Androgen responses to adrenocorticotropic hormone infusion among individual women with polycystic ovary syndrome

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Objective: To compare androgen responses during ACTH infusion among women with polycystic ovary syndrome (PCOS) and healthy women.
Design: Cross-sectional study.
Setting: Academic medical center.
Patient(s): Women with PCOS (n = 13) and healthy controls (n = 15).
Intervention(s): Blood samples were obtained frequently during a 6-hour dose-response ACTH infusion.
Main Outcome Measure(s): Comparison of basal and stimulated levels of 17α-hydroxyprogesterone (17-OHP), androgens, and cortisol (F) during ACTH infusion with those after hCG injection within individual subjects.
Result(s): In women with PCOS increased 17-OHP, androstenedione (A), and DHEA responses during ACTH infusion were comparable to those observed in healthy controls. The magnitude of responses was highly variable among women with PCOS. Within individual women with PCOS adrenal responses to ACTH and ovarian responses to hCG were significantly correlated. Cortisol responses to ACTH were similar in women with PCOS and healthy controls.
Conclusion(s): Within individual women with PCOS, enhanced androgen responses to ACTH are accompanied by comparable androgen responsiveness to hCG. These findings suggest that dysregulated steroidogenesis leading to hyperandrogenemia in this disorder is likely present in both adrenal and ovarian tissues.
Clinical Trial Registration Number: NCT00747617. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: 17-OHP, androgen, ACTH, polycystic ovary syndrome

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One of the hallmark features of polycystic ovary syndrome (PCOS) is excess androgen production. It has been well established that the primary source of androgen overproduction in women with PCOS is the ovary (1–3). The contribution from the adrenal has varied from 20%–50% (4–7). In addition, adrenal androgen production has not been particularly associated with ovarian androgen excess in this disorder. Recently, it was demonstrated that androgen responses to gonadotropin stimulation were exaggerated in some women with PCOS, whereas in other women androgen responses were similar to that of healthy women (8). Of note, androstenedione (A) and DHEA responses to ACTH stimulation did not distinguish between exaggerated and normal responder women with PCOS. By comparison, Ehrmann et al. (9, 10) reported that in hyperandrogenic women with exaggerated ovarian androgen responses to gonadotropin stimulation, 57% had functional adrenal hyperandrogenism based on ACTH-dependent 17-ketosteroid excess, whereas 43% had normal responses. Conversely, in hyperandrogenic women with normal gonadotropin-stimulated androgen responses, 59% had hyperresponsiveness to ACTH and 41% exhibited normal responses. These findings

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underscore variable androgen production by the adrenal, much like that reported for ovarian androgen production in women with PCOS. In addition, ovarian hyperandrogenemia may arise from an inherent defect of theca cell steroidogenesis, which incriminates a similar dysfunction of adrenal androgen production in this disorder. To examine whether excess androgen production by the ovary is associated with altered androgen production by the adrenal within individuals, we used a 6-hour dose-response ACTH infusion in women with PCOS and healthy women who had previously undergone hCG stimulation, as reported previously (11).

MATERIALS AND METHODS

Subjects

There were 13 women with PCOS and 15 women with regular menstrual cycles recruited for the study. All individuals with PCOS were oligomenorrheic or amenorrheic and demonstrated either biochemical or clinical evidence of hyperandrogenism. All study participants underwent a three-dimensional pelvic ultrasound. Patients with PCOS demonstrated evidence of bilaterally enlarged ovaries with >12 antral follicles per ovary. Circulating TSH and PRL levels were within the normal range and not significantly different between the two groups of participants. Congenital adrenal hyperplasia was excluded based on a basal serum 17α-hydroxyprogesterone (17-OHP) of <2 ng/mL. No participant received any hormone medication or metformin within 2 months of study enrollment. The study was approved by the Human Research Protection Program at the University of California, San Diego, and written informed consent was obtained for each individual before participation.

Procedures

Subjects were admitted to the Clinical and Translational Research Institute at the University of California, San Diego, on the day of hCG stimulation. Healthy subjects were studied during the midfollicular phase (cycle days, 5–8), whereas patients with PCOS were anovulatory and studied on a random day. The 17-OHP responses to recombinant hCG in 13 women with PCOS and 14 healthy controls in this study have been previously reported (11). Briefly, each subject received IV administration of recombinant hCG (25 μg). Blood samples were collected before and 24 hours after the recombinant hCG injection.

Adrenal stimulation was performed in a subsequent month on the same patient. All study participants were instructed to begin fasting the midnight before the planned study day, and received 1 mg dexamethasone at 11 PM the night before and at 7 AM on the morning of the study. On the day of study, an infusion of ACTH was initiated at 8 AM with a starting rate of 0.1 μg/hr, and increased at hourly intervals (0.25, 1, 2.5, 10, and 25 μg/hr) during a 6-hour period. Baseline serum was obtained and subsequent blood sampling was performed every 30 minutes for the duration of the infusion. For all portions of the study, none of the subjects with PCOS experienced recent ovulation as evidenced by absence of recent menstrual bleeding for 2 months before study and serum P <1.0 ng/mL in the baseline sample.

Assays

Serum concentrations of LH and FSH were measured by RIA with intra-assay and interassay coefficients of variation (CV) of 5.4% and 8.0%, respectively, for LH and 3.0% and 4.6%, respectively, for FSH (Diagnostic Products Corp). Serum concentrations of E_2, A, T, and DHEA were measured by well-established RIA with intra-assay CV <7%. Serum levels of 17-OHP, P, DHEA, and DHEAS were measured by RIA with intra-assay CV <7% (Diagnostic Systems Laboratories, Inc.). Serum P, DHEAS were measure by RIA (Diagnostic Systems Laboratories, Inc.) with an intra-assay CV less than 7%. The detection limit for T, A, DHEA, and 17-OHP were 3.4, 10.4, 50, and 25 pg, respectively.

Statistics

For continuous data, normal distribution was determined visually using normal quantile plots. For cases where normal distribution was still in question, the Shapiro-Wilk test was used with a W<0.05 establishing non-normal distribution. For normally distributed continuous data, a two-sided Student's *t* test was used to establish statistical significance between two groups. For non-normally distributed continuous data, Wilcoxon ranked sums were used to establish statistical significance between the two groups. To account for body mass index (BMI), analysis of covariance was performed.

To compare the cumulative steroid response to ACTH infusion, the Riemann Sums method was used to approximate the area under the curve. Given baseline differences for steroid level among control and participants with PCOS, we calculated the delta area under the curve by subtracting the baseline from all Riemann Sum measurements. To determine whether there was an association between previously characterized ovarian theca cell responses to hCG and adrenal responses to ACTH infusion, Pearson correlations and *P* values were obtained for comparison between continuous variables.

RESULTS

Clinical Features and Basal Hormone Levels in Women with PCOS and Healthy Controls

There was no difference in mean (±SE) age between women with PCOS and healthy women. There was a trend toward more BMI among women with PCOS but it did not reach statistical significance ($P=.06$). As shown in Table 1, elevated circulating LH, A, T, and 17-OHP levels in women with PCOS were significantly higher compared with those observed for healthy controls. Levels of serum FSH, DHEA, DHEAS, E_2, and F were similar between groups. These comparisons did not change after adjusting for BMI.