Title

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

Journal

Fertility and Sterility, 106(5)

ISSN

0015-0282

Authors

Maas, Kevin H
Chuan, Sandy
Harrison, Evan
et al.

Publication Date

2016-10-01

DOI

10.1016/j.fertnstert.2016.06.039

Peer reviewed

HHS Public Access

Author manuscript

Fertil Steril. Author manuscript; available in PMC 2017 October 01.

Published in final edited form as:

Fertil Steril. 2016 October ; 106(5): 1252–1257. doi:10.1016/j.fertnstert.2016.06.039.

Androgen Responses to ACTH Infusion among Individual Women with Polycystic Ovary Syndrome

Kevin H. Maas, M.D., Ph.D., **Sandy Chuan, M.D.**, **Evan Harrison, M.D.**, **Heidi Cook-Andersen, M.D., Ph.D.**, **Antoni J Duleba, M.D.**, and **R. Jeffrey Chang, M.D.**

Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA 92093

Abstract

Objective—To compare androgen responses during ACTH infusion among women with PCOS and normal women.

Design—Cross-sectional study.

Setting—Research center at an academic medical center.

Participants—Women with PCOS (n=13) and normal controls (n=15).

Interventions—Blood samples were obtained frequently during a 6-hour dose-response ACTH infusion.

Main Outcome Measures—Comparison of basal and stimulated levels of 17-OHP, androgens, and cortisol during ACTH infusion with those following hCG injection within individual subjects.

Results—In women with PCOS increased 17-OHP, A4, and DHEA responses during ACTH infusion were comparable to those observed in normal controls. The magnitude of responses was highly variable among PCOS women. Within individual women with PCOS adrenal responses to ACTH and ovarian responses to hCG were significantly correlated. Cortisol responses to ACTH were similar in PCOS and normal controls.

Conclusion—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.

Keywords

17-OHP; androgen; ACTH; Polycystic Ovary Syndrome

Corresponding author: R. Jeffrey Chang, M.D., Department of Reproductive Medicine, University of California, San Diego, School of Medicine, 9500 Gilman Drive, La Jolla, California 92093-0633, Telephone: (858) 534-8930, Fax: (868) 534-8856, rjchang@ucsd.edu.

Clinical Trial Registration Number: NCT00747617

Disclosure Statement: The authors have nothing to disclose

Introduction

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) while contribution from the adrenal has varied from 20–50% of cases(4–7). Moreover, 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 PCOS women whereas in others androgen responses were similar to that of normal women(8). Of note, androstenedione and DHEA responses to ACTH stimulation did not distinguish between exaggerated and normal responder PCOS women. By comparison, Ehrmann *et al.* 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(9, 10). Conversely, in hyperandrogenic women with normal gonadotropin stimulated androgen responses, 59% had hyperresponsiveness to ACTH and 41% exhibited normal responses. These findings underscore variable androgen production by the adrenal much like that reported for ovarian androgen production in women with PCOS. Moreover, that ovarian hyperandrogenemia may arise from an inherent defect of theca cell steroidogenesis incriminates 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 employed a 6-hour dose-response ACTH infusion in PCOS and normal women who had previously undergone hCG stimulation as reported previously(11).

Subjects and Methods

Subjects

There were 13 women with PCOS and 15 women with regular menstrual cycles recruited for the study. All PCOS individuals were oligo- or amenorrheic and demonstrated either biochemical or clinical evidence of hyperandrogenism. All study participants underwent 3D pelvic ultrasound. Patients with PCOS demonstrated evidence of bilaterally enlarged ovaries with more than 12 antral follicles per ovary. Circulating TSH and prolactin levels were normal and not significantly different between the two groups. Congenital adrenal hyperplasia was excluded based on a basal serum 17-OHP of less than 2 ng/ml. No participant received any hormone medication or metformin within two 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 prior to participation.

Procedures

Subjects were admitted to the Clinical and Translational Research Institute (CTRI) at the University of California, San Diego on the day of hCG stimulation. Normal subjects were studied during the midfollicular phase (cycle days 5–8), while PCOS patients were anovulatory and studied on a random day. The 17-OHP responses to r-hCG in 13 women with PCOS and 14 normal controls in this study have been previously reported(11). Briefly,

each subject received intravenous (iv) administration of recombinant hCG (r-hCG), 25 μg. Blood samples were collected prior to and 24 hr following r-hCG injection.

Adrenal stimulation was performed in a subsequent month in the same patients. All study participants were instructed to begin fasting the midnight before the planned study day, and received 1 mg dexamethasone both at 11 pm the night before and at 7 am 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, respectively) over 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 PCOS subjects experienced recent ovulation as evidenced by absence of recent menstrual bleeding for 2 months before study and serum progesterone (P_4) less than 1.0 ng/ml at in baseline sample.

Assays

Serum concentrations of LH and FSH were measured by radioimmunoassay (RIA) with intra-and inter-assay 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., Los Angeles, CA). Serum concentrations of estradiol (E_2), A4, T, and dehydroepiandrosterone (DHEA) were measured by well-established RIA with intra-assay CV less than 7%. Serum levels of 17-OHP, P_4, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEA-S) were measured by RIA with intra-assay CV less than 7% (Diagnostic Systems Laboratories, Inc., Webster, TX). Serum P_4, 17-hydroxyprogesterone (17-OHP), and dehydroepiandrosterone sulfate (DHEAS) were measure by RIA (Diagnostic Systems Laboratories, Inc., Webster, TX) with an intra-assay CV less than 7%. The detection limit for T, A4, DHEA, and 17-OHP were 3.4 pg, 10.4 pg, 50 pg, 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 two groups. To account for BMI, analysis of covariance was performed.

In order to compare the cumulative steroid response to ACTH infusion, the Riemann Sums method was used to approximate the area under the curve (AUC). Given baseline differences for steroid level amongst control and PCOS participants, we calculated the delta AUC (ΔAUC) by subtracting the baseline from all Riemann Sum measurements.

In order to determine if 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 PCOS women and normal controls

There was no difference in mean (±SE) age between PCOS and normal women. There was a trend towards greater BMI among women with PCOS but it did not reach statistical significance (p=0.06). As shown in Table 1, elevated circulating LH, A4, T and 17-OHP levels in PCOS women were significantly higher compared to those observed for normal controls. Levels of serum FSH, DHEA, DHEA-S, E_2 and cortisol were similar between groups. These comparisons did not change after adjusting for BMI.

17-OHP and androgen responses to hCG in PCOS women and normal controls

The 17-OHP response to hCG in PCOS women, as measured by percent change above baseline, was significantly greater ($p<0.03$) compared to that observed in normal women. However, after adjusting for BMI this difference as well as those for A4, DHEA, and T following hCG were not significant between groups.

17-OHP and androgen responses to ACTH in normal controls and women with PCOS

During ACTH infusion, increased serum levels of 17-OHP and DHEA were observed for both PCOS and normal women compared to subtle rises of A4 and T for each group (Figure 1). These incremental changes of 17-OHP, DHEA, A4 and T between groups were not statistically significant.

Comparison of steroid hormone responses between ovarian (hCG) and adrenal (ACTH) stimulation

Within individual women with PCOS, the 17-OHP response to ACTH infusion, as measured by the net change of area under the curve (Δ AUC), was significantly correlated with the incremental 17-OHP response to hCG ($R^2=0.38$; P=0.03). A corresponding association was also evident for ACTH-stimulated DHEA (P=0.04) and A4 (P=0.003) responses (Figure 2). Serum T responses during ACTH and after hCG were not correlated in PCOS women. In the normal control group, no correlations were observed between ACTH and hCG stimulated 17-OHP and androgen responses.

Discussion

The results of this study have demonstrated that in women with PCOS androgen dose-responsiveness during ACTH infusion was similar to that observed in normal controls. However, androgen responses among PCOS women were highly variable and, notably, correlated significantly with ovarian androgen production following hCG stimulation.

Our findings are consistent with previous published reports that showed in PCOS and normal women minimal and non-significant changes of 17-OHP and adrenal androgens following acute injection of ACTH(8, 12, 13). In contrast, Lachelin et al observed increases of 17-OHP and DHEA following a 2 hour infusion of ACTH in women with PCOS that were significantly greater than those noted for normal controls(13).

Among women with PCOS 17-OHP, A4 and DHEA responses during ACTH infusion were highly variable. In some subjects adrenal androgen responses were similar to those observed in normal women whereas in others, enhanced androgen production was obvious. The differing magnitudes of response suggest that adrenal androgen production is not the same in all women with PCOS and may relate to the inconsistent prevalence of increased serum DHEA-S levels reported in this disorder(4–6, 14). Notably, in our study mean levels of DHEA-S were greater PCOS women compared to normal women although a wide range of values likely precluded statistical significance.

The notion that dysregulated CYP17, 17-hyddroxylase, and 17–20-lyase activities may be present in both the ovary and adrenal of women with PCOS has been suggested by Ehrmann *et al.* (10). In a prospective study of 40 hyperandrogenic women with hirsutism or irregular menstrual bleeding that underwent both ovarian and adrenal stimulation with GnRH agonist (nafarelin, 100 μg, sq) and ACTH (10 μg/m^2, iv) 23 individuals exhibited functional ovarian hyperandrogenism (10). Of these, 13 (57%) had abnormal 17-OHP responses to ACTH indicative of functional adrenal hyperandrogenism. These results tended to imply co-existent abnormalities of ovarian and adrenal androgen overproduction in women with PCOS.

However, there have been few efforts to examine corresponding androgen responses to gonadotropin and ACTH stimulation within individual women with PCOS. In the current study, we detected positive correlations between ovarian and adrenal stimulated responses for 17-OHP, DHEA, and A4 in women with PCOS that suggested a common dysregulation of steroid biosynthesis involving CYP17 activities in both these tissues. Consequently, it follows that individual PCOS women with the highest DHEA and A4 responses to hCG stimulation also demonstrated highest responses during ACTH infusion. This commonality of dysregulated CYP17 activities underlying androgen excess of the ovary and adrenal in PCOS has been suggested by others as well(10, 15).

Additionally, other alterations of steroid synthesis may contribute to androgen overproduction in women with PCOS. Studies conducted with human PCOS theca cells demonstrated increased expression of CYP11A and CYP17 mRNA as well as enzyme activities for 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase on a per cell basis that suggested altered steroidogenesis in this disorder may involve multiple steps(16). In hyperandrogenic women 17-hydroxylase and 17, 20-lyase activities were examined by comparing precursor/product ratios before and after ACTH stimulation. There was no association between the estimated enzyme activities and circulating adrenal androgen levels which led the investigators to propose that a generalized alteration of adrenocortical biosynthesis existed in these women(14). While our results may be attributed to an inherent dysregulation of CYP17 activities in both ovarian and adrenal steroidogenic pathways, alterations of other enzyme activities may also exist. Overall, our findings suggest that in women with PCOS an inherent theca cell defect may reflect a general dysregulation of androgen steroidogenesis.

Our study did not assess insulin secretion in women with PCOS which has been shown, *in vitro* and *in vivo*, to enhance gonadotropin-stimulated androgen production in hyperandrogenic women with PCOS(17–19). Evidence to show an effect of insulin on

adrenal androgen production is limited. It has been reported that insulin augmented ACTH stimulated A4 production in bovine adrenal tissue(20). However, in minced human adrenal tissue insulin failed to show a consistent effect on androgen production(21). Further studies using a human adrenocortical cell line, co-incubation with insulin was not associated with increases of T and DHEAS(22). In contrast, there is clinical evidence in humans to suggest that hyperinsulinemia or hyperglycemia may decrease adrenal androgen levels(23, 24). Our subjects with PCOS had higher BMI than that of normal controls that would suggest greater hyperinsulinemia in these women. Despite this consideration adrenal androgen responses to ACTH were not different between PCOS and normal women.

In summary, we have shown that within individuals with PCOS enhanced androgen responses to hCG are accompanied by comparable androgen responsiveness to ACTH. These findings suggest that dysregulated steroidogenesis leading to hyperandrogenemia is likely present in both the adrenal and ovary.

Acknowledgments

This research was supported by the Eunice Kennedy Shriver NICHD/NIH through cooperative agreement (U54 HD12303-28) as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research, NIH T32 HD007203, and in part by NIH grant MO1 RR00827.

References

1. Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F. Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest. 1976; 57:1320–9. [PubMed: 770505]
2. Chang RJ, Laufer LR, Meldrum DR, DeFazio J, Lu JK, Vale WW, et al. Steroid secretion in polycystic ovarian disease after ovarian suppression by a long-acting gonadotropin-releasing hormone agonist. J Clin Endocrinol Metab. 1983; 56:897–903. [PubMed: 6403570]
3. Gilling-Smith C, Willis DS, Beard RW, Franks S. Hypersecretion of androstenedione by isolated thecal cells from polycystic ovaries. J Clin Endocrinol Metab. 1994; 79:1158–65. [PubMed: 7962289]
4. Hoffman DI, Klove K, Lobo RA. The prevalence and significance of elevated dehydroepiandrosterone sulfate levels in anovulatory women. Fertil Steril. 1984; 42:76–81. [PubMed: 6327404]
5. Steinberger E, Rodriguez-Rigau LJ, Smith KD. The prognostic value of acute adrenal suppression and stimulation tests in hyperandrogenic women. Fertil Steril. 1982; 37:187–92. [PubMed: 6277699]
6. Carmina E, Rosato F, Janni A. Increased DHEAs levels in PCO syndrome: evidence for the existence of two subgroups of patients. J Endocrinol Invest. 1986; 9:5–9. [PubMed: 3009597]
7. Azziz R, Black V, Hines GA, Fox LM, Boots LR. Adrenal androgen excess in the polycystic ovary syndrome: sensitivity and responsivity of the hypothalamic-pituitary-adrenal axis. J Clin Endocrinol Metab. 1998; 83:2317–23. [PubMed: 9661602]
8. Pasquali R, Patton L, Pocognoli P, Cognigni GE, Gambineri A. 17-hydroxyprogesterone responses to gonadotropin-releasing hormone disclose distinct phenotypes of functional ovarian hyperandrogenism and polycystic ovary syndrome. J Clin Endocrinol Metab. 2007; 92:4208–17. [PubMed: 17785360]
9. Ehrmann DA, Rosenfield RL, Barnes RB, Brigell DF, Sheikh Z. Detection of functional ovarian hyperandrogenism in women with androgen excess. N Engl J Med. 1992; 327:157–62. [PubMed: 1319000]

10. Ehrmann DA, Barnes RB, Rosenfield RL. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. Endocr Rev. 1995; 16:322–53. [PubMed: 7671850]
11. Maas KH, Chuan SS, Cook-Andersen H, Su HI, Duleba AJ, Chang RJ. Relationship between 17-hydroxyprogesterone responses to human chorionic gonadotropin and markers of ovarian follicle morphology in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2015; 100:293–300. [PubMed: 25313914]
12. Hirshfeld-Cytron J, Barnes RB, Ehrmann DA, Caruso A, Mortensen MM, Rosenfield RL. Characterization of functionally typical and atypical types of polycystic ovary syndrome. J Clin Endocrinol Metab. 2009; 94:1587–94. [PubMed: 19240152]
13. Lachelin GC, Barnett M, Hopper BR, Brink G, Yen SS. Adrenal function in normal women and women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1979; 49:892–8. [PubMed: 229120]
14. Azziz R, Bradley EL Jr, Potter HD, Boots LR. Adrenal androgen excess in women: lack of a role for 17-hydroxylase and 17,20-lyase dysregulation. J Clin Endocrinol Metab. 1995; 80:400–5. [PubMed: 7852496]
15. Rosenfield RL, Mortensen M, Wroblewski K, Littlejohn E, Ehrmann DA. Determination of the source of androgen excess in functionally atypical polycystic ovary syndrome by a short dexamethasone androgen-suppression test and a low-dose ACTH test. Hum Reprod. 2011; 26:3138–46. [PubMed: 21908468]
16. Nelson VL, Legro RS, Strauss JF 3rd, McAllister JM. Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol. 1999; 13:946–57. [PubMed: 10379893]
17. Barbieri RL, Makris A, Randall RW, Daniels G, Kistner RW, Ryan KJ. Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrinol Metab. 1986; 62:904–10. [PubMed: 3514651]
18. Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab. 1998; 83:2001–5. [PubMed: 9626131]
19. Tosi F, Negri C, Perrone F, Dorizzi R, Castello R, Bonora E, et al. Hyperinsulinemia amplifies GnRH agonist stimulated ovarian steroid secretion in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2012; 97:1712–9. [PubMed: 22419715]
20. Kramer RE, Buster JE, Andersen RN. Differential modulation of ACTH-stimulated cortisol and androstenedione secretion by insulin. J Steroid Biochem. 1990; 36:33–42. [PubMed: 2163473]
21. Hines GA, Smith ER, Azziz R. Influence of insulin and testosterone on adrenocortical steroidogenesis in vitro: preliminary studies. Fertil Steril. 2001; 76:730–5. [PubMed: 11591406]
22. Kumar A, Magoffin D, Munir I, Azziz R. Effect of insulin and testosterone on androgen production and transcription of SULT2A1 in the NCI-H295R adrenocortical cell line. Fertil Steril. 2009; 92:793–7. [PubMed: 18684447]
23. Nestler JE, Clore JN, Strauss JF, Blackard WG. The effects of hyperinsulinemia on serum testosterone, progesterone, dehydroepiandrosterone sulfate, and cortisol levels in normal women and in a woman with hyperandrogenism, insulin resistance, and acanthosis nigricans. J Clin Endocrinol Metab. 1987; 64:180–4. [PubMed: 2946716]
24. Yamauchi A, Takei I, Nakamoto S, Ohashi N, Kitamura Y, Tokui M, et al. Hyperglycemia decreases dehydroepiandrosterone in Japanese male with impaired glucose tolerance and low insulin response. Endocr J. 1996; 43:285–90. [PubMed: 8886622]

Capsule

Among individual women with polycystic ovary syndrome androgen responses to ACTH infusion are variable and positively correlated with those following hCG injection.

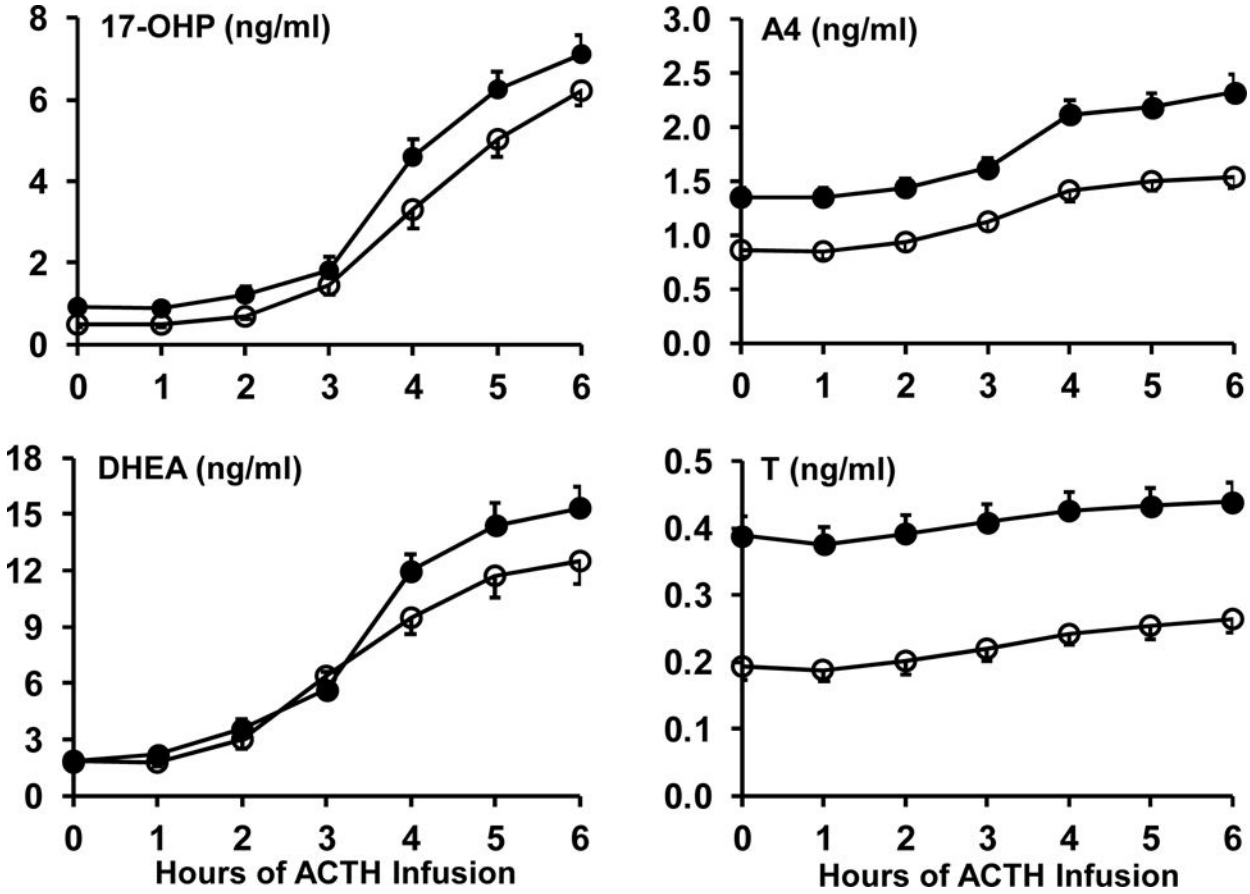

Figure 1.

Mean (± SE) serum 17-OHP, DHEA, A4, and T levels during 6 hr ACTH step-wise, dose-response infusion in women with PCOS (filled circles) and normal controls (open circles). ACTH doses: 0.1, 0.25, 1.0, 2.5, 10, and 25 μg/hr.

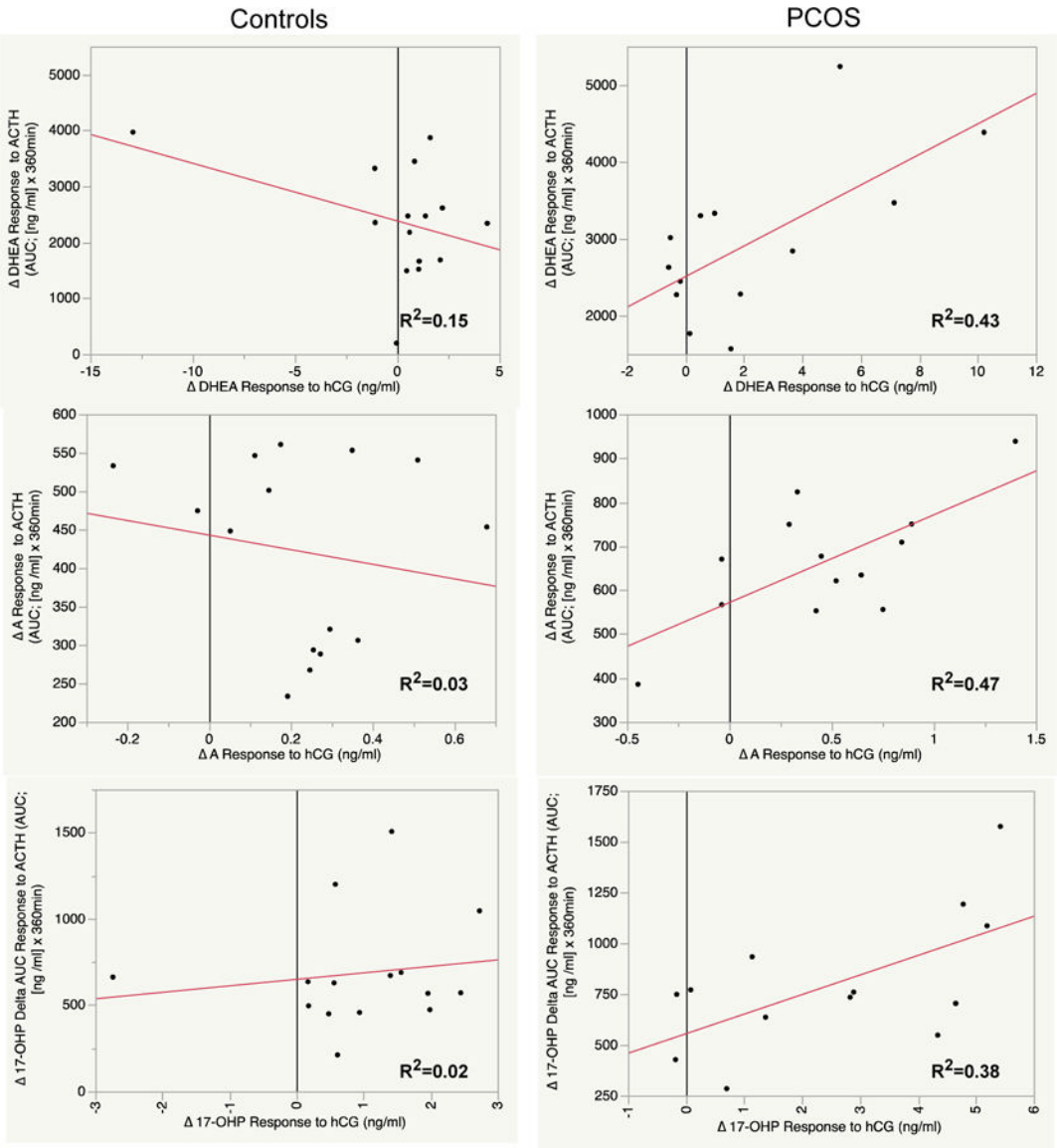

Figure 2.

Correlation of hormone responses to ACTH infusion with 24 hr responses to r-hCG for DHEA (upper panels), A4 (middle panels) and 17-OHP (lower panels) in individual control (left) and PCOS (right) subjects. The response to ACTH was determined by area under the curve above baseline during 6 hr infusion. The response to hCG was determined by the net change from baseline.

Table 1

Mean (± SD) clinical and basal hormone values for PCOS and normal women

Measure	PCOS (n=13)	Control (n=15)	P-value
Age (yrs)	26.1 ± 1.3	27.2 ± 1.3	0.60
BMI	31.3 ± 1.4	26.6 ± 1.7	0.06
LH (mIU/mL)	10.0 ± 1.0	4.1 ± 2.5	<0.001
FSH (mIU/mL)	5.7 ± 0.3	5.2 ±1.8	0.46
17-OHP (ng/mL)	0.9 ± 0.1	0.5 ± 0.2	0.001
A4 (ng/mL)	1.4 ± 0.1	0.9 ± 0.3	<0.001
T (ng/mL)	0.4 ± 0.03	0.19 ± 0.08	<0.001
DHEA (ng/mL)	1.9 ± 0.2	1.8 ± 1.0	0.51
DHEAS (ng/mL)	2933 ± 516	2145 ± 1780	0.30
E_2 (pg/mL)	58 ± 4	83 ± 66	0.26
Cortisol (μg/dL)	2.5 ± 0.3	2.8 ± 2.3	0.71

To convert to SI units multiply by the following conversion factor: 17-OHP (3.03); A4 (3.49); T (3.47); DHEA (3.47): DHEA-S (0.0027); E_2 (3.67)