ACTH stimulation test in lean polycystic ovary syndrome patients with insulin resistance

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Objective: To determine whether insulin resistance in nonobese polycystic ovarian syndrome (PCOS) patients is associated with a higher response to ACTH stimulation.
Design: Controlled prospective study.
Setting: University hospital ambulatory care unit.
Patient(s): Twenty nonobese PCOS syndrome patients with insulin resistance and a body mass index–matched control group of 20 nonobese PCOS patients without insulin resistance.
Intervention(s): A 75-g oral glucose tolerance test and an ACTH stimulation test.
Main Outcome Measure(s): Blood insulin and glucose levels after ingestion of 75 g oral glucose; blood androstenedione (A), 17(OH)-progesterone [17(OH)-P], free and total T, DHEAS, and progesterone (P) response to ACTH stimulation.
Result(s): The proportional increases in the blood levels of total and free T, DHEAS, A, 17(OH)-P, and P following ACTH stimulation were similar in the two groups. Only the insulin area under the curve (AUC) values were significantly different among the two groups. The insulin AUC values were positively correlated to the free T blood levels and the homeostasis model assessment scores.
Conclusion(s): Within the population of nonobese PCOS patients, insulin resistance is not associated with a more pronounced response to ACTH stimulation. The pathogenesis and management of PCOS in nonobese patients may be different from in obese patients. (Fertil Steril® 2007;88:670–4. ©2007 by American Society for Reproductive Medicine.)

Key Words: PCOS, nonobese PCOS patients, hyperinsulinemia, insulin resistance, ACTH test

Polycystic ovarian syndrome (PCOS) defined by the Rotterdam Criteria not only comprises 80%–90% of infertility causes due to ovulatory dysfunction (World Health Organization Classification II), but also is associated with long-term morbidity, including type II diabetes mellitus, dyslipidemia, hypertension, and coronary artery disease (1). Despite the presence of a definitive framework formed by a set of criteria to define the syndrome, its limits are still controversial (2). Insulin resistance, obesity, and adrenal androgenemia are factors associated with PCOS. Obesity and insulin resistance are common among PCOS patients; they have been shown to be potentiators of ovulatory dysfunction, clinically and/or biochemically evident hyperandrogenemia, and the metabolic syndrome (3, 4).

Adrenal androgenemia is defined in 20%–30% of PCOS patients and is proposed to play an important role in its pathogenesis (5): the sequence of an early and exaggerated pubarche, insulin resistance, and a history of low birth weight followed by catch-up growth in the infant period have been shown to be strong predictors of the appearance of the PCOS phenotype after puberty (6). Insulin sensitizer treatment at this earlier period has been shown to slow the progression of the condition to PCOS; however, following the cessation of medical treatment, this favorable effect had disappeared (7). Adrenal androgenesis has been more thoroughly studied in PCOS adults. It has been shown to be more responsive to ACTH stimulation if insulin resistance is present. Insulin sensitizing treatment has diminished this effect. Interestingly, the effect of insulin resistance on adrenal response to ACTH stimulation is mostly studied in overweight and obese populations. About 30%–60% of PCOS patients are obese. In the presence of obesity, menstrual irregularity, hyperandrogenemia, and the metabolic syndrome are more common in PCOS patients (3). In the present study, the effect of insulin resistance on the adrenal response to ACTH stimulation was studied in a nonobese group of PCOS patients—to our knowledge, for the first time.

PATIENTS AND METHODS

Forty PCOS patients who applied to the ambulatory care unit of Uludag University Obstetrics and Gynecology department diagnosed as PCOS according to the Rotterdam 2003

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criteria and with body mass index (BMI) equal to or lower than 28 kg/m^2 were included in this study 20 patients with homeostasis model assessment (HOMA) scores higher than 3.77 (group A) and 20 patients with HOMA scores less than or equal to 3.77 (group B) BMI matched with the study group. The two groups were similar in all aspects. Ferriman-Gallwey scoring system was used to define hirsutism (score >8) (8). Informed consent was obtained from all of the patients, and the study was approved by the Research Ethics Commitee. Adrenal tumors, nonclassic adrenal hyperplasia, hyperprolactinemia (or prolactinoma), diabetes mellitus, or thyroid dysfunction were eventually ruled out.

In this prospective descriptive study, the ACTH test response and 75 g oral glucose tolerance test (OGTT) results of the women were analyzed. After the initial physical exam, vaginal sonography was performed to rule out other pelvic pathologies. For those women sexually active and with amenorrhea for longer than 28 days, urine hCG tests were performed to rule out pregnancy. After the oral ingestion of 75 g glucose, blood samples were taken at 0, 30, 60, 90, and 120 minutes.

Blood glucose levels and insulin levels were measured as well. Glucose levels were measured with the glucose-oxidase method.

All hormones were measured with commercial available radioimmunoassay kits. Homeostasis model assessment of insulin resistance (HOMA-IR) was used to assess insulin sensitivity [fasting insulin (μU/mL) $\times$ fasting glucose (mmol/L)/22.5] (normal value <3.77). The HOMA test has been shown to correlate, with glucose clamp technique, with insulin resistance but not with insulin secretion by Phillips et al. (9). In the present study, however, the values of the area under curve (AUC) for insulin during the OGTT, which is a reliable expression for insulin secretion, were significantly different among the two groups. According to Mohlig et al. (10), HOMA score or fasting insulin measurement has been shown to be the best assessment method for metabolic screening of PCOS women.

The ACTH stimulation test was conducted after obtaining blood for total and free T, DHEA-S, androstenedione (A), 17(OH)-progesterone [17(OH)-P] and progesterone (P) by IV administration of ACTH (1 mg Synacthen). Blood sampling was made 1 hour later to measure the blood levels of these same hormones to quantify the response to ACTH stimulation. The AUCs for blood insulin levels during the 75 g OGTT were calculated using the trapezoid rule. Statistical analysis of the data was conducted with the SPSS 11.0 statistical package (SPSS, Chicago, IL). Comparisons of the groups were conducted using the Student *t* test where normal distribution was observed; otherwise nonparametric tests were performed.

RESULTS

There were 40 patients fulfilling the criteria for inclusion in the study. All of these cases had polycystic ovaries observed with vaginal sonography. Seven of the patients (17.5%) were infertile; 35 (87.5%) were hirsute according to the Ferriman-Gallwey scoring system, 14 (35%) had regular cycles, 25 (62.5%) were oligomenorrheic, 1 (2.5%) was hypermenorrheic, and 4 (10%) were galactorrheic.

TABLE 1

PCOS phenotypes of the patients in the two groups according to the Rotterdam Criteria (2003).

Phenotype	Group A, n (%)	Group B, n (%)
a	10 (50)	10 (50)
b	0 (0)	0 (0)
c	3 (15)	2 (10)
d	7 (35)	8 (40)

Note: Group A: HOMA score >3.77; group B: HOMA score $\leq$3.77. Phenotype a: ovulatory dysfunction, hirsutism and/or hyperandrogenemia, polycystic ovaries; b: ovulatory dysfunction, hirsutism and/or hyperandrogenemia; c: ovulatory dysfunction, polycystic ovaries; d: hirsutism and/or hyperandrogenemia, polycystic ovaries. HOMA = homeostasis model assessment of insulin resistance.

Uncu. ACTH test in lean PCOS patients with ınsulin resistance. Fertil Steril 2007.

The PCOS phenotypes of individuals in the two groups were as in Table 1. Twenty-three of the patients (57.5%) had elevated adrenal androgen blood levels. The patient groups were formed according to the HOMA scores. For 20 patients (50%), the HOMA score was over 3.77, and for 20 (50%) the score was equal to or less than 3.77. Only two patients (one from each group) also showed impaired glucose tolerance. The groups were compared with reference to the response of blood androgen levels to ACTH stimulation. This comparison was made in terms of the proportional increase in the blood levels of these hormones (Table 2). The proportional increases in the blood levels of total and free T, DHEAS, A, and 17(OH)-P after ACTH stimulation were similar in the two groups. Only the AUC values for insulin were different between the two groups. Only the insulin AUC values were, among all the hormone values, positively correlated to the free T blood levels. The insulin AUC values did not show any correlation with the androgen responses to ACTH stimulation and were significantly correlated to the HOMA scores.

DISCUSSION

Diagnosis of PCOS requires that two of the three following criteria be fulfilled: chronic anovulation, clinical and/or biochemical evidence of hyperandrogenism, and visualization of polycystic ovaries (11). Obesity, insulin resistance, infertility, or menstrual irregularities may accompany the clinical picture. The excessive androgen load in PCOS patients originates from the ovary and the adrenal glands.

The adrenal androgens include DHEAS and A, which have been reported to be elevated in 25%–60% of PCOS

TABLE 2

Patient characteristics.

	HOMA test, mean ± SD	
	Group A	**Group B**
Age (yrs)	21.3 ± 4.3	26.2 ± 5.8
Age of menarche (yrs)	13.4 ± 1.6	13.3 ± 1.3
Body mass index (kg/m^2)	23.7 ± 3.1	23.9 ± 3
LH/FSH	3.2 + 2.6	2.3 ± 1.6
Blood E_2 (pg/mL)	90.7 ± 7.8	85 ± 20.2
P (ng/mL)	2.9 ± 3.8	1.2 ± 1.1
Total T (ng/mL)	108.8 ± 5.7	123.1 ± 7.1
Free T (ng/mL)	4.2 ± 9.8	3.7 ± 2.6
DHEAS (ng/mL)	310.7 ± 32.4	233 ± 31.3
A (ng/mL)	1.5 ± 0.8	3.37 ± 2.3
17(OH)-P (ng/mL)	2.1 ± 1.2	3.1 ± 0.5
Factorial change in 17(OH)-P[a]	2.5 ± 1.2	3 ± 1.1
Factorial change in P[a]	2.3 ± 0.7	1.9 ± 0.9
Factorial change in DHEAS[a]	1.3 ± 0.3	1.39 ± 0.56
Factorial change in T[a]	1.3 ± 0.6	1.4 ± 0.8
Factorial change in free T[a]	1.3 ± 0.8	1.2 ± 0.2
Insulin (area under the curve)[b,c]	6,818.9 ± 5,516.6	16,427 ± 315.1

Note: Group A: HOMA score >3.77; group B: HOMA score ≤3.77.
[a] Blood hormone level 1 h after ACTH stimulaton/blood hormone level just before ACTH stimulation).
[b] Insulin blood levels in 75 g oral glucose tolerance test calculated with the trapezoid method.
[c] $P < .05$.

Uncu. ACTH test in lean PCOS patients with ınsulin resistance. Fertil Steril 2007.

patients (5). A further pathogenetic role has been suggested for adrenal androgenesis by Ibanez et al. (6, 7) showed in successive studies that low birth weight followed by catch-up growth in the infant period, premature pubarche, insulin resistance, oligo-(an)ovulation, and polycystic ovarian morphology form a sequence which progresses later to PCOS and that this sequence can be interfered with by insulin sensitizers although the favorable effect is limited only to the medical treatment interval. Later in the adult life, the blood levels of the adrenal androgens are age dependent and decline with time after the second decade of life in normal individuals. In PCOS individuals with hyperandrogenemia, adrenal androgens are more stable in the long run, owing most probably to the persistence of the reticularis zone (12).

A BMI greater than 30 kg/m^2 is found in 35%–50% of PCOS patients. Obese patients are more likely to be menstrually irregular, infertile, and hirsute (13). Obesity is also the most important risk factor for insulin resistance with relative risks up to 40 (14). Insulin resistance is common in PCOS (approximately 30%–35%), yet the mechanisms are not yet clear (15). Insulin acts synergistically with LH and insulin-like growth factors to cause increased androgen production from the theca cells and the adrenal gland. During adolescence, ACTH and LH have trophic effects on the adrenal gland both directly and indirectly through insulin-like growth factors (16).

The interaction of insulin resistance and ovarian androgen excess is well defined. The ovarian androgen load can be treated with weight loss and insulin sensitizers. Similarly, the adrenal compartment has been suggested to be treatable as well with insulin sensitizers by some authors (17, 18), although the relation between the adrenal androgens and insulin resistance is still not well defined. Some studies have reported a positive correlation between adrenal response to ACTH with hyperinsulinemia, which may be induced with glucose administration or insulin infusion, detected at the fasting state, or attenuated with insulin sensitizers (19–21). Some other studies have not found any difference in the response to ACTH stimulation in the presence of or after the treatment of hyperinsulinemia (18, 22–24).

Silfen et al., Ibanez et al., and Nestler et al. (7, 25–27) have previously put forward that the metabolic and endocrinologic characteristics and the response to insulin sensitizers of lean PCOS patients may be different from those of obese patients. Guven et al. have suggested that obese girls with premature adrenarche had higher blood levels of leptin and 17(OH)-P as well as higher ACTH-stimulated 17(OH)-P than lean girls (28). To our knowledge, the present study is the first to examine the influence of insulin resistance on the response to ACTH stimulation within the population of nonobese PCOS patients.

The absence of a positive influence of insulin resistance on the response to ACTH stimulation, as we concluded, is in accordance with some similar studies conducted on PCOS populations which comprised mostly nonobese individuals (18, 29). Common to other studies that dispute our findings is that they almost all comprised insulin resistant/hyperinsulinemic or hyperandrogenemic patients with high mean BMIs (30, 31).

How could we explain a potential difference between the adrenal steroidogenesis of lean and obese PCOS patients? Gambineri et al. (32) recently reported that genetic variation in 11β-HSD1 contributed to enhanced cortisol clearence and compensatory adrenal hyperandrogenism in lean patients with PCOS but may be protective against obesity and some other metabolic features, including hyperinsulinemia. Those findings were in sharp contrast with findings reported by San Millan et al. (33), who stated that no association was present between 11β-HSD1 genotype and PCOS within an obese patient population. In both of those earlier studies, the activity of the 11β-HSD1 variant, similar to what we have observed in our study group, was not related to insulin resistance as detected at the fasting state. The expression of variations in enzyme systems may be dependent on geographic or ethnic variations as well as how insulin resistance is measured. For instance, in an earlier study conducted on a large population of Pima Indians, genetic polymorphisms in the 11β-HSD1 gene were found to be correlated to insulin sensitivity as measured with a euglycemic hyperinsulinemic clamp instead of other modalities (34).

Another issue that may be causing dispute is how we measure the response to ACTH. Some studies express factorial changes in the precursor concentrations (as we have), some express parametric changes in concentrations, and some measure enzymatic influence with the (product/precursor) quotient (35, 36). The contribution of insulin resistance to the pathogenesis of PCOS may differ among patients with various body mass profiles and therefore with various metabolic characteristics, such as the activity of adiponectin or leptins (37).

In conclusion, the pathogenesis and management of nonobese PCOS patients may be different from those of obese PCOS patients. The final clinical picture of PCOS may develop through different routes in patients from different geographic locations or ethnic populations. Differential analysis of the population characteristics of PCOS should be reanalyzed with respect to these factors.

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