CLINICAL STUDY

Ovarian–adrenal cross-talk in polycystic ovary syndrome: evidence from wedge resection

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Abstract

Objective: To determine whether the ovary influences adrenal androgen secretion in women with polycystic ovary syndrome (PCOS).

Design: Six PCOS-affected patients with clomiphene resistance and gonadotrophin hyperresponsivity, and six controls with regular ovulatory cycles, matched for age and body mass index.

Methods: Bilateral ovarian wedge resection was performed to induce ovulation surgically for these refractory women with PCOS. The adrenal androgen secretions were evaluated in PCOS patients before and again 6 months after this surgery, and in the controls, using an ACTH stimulation test (0.25 mg synthetic ACTH(1–24)).

Results: Biochemically, basal levels and the maximum net increases (Δ) of 17-hydroxyprogesterone (17-OHP) and androstenedione, Δ 17-OHP/ Δ progesterone and Δ androstenedione/ Δ 17-OHP ratios in response to exogenous ACTH were significantly higher in PCOS patients before operation than those of controls. This purely ovarian surgery in women with PCOS was found to significantly reduce their basal androstenedione, testosterone and LH levels, insulin/glucose ratio, and post-corticotrophic Δ 17-OHP, Δ androstenedione, Δ 17-OHP/ Δ progesterone and Δ androstenedione/ Δ 17-OHP, without obvious changes in FSH, oestradiol, sex hormone-binding globulin, Δ dehydroepiandrosterone, Δ dehydroepiandrosterone sulphate, Δ aldosterone and Δ cortisol values.

Conclusions: Ovarian hyperandrogenicity from polycystic ovary may contribute to the enhanced adrenal P450c17 α activity and subsequent Δ^4 androgen reserve revealed by the pharmacological corticotrophin stimulation in our special PCOS cases.

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Introduction

Increased androgen production is the biochemical hallmark of polycystic ovary syndrome (PCOS), and is central to the pathogenesis of the morphological changes in the ovaries and the clinical syndrome. Numerous investigators have found elevated androgen synthesis by the adrenal gland in patients with this syndrome. Both basal levels of dehydroepiandrosterone sulphate (DHEAS) and 11 β -hydroxyandrostenedione, the exclusive products of the adrenal, and responses of adrenal androgens to adrenocorticotrophic hormone (ACTH) have been found to be elevated in 70–75% of affected patients, independent of their ethnic diversity (1). Adrenal hyperandrogenism, therefore, constitutes a main endocrine component of PCOS.

Adrenal enzyme dysfunction characterized by a deficiency is a relatively rare phenomenon, which has many biochemical and clinical overlaps with PCOS, and

should be differentiated from this entity (1, 2). In contrast, enhanced adrenal enzymatic activity has been proposed to occur in association with PCOS. Some of the adrenal enzymes which explain this association between PCOS and adrenal hyperandrogenism include both arms of the cytochrome P450c17 α enzyme, 17-hydroxylase and 17,20-lyase, as well as 11 β -hydroxysteroid dehydrogenase, etc. (3, 4).

Recently, available data from treatment with gonadotrophin-releasing hormone (GnRH) agonists and insulin (INS)-sensitizing drugs in PCOS have further suggested that ovary functionality and INS resistance may be involved in the altered adrenal enzyme activity and subsequent steroid production (4–6). To our knowledge, there have been no studies evaluating the effect of bilateral ovarian wedge resection (OWR) on steroidogenic responses of the adrenal gland in PCOS women of Asian background, especially with failed drug ovulation induction. We hypothesized

that ovarian androgen excess, a main endocrine feature in this syndrome, may contribute to adrenal dysfunction in PCOS.

Materials and methods

Subjects

The University Ethics Committee approved this study, and informed consent was obtained from each subject. Six PCOS-affected women with both clomiphene resistance and gonadotrophin hyperresponsivity were selected for the study. Before OWR, they all exhibited the classic features of oligomenorrhoea, anovulation, elevated total testosterone levels (>2.5 nmol/l) and a ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH) greater than 2. Enlarged polycystic ovaries containing multiple small follicles and increased stroma were confirmed by transvaginal sonography and diagnostic laparoscopy. Their mean ($\pm$ s.d.) age was 27.4 ± 4.3 years (range, 24–31 years) and their mean ($\pm$ s.d.) body mass index (BMI) was 21.9 ± 2.2 kg/m². Six women with normal ovulation, four of whom previously had tubal infertility and two of whom had infertility due to a male factor, were recruited as the control group because they demonstrated a biphasic basal body temperature and a luteal phase progesterone level of >19.2 nmol/l. The mean age and BMI for these controls was 26.8 ± 4.7 years (range, 23–30 years) and 22.6 ± 1.7 kg/m² respectively.

Clomiphene resistance was defined as the absence of follicular development for three cycles on transvaginal ultrasonographic examination with concomitant failure of oestradiol (E₂) levels to rise after treatment with clomiphene citrate, 150 mg, for 5 days. Six patients who met the inclusion criteria were further determined to be gonadotrophin hyperresponsive. Three patients had a previous cycle complicated by moderate ovarian hyperstimulation syndrome according to the modified criteria (7). The other three patients had a previous ovulation induction with human gonadotrophin cancelled because of hyperresponsivity (E₂ level >9.18 nmol/l or more than four follicles of >20 mm in average diameter with a large number of small to intermediate-sized follicles). Only diagnostic laparoscopy was available at that time in our department and these patients refused a trial treatment because of no experience by the physicians. As the last step for their long-lasting anovulatory infertility, bilateral OWR was performed in these refractory patients with PCOS in the manner described previously (8).

Since obesity is a well-recognized cause of either resistance or hyperresponsiveness to drug ovulation induction, only non-obese patients were enrolled (7). None of the subjects in either group was obese (BMI <25 kg/m²) or had taken any medication, including hormonal contraception for the previous 3 months. Subjects in both groups were matched for age and BMI.

The waist/hip ratio (WHR), a simple anthropometric method, was further used to determine the pattern of body fat distribution. The waist and hip circumferences were measured as previously described (9). Thyroid dysfunction, hyperprolactinaemia, congenital adrenal hyperplasia (CAH) and androgen-secreting tumours were specifically excluded by appropriate tests.

Protocol

The ACTH test was performed in the midfollicular phase (days 5–7) of the cycle for menstruating subjects (such as all controls and the PCOS patients after OWR). In cases where patients with PCOS were amenorrhoeic before operation, data were collected when the serum progesterone level was <8.0 nmol/l. Days 5–7 were chosen on the basis of observations that there is no difference in the adrenocortical response to acute ACTH stimulation between tests arranged on days 3–5 and tests arranged on days 6–8 of the menstrual cycle (10). All women were asked to attend the hospital at 08.00 h after an overnight fast, and i.v. cannulas with heparin locks were placed in antecubital veins, followed by a 30 min rest to minimize the effects of physical activity and non-specific stress. Immediately after the baseline blood collection, all women received an i.v. bolus of 0.25 mg synthetic ACTH(1–24) (Biochemical Company, Shanghai, China) over 60 s, and blood was sampled every 30 min for 2 h. Then serum was separated and stored at -80°C until assayed. In PCOS patients, this adrenal dynamic test was carried out at an appropriate date before the OWR operation (PCOS-BO patients) and repeated 6 months after the operation (PCOS-AO patients), while in controls ACTH was administered once only. Dexamethasone was not given to any women of the three groups on the evening before the study day, in order to assess the resting basal steroid levels.

Determinations

Using commercially available kits, serum responsive levels of FSH, LH, E₂, progesterone, testosterone, aldosterone (ALD), cortisol, INS (kits from NT Biomedical, Tianjin, China), androstenedione, dehydroepiandrosterone (DHEA), DHEAS (kits from Diagnostic Products Corporation, Los Angeles, CA, USA), and 17-hydroxyprogesterone (17-OHP) (kits from Biosource, Fleurus, Belgium) during the ACTH test, as well as basal sex hormone-binding globulin (SHBG) (kits from Wallac Oy, Turku, Finland), glucose and endogenous ACTH (kits from Amersham, Arlington Heights, IL, USA) were evaluated in all subjects of the three groups with well-determined procedures (8–10). The intra- and inter-assay precision coefficients of variation were 2.3 and 7.5% for LH, 2.5 and 8.7% for FSH, 7.4 and 6.1% for testosterone, 9.4 and 14.8% for androstenedione, 8.0 and 7.7% for E₂, 7.2 and 8.9% for progesterone, 3.4 and

6.7% for INS, 8.6 and 7.3% for cortisol, 6.6 and 7.9% for ALD, 4.4 and 7.4% for 17-OHP, 5.3 and 6.8% for DHEA, 9.8 and 9.5% for DHEAS, 4.3 and 6.5% for SHBG, and 6.9 and 7.9% for ACTH. All samples from the same patients were assayed in the same batch. Anthropometric data (BMI and WHR) were also obtained just before the adrenal dynamic test in each woman of the three groups.

Statistics and calculations

The extra-adrenal contribution to circulating levels of adrenal products could affect the evaluation of the response to ACTH. To avoid this, the analysis of the release of steroids after the exogenous ACTH stimulation test was performed on data normalized as net increase over basal values. The maximum net increases (Δ) over baseline values in terms of androstenedione, DHEA, and DHEAS after this pharmacological ACTH were calculated to evaluate the adrenal androgen reserve. The activities of 17 α -hydroxylase and 17,20-lyase were represented by the mean ratios of Δ 17-OHP to Δ progesterone (Δ 17-OHP/ Δ progesterone) and Δ androstenedione to Δ 17-OHP (Δ androstenedione/ Δ 17-OHP) respectively. INS resistance was assessed by the ratio of fasting INS (mU/l) to fasting glucose (mmol/l), a simple and accurate indicator as reported by Legro *et al.* (11). The results are reported as means $\pm$ S.D. In women with PCOS, we compared the results before operation with those after operation by using the Wilcoxon signed-rank test. Between the PCOS and control subjects the results were compared by using

the Mann–Whitney U test. Pearson's product moment correlation was used to estimate linear relationships between variables. $P < 0.05$ was regarded as statistically significant.

Results

The clinical and basal hormonal characteristics of PCOS and control subjects are shown in Table 1. As compared with controls, PCOS-BO patients demonstrated significantly higher mean serum levels of LH, testosterone, androstenedione, 17-OHP and INS, while BMI, WHR and serum FSH, E_2 , progesterone, glucose, ALD, DHEA, SHBG, ACTH and cortisol levels were similar, resulting in higher mean LH/FSH and INS/glucose ratios. As for PCOS-AO patients, OWR significantly reduced their mean levels of testosterone, androstenedione, 17-OHP, LH and INS without obvious changes in BMI, WHR, FSH, E_2 , progesterone, glucose, ALD or cortisol, followed then by normal LH/FSH and INS/glucose ratios. Furthermore, no significant variations were found in subjects of the three groups for mean LH, FSH, E_2 and testosterone levels in response to the ACTH stimulation test, with persistently higher LH and testosterone values in PCOS-BO patients than in the other two groups (data not shown).

Figure 1 summarizes the maximum net incremental responses of INS and some adrenal steroids during adrenal dynamic tests in the three groups. After ACTH stimulation, no significant differences existed among the three groups for Δ progesterone, Δ DHEA, Δ DHEAS, Δ cortisol and Δ ALD. However, PCOS-BO patients

Table 1 Clinical and basal hormonal characteristics (means $\pm$ S.D.) of PCOS patients before (PCOS-BO) and after (PCOS-AO) bilateral OWR, and in control subjects.

Determination	PCOS ($n = 6$)		Controls ($n = 6$)
	BO	AO	
BMI (kg/m ²)	21.9 $\pm$ 2.2	21.8 $\pm$ 2.3	22.6 $\pm$ 1.7
WHR	0.80 $\pm$ 0.06	0.81 $\pm$ 0.06	0.81 $\pm$ 0.05
Testosterone (nmol/l)	4.78 $\pm$ 0.42**	2.27 $\pm$ 0.36†	2.08 $\pm$ 0.38
Androstenedione (nmol/l)	13.9 $\pm$ 2.5**	9.0 $\pm$ 1.8†	8.8 $\pm$ 1.7
E_2 (pmol/l)	341 $\pm$ 44	365 $\pm$ 49	334 $\pm$ 66
Progesterone (nmol/l)	0.99 $\pm$ 0.32	0.95 $\pm$ 0.45	0.92 $\pm$ 0.29
17-OHP (nmol/l)	3.5 $\pm$ 0.15**	2.0 $\pm$ 0.36†	1.4 $\pm$ 0.9
DHEA (μ g/l)	8.3 $\pm$ 1.9	9.5 $\pm$ 2.2	8.4 $\pm$ 2.3
DHEAS (μ mol/l)	8.0 $\pm$ 1.9	9.0 $\pm$ 2.6	8.5 $\pm$ 1.8
Cortisol (μ g/dl)	8.7 $\pm$ 2.5	8.3 $\pm$ 2.6	6.7 $\pm$ 0.8
ALD (pg/l)	10.6 $\pm$ 1.7	10.4 $\pm$ 1.2	9.5 $\pm$ 1.4
SHBG (nmol/l)	42.1 $\pm$ 9.7	51.6 $\pm$ 8.8	47.8 $\pm$ 8.7
LH (IU/l)	15.7 $\pm$ 3.7**	8.8 $\pm$ 1.9††	8.0 $\pm$ 0.9
FSH (IU/l)	6.1 $\pm$ 1.0	4.9 $\pm$ 1.8	4.5 $\pm$ 0.9
LH/FSH	3.3 $\pm$ 0.61*	1.1 $\pm$ 0.33††	1.3 $\pm$ 0.21
ACTH (ng/l)	15.6 $\pm$ 2.3	14.9 $\pm$ 3.4	16.0 $\pm$ 2.9
INS (mU/l)	12.9 $\pm$ 1.4*	9.0 $\pm$ 2.1†	8.1 $\pm$ 1.7
Glucose (mmol/l)	4.2 $\pm$ 0.38	4.4 $\pm$ 0.33	4.5 $\pm$ 0.35
Insulin/glucose (U/mol)	2.9 $\pm$ 0.3**	2.0 $\pm$ 0.3†	2.0 $\pm$ 0.2

* $P < 0.05$, ** $P < 0.01$ vs controls; † $P < 0.05$, †† $P < 0.01$ vs PCOS-BO.

Figure 1 (a) The maximum net increase (Δ) of progesterone (P), 17-OHP and androstenedione (A) and the ratios Δ 17-OHP/ Δ P and Δ A/ Δ 17-OHP; and (b) Δ of DHEAS, DHEA, cortisol (F), ALD and INS during the ACTH stimulation test in PCOS-BO (shaded columns) and PCOS-AO (filled columns) patients and controls (open columns). * $P < 0.05$, ** $P < 0.01$ vs controls; † $P < 0.05$, ‡ $P < 0.01$ vs PCOS-BO patients.

demonstrated much higher mean post-corticotrophic Δ 17-OHP and Δ androstenedione, and their ratios, Δ 17-OHP/ Δ progesterone and Δ androstenedione/ Δ 17-OHP, with respect to controls. OWR modified the post-corticotrophic Δ 17-OHP and Δ androstenedione secretions, and Δ 17-OHP/ Δ progesterone and Δ androstenedione/ Δ 17-OHP ratios in women with PCOS, as the differences of these indices disappeared between PCOS-AO patients and controls. INS levels were elevated during ACTH stimulation in all subjects of the three groups, the net responses being higher in controls, lower in PCOS-BO patients. The surgical approach of OWR reduced the ACTH-induced Δ INS in women with PCOS. Linear regression analysis found positive correlations between Δ INS and Δ 17-OHP/ Δ progesterone ($r = 0.62$, $P < 0.05$), and between Δ INS and

Δ androstenedione/ Δ 17-OHP ($r = 0.47$, $P < 0.05$), when all data for both PCOS-BO and PCOS-AO patients were put together as a group.

Discussion

Clomiphene citrate is an oestrogen antagonist that is widely used to induce ovulation by competing oestrogen receptors at the hypothalamic level, while gonadotrophins work directly on the ovarian receptors for follicle development. The mechanism of anovulation behind both clomiphene-resistant and gonadotrophin-hyperresponsive patients with PCOS in the present study seems to lie at the level of a defective pituitary gland. Our findings that reduced androgen levels after the bilateral OWR were associated with appropriate gonadotrophin sections and then spontaneous ovulation suggest a detrimental effect of androgens on the pituitary release of gonadotrophins and ovarian performance of normal functionality (12). On the other hand, it seems likely that adrenal hyperandrogenism is acting to maintain the ovulatory dysfunction in some patients with PCOS. Approximately 50–70% of PCOS patients have excessive adrenal androgen levels and treatment with glucocorticoids only has been reported to improve menstrual regularity in 30–66% of oligo-ovulatory patients (13). Further, corticosteroid suppression may enhance the ovulatory response to clomiphene citrate, particularly in those patients with higher DHEAS levels (14). Although no differences were found for basal levels and maximum net increases of DHEA and DHEAS among three groups, higher Δ androstenedione in response to ACTH stimulation in PCOS-BO patients suggests its adrenal origin. As far as we know, no previous studies have evaluated the adrenal function in such special cases with PCOS.

The ACTH stimulation test can be performed in several ways depending on the intended purpose. If a pharmacological dose of ACTH is administered without pretreatment with dexamethasone, the maximal incremental rise between the pre- and post-stimulation values of the various corticosteroids is an indicator of adrenal reserve. In hyperandrogenic women the ACTH stimulation test is able to show changes in adrenal secretion as well as subtle enzymatic alterations that a basal hormonal evaluation fails to show (15). Thirty- or 60-min ACTH stimulation tests have been widely used for the diagnosis of late-onset CAH or heterozygote carriers of CAH. 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. Recently, it has also been reported that steroid levels reach a plateau 60–90 min after ACTH stimulation (10). Therefore, we extended blood sampling time to 120 min with 30 min intervals to observe the long-term changes in serum progesterone, 17-OHP levels and androstenedione as well as 17-OHP/progesterone and androstenedione/17-OHP ratios. In

the present study, before operation women with PCOS demonstrated significantly higher post-stimulation $\Delta 17$ -OHP and Δ androstenedione, and higher ratios of $\Delta 17$ -OHP/ Δ progesterone and Δ androstenedione/ $\Delta 17$ -OHP, indicating a greater androgen reserve in terms of androstenedione. Our results are in accord with data (16) in which only a physiological ACTH dose was used instead of the pharmacological amount for stimulation to evaluate adrenal cytochrome P450c17 α activity, and are at variance with a study (17) which failed to find enhanced activity of this key limiting enzyme in adrenal steroidogenesis.

As shown in this study, hyperinsulinaemia and INS resistance is another component of the endocrine pattern of PCOS and may have a pathogenic role for the adrenal alteration (1, 5). Furthermore, in patients with PCOS, positive correlations were found after ACTH stimulation between maximum net INS changes and $\Delta 17$ -OHP/ Δ progesterone and Δ androstenedione/ $\Delta 17$ -OHP ratios. These data suggest an important influence of INS on adrenal Δ^4 androgen production, probably by the activation of both arms of P450c17 α , namely 17-hydroxylase and 17,20-lyase. Furthermore, metformin therapy in some PCOS patients has been reported to improve INS sensitivity and reduce the basal and post-glucose load INS levels as well, but it fails to alter 17-OHP and androstenedione responses to ACTH and cytochrome P450c17 α activity (18). Ethnic and presumably underlying genetic differences in INS resistance may explain such a finding, as metformin appears effective for androgen suppression in certain populations, such as Venezuelan (19) and Finnish (20), but ineffective in others, such as American (21) and Turkish (22). It has been stated that an INS-sensitizing agent is effective for endocrine abnormalities and ovulatory performance in PCOS women of Asian background (23). Therefore, therapeutic roles of INS-lowering drugs for adrenal enzyme alteration and steroid biosynthesis warrant further investigation in the future.

Many studies have been dedicated to evaluating the possibility that altered ovarian function is the cause of adrenal androgen excess in PCOS. GnRH agonists, which inhibit the ovary selectively, without discernible direct effects on adrenal function, have been used to suppress ovarian activity (24). Indeed, studies with GnRH administration have shown a reduction of adrenal androgens and adrenal androgen response to ACTH in some PCOS patients (6, 16). Among the ovarian factors which may be involved, hyperoestrogenism and androgen excess have attracted most attention. Recently, it has been shown that oestrogen administration to PCOS women treated with GnRH agonist results in an enhancement of some adrenal enzymatic activities (6). More recently, in PCOS patients, serum unbound E_2 has also been demonstrated to correlate positively with basal serum DHEAS, DHES and 17-hydroxypregnenolone (25). This suggests a positive

influence of oestrogens on the Δ^5 pathway, predominantly resulting in an enhancement of DHEA and DHEAS production. In contrast, we were not able to demonstrate any differences in basal E_2 level, or basal values and post-corticotrophic maximum net increases of DHEA and DHEAS between PCOS-BO patients and controls. It seems unlikely that oestrogen alone is sufficient to cause adrenal hyperandrogenism here. Conversely, our work on purely ovarian surgery by OWR, showing decreased ovarian testosterone and androstenedione, accompanied by normalized basal and ACTH-induced adrenal steroid responses, suggests a possible role of ovarian androgens in adrenal steroidogenesis. This concept that some changes in adrenal steroidogenesis might be secondary to the ovarian hyperandrogenicity is supported by the findings that hyperoestrogenism and INS resistance may come from the extra-glandular aromatization of ovarian androgen excess and the direct impact of androgens on INS sensitivity (26).

Available data have also suggested a possible direct role of corticotrophin-releasing hormone (CRF)/ACTH in ovary physiology. It is believed that a human intra-ovarian CRF system may function as an inhibitory regulator for androgen biosynthesis within the thecal cell compartment, and is mediated through CRF receptors and its binding proteins (27). ACTH stimulation of E_2 and progesterone production from rat luteal cells has been defined to relate to its specific binding sites on cell surfaces and increased prostaglandin E_2 production (28). In consideration of these factors, we performed adrenal dynamic tests for all subjects only during the mid-follicular phase of their menstrual cycles by using synthetic ACTH instead of CRF. Another possibility why adrenal dysfunction in patients with PCOS resolved after ovarian surgery in the present study may be the decreased LH levels. LH receptors have recently been identified in adult human reticularis and lower fasciculated cells, which are proven sites of adrenal androgen production (29). Human chorionic gonadotrophin, a structural and functional homologue of LH, has been shown to stimulate adrenal androgen secretion in guinea pig adrenocortical cells (30). However, it seems unlikely as no obvious variations in both gonadotrophin levels were observed in these PCOS-affected women during this adrenal dynamic test.

As the number of patients in our pilot study is small and use of steroid ratios to estimate true enzyme activities should be interpreted with caution, the present results only suggest that ovarian function may influence adrenal androgen reserve in some highly selected cases with PCOS, mainly through Δ^4 cytochrome P450c17 α hyperactivity.

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