stimulation in women with PCOS provides a reasonable explanation for why the present study finds interaction between insulin resistance/hyperinsulinemia and the presence of PCOS in modulation of plasma testosterone concentrations.

The results in Fig. 1B indicate that free testosterone concentrations were also higher, as a function of both disease state (PCOS vs normal) and insulin sensitivity (IR vs IS); but in this instance, we could not discern a significant degree of interaction between disease state and insulin sensitivity. Thus, although these results are similar to the total testosterone findings in terms of the significant effects on androgen concentrations of disease state and insulin sensitivity, they differ in the lack of evidence of significant interaction. The reason for this latter discrepancy

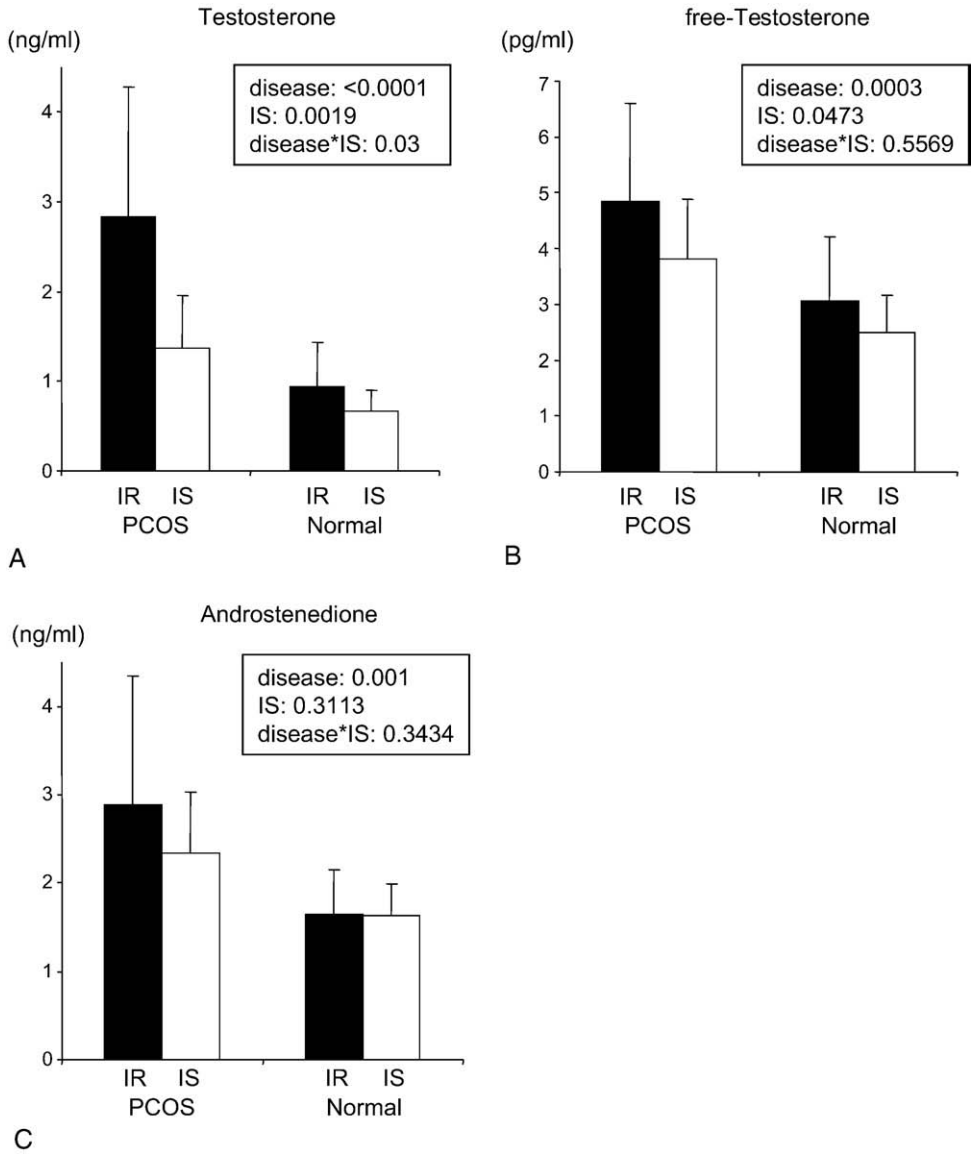

Fig. 1. Androgen concentrations. A, Plasma testosterone concentrations. B, Plasma free testosterone concentrations. C, Plasma androstenedione concentrations. PCOS-IR, n = 11; PCOS-IS, n = 12; normal-IR, n = 12; and normal-IS, n = 8. Disease: comparison between PCOS and normally ovulating women; IS: comparison between insulin-sensitive and insulin-resistant women; disease∗IS: interaction between disease state (PCOS vs normal) and insulin sensitivity. The statistical significance was calculated by 2-way ANOVA.

is not clear, but might result from something as simple as our use of a free testosterone assay that does not have the precision required to discern interaction between disease and insulin sensitivity [29].

It should be noted that total and free testosterone concentrations were higher in PCOS-IS women than in either of the normal groups and that despite being considered to be overweight/obese, with a mean BMI of 28.4 kg/m^2, these patients remained quite insulin sensitive (mean SSPG concentration = 80 mg/dL). Thus, in contrast to some published reports [13], the results of this study demonstrate that obese women with PCOS can also be insulin sensitive and, as a corollary, that normal insulin sensitivity in PCOS is not confined to nonobese women. As such, our results reinforce previous findings that obese women, in general, can be insulin sensitive, with low levels of insulin [7-9].

In contrast to the impact of differences in insulin sensitivity on testosterone and free testosterone concentrations, androstenedione concentrations, as shown in Fig. 1C, only varied whether or not subjects had PCOS. Thus, androstenedione concentrations were significantly higher in PCOS-IR and PCOS-IS than in normal-IR and normal-IS (P = .001), but were comparable within each disease group. These data are consistent with in vitro studies showing that luteinizing hormone (LH)-stimulated androstenedione secretion from cultured theca cells obtained from women with PCOS is enhanced as compared with cells from normal individuals [25,28]. However, it is not clear if insulin

stimulation of androstenedione is greater in ovaries obtained from women with PCOS [28,30]. Similarly, there is disparity between our finding that androstenedione concentrations did not differ between PCOS-IR and PCOS-IS women, despite the marked difference in insulin resistance and insulin concentration, and the observation by Baillargeon and Carpentier [24] that androstenedione concentrations fell in insulin-sensitive women with PCOS in association with diazoxide inhibition of insulin secretion. The explanation for this disparity concerning the relationship between insulin and androstenedione concentrations is not readily apparent. In fact, the percentage of change in androstenedione concentrations after diazoxide in the 9 patients studied by Baillargeon and Carpentier [24] was comparable with the difference we saw between PCOS-IR (n = 11) and PCOS-IS (n = 12) women. The fact that our comparison was cross-sectional and theirs was before and after the intervention in the same subject may explain why they perceived a statistically significant difference and we did not. In any event, this difference should not obscure the fact that we both came to the conclusion that there is an interaction between the metabolic effects of insulin on ovarian androgen secretion and the presence of PCOS.

A major strength of the results of this study is that they are based on the use of a specific method to quantify IMGU, rather than such surrogate estimates as fasting plasma insulin concentration, plasma glucose × plasma insulin concentration, homeostasis model assessment of insulin resistance, quantitative insulin sensitivity check index, etc [31,32]. Although these surrogate estimates of insulin action are correlated to specific measures of IMGU, the relationships are relatively modest in magnitude, with correlation coefficients of ~0.6 [31,32], thereby accounting for no more that 36% of the actual variability in IMGU in the population studied. In contrast, the IST used in this study and the hyperinsulinemic, euglycemic clamp technique, often referred to as the *criterion standard* for quantifying IMGU, are highly correlated ($r > 0.9$) in normal women and patients with type 2 diabetes mellitus, both normal and obese [17]. Thus, the method used to separate the subjects in this study on the basis of their degree of insulin sensitivity is quite precise, an advantage that is further accentuated by enrolling only the most insulin-resistant and insulin-sensitive individuals, excluding those intermediate in terms of IMGU.

On the other hand, quantities of subjects in each of the 4 groups are relatively small, which may limit the ability to generalize from our results. However, our precise division of participants as to degree of insulin sensitivity, while also carefully selecting for a comparable degree of obesity in all 4 groups, increases the chances that our results can be confirmed in larger studies. Despite these shortcomings, we believe that the results presented provide useful information concerning the relationship between obesity, insulin resistance, and PCOS.

In conclusion, our results suggest that obesity, insulin resistance, and compensatory hyperinsulinemia are neither necessary nor sufficient for the development of PCOS. Thus, women who are extremely insulin resistant/hyperinsulinemic can have a normal menstrual history. Furthermore, the diagnosis of PCOS in the presence of normal insulin sensitivity is not confined to nonobese women; and some patients with PCOS, despite being overweight/obese, remain very insulin sensitive with low plasma insulin concentrations (PCOS-IS). The simplest interpretation of these data is that the ovaries of women with PCOS are hypersensitive to the ability of insulin to increase testosterone production and that the more insulin resistant/hyperinsulinemic an individual, the higher will be the total testosterone and free testosterone concentrations. The ovaries of women with PCOS also seem to be hyperresponsive to LH stimulation of androstenedione secretion, but this abnormality seems to be independent of differences in insulin resistance/hyperinsulinemia. These results emphasize the need to increase understanding of the factors that modulate ovarian androgen secretion.

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