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Stability of Adrenocortical Steroidogenesis over Time in Healthy Women and Women with Polycystic Ovary Syndrome

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Adrenocortical secretion is up-regulated in women with polycystic ovary syndrome (PCOS), and absolute adrenal androgen (AA) excess is evident in ~25% of these patients. We hypothesized that AA biosynthesis is an inherited trait and that, as for other inherited traits, AA biosynthesis remains stable over time. To test this hypothesis, we prospectively studied 23 off-treatment PCOS patients and seven age- and body mass index-matched control women on two separate occasions 3–5 yr apart (45.0 ± 19.0 months and 47.4 ± 21.3 months, respectively; $P > 0.05$). All subjects underwent an acute adrenal stimulation using 0.25 mg ACTH-(1–24), and dehydroepiandrosterone (DHEA), androstenedione, and cortisol (F) were measured 0 and 60 min post ACTH; basal levels of total and free testosterone (T), SHBG, and DHEA sulfate (DHEA-S) were also assessed. Among PCOS patients, the mean DHEA-S levels during the repeat study were significantly lower when compared with the initial assessment (170 ± 107 μg/dl *vs.* 134 ± 79 μg/dl, respectively; $P = 0.02$). However, only patients with initial DHEA-S levels above the median (high DHEA-S) experienced a net decrease in the levels of this metabolite (252.5 ± 99.2 μg/dl *vs.* 174.3 ± 82.5 μg/dl; $P = 0.001$) over the time of the study; patients with initial DHEA-S levels in the lower half (low DHEA-S) did not experience a change in DHEA-S (94.6 ± 28.9 μg/dl *vs.* 97.7 ± 56.5 μg/dl; $P = 0.85$).

In patients, the total T levels tended to be higher at the second study, although SHBG levels were also higher, resulting in unchanged free T levels over time. Among controls, no significant changes in basal androgens were observed over the time of the study. There were no significant differences in either the basal or ACTH-stimulated levels of DHEA, androstenedione, or F over the time of the study in either PCOS or control women.

We conclude that the adrenocortical secretion of AAs or F in PCOS and control women remains stable over time, supporting the hypothesis that the adrenal response to ACTH may be an inherited trait. Alternatively, a decrease in DHEA-S levels over time was observed but only among PCOS patients whose initial levels of this metabolite were above the group median, suggesting that the activity of sulfotransferase in these patients may be up-regulated by factors other than those affecting adrenocortical biosynthesis and that such regulatory influences attenuate over time. (*J Clin Endocrinol Metab* 89: 5558–5562, 2004)

POLYCYSTIC OVARY SYNDROME (PCOS) is a common endocrine disorder of reproductive-aged women, with an estimated prevalence of 6–7% (1–3). The syndrome represents a complex trait with clinical and biochemical heterogeneity (4). Adrenocortical secretion in PCOS is up-regulated (5–9), and absolute adrenal androgen (AA) excess is evident in about 25% of these patients (10). Whether adrenal secretion in response to ACTH in PCOS represents an inherited trait or whether it simply reflects the effects of extraadrenal factors remains unclear.

In normal women, circulating AA levels and their response to ACTH are highly individualized compared with their secretion of glucocorticoids (11). For example, both basal and ACTH-stimulated levels of dehydroepiandrosterone (DHEA) demonstrated a high level of between-subject variability (~60–70%) compared with the between-subject variability for cortisol (F; ~15–40%) (11). Supporting the concept that AA secretion is highly individualized and perhaps genetically predetermined, various investigators have observed a significant degree of heritability for the AA metabolite DHEA sulfate (DHEA-S) (12), with the relative basal levels of DHEA and its metabolite DHEA-S varying little over time (13, 14). These data suggest that AA secretion is potentially an inherited trait and may represent an inherited risk factor for PCOS. For example, girls with premature pubarche and exaggerated AA secretion appear to be at increased risk for developing PCOS in adulthood (15).

Taken together, these data suggest that AA secretion is an inherited trait in PCOS and may represent a risk factor for PCOS. We hypothesized that, as for other inherited traits, AA biosynthesis remains stable over time. To test this hypothesis, we prospectively studied PCOS patients and matched controls on two separate occasions approximately 3–5 yr apart, assessing the basal AA levels and their response to ACTH stimulation.

Abbreviations: A4, Androstenedione; AA, adrenal androgen; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; F, cortisol; NCAH, nonclassic adrenal hyperplasia; PCOS, polycystic ovary syndrome; T, testosterone.

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Subjects and Methods

Subjects

We studied 23 off-treatment PCOS patients and seven healthy matched controls. The diagnostic criteria for PCOS included hyperandrogenism and/or hyperandrogenemia; oligoovulation; and exclusion of other known disorders such as Cushing's syndrome, hyperprolactinemia, congenital nonclassic adrenal hyperplasia (NCAH), hypothyroidism, or hyperprolactinemia (16). Clinical hyperandrogenism was defined as the presence of hirsutism (*i.e.* modified Ferriman-Gallwey score ≥ 6) (17). Hyperandrogenemia was defined as a total and/or free testosterone (T), and/or androstenedione (A4) level above the upper 95th percentile of 98 healthy nonhirsute eumenorrheic women, as previously reported (18). Specifically, the upper normal limits were as follows: total T, 84.7 ng/dl (2.94 nmol/liter); free T, 0.75 ng/dl (0.026 nmol/liter); and A4, 2.5 ng/ml (8.73 nmol/liter). Ovulatory dysfunction was defined as menstrual cycles greater than 45 d in length or, if vaginal bleeding occurred at less than 45-d intervals, by a midluteal-phase progesterone level of less than 4 ng/ml (12.7 nmol/liter). We excluded hyperprolactinemia and thyroid dysfunction by the measurement of a basal prolactin and TSH, respectively, and we excluded NCAH by the measurement of a basal 17-hydroxyprogesterone measure of less than 2 ng/ml (6.0 nmol/liter) and Cushing's syndrome and androgen-secreting tumors using clinical and hormonal parameters.

Seven healthy eumenorrheic women without evidence of hyperandrogenism or endocrine disorders were recruited as controls. None of the study subjects were taking any hormonal medication including oral contraceptive pills for at least 3 months before the study. Groups were matched for age and body mass index.

The study was approved by the Institutional Review Board of the University of Alabama at Birmingham, and all subjects gave written informed consent.

Clinical protocol

All subjects underwent acute adrenal stimulation twice, approximately 3–5 yr apart. Patients were off medication for at least 3 months before the tests. Acute adrenal stimulation was performed as previously described (19). In brief, all studies were performed between 0800 and 1030 h after an overnight fast in the follicular phase (d 3–8) of the menstrual cycle. Dexamethasone was not administered before the study to assess the resting basal steroid levels. Three baseline samples were obtained 15 min apart and mixed to form the 0 min (basal) sample. Immediately afterward, 0.25 mg ACTH-(1–24) (Cortrosyn; Organon Co., Orange, NJ) was administered iv over 60 sec, and blood was sampled 60 min later. Serum was separated and stored at −70 C until assayed.

Assays

The initial and repeat test samples from an individual subject were assayed in the same run to minimize the effect of interassay variability. A4, DHEA, and total T were quantified by previously described validated RIAs (20–23). Before RIA, steroids were extracted from serum with ethyl acetate-hexane (3:2). A4, DHEA, and T were then separated by Celite column partition chromatography, using ethylene glycol as stationary phase. The three androgens were eluted in 0, 15, and 35% toluene in isooctane, respectively. The sensitivities of these RIAs were 10, 20, and 10 pg/ml, respectively. The intraassay and interassay coefficients of variation ranged from 3.0–6.7% and 7.3–13.2%, respectively.

DHEA-S and F were measured by direct, sold-phase, competitive, chemiluminescent enzyme immunoassays, and SHBG was quantified by a solid-phase, two-site chemiluminescent immunoassay using the Immulite analyzer (Diagnostic Products Corporation, Inglewood, CA). The sensitivities of these assays were 30 and 2 ng/ml and 0.2 nmol/liter, respectively. The interassay coefficients of variation were 8.1, 7.6, and 6.2%, respectively.

The calculation of free and bioavailable T was based on use of an algorithm derived from equations described by Sodergard *et al.* (24) and Vermeulen *et al.* (25). Reliability of the published method has been reported (26).

Data analysis

All parameters are given as mean ± SD. Within-group comparisons for the initial and repeat tests were performed using the paired *t* test. Pearson test was used for correlation analyses. $P < 0.05$ was considered statistically significant. Data analysis was performed using the SAS 9.0 statistical software (SAS Institute, Inc., Cary, NC).

Results

Clinical and hormonal characteristics of PCOS and control women

The baseline characteristics of the subjects are shown in Table 1. There were no differences between controls and PCOS patients in mean initial age, body mass index, mean interval between the initial and repeat tests, and mean DHEA-S, SHBG, and F levels. The mean levels DHEA, A4, total T, and free T were significantly higher among PCOS women than control women.

Changes in basal androgen levels over time

The basal androgen levels at the initial and repeat studies are depicted in Table 2. Among controls, no significant change in androgens occurred over the interval of the study. Alternatively, among PCOS patients, the mean total T levels tended to be higher at the repeat study. However, the mean SHBG levels also increased, resulting in unchanged free T over time.

Among PCOS patients, the mean DHEA-S levels during the repeat test were significantly lower when compared with the initial test (Table 2). However, only patients with initial DHEA-S levels above the median (high DHEA-S) experienced a net decrease in the levels of this metabolite from their initial to repeat study (252.5 ± 99.2 μg/dl *vs.* 174.3 ± 82.5 μg/dl, respectively; $P = 0.001$); patients with initial DHEA-S levels in the lower half (low DHEA-S) did not experience a change in DHEA-S (94.6 ± 28.9 μg/dl *vs.* 97.7 ± 56.5 μg/dl, respectively; $P = 0.85$). As before, there was no change over time in basal or ACTH-stimulated DHEA levels, even when low DHEA-S and high DHEA-S PCOS patients were considered separately (data not shown).

Next, we determined whether the differences observed

TABLE 1. Baseline clinical and hormonal characteristics of PCOS and control women

	PCOS (n = 23)	Controls (n = 7)	*P*
Age (yr)	29.3 ± 8.1	32.0 ± 7.2	NS
BMI (kg/m^2)	33.5 ± 6.3	31.8 ± 8.3	NS
Mean interval between studies (months)	45.0 ± 19.0	47.4 ± 21.3	NS
Total T (ng/dl)	58.2 ± 25.9	33.2 ± 6.7	0.01
SHBG (nmol/liter)	24.1 ± 12.2	36.4 ± 14.6	NS
Free T (ng/dl)	1.59 ± 0.66	0.73 ± 0.14	<0.001
DHEA-S (μg/dl)	170 ± 107	99 ± 47	NS
DHEA (ng/ml)	5.3 ± 3.5	2.8 ± 1.0	0.01
A4 (ng/ml)	2.0 ± 0.9	1.0 ± 0.2	0.002
F (μg/dl)	11.0 ± 5.3	9.6 ± 2.7	NS

Data are presented as mean ± SD. Due to the small number of controls, nonparametric analysis was performed using the NPAR1WAY procedure for the median one-way analysis. NS (not significant) is $P > 0.05$. BMI, Body mass index. Conversion to SI units, total and free T × 3.467 (nmol/liter), DHEAS × 0.02714 (μmol/liter), DHEA × 3.47 (nmol/liter), A4 × 3.49 (nmol/liter), F × 27.59 (nmol/liter).

TABLE 2. Basal androgen levels in PCOS and control women: a comparison of the initial and repeat study results

	PCOS (n = 23)			Controls (n = 7)		
	Initial	Repeat	*P*	Initial	Repeat	*P*
Total T (ng/dl)	58.2 ± 25.9	70.8 ± 41.3	0.052	33.2 ± 6.7	33.1 ± 10.8	NS
SHBG (nmol/liter)	24.1 ± 122	29.8 ± 19.0	0.04	36.4 ± 14.6	33.9 ± 13.2	NS
Free T (ng/dl)	1.59 ± 0.66	1.82 ± 1.14	NS	0.73 ± 0.14	0.75 ± 0.21	NS
DHEA-S (μg/dl)	170 ± 107	134 ± 79	0.02	99 ± 47	128 ± 45	NS

Data are presented as mean ± SD. Comparisons were performed using the paired *t* test. NS, Not significant.

TABLE 3. Basal and ACTH-stimulated[a] steroid levels in PCOS and control women: a comparison of the initial and repeat stimulation results

	PCOS (n = 23)			Controls (n = 7)		
	Initial	Repeat	*P*	Initial	Repeat	*P*
$DHEA_0$ (ng/ml)	5.3 ± 3.5	4.7 ± 2.7	NS	2.8 ± 1.0	2.6 ± 1.1	NS
$DHEA_{60}$ (ng/ml)	11.8 ± 7.3	10.5 ± 4.6	NS	7.1 ± 3.5	6.5 ± 3.7	NS
$A4_0$ (ng/ml)	2.0 ± 0.9	2.0 ± 1.2	NS	1.0 ± 0.2	0.9 ± 0.3	NS
$A4_{60}$ (ng/ml)	2.7 ± 1.3	2.8 ± 1.9	NS	1.5 ± 0.5	1.3 ± 0.4	NS
F_0 (μg/dl)	11.0 ± 5.3	9.0 ± 4.6	NS	9.6 ± 2.7	7.8 ± 1.4	NS
F_{60} (μg/dl)	27.1 ± 8.0	29.7 ± 5.5	NS	30.4 ± 10.9	27.5 ± 9.1	NS

Data are presented as mean ± SD. Comparisons were performed using the paired *t* test. NS, Not significant.
[a] Stimulated hormone levels ($steroid_{60}$) were measured 60 min after administration of 0.25 mg ACTH-(1–24).

over time between low DHEA-S and high DHEA-S PCOS patients was possibly due to differences in age or duration of study interval. There was no correlation between the duration of study interval or the initial age of the subjects and the net change in DHEA-S over time (r = 0.353, $P = 0.116$, and r = 0.221, $P = 0.378$; respectively). Furthermore, low DHEA-S and high DHEA-S PCOS patients did not differ in initial age (30.4 ± 5.0 yr *vs.* 28.0 ± 10.7 yr, respectively; $P = 0.55$). Because there was a trend toward a longer interval of study for high DHEA-S compared with low DHEA-S PCOS patients (53.2 ± 18.5 months *vs.* 37.3 ± 18.8 months, respectively; $P = 0.058$), we compared the net change in DHEA-S per month of study between the groups. The previously observed differences between the groups remained, such that low DHEA-S patients experienced a net gain in DHEA-S levels, whereas high DHEA-S PCOS patients experienced a decrease (1.67 ± 1.24 μg/dl *vs.* −0.73 ± 2.29 μg/dl, respectively; $P = 0.008$).

We performed Pearson correlation analyses to assess whether there was a relationship between changes in T and DHEA-S levels. The changes in these hormones were not correlated in PCOS patients as a whole (r = −0.21, $P = 0.27$) or in low DHEA-S or high DHEA-S PCOS patients (r = 0.38, $P = 0.23$; and r = −0.37, $P = 0.26$, respectively).

Comparison of the basal and ACTH-stimulated steroid levels over time

There were no significant differences in any of the basal or stimulated levels of DHEA, A4, or F over the duration of the study in either PCOS or control women (Table 3). To assess the relative variance of the response to ACTH stimulation, we determined the number of repeat ACTH stimulation tests of which the results were on the same side of the median as the initial study. For the vast majority of parameters studied, the repeat stimulation results fell on the same side of the median as the first results more than 50% of the time (Table 4).

The robustness of our data were supported by a power analysis indicating that, with an α level of 0.05 and a β level of 0.20, between 450 and 660 controls and between 350 and 430 PCOS women would need to be studied to detect a difference in either the basal or ACTH-stimulated DHEA levels.

TABLE 4. The number of repeat ACTH stimulation results on the same side of the median in PCOS and control women[a]

Hormone	PCOS (%)	Controls (%)
DHEA-S	14 (66.7)	4 (66.7)
$DHEA_0$	16 (80.0)	3 (50.0)
$DHEA_{60}$	15 (71.4)	4 (66.7)
$A4_0$	14 (73.7)	3 (60.0)
$A4_{60}$	15 (75.0)	5 (71.4)
F_0	13 (61.9)	7 (100.0)
F_{60}	15 (68.2)	3 (50.0)

Stimulated hormone levels ($steroid_{60}$) were measured 60 min after administration of 0.25 mg ACTH-(1–24).
[a] Same side of the median indicates that the hormone value is either below or above the group median in both initial and repeat tests. Test results that equaled the median were excluded from the analysis.

Discussion

We hypothesized that AA biosynthesis is an inherited trait in PCOS and that, as for other inherited traits, the AA biosynthesis remains stable over time. To test this hypothesis, we prospectively studied 23 PCOS patients and seven matched controls on two separate occasions approximately 3–5 yr apart. Our data indicate that the adrenocortical secretion of AAs or F in both PCOS and control women remains stable over time, supporting the hypothesis that the adrenal response to ACTH may be an inherited trait. Alternatively, a decrease in DHEA-S levels over time was observed but only among PCOS patients whose initial levels of this metabolite were in the upper half of such values, suggesting that the activity of sulfotransferase in these patients may be under different regulation than the adrenocortical secretion of AAs and that such regulatory influences attenuate over time.

Increasing evidence suggests that inheritance plays a sig-

nificant role in determining the circulating AA levels in normal individuals (12, 27–29). Previous studies have also shown that basal and ACTH-stimulated F levels appear to have significant heritability (28, 30). In agreement, our data indicate that F secretion, basally and in response to acute ACTH stimulation, remains highly stable in both PCOS and healthy women. In addition, notwithstanding the previously reported high degree of between-subject variance in DHEA levels (12), the secretion of DHEA and A4 remained relatively unchanged over the approximately 4 yr of our study. The high degree of stability over time of adrenocortical secretion, basally or in response to ACTH, is consistent with its being an inherited trait, although this remains to be determined in specific family studies. Previous family studies examining siblings of PCOS patients suggested that ovarian hyperandrogenism (31) and defects in insulin action (32) appear to be genetically inherited. Moreover, the preliminary findings of increased DHEA-S levels in sisters (31) and brothers (33) of PCOS patients pave the way for specific family studies to test the hypothesis that adrenal androgen secretion is also genetically inherited in PCOS.

Circulating DHEA-S levels are used as a marker of AA production (34). However, it is important to note that DHEA-S is an AA metabolite and its levels could change due to alterations in sulfotransferase activity without concomitant changes in AA production. For example, we (35) and others (36) have reported that DHEA-S levels are not invariably elevated in patients with NCAH. Evidence of this discordance between AA production and DHEA-S levels was also observed in the present study where the levels of this metabolite decreased over the study period in PCOS patients who initially had DHEA-S levels above the median (labeled as high DHEA-S) without concomitant change in AA secretion, either basally or in response to ACTH, in these patients. It was also evident that the observed decrease in DHEA-S levels among high DHEA-S PCOS patients was not due to differences in mean age or duration of study.

For example, when oophorectomized women are treated with exogenous T, the DHEA-S to DHEA ratio increases without an accompanying change in the adrenocortical secretion of DHEA or A4 in response to ACTH stimulation (37). In addition, PCOS women treated with GnRH analog suppression experienced a decrease in basal DHEA-S levels without accompanying changes in basal DHEA levels or the steroid response to ACTH stimulation (38). *In vitro* studies also support the dichotomy of DHEA-S and DHEA production. For example, the production of DHEA-S, in contrast to that of DHEA, was highly affected by the type of tissue preparation (adrenocortical slices, minces, or cell suspensions) (39). *In vitro* insulin within physiologic levels increased DHEA-S production while alternatively decreasing the secretion of DHEA (40). Consequently, caution should be used in using DHEA-S as a marker of AA secretion because AA metabolism (*e.g.* via DHEA sulfotransferase) may be altered by factors that do not affect AA biosynthesis.

That DHEA-S decreased primarily in PCOS patients with high DHEA-S levels, but not in controls or in PCOS patients with lower DHEA-S levels, is supported by previous studies documenting the effect of GnRH analogs on circulating DHEA-S levels. For example, in a study of 18 PCOS patients, we observed that patients with high DHEA-S levels had a higher net decrease in basal DHEA-S and DHEA levels with treatment with a GnRH analog for 6 months compared with PCOS women with normal DHEA-S levels. Our results are in agreement with those of other investigators using a similar study design (41). Presumably, sulfotransferase in PCOS patients with higher DHEA-S levels is under greater stimulation by extraadrenal factors, including ovarian androgens, compared with PCOS patients with normal or low DHEA-S values. Although the baseline and repeat studies were performed when patients were off medication for at least 3 months, it is possible that intervening therapies could have served to reduce these effects on the production of DHEA-S, including oral contraceptives (42), spironolactone (43), and insulin sensitizers (44).

One potential limitation of our study is sample size. However, power analysis for detecting a difference in $DHEA_0$ or $DHEA_{60}$ singly, with an α level of 0.05 at a power of 0.80, indicated that between 450 and 660 controls and between 350 and 430 PCOS women would be needed. The high number of subjects required to demonstrate a difference between the first and repeat tests, taking into account present data, supports the robustness of the conclusions obtained. Another potential limitation is the length of the time of the study. Although the approximately 4-yr interval of the study is one of the longest reported in the literature, it is also possible that longer periods of observation would have demonstrated differences in adrenocortical steroidogenesis. However, we should also note that intervals much greater than that of this study would have introduced the age-related decline in AA production as a confounder.

We conclude that the adrenocortical secretion of AAs or F in PCOS and control women remains stable over time. The high degree of stability of adrenocortical secretion over time, basally or in response to ACTH, is consistent with its being an inherited trait, although this remains to be determined in specific family studies. Alternatively, a decrease in DHEA-S levels over time was observed but only among PCOS patients whose initial levels of this metabolite were above the group median. This suggests that the activity of sulfotransferase in these patients may be up-regulated by factors other than those affecting adrenocortical biosynthesis (*e.g.* ovarian androgens) and that such regulatory influences attenuate over time.

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