Gynecol Endocrinol 1998;12:223–229

Adrenal androgenic response to 2-hour ACTH stimulation test in women with PCOS

C. T. Erel, L. M. Senturk, E. Oral, U. Colgar and E. Ertungealp

Istanbul University, Cerrahpasa School of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Istanbul, Turkey

Key words: POLYCYSTIC OVARY SYNDROME, ACTH STIMULATION TEST, 21-HYDROXYLASE DEFICIENCY, HIRSUTISM

ABSTRACT

Adrenal function may be abnormal in women with polycystic ovary syndrome (PCOS). This study aims to evaluate adrenal steroid response to the adrenocorticotropic hormone (ACTH) stimulation test and to find out the effect of high serum testosterone levels on adrenal response. We have also investigated any subtle enzyme deficiency by extending blood sampling to 2 h with 30 min intervals following ACTH administration. Twenty-eight women with PCOS and 18 healthy controls without hirsutism and oligomenorrhea were included in the study. After determining their serum basal levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, dehydroepiandrosterone sulfate (DHEAS), 17-hydroxyprogesterone (17-OHP), and progesterone, ACTH stimulation test was performed. The change in serum 17-OHP and the summed rate of change in serum 17-OHP and progesterone levels were estimated and 95th percentile for each value was computed. Women with PCOS were heavier and more hirsute than controls ($p < 0.01$, $p < 0.001$, respectively). Serum basal LH, LH : FSH ratio, testosterone ($p < 0.001$, for all), DHEAS ($p < 0.01$), and 17-OHP ($p < 0.05$) were higher in women with PCOS. All of the 17-OHP measurements, including basal and each 30 min interval after the administration of ACTH, were higher in women with PCOS than those of healthy controls ($p < 0.05$, $p < 0.002$, $p < 0.001$, $p < 0.015$, $p < 0.018$, respectively). However, the incremental changes in serum 17-OHP_{30-0}, 17-OHP_{60-0}, 17-OHP_{90-0}, 17-OHP_{120-0}, and the summed rate of change in serum 17-OHP and progesterone in women with PCOS were not different from those in healthy controls. The incremental response in terms of serum progesterone, DHEAS, and testosterone levels to the ACTH stimulation test for each 30 min interval was not different in women with PCOS than in healthy controls. We were not able to show any critical value for serum basal testosterone and DHEAS levels that would effect response to ACTH stimulation in terms of 17-OHP levels. We have concluded that extending the duration of blood sampling up to 2 h has no advantage in evaluating adrenal steroid response to ACTH stimulation. Since serum 17-OHP levels remain within normal limits in response to ACTH stimulation, the origin of elevated serum basal 17-OHP levels may be polycystic ovaries. Elevated serum testosterone level does not have any adverse effect on adrenal function. Serum progesterone measurement seems to have no place in the diagnosis of 21-hydroxylase deficiency. Adrenal androgenic response to ACTH stimulation is normal in women with PCOS.

Correspondence: Dr C. T. Erel, Istanbul University, Cerrahpasa School of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Istanbul, Turkey

INTRODUCTION

Since the association of polycystic ovaries with amenorrhea, hirsutism and obesity was first reported by Stein & Leventhal in 1935[1], various endocrinologic aspects have been found to be interrelated in polycystic ovary syndrome (PCOS) characterized by hyperandrogenism. Androgen excess, especially serum testosterone and androstenedione, is the most common abnormal hormonal change and a central part of the clinical expression of PCOS[2,3]. Although the main source of hyperandrogenism in PCOS is believed to be due to ovarian production, there is some evidence of adrenocortical hyperfunction. Dehydroepiandrosterone sulfate (DHEAS), an androgen formed primarily by the adrenal cortex, is elevated in over 50% of women with PCOS[4]. Some investigators have suggested adrenal androgen hyperresponsiveness after adrenocorticotropic hormone (ACTH) stimulation[5,6].

Late-onset congenital adrenal hyperplasia (LO-CAH) due to 21-hydroxylase enzyme deficiency can mimic the clinical and hormonal characteristics of PCOS[7–12]. Although the change in serum 17-hydroxyprogesterone (17-OHP) levels after ACTH stimulation test is the diagnostic hallmark for LO-CAH due to 21-hydroxylase enzyme deficiency, women with PCOS may respond to this test with a moderate increase in serum 17-OHP levels[5,13,14]. It is still debatable whether adrenal disorders in PCOS are a consequence of elevation of androgens of ovarian origin or adrenal overproduction of androgens *per se* may precede PCOS.

Since the studies assessing ovarian and adrenal function in PCOS are contradictory, this study aims to evaluate the ovarian and adrenal function in women with PCOS by measuring serum basal testosterone, DHEAS, 17-OHP and progesterone levels[5,6,13–20]. We then performed an ACTH stimulation test to detect the changes in serum 17-OHP and progesterone levels, as well as adrenal androgenic response by focusing on the changes in serum DHEAS levels. Meanwhile, we have also tried to find any possible effect of high serum testosterone levels on adrenal steroid production during ACTH stimulation tests performed on women with PCOS. In the present study, we extended the duration of blood sampling to 2 h with 30 min intervals after ACTH stimulation in order to reveal any enzyme deficiency that cannot be noticed by the routine 30 min test.

MATERIALS AND METHODS

Patients

Twenty-eight women with PCOS and 18 healthy control women without hirsutism and oligomenorrhea were included into the study. Informed consent was obtained from each woman. A woman was considered to have hirsutism when she had a score above 8 on the Ferriman–Gallwey scale[21]. Oligomenorrhea was defined as menstrual cycle greater than 40 days in length and body mass index (BMI) was computed as kg/m^2. PCOS was defined as having elevated serum testosterone and/or DHEAS levels, serum basal luteinizing hormone (LH) concentration, increased LH : follicle-stimulating hormone (FSH) ratio[2,3] and the appearance of polycystic ovaries on ultrasonography according to the criteria of Adams and colleagues[22]. Transvaginal ultrasonography was performed with a 5-MHz, 200 degrees phased-array sector vaginal probe (Kretz, Tiefenbach, Zips, Germany) on all women.

ACTH stimulation test

No woman received hormonal medication for at least 3 months before the test. ACTH stimulation test was performed on all women in the fasting state and in the supine position between 08.00 and 09.00 am of their cycle day 3–5. A heparin lock was placed in the forearm. After baseline blood was sampled, 0.25 mg synthetic ACTH (Syncaine®, Synacten, CIBA, Basel, Switzerland) was injected intravenously and blood samples were obtained every 30 min for 2 h. Then serum was separated and stored at −20°C until it was assayed for testosterone, DHEAS, 17-OHP and progesterone determinations.

In addition, the 17-OHP level 30 min after ACTH administration (17-OHP$_{30}$), the changes in 17-OHP levels (17-OHP$_{30-0}$) and the summed rate of change in 17-OHP and progesterone [(P$_{30-0}$) + (17-OHP$_{30-0}$)/30 min], which was originally proposed by Gutai and colleagues[23] for the detection of heterozygous congenital adrenal hyperplasia carrier, were calculated in both groups.

Hormone assays

Serum LH and FSH levels were measured by double antibody radioimmunoassay (RIA)

techniques (Diagnostic Products Corporation, Los Angeles, CA). Serum testosterone, DHEAS, 17-OHP and progesterone levels were measured by the Coat-a-Count RIA kit (Diagnostic Products Corporation, Los Angeles, CA).

The intra-assay coefficients of variation (CVs) for the kits were 4.0%, 5.0% and 5.6% for low, medium and high 17-OHP and 2.6%, 5.1% and 6.4% for low, medium and high progesterone values, respectively. The intra-assay CVs for the measurements of testosterone were 9.2%, 10.4% and 12.9% for low, medium and high values, respectively. For DHEAS the intra-assay CVs were 3.9%, 4.1% and 5.3% for low, medium and high values, respectively. The intra-assay CVs for the kits were 2.3%, 3.6% and 7.0% for low, medium and high LH values and 3.1%, 3.9% and 6.5% for low, medium and high FSH values, respectively. The upper ranges for serum testosterone, DHEAS, 17-OHP and progesterone levels in the early follicular phase in our laboratory were 0.85 ng/ml, 430 μg/dl, 1.2 ng/ml, and 0.3 ng/ml, respectively.

Statistical analysis

The results were compared between the group of women with PCOS and the healthy controls for each time interval using ANOVA or Kruskal-Wallis test where appropriate. Mann-Whitney rank sum test or *t*-tests were used for comparison of the two groups depending on the distribution of the data. Normality of data was tested with the Kolmogorov-Smirnov test. Spearman rank order correlation was used for testing correlation between two parameters. Statistical calculations were performed using Statistical Package for Social Sciences (SPSS) for Windows, version 6.0 (SPSS, Chicago, IL) and Sigmastat for Windows, version 2.0 (Jandel Scientific Corporation, San Rafael, CA, USA).

RESULTS

The mean age and BMI were 20.8 ± 3.7 years (range 17–26) and 24.5 ± 4.3 kg/m^2 for women with PCOS, compared with 22.1 ± 5.6 years (16–35) and 21.0 ± 1.5 kg/m^2 for normal healthy controls. The groups were comparable in terms of age, but women with PCOS were heavier than the controls ($p < 0.01$). Of 28 women with PCOS, 20 had oligomenorrhea. All women with PCOS were more hirsute than healthy controls with mean Ferriman–Gallwey score of 17.3 ± 8.5 *vs.* 3.1 ± 1.6 ($p < 0.001$). Serum basal testosterone, DHEAS, 17-OHP, LH levels and the LH : FSH ratio were significantly higher in women with PCOS than in controls (Table 1).

The mean serum basal 17-OHP and 30 min ACTH-stimulated levels for controls were found to be 1.04 ± 0.36 ng/ml and 1.55 ± 0.49 ng/ml, respectively. Change in 17-OHP (17-OHP$_{30-0}$) and summed rate of change of 17-OHP and progesterone [(P$_{30-0}$) + (17-OHP$_{30-0}$)/30] were 0.51 ± 0.32 ng/ml and 3.15 ± 1.80 ng/dl/min,

Table 1 Clinical and hormonal characteristics of the study and control groups (mean ± SE)

	Study group (n = 28)	*Control group* (n = 18)
Age (years)	20.8 ± 3.7	22.1 ± 5.6
BMI (kg/m^2)	24.5 ± 4.3*	21.0 ± 1.5
Oligomenorrhea (%)	20/28 (71%)	0
Hirsutism (Ferriman–Gallwey)	17.3 ± 8.5**	3.1 ± 1.6
LH (mIU/ml)	12.1 ± 3.9**	4.3 ± 1.8
FSH (mIU/ml)	4.0 ± 1.2**	5.7 ± 1.5
LH : FSH	3.3 ± 1.4**	0.8 ± 0.3
17-hydroxyprogesterone (ng/ml)	1.6 ± 0.7***	1.0 ± 0.4
Progesterone (ng/ml)	0.8 ± 0.3	1.0 ± 0.4
Total testosterone (ng/ml)	1.0 ± 0.3**	0.5 ± 0.1
DHEAS (μg/dl)	317 ± 120	230 ± 57

*$p < 0.01$; **$p < 0.001$; ***$p < 0.05$

BMI, body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; DHEAS, dehydroepiandrosterone sulfate

Table 2 17-hydroxyprogesterone (17-OHP$_{30-0}$) and Gutai values of study and control groups (mean ± SE)

	Study group (n = 28)	*Control group* (n = 18)
17-OHP$_{30-0}$ (ng/ml)	0.84 ± 0.59*	0.51 ± 0.32
[(P$_{30-0}$) + (17-OHP$_{30-0}$)]/30 (ng/dl/min)	4.22 ± 2.90*	3.15 ± 1.80

*$p > 0.05$

respectively (Table 2). The 95th percentile values for 17-OHP$_{30}$, 17-OHP$_{30-0}$ and [(P$_{30-0}$) + (17-OHP$_{30-0}$)/30] measurements were selected as the upper normal limit in our study: 2.53 ng/ml, 1.15 ng/ml and 6.75 ng/dl/min, respectively. Azziz & Zacur[12] have claimed that levels that are more than three-fold the 95th percentile value, designate LO-CAH as being due to 21-hydroxylase enzyme deficiency. None of the women in our study group fulfilled this criterion.

The mean hormonal values in women with PCOS were 1.61 ± 0.67 ng/ml for basal 17-OHP, 2.46 ± 0.9 ng/ml for 17-OHP$_{30}$, 0.84 ± 0.59 ng/ml for 17-OHP$_{30-0}$ and 4.22 ± 2.9 ng/dl/min for [(P$_{30-0}$) + (17-OHP$_{30-0}$)/30] (Tables 1 and 2). The measurements of serum 17-OHP levels for basal, 30, 60, 90, and 120 min after ACTH stimulation test are shown in Figure 1. All of the 17-OHP measurements, including basal and at each 30 min interval following administration of ACTH, were significantly higher in women with PCOS than healthy controls ($p < 0.05$, $p < 0.002$, $p < 0.001$, $p < 0.15$, $p < 0.018$, respectively). In study and control groups, serum 17-OHP levels increased with 30 min intervals following ACTH stimulation ($p < 0.05$). However, the incremental changes in serum 17-OHP$_{30-0}$, 17-OHP$_{60-0}$, 17-OHP$_{90-0}$, 17-OHP$_{120-0}$ and the summed rate of change in serum 17-OHP and progesterone in women with PCOS did not differ from those in healthy controls (Table 2). The incremental response of serum progesterone levels to ACTH stimulation test for each 30 min interval was not different in the two groups (Figure 2).

The mean serum DHEAS levels for basal, 30, 60, 90, and 120 min after ACTH stimulation test were higher in women with PCOS than healthy controls (Figure 3) ($p < 0.012$, $p < 0.011$, $p < 0.012$, $p < 0.021$, and $p < 0.008$, respectively). Adrenal androgenic response in terms of incremental values in serum DHEAS levels following ACTH stimulation test for each 30 min interval in women with PCOS was not different from those in healthy controls. Serum DHEAS levels did not show any correlation with serum 17-OHP levels either at basal state or following ACTH stimulation.

The mean serum testosterone levels at 0, 30, 60, 90, and 120 min were significantly higher in women with PCOS than normal women ($p < 0.001$ for each value) and this finding confirmed the selection criteria for women with

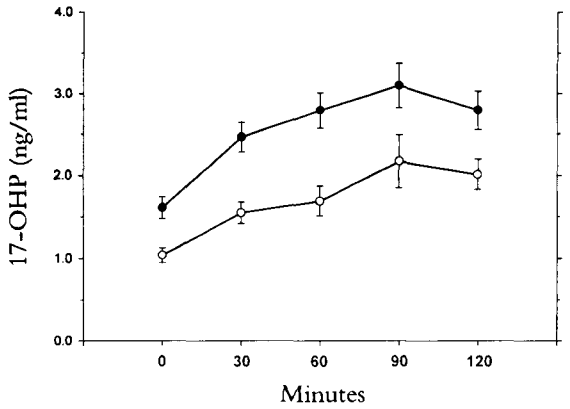

Figure 1 Serum 17-hydroxyprogesterone (17-OHP) levels in women with PCOS (-●-) and controls (-○-) after ACTH stimulation test. Values are mean ± SE

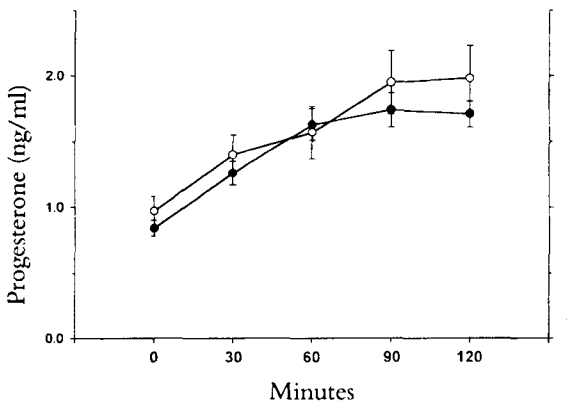

Figure 2 Serum progesterone levels in women with PCOS (-●-) and controls (-○-) after ACTH stimulation test. Values are mean ± SE

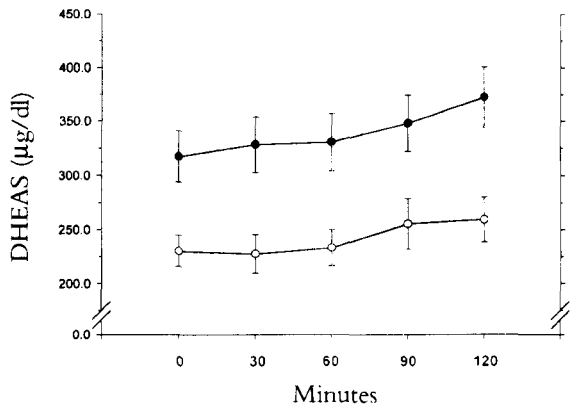

Figure 3 Serum DHEAS values in women with PCOS (-●-) and controls (-○-) following ACTH stimulation test. Values are mean ± SE

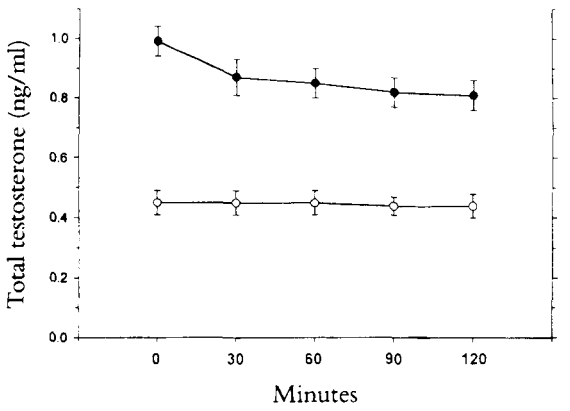

Figure 4 Serum testosterone levels in women with PCOS (-●-) and controls (-○-) after ACTH stimulation test. Values are mean ± SE

PCOS. We found a significant decrease in serum testosterone levels between basal and 30 min after the test in women with PCOS ($p < 0.05$). Following ACTH stimulation, serum testosterone levels did not change in both groups during the last 90 min (Figure 4). There was no correlation between serum testosterone and 17-OHP levels.

DISCUSSION

In the present study, we performed a 2 h ACTH stimulation test to evaluate adrenal steroidogenesis in women with PCOS. Thirty or 60 min ACTH stimulation tests have been widely used for the diagnosis of LO-CAH or heterozygote carriers of CAH[8,10,12,23,24]. It has been suggested that it is possible to evaluate adrenal steroid production by determining serum cortisol and 17-OHP levels with longer time intervals[25]. Recently, it has been also reported that steroid levels reach a plateau 60–90 min after ACTH stimulation[15]. Therefore, we extended blood sampling time to 120 min with 30 min intervals to observe the long-term changes in serum 17-OHP and progesterone levels as well as to detect the adrenal androgenic response by determining serum DHEAS levels. However, we were not able to find any advantages of extending the duration of blood sampling up to 120 min either in women with PCOS or healthy controls.

Obesity, oligomenorrhea and hirsutism are well known clinical characteristics of women with PCOS, as confirmed in our study (Table 1)[2,3]. Since women with PCOS had a higher BMI in our study, we had some concerns about the effect of BMI on adrenal steroid response to ACTH stimulation. However, it has been shown that subject weight does not influence ACTH test results[15,26].

We were able to demonstrate elevated basal and ACTH-stimulated serum levels of 17-OHP in women with PCOS. However, the incremental change in serum 17-OHP levels during 2 h did not show a difference between women with PCOS and healthy controls. It has been reported that some women with hyperandrogenism, who are not sufferers from LO-CAH, showed mild 21-hydroxylase enzyme deficiency by ACTH stimulation test[12]. Two different hypotheses might be helpful in explaining the deficiency in 21-hydroxylase activity and elevated serum levels of 17-OHP in women with PCOS. (1) It has been stated that in non-LO-CAH hyperandrogenic women, elevated serum 17-OHP levels after ACTH stimulation are most likely to represent a normal 17-OHP adrenocortical response superimposed on an elevated circulating 17-OHP level of non-adrenal origin[14]. Possibly, the origin of 17-OHP might be polycystic ovaries[13]. The study of Lachelin and colleagues[5] also supports our results. (2) Since it is well known that one of the major androgens elevated in PCOS is serum testosterone, high serum testosterone levels may result in subtle inhibition of 21-hydroxylase enzyme deficiency in the adrenal steroidogenesis[16]. Fruzetti and colleagues[17] investigated whether high serum testosterone levels of ovarian origin affect adrenal steroid production and evaluated 39 hyperandrogenic women with normal or high serum testosterone levels and 10 normal controls.

17-OHP response to the ACTH stimulation test was significantly higher in hyperandrogenic women with high serum testosterone levels. However, they could not confirm this finding when they administered a gonadotropin-releasing hormone (GnRH) agonist to suppress ovarian testosterone production. Azziz *et al.*[18] also concluded that adrenocortical biosynthetic abnormalities noted in women with hyperandrogenism did not appear to result from the elevated circulating testosterone levels. Although serum testosterone levels in women with PCOS were above the upper normal limit in our study, we were not able to show any significant correlation between serum testosterone and 17-OHP levels. In addition, basal serum testosterone levels were not indicative for 17-OHP response to ACTH stimulation. All these data, including our study, do not seem to prove the theory of subtle inhibition of adrenals in women with PCOS.

Basal serum progesterone levels and progesterone response to ACTH stimulation were not different in women with PCOS and controls. Conclusively, we believe that serum progesterone measurements and the related formula proposed by Gutai *et al.*[23] have no place in determining 21-hydroxylase enzyme deficiency.

Since the subject is still under debate, we have evaluated adrenal androgenic response to ACTH stimulation by focusing on serum DHEAS levels. We found that although serum DHEAS level was higher in women with PCOS during 2 h, the response to ACTH stimulation test in terms of DHEAS was not different in women with PCOS and healthy controls. Siegel and colleagues[20] undertook a study to reveal a relationship between the basal serum DHEAS levels and the hormonal response to ACTH. They were not able to show any significant correlation between the basal serum DHEAS levels and the hormonal response, including DHEAS levels, to ACTH. Consistent with the findings of Siegel's study[20], we could not show any correlation between basal DHEAS levels and the response of adrenal steroids in the present study. In contrast to this, a recent study[19] divided women with PCOS into two groups according to their basal serum DHEAS levels, normal or high, and performed the ACTH stimulation test. The PCOS women with high basal serum DHEAS levels showed a higher increase in serum DHEAS level in response to the ACTH stimulation test than the PCOS women with normal basal serum DHEAS levels and normal controls. In our study, women with PCOS had normal serum basal DHEAS levels. Therefore, this might be the reason that we were not able to show a significant increase in serum DHEAS levels in response to ACTH stimulation.

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