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EFFECT OF BILATERAL OOPHORECTOMY ON ADRENOCORTICAL FUNCTION IN WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS)

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Abstract

Objective—To determine the impact of ovary-secreted products on adrenocortical function in women with PCOS by studying the adrenocortical response to acute adrenocorticotrophic-stimulating hormone (ACTH) stimulation before and after bilateral oophorectomy.

Design—Prospective study.

Setting—Tertiary care medical center

Participants—Fourteen women with PCOS scheduled for bilateral oophorectomy for benign indications, on transdermal estradiol (E2) postoperatively.

Interventions—Physical exam, blood sampling before and after oophorectomy, measurement of hormone levels. Basal (Steroid₀), maximum stimulated (Steroid₆₀), and net increment (Δ Steroid) levels of androstenedione (A4), dehydroepiandrosterone (DHEA), and cortisol (F) before and after ACTH-1–24 stimulation were assessed.

Main Outcome Measures—Pre- and post-operative basal and ACTH(1–24)-stimulated hormonal levels.

Results—Total testosterone, free testosterone, and estrone levels decreased, and FSH levels increased significantly following oophorectomy. No significant differences in E2, DHEA sulfate (DHEAS) or sex hormone binding globulin levels were detected. Basal and ACTH-stimulated A4 levels decreased significantly following oophorectomy, and Δ A4 was significantly increased. No significant differences in DHEA₀, DHEA₆₀, or F₀ levels were detected; F₆₀ and Δ F levels tended to increase following oophorectomy, but the differences did not reach significance.

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Conclusions—Ovarian factors do not appear to contribute significantly to the adrenocortical dysfunction of PCOS.

Key terms

Polycystic ovary syndrome; adrenal androgen; oophorectomy

INTRODUCTION

The polycystic ovary syndrome (PCOS) is a complex, heterogeneous disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries (1). PCOS is one of the most common endocrine disorders, affecting approximately 6% to 10% of reproductive aged women. In the majority of cases, the ovary is the primary source of androgen excess. However, androgen biosynthesis in the adrenal is also upregulated and exaggerated in PCOS, and 25% to 50% of PCOS patients demonstrate excess adrenal androgen (AA) secretion (2).

The mechanisms underlying AA excess in PCOS are unclear. AAs are primarily secreted by the zona reticularis of the adrenal cortex, and include dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), and Δ^5 -androstene-3 β ,17 β -diol (androstenediol). Although less specific, A4 can also be considered an AA. It is possible that ovarian-secreted factors, such as testosterone (T), may contribute to the adrenal hyperandrogenism of these patients. For example, elevated T levels in men, and the administration of T to oophorectomized women, have been associated with elevations in the ratio of DHEAS to DHEA (3), suggesting that ovarian hyperandrogenemia may increase DHEA sulfation. However, we found that T had no predictable effect on the production of DHEA, DHEAS or F in human adrenal tissue in vitro (4). Alternatively, studies in NCI-H295R adrenocortical cell lines indicated that while T increased the production of DHEA, it appeared to decrease the secretion of DHEAS (5). Overall, T appears to have only a modest effect on AA secretion, primarily increasing the sulfation of DHEA to DHEAS, and does not result in an exaggerated secretion of active androgens.

In this study, we aimed to definitively determine the impact of ovary-secreted products on adrenocortical function in PCOS, by examining differences in AA secretion in affected women before and after bilateral oophorectomy. We controlled for the effects of postoperative estrogen deprivation through the administration of transdermal E2 to the study subjects, because; i) estrogens have been reported to affect adrenocortical function in PCOS (6), although we did not find such an effect in menopausal women (7); ii) the stress and insomnia arising from hypoestrogenic vasomotor flushing could affect adrenocortical function; and iii) our primary interest was to examine the role of ovarian androgens and other factors, not estrogens, on adrenal function.

MATERIALS AND METHODS

Subjects

Fourteen premenopausal PCOS patients and four healthy eumenorrheic control women awaiting abdominal oophorectomy for a variety of benign conditions were recruited. PCOS was diagnosed according to the NIH 1990 criteria, such that all subjects had: 1) hyperandrogenism (biochemical or hirsutism), 2) oligo-ovulation, and 3) the exclusion of related disorders. All subjects were aged between 25–45 years and had not taken hormonal and/or anti-androgenic medications for at least six weeks prior to the study. Women with ovarian or adrenal tumors or non-classic congenital adrenal hyperplasia (NCAH) were excluded. All patients were recruited and followed prospectively. The study was approved

by the institutional review boards of the University of Alabama at Birmingham and Cedars-Sinai Medical Center, and all subjects gave written informed consent.

Study Protocol

All patients underwent a complete history and physical evaluation, and the degree of hirsutism was documented using a modified Ferriman-Gallwey (mFG) score (8). Subjects were deemed hirsute if their mFG score was ≥ 6 . Patients underwent acute adrenal testing during the initial preoperative period, and two to six weeks following surgery. For the preoperative test, patients were studied between days 3 and 10 of their menstrual cycles. Oligomenorrheic patients were studied following the induction of a withdrawal bleed after oral or intramuscular administration of progesterone. Following surgery, all patients were placed on transdermal E2 (Estraderm, Ciba-Geigy, 0.1 mg/day) continuously until completion of their postoperative evaluation.

ACTH tests were performed between 0700 and 1000 hrs. An intravenous catheter was placed in the patient's forearm. Following a 30 – 45 minute rest period, 20 ml aliquots of blood were drawn at 30, 15, and 5 minutes (baseline samples) prior to the intravenous administration of 0.25 mg of ACTH(1–24) (Cortrosyn, Organon Co.) over 60 seconds. An additional 40 ml blood sample was drawn 60 minutes after the ACTH injection.

Hormonal Assays

Total and free T, estrone (E1), E2, FSH, DHEAS, and SHBG were measured in basal samples; A4, DHEA and F were measured in basal (Steroid₀) and ACTH-stimulated (Steroid₆₀) samples, and the net change (Δ Steroid) was calculated. A4, DHEA and T were quantified using validated radioimmunoassays (RIAs) (9, 10). Prior to RIA, steroids were extracted from serum with ethyl acetate:hexane (3:2), and A4, DHEA and T separated by Celite column partition chromatography, using ethylene glycol as the stationary phase. The three androgens were eluted in 0%, 15% and 35% toluene in isooctane, respectively. RIA sensitivities are 10, 20 and 10 pg/ml, respectively. The intra-assay coefficients of variation ranged from 3.0% to 6.7%, and the inter-assay coefficients of variation ranged from 7.3% to 13.2%. DHEAS and F were measured by direct, solid-phase, competitive, chemiluminescent enzyme immunoassays, and SHBG by a solid-phase, two-site chemiluminescent immunoassay, using the Immulite analyzer (Diagnostic Products Corporation, Inglewood, CA). Assay sensitivities are 30 ng/ml, 2 ng/ml and 0.2 nmoles/L, respectively. The interassay coefficients of variation (CVs) are 8.1%, 7.6% and 6.2% respectively. The calculation of free and bioavailable T is based an algorithm derived from equations described previously (11).

Estradiol and E1 were quantified by validated RIA with preceding organic solvent extraction and Celite column partition chromatography (9). Chromatographic separation of the estrogens is achieved by use of different concentrations of ethyl acetate in isooctane. The assay sensitivities are 2 pg/ml and 4 pg/ml for the E2 and E1 assays, respectively. The CVs are 10.3% and 9.6% at 18 pg/ml and 42 pg/ml, respectively, for E2, and 11.1% and 9.1% at 28 pg/ml and 69 pg/ml, respectively, for E1. FSH was measured by a solid-phase, two-rate by chemiluminescent immunometric assay on the Immulite analyzer. The assay sensitivity is 1 mIU/ml, and the interassay CVs are 4.1%, 4.8% and 4.1% at 6.8, 22.9 and 44.9 mIU/ml, respectively.

Statistical Analyses

Statistical analysis was performed using the Statistical Analysis System program (SAS Institute, Inc., Cary, NC). The Shapiro-Wilks statistic was computed to assess conformity with the normal distribution. A value of this statistic closer to 1.0 implies that the data

distribution follows a normal distribution. As this was closer to 1.0 for log scale data, all values were log-transformed to achieve a more normal distribution, and comparisons were carried out parametrically using t-tests if paired and means were compared on the log scale using a repeated measure analysis of variance model if multiple groups.

RESULTS

PCOS (n=14) and control (n=4) women had a mean age of 38.0 ± 6.8 years and 32.5 ± 8.9 years, a mean waist-hip ratio (WHR) of 0.86 ± 0.07 and 0.82 ± 0.06 , and a mean body mass index (BMI) of $32.8 \pm 8.2 \text{ kg/m}^2$ and $34.6 \pm 7.1 \text{ kg/m}^2$. PCOS women had a mean mFG score of 8.15 ± 3.76 . We should note that in this study, as in most of our clinical studies, we first recruited affected subjects (cases) and then began to recruit controls to ensure as close a match between cases and controls. However, by the time we start recruiting controls, our data had already indicated that there were no significant changes in the adrenal response of PCOS subjects with oophorectomy, and recruitment of controls was discontinued. Hence, the small number of controls included in this report, which primarily should be used for qualitative, not quantitative, purposes. Consequently, their data is included in the text, but not in the tables, in order to not mislead the reader that somehow their data is to be used quantitatively.

Comparisons of baseline and ACTH-stimulated hormone profiles in PCOS patients before and after oophorectomy are presented in Tables 1 and 2. In our PCOS subjects the levels of total and free T, and E1, decreased and FSH increased significantly following oophorectomy. No significant differences in E2, DHEAS or SHBG levels were detected (Table 1). Baseline levels (A_{40}) and ACTH(1–24)-stimulated (A_{460}) levels of A4 decreased significantly with oophorectomy; alternatively, the net increment in A4 levels (ΔA_4) increased (Table 2). No significant differences in $DHEA_{40}$, $DHEA_{60}$ or $\Delta DHEA$ were observed with oophorectomy. Furthermore, no changes in F_0 levels were detected; although F_{60} and ΔF tended to be higher post-operatively, the differences did not reach statistical significance (Table 2). As in the PCOS patients, the controls studied demonstrated few changes in adrenal response, with the exception that baseline cortisol levels decreased ($19.2 \pm 3.8 \text{ } \mu\text{g/dl}$ vs. $7.9 \pm 1.6 \text{ } \mu\text{g/dl}$, $p=0.026$) and peak (60 min.) ACTH-stimulated DHEA levels increased ($2.5 \pm 0.5 \text{ ng/ml}$ vs. $3.4 \pm 0.7 \text{ ng/ml}$, $p=0.0143$) with oophorectomy.

Finally, with few exceptions, no differences were observed when we subdivided the PCOS cohort into those with DHEAS values above (Hi-DHEAS n=7) and below (Lo-DHEAS n=7) the median DHEAS value ($92.3 \text{ } \mu\text{g/dl}$). The exception was, as before, the baseline A4, which decreased with surgery in both Hi-DHEAS and Lo-DHEAS PCOS patients (A4 pre vs. A4 post in Hi-DHEAS PCOS: $1306.5 \pm 284.9 \text{ pg/ml}$ vs. $682.6 \pm 148.9 \text{ pg/ml}$, $p=0.0236$; and A4 pre vs. A4 post in Lo-DHEAS PCOS: $1112.3 \pm 242.6 \text{ pg/ml}$ vs. $498.0 \pm 108.6 \text{ pg/ml}$, $p=0.0057$).

A post-hoc power analysis was performed to determine the number of subjects that would have been necessary to establish a difference between the pre and post-oophorectomy levels of DHEA (the most representative of the adrenal androgens), if a difference was present. Our data indicated that at an 80% power, the number of subjects required to establish an absolute difference in baseline (0 min.) and stimulated (60 min.) DHEA levels would have been 594 and 193, respectively. Alternatively, if a 25% difference is considered to be clinically relevant, the number of subjects necessary to detect a difference, with an 80% power, is only 8 and 10 (for baseline and stimulated DHEA levels, respectively). Overall, these data suggest that absolute differences in these values pre and post-oophorectomy, if any, are very small. These data also indicate that our study has sufficient power to detect a clinically relevant difference (i.e. 25%), if a difference had existed.

DISCUSSION

Adrenocortical steroidogenesis and AA secretion are exaggerated in PCOS, both basally and following stimulation with ACTH. As AA excess may contribute to the development of PCOS, particularly when occurring during puberty (12), identifying the underlying causes is of key clinical importance. Previous studies have indicated that AA excess in PCOS is not the result of 21-OH, 3 β -HSD, or 11-OH deficient NCAH; or of heterozygosity for mutations of CYP21 (13–15). Further, AA excess in PCOS is not the result of altered sensitivity of the pituitary to CRH, or of the adrenal cortex to ACTH (16). To determine whether adrenocortical dysfunction in PCOS results from ovary-secreted products, excluding estrogen, we examined the effects of oophorectomy on AA secretion in women with PCOS.

We found that patients experienced the expected decreases in ovarian androgen levels (TT, FT, and E1), and increases in FSH levels, following oophorectomy. However, there was no effect on basal DHEAS levels or DHEA levels, or on the secretion of DHEA in response to ACTH stimulation. This group of patients studied was older, as would be expected for women undergoing an elective oophorectomy, and hence had lower DHEAS levels, as previously reported (17). Nonetheless, as we and others have reported, the relative level of adrenal androgen secretion remains the same over time (18–20), we also compared women with higher DHEAS (above the median) to lower DHEAS (below the median) levels and still found no difference in the adrenal response to oophorectomy. However, it is possible that an ovarian effect may be observable in PCOS patients at the highest levels of DHEAS excess, as suggested by some investigators exploring the effects of medical oophorectomy (21).

Baseline A4 levels decreased by ~50% with oophorectomy, as would be expected since ~50% of circulating A4 is the product of ovarian theca cells. A lower post-oophorectomy baseline A4 level resulted in lower post-surgery peak (60 min) A4 levels. However, the post-oophorectomy peak A4 value was only 26% (and not 50%) lower than pre-oophorectomy levels, a difference accounted for by a greater production of A4 by the adrenal cortex in response to ACTH stimulation (i.e. a greater responsivity of the adrenal for A4) with surgery. The cause for this finding is unclear. It may represent simply an artifact of small numbers, or may suggest that the adrenal cortex is able to compensate in some manner for the decrease in ovarian A4 production, paralleling that observed for F. Further studies will be needed to assess this question. However, these findings do not support a major role for ovarian factors in the AA excess of PCOS.

We did find a trend towards increased ACTH-stimulated F and net response levels following oophorectomy. The increased F production cannot be related to development of hypoestrogenic vasomotor symptoms, as these differences in cortisol secretion with ACTH occurred despite the administration of transdermal E2. F metabolism has been suggested to be accelerated in PCOS, and that this dysregulation may play a role in the development of AA excess (22). Increased F metabolism in PCOS may result from enhanced F inactivation by 5 α -reductase (5 α -RA) type 1, and decreased activity of 11 β -hydroxysteroid dehydrogenase (11 β -HSD) type 1, which catalyzes the conversion of the weaker glucocorticoid cortisone to the stronger F (22). Our results suggest that ovarian factors may play a role, albeit minor, in the altered F metabolism observed in some PCOS patients.

Some investigators (23, 24), but not all (25), evaluating the effect of ovarian suppression with a long-acting GnRH analogue reported decreased DHEAS levels, suggesting an effect of the ovary on the adrenal in PCOS. However, we have observed that T administration does not alter the response of A4, DHEA, or F to ACTH stimulation, although it appears to increase the sulfation of DHEA to DHEAS (3). In cadaveric adrenocortical minces, T has no

predictable effect on the ACTH-stimulated production of DHEA or F (4). In addition, while T increases the production of DHEA in adrenocortical cell lines, it decreases DHEAS secretion (5). Our current finding that DHEAS and DHEA levels, and ACTH-stimulated DHEA secretion, are unchanged following oophorectomy are consistent with these results, and suggest that ovarian factors play a limited role in the adrenocortical dysfunction and adrenal androgen excess of PCOS.

Of note, the fall in testosterone levels, along with a limited change in circulating estrogen levels, was not associated with any significant change in SHBG levels. This likely reflects the relatively limited role that T levels within the physiologic range play (as opposed to insulin) in modifying SHBG levels (26–29). Experiments demonstrating the suppressive effect of T on SHBG levels primarily used supraphysiologic doses of exogenous androgens (30, 31).

This study has potential limitations. Firstly, as noted previously, these women are somewhat older than the average PCOS patient, and thus have lower DHEAS levels (17). Nonetheless, no difference in response was observed when comparing women with higher vs. lower DHEAS levels. Furthermore, if we use data published by our laboratory (32), we can note that 64% of PCOS subjects demonstrated prestudy screening DHEAS levels (data not shown) above the upper normal limit of controls ages 35–45 years.

Secondly, we preferred to avoid the effect of stress associated with hypoestrogenic vasomotor flushing and insomnia on adrenocortical function by administering transdermal estrogen. However the use of this medication precluded analysis of the effect of this steroid on adrenal function, although this was not part of the study's aim. Furthermore, the effect of changes in metabolic parameters on adrenal function was not assessed, although as the tests were performed within a short period of time and it is unlikely that oophorectomy alone resulted in major changes in metabolic status. Other investigators have noted that oophorectomy alone or with postoperative transdermal estradiol did not alter circulating insulin values or insulin resistance assessed by the homeostasis model assessment method (HOMA-IR) (33).

Finally, the number of controls (healthy women) included is small, and serve only for illustrative (qualitative) purposes, although in this study the PCOS subjects essentially acted as their own controls for the question at hand. We should note that the principal value of this small number of controls (whose recruitment was truncated since the findings in the PCOS women were negative) is the observation that the changes in adrenal response with oophorectomy observed were relatively similar, with few exceptions, with those observed in our PCOS subjects.

In conclusion, we were unable to demonstrate a major ovarian effect on the adrenocortical function in PCOS, excluding any effect there may be from the resulting hypoestrogenemia, consistent with our previous studies using gonadotropin suppression, although it is possible that such an effect may be observable in women with the highest levels of DHEAS. Paradoxically, oophorectomy in PCOS women resulted in an exaggerated, not suppressed, response of A4, and possibly F, to ACTH stimulation. These data suggest that the adrenocortical dysfunction observed in a fraction of PCOS women is likely a primary, possibly inherited, factor (34).

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Figure 1.

Scattergram showing raw untransformed data of 0 min., and 60 min. DHEA values, pre and post oophorectomy (p value >0.05 for all pre- vs. post-oophorectomy comparisons).

Table 1

Comparison of pre- and post-operative hormonal levels in PCOS subjects.

Steroid	Before BSO		After BSO		Direction	P value
	mean	CV(%)	mean	CV(%)		
Total T (ng/dl)	37.0	6.8	19.86	4.8	↓	0.0061
Free T (pg/ml)	8.21	7.6	4.54	3.0	↓	0.0159
E1 (pg/ml)	96.4	4.8	69.60	5.1	↓	0.0170
E2 (pg/ml)	100.7	8.4	78.47	9.1	→	NS
FSH (mIU/ml)	3.77	3.4	10.53	10.9	↑	0.0014
DHEAS (μg/dl)	84.6	6.9	84.75	7.1	→	NS
SHBG (nmol/L)	32.5	6.3	30.29	6.0	→	NS

Values depicted represent the anti-log of the geometric means based on the log scale used for analysis

Abbreviations: E1 is estrone, E2 is estradiol, T testosterone, and SHBG is sex hormone-binding globulin; and CV(%) is percent coefficient of variation, equal the SD divided by the mean (x 100).

Table 2

Comparison of pre- and post-operative basal and ACTH-stimulated hormonal levels in PCOS subjects.

Steroid	Before BSO		After BSO		Direction	P value
	mean	CV(%)	mean	CV(%)		
Baseline						
DHEA ₀ (ng/ml)	2.62	2.6	2.55	5.7	→	NS
A4 ₀ (pg/ml)	1205.5	6.4	583.06	5.2	↓	0.0005
F ₀ (μg/dl)	11.0	6.3	10.27	4.5	→	NS
Stimulated						
DHEA ₆₀ (ng/ml)	6.80	5.1	6.42	5.6	→	NS
A4 ₆₀ (pg/ml)	1464.3	9.3	1158.6	4.3	↓	0.0419
F ₆₀ (μg/dl)	26.8	6.9	33.1	2.0	↑	0.0803
Ratios of net increments						
Δ DHEA	2.59	5.0	2.52	5.5	→	NS
Δ A4	1.21	9.2	1.99	6.2	↑	0.0026
Δ F	2.45	8.3	3.23	5.0	↑	0.0527

Values depicted for baseline and for stimulated values represent the anti-log of the geometric means based on the log scale used for analysis. Values depicted for ratios of net increments represent the ratios of the 60 min/base values obtained using the log scale 60 min value minus base value.

Abbreviations: A4 is androstenedione; BSO is bilateral salpingo-oophorectomy; DHEA is dehydroepiandrosterone; F is cortisol; Steroid₀ represents baseline level; Steroid₆₀ represents post-ACTH stimulation level; and ΔSteroid represents the net change in steroid levels in response to acute ACTH(1–24) stimulation, i.e. basal minus stimulated level; and CV(%) is percent coefficient of variation, equal the SD divided by the mean (x 100).