CLINICAL STUDY

A comparison between the effects of low dose (1 µg) and standard dose (250 µg) ACTH stimulation tests on adrenal P450c17α enzyme activity in women with polycystic ovary syndrome

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Abstract

Objective: Numerous studies have found elevated androgen production by the adrenal glands in patients with polycystic ovary syndrome (PCOS). However, the role and the mechanisms responsible for the adrenal androgen excess in women with PCOS are not well understood.

Design: Our aim was to compare 17-hydroxyprogesterone (17-OHP), androstenedione, dehydroepiandrosterone sulfate (DHEAS) and cortisol responses to a low dose (1 µg) ACTH stimulation test (LDT) with the responses to a standard dose (250 µg) ACTH stimulation test (SDT) in patients with PCOS.

Methods: Fifty women with PCOS (mean age 25.4±0.7 years) and 20 healthy women (mean age 27.3±2.2 years) were included in the study. The patients and controls underwent ACTH stimulation tests with 1 µg and 250 µg synthetic ACTH in the follicular phase of their cycles. Venous blood was drawn at 0, 30 and 60 min for determination of serum cortisol, 17-OHP, androstenedione and DHEAS levels.

Results: In PCOS subjects, peak and area under the curve (AUC) 17-OHP (9.3±0.3 nmol/l, 378.4±61 nmol/l×60 min), androstenedione (15.6±0.6 nmol/l, 806.4±52 nmol/l×60 min) and DHEAS (7.5±0.4 µmol/l, 385.6±25.5 µmol/l×60 min) responses to SDT were significantly higher than the levels in healthy women (respectively 5.7±0.3 nmol/l and 249.4±52.2 nmol/l×60 min for 17-OHP; 9.1±0.3 nmol/l and 413.7±31.6 nmol/l×60 min for androstenedione; 4.3±0.4 µmol/l and 224.9±24.5 µmol/l×60 min for DHEAS) ($P < 0.05$). Peak and AUC cortisol responses to SDT were similar in PCOS and control subjects. Peak and AUC cortisol and 17-OHP responses to LDT in women with PCOS were similar to the values obtained in healthy women. Peak androstenedione (12.5±0.6 nmol/l) and peak (6.5±0.5 nmol/l) and AUC (336.3±22.4 µmol/l×60 min) DHEAS responses to LDT were significantly higher in women with PCOS.

Conclusions: These results show that LDT is capable of revealing the adrenal hyperactivity in women with PCOS. Adrenal P450c17α enzyme dysregulation in PCOS is revealed by ACTH stimulation at a pharmacological dose (250 µg) but not by a physiological dose (1 µg). LDT is able to demonstrate adrenal hyperactivity characterized by an increase in DHEAS levels.

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Introduction

Polycystic ovary syndrome (PCOS) is a common and heterogeneous disease which is characterized by menstrual abnormalities, hirsutism and obesity and it affects approximately six percent of women of reproductive age (1). Androgen excess is central to the pathophysiological changes and clinical expression of PCOS (2). PCOS is thought to be caused by increased activity of steroidogenesis through 17 hydroxylation and/or increased but relatively inefficient activity of 17–20 lyase. Both 17 hydroxylation and 17–20

lyase activity arise from the action of the same enzyme, cytochrome P450c17α (3, 4). Cytochrome P450c17α is the enzyme that is important for both ovarian and adrenal steroidogenesis. This enzyme is encoded by a single gene on chromosome 10 and is expressed in both the adrenal gland and ovarian theca cells (3).

Although the ovary is the principal source of androgen excess in women with PCOS, about 40–70% of patients also demonstrate elevated levels of adrenal androgens and the adrenal glands may play a role in the genesis of PCOS (5, 6). However, the factors and

the mechanisms that cause the excess adrenal androgen levels in a disorder considered primarily due to altered ovarian steroidogenesis are not very clear.

Adrenal stimulation with adrenocorticotropin (ACTH) has been the principal challenge test for estimating the relative activity of adrenocortical enzymes (7). However, during the last years it has been reported that the 250 µg ACTH stimulation test is a supra-physiological test and it may cause misdiagnosis in secondary and primary adrenal failure (8–11). The sensitivity of the low dose (1 µg) test (LDT) is higher than that of the standard dose (250 µg) test (SDT) in the assessment of the hypothalamo–pituitary–adrenal (HPA) axis, particularly in pituitary disease (12, 13). There are not enough data evaluating adrenal steroidogenesis, particularly P450c17 α enzyme activity, with LDT in women with PCOS. Our aim was to investigate the activity of adrenal cytochrome P450c17 α enzyme activity and adrenal androgen responses to the LDT to simulate normal circulating levels of ACTH and to compare the results obtained with those from the SDT in women with PCOS.

Patients and methods

Patients

The study was approved by the Ethical Committee of Erciyes University Medical School, and informed consent was obtained from each woman. Fifty women with PCOS (aged 16–40 years, mean age 25.4 ± 0.7 years) and 20 healthy women (aged 17–40 years, mean age 27.3 ± 2.2 years) were included in the study. The diagnosis of PCOS was made according to National Institute of Health/NICHHD conference criteria (14). All PCOS subjects were hyperandrogenemic: serum free testosterone > 11.1 pmol/l, (normal range 2.8–11.1) and the free androgen index (FAI = $100 \times$

testosterone/sex hormone binding globulin) was higher than the highest value (5.1) of the control subjects. Mean FAI values of the patients and control subjects are shown in Table 1. Although it was not an inclusion criteria, all women had polycystic ovarian morphology (the presence of 10 or more cysts, 2–10 mm in diameter, arranged around a dense stroma or scattered through an increased amount of stroma). Pelvic ultrasound examinations were performed by the same investigator (Y S) using a 3.5 MHz abdominal transducer and 6.5 MHz vaginal endoprobe (Hitachi EMB 450, Japan). Hirsutism was the main complaint of the women with PCOS and the modified Ferriman–Gallwey score (15) was higher than 8 in all subjects. All the patients had had spontaneous puberty onset and normal sexual development. Cushing's syndrome, hyperprolactinemia, androgen secreting tumors and non-classical congenital adrenal hyperplasia (16) were excluded by appropriate tests. None of the patients had any other disease nor had they received any medication for at least one year before the study.

Forty-five (90%) women were oligomenorrheic (intermenstrual interval > 35 days) and 5 (10%) were amenorrheic (no menstrual period for > 6 months). All the patients were studied in the follicular phase of their cycles or in the event that the patient was amenorrheic when serum progesterone concentration was < 8.0 nmol/l.

After an overnight fast, blood samples were obtained for measurement of basal hormone levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, 17-hydroxyprogesterone (17-OHP), cortisol, androstenedione, free testosterone, dehydroepiandrosterone sulfate (DHEAS) and sex hormone binding globulin (SHBG). After the basal hormone levels were obtained, ACTH stimulation tests were performed.

Methods

LDT and SDT ACTH stimulation tests were performed by using Tetracosactrin (1–24) (Synacthen 0.25 mg, Novartis Pharma, Nürnberg, Germany). For LDT, Tetracosactrin (1–24) was kept in 50 ml 0.9% NaCl at 4 °C, for a maximum period of four months and 1 µg ACTH was prepared in 0.2 ml of this solution (17).

The LDT and SDT were performed by administration of a single i.v. bolus of 1 µg or 250 µg synthetic ACTH at 0800 h. Venous blood was drawn through an indwelling catheter at 0, 30 and 60 min for the determination of serum 17-OHP, androstenedione, cortisol, and DHEAS levels. LDT and SDT were performed with a three-day interval between the tests, and the hormonal responses to each test were also expressed as area under the curve (AUC) estimated by the trapezoidal rule including the basal value.

Serum cortisol (Immunotech, Marseille, France), androstenedione (DOC, Los Angeles, CA, USA), free

Table 1 Clinical and basal hormonal characteristics of PCOS and control subjects.

	PCOS (n = 50)	Control (n = 20)	P value
Age (year)	25.4 ± 0.7	27.3 ± 2.2	NS
BMI (kg/m ²)	29.3 ± 1.4	28.1 ± 0.9	NS
Ferriman–Gallwey score	16.5 ± 1.2	2.5 ± 0.1	< 0.0001
FAI	13.0 ± 1.5	4.3 ± 0.2	< 0.001
LH (IU/l)	8.2 ± 1.1	3.4 ± 0.8	< 0.05
FSH (IU/l)	4.6 ± 0.5	4.0 ± 0.3	NS
LH/FSH	1.7 ± 0.3	0.85 ± 0.1	< 0.05
Free testosterone (nmol/l)	16.7 ± 1.2	6.8 ± 0.3	< 0.0001
Androstenedione (nmol/l)	10.4 ± 0.8	7.4 ± 0.5	< 0.05
DHEAS (µmol/l)	5.7 ± 0.6	4.0 ± 0.3	NS
Cortisol (nmol/l)	440.7 ± 22.1	542.1 ± 48.3	NS
17-OHP (nmol/l)	3.3 ± 0.3	2.0 ± 0.3	NS
SHBG (nmol/l)	35.1 ± 2.3	68.1 ± 4.3	< 0.05

NS, not significant.

Table 2 Cortisol, 17-OHP, androstenedione and DHEAS responses to LDT in PCOS and control women.

	PCOS (n = 50)	Control (n = 20)	P value
Peak cortisol (nmol/l)	772.7±33.0	825±68.7	NS
AUC cortisol (nmol/l × 60 min)	36 680.4±1573.2	38 010.7±1851.9	NS
Peak 17-OHP (nmol/l)	4.8±1.6	5.1±0.6	NS
AUC 17-OHP (nmol/l × 60 min)	233.7±48.9	236.4±32.0	NS
Peak androstenedione (nmol/l)	12.5±0.6	11.2±0.6	< 0.05
AUC androstenedione (nmol/l × 60 min)	661.3±52.3	541.6±41.8	NS
Peak DHEAS (μmol/l)	6.5±0.5	4.4±0.7	< 0.05
AUC DHEAS (μmol/l × 60 min)	336.3±22.4	232.2±36.6	< 0.05

NS, not significant.

testosterone (DSL, Webster, Texas, USA), total testosterone (DSL), 17-OHP and DHEAS (ICN, Costa Mesa, CA, USA) were measured by RIA, and FSH, LH (DSL) and SHBG (Radim, Roma, Italy) were measured by IRMA, using commercial kits. The intra-assay and interassay coefficients of variation were 3.6/7.9% for FSH, 6.8/7.3% for LH, 3.7/7.9% for free testosterone, 9.6/8.6% for total testosterone, 3.7/4.5% for androstenedione, 9/9.5% for DHEAS, 7.8/9.8% for 17-OHP, 4.6/5.1% for SHBG and 9%/10.3% for cortisol. Samples were stored at -20 °C until assay.

Data analysis

The results are reported as means±S.E.M. The results of LDT and SDT were compared by using the paired *t*-test within the PCOS and control subjects and the unpaired *t*-test was used for the comparison of LDT or SDT in the PCOS group versus control subjects. A *P* value of <0.05 was regarded as statistically significant.

Results

PCOS women and control subjects did not differ in mean age and body mass index (BMI). Women with PCOS had higher serum androgen levels, higher FAI values and lower SHBG levels than control subjects. Some of the clinical and basal hormonal characteristics of PCOS and control subjects are shown in Table 1.

Peak and AUC 17-OHP responses to LDT were not significantly different in PCOS and control women (Table 2). In PCOS women, peak 17-OHP and AUC 17-OHP responses to SDT were significantly (*P* < 0.05) higher than in control subjects (Table 3). Peak 17-OHP responses to SDT (9.3±0.3 nmol/l) were significantly (*P* < 0.05) higher in women with PCOS than the responses to LDT (4.8±1.6 nmol/l). In the control group, peak 17-OHP responses to LDT (5.1±0.6 nmol/l) and SDT (5.7±0.3 nmol/l) were not significantly different.

Peak but not AUC androstenedione responses to LDT were significantly different in PCOS and control women (Table 2). Peak and AUC androstenedione responses to SDT were significantly (*P* < 0.05) higher in women with PCOS than in control subjects (Table 3).

We have also determined the cortisol and DHEAS responses to LDT and SDT in both PCOS and control subjects. There were no significant differences in peak and AUC cortisol responses to LDT (Table 2) and SDT (Table 3) between PCOS and control subjects. Peak and AUC DHEAS responses to LDT (Table 2) and SDT (Table 3) were significantly (*P* < 0.05) higher in PCOS subjects.

Discussion

PCOS is the most common endocrine disorder among premenopausal women and the pathogenesis of the disease is not well known. A number of studies have found

Table 3 Cortisol, 17-OHP, androstenedione and DHEAS responses to SDT in PCOS and control women.

	PCOS (n = 50)	Control (n = 20)	P value
Peak cortisol (nmol/l)	924±49.5	910.2±49.5	NS
AUC cortisol (nmol/l × 60 min)	44 378±1573.2	41 173.6±1912.6	NS
Peak 17-OHP (nmol/l)	9.3±0.3	5.7±0.3	< 0.05
AUC 17-OHP (nmol/l × 60 min)	378.4±61	249.4±52.2	< 0.05
Peak androstenedione (nmol/l)	15.6±0.6	9.1±0.3	< 0.05
AUC androstenedione (nmol/l × 60 min)	806.4±52	413.7±31.6	< 0.05
Peak DHEAS (μmol/l)	7.5±0.4	4.3±0.4	< 0.05
AUC DHEAS (μmol/l × 60 min)	385.6±25.5	224.9±24.5	< 0.05

NS, not significant.

elevated adrenal androgen production in women with PCOS and have suggested that the adrenal glands may play a role in the genesis of PCOS (5, 6). Using selective catheterization of the adrenal and ovarian vessels, women with PCOS demonstrate significant adrenal androgen hypersecretion (5). Gross and colleagues (18) observed a higher uptake of iodocholesterol by the adrenal gland of patients with PCOS compared with that in normal subjects. We have also previously shown that the responses of adrenal androgens and their precursors such as 17-OHP to an ACTH stimulation test are higher in PCOS women than in control women (19, 20).

The mechanisms for the adrenal hyperandrogenism remain unclear and several hypotheses have been put forward to explain the association between PCOS and adrenal hyperandrogenism: enhanced ovarian and adrenal P450c17 α enzyme activity (21), increased activity of enzymes involved in cortisol clearance such as 11 β -hydroxysteroid dehydrogenase (22) and the possible role of hyperinsulinemia (5, 23). Additionally, the role of the ovary in adrenal androgen hypersecretion is a matter of debate. Gonzalez *et al.* (24) showed that there are subgroups of women with PCOS: one is characterized by increased intrinsic adrenal 17,20 lyase activity that persisted after suppression of gonadal functions with chronic gonadotropin-releasing hormone (GnRH) treatment; the other subgroup is characterized by adrenal androgen hyper-responsiveness to ACTH that is resolved after GnRH-induced ovarian suppression. On the other hand some studies have suggested that ovarian hormone secretion does not affect adrenal steroidogenesis and an intrinsic abnormality of adrenal steroidogenesis may be an independent abnormality (25, 26).

In hyperandrogenic women with PCOS the ACTH stimulation test is able to show changes in adrenal androgen secretion as well as subtle enzymatic alterations which may not be demonstrated by basal hormonal evaluation (27). Adrenal steroid enzyme deficiency is a rare phenomenon and it may mimic PCOS biochemically and clinically (28). Apart from specific enzyme dysfunctions, most patients with PCOS show enhanced P450c17 α enzyme activity (20, 29). In this study, 50 women with PCOS have been investigated to see whether the low dose ACTH stimulation test, which is widely accepted as a physiological test, is also capable of revealing adrenal steroidogenic enzyme dysfunctions. In addition to 17-OHP which is a marker of P450c17 α enzyme activity, the responses of DHEAS to LDT and SDT have also been measured in women with PCOS and compared with those of the control group. This study showed that 17-OHP and androstenedione responses to the 250 μ g ACTH stimulation test are significantly higher in PCOS women than in controls. These results are comparable with our previous report (20) and indicate adrenal P450c17 α enzyme dysregulation.

Additionally, it has been shown that plasma ACTH concentrations in women with PCOS are similar to those in healthy women (30, 31). This implies that increased adrenal androgen production is not due to abnormal ACTH secretion. In contrast to the other studies, we have also used a low dose of ACTH to simulate the physiological stimulation of adrenal glands to assess adrenal P450c17 α enzyme activity in women with PCOS. Our results show that there is a dysregulation of cytochrome P450c17 α enzyme activity in women with PCOS which is established by SDT but not by LDT. Gonzalez *et al.* (32) have also investigated adrenal P450c17 α enzyme activity with low and standard doses of ACTH in 6 patients with PCOS. The authors suggest that there is an increase in adrenal androgen secretion and an increase in the activity of P450c17 α enzyme after low dose ACTH stimulation. These results are not comparable with ours. We found that control and PCOS subjects showed similar adrenal P450c17 α enzyme activity to the LDT. The discrepancies between our data and those of Gonzalez and colleagues may be explained by the different study protocols and study populations. In the study of Gonzalez *et al.* (32) the number of patients is too small (6 patients and 4 control subjects) to draw a definitive conclusion. Additionally, they used two 0.2 μ g i.v. ACTH injections at 2-h intervals and the standard dose ACTH test was performed only 2 h after the last dose of 0.2 μ g i.v. ACTH injection. The results of the low dose test might interfere with the standard dose ACTH test since both tests were performed within a small time interval.

In several studies both basal and ACTH-stimulated DHEAS levels have been found to be elevated in women with PCOS (6, 31, 33, 34). A partial defect of 3 β -hydroxysteroid dehydrogenase may be the reason for elevated DHEAS levels. But it is difficult to arrive at an exact diagnosis with modest elevations or without measurement of other Δ 5 steroids. In contrast to other steroids and precursor hormones, DHEAS responses (peak and AUC values) to LDT in PCOS women were significantly higher than the results obtained from control subjects. This result implies that although LDT could not demonstrate adrenal P450c17 α enzyme dysregulation, it can reveal adrenal androgen hypersecretion in women with PCOS. However, since we have not measured 17-hydroxypregnenolone responses to LDT/SDT, we could not exclude the possible role of 3 β -hydroxysteroid dehydrogenase enzyme dysfunction in these patients.

These results show that the LDT is strong enough to elicit similar cortisol levels as the SDT but not strong enough to elicit mild enzyme dysfunctions in adrenal steroidogenesis in women with PCOS. We think that a powerful stimulus is needed to establish adrenal P450c17 α enzyme dysregulation.

In conclusion, these results show that the LDT is capable of revealing adrenal hyperactivity in women with PCOS. Adrenal P450c17 α enzyme dysregulation

in PCOS is revealed by pharmacological doses of ACTH (250 µg) but not by physiological doses of ACTH (1 µg). LDT is able to demonstrate adrenal hyperactivity characterized by an increase in DHEAS.

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