Decreased inhibin B responses following recombinant human chorionic gonadotropin administration in normal women and women with polycystic ovary syndrome

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Objective: To determine whether granulosa cells contribute to excess androgen production, by assessing inhibin B (Inh B) responses to hCG in women with polycystic ovary syndrome (PCOS) and in normal women.
Design: Prospective study.
Setting: Academic medical center.
Patient(s): Twenty women with PCOS and 16 normal women.
Intervention(s): Blood samples obtained before and 24 hours after injection of 25 μg recombinant hCG (r-hCG).
Main Outcome Measure(s): Basal and stimulated Inh B, E_2, androstenedione (A), and T responses after r-hCG administration.
Result(s): In normal and PCOS women, r-hCG induced a significant reduction of Inh B levels. Lowered Inh B responses were not related to body mass index, PCOS status, or age by multivariate regression. Recombinant hCG significantly increased serum A and E_2 in both normal and PCOS women.
Conclusion(s): In normal and PCOS women, Inh B production was decreased following r-hCG administration. These findings strongly suggest that in PCOS women androgen excess is not enhanced by LH-stimulated Inh B production.
Clinical Trial Registration Number: NCT00747617. (Fertil Steril® 2014;101:275–9. ©2014 by American Society for Reproductive Medicine.)
Key Words: Inhibin B, hCG, polycystic ovary syndrome

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Received May 6, 2013; revised September 10, 2013; accepted September 26, 2013; published online November 1, 2013.
R.F.S. has nothing to disclose. M.A.R. has nothing to disclose. S.S.C. has nothing to disclose. H.C.-A. has nothing to disclose. W.E.R. has nothing to disclose. H.I.S. has nothing to disclose. S.S. has nothing to disclose. R.J.C. has nothing to disclose.
Supported by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (NIH), through cooperative agreement (U54 HD12303–28) as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research and in part by NIH grant MO1 RR00827.
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Fertility and Sterility® Vol. 101, No. 1, January 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.09.037

In women with polycystic ovary syndrome (PCOS), excessive ovarian androgen production is a major pathophysiologic feature. The basis for androgen overproduction has been attributed to altered theca cell responsiveness to gonadotropin stimulation in association with increased pituitary LH secretion (1–3). In particular, hyperandrogenemic women with PCOS have exhibited exaggerated 17-hydroxyprogesterone

(17OH-P) production in response to hCG compared with normal women (4). In addition, studies have shown that women with PCOS exhibit significant increases in circulating androgens after an acute injection of FSH, which suggests that ovarian androgen production may also be subject to paracrine regulation by factors derived from granulosa cells (GCs) (5). Early in vivo and in vitro animal reports have suggested an interaction between adjacent granulosa and theca cells, because reduction of androgen production was observed after removal of GCs from theca tissue cultures (6, 7). Subsequently, it was shown that ovine theca cells coincubated with conditioned media from FSH-stimulated GC cultures produced significantly more LH-induced androgen than did theca cells incubated with untreated media (8). In addition, LH-stimulated androgen production from cultured rat theca cells pretreated with FSH was substantially greater than that produced by rat theca cells treated with vehicle (9).

Among GC-derived proteins, inhibin appears to enhance LH-mediated androgen production. In cultured human ovarian theca cells, the presence of inhibin was clearly associated with greater production of androgen compared with that observed in the absence of inhibin (10, 11). In addition, inhibin was dose-dependently able to negate the inhibitory effect of activin on human theca cell androgen production (12). In women with PCOS, significant increases in ovarian androgens stimulated by FSH were accompanied by similar significant increments in FSH-stimulated inhibin B (Inh B) levels compared with those of normal women (5).

Granulosa cells are also known to possess LH receptors. During normal follicular development, acquisition of LH receptors by GCs occurs with advanced stages of growth and antrum formation (13–15). However, in GCs obtained from ovaries of anovulatory PCOS women, LH receptor mRNA expression was abundant in small antral follicles of 4–8 mm (16). This suggests that inhibin production may be enhanced by increased LH secretion in women with PCOS, which may provide an indirect mechanism of androgen production beyond that of direct theca cell stimulation by LH. We have previously demonstrated that women with PCOS exhibit a marked androgen production in response to hCG administered intravenously (4). To further explore whether excess androgen production may be coupled to corresponding inhibin responses to hCG, Inh B, E_2, and androgen levels were assessed before and after intravenous administration of hCG to women with PCOS and normal women.

MATERIALS AND METHODS

Participants

Twenty women with PCOS and 16 normal women were recruited. The diagnosis of PCOS was based on 1992 National Institutes of Health criteria: clinical and/or biochemical evidence of hyperandrogenism and irregular menstrual bleeding, either oligomenorrhea or amenorrhea (17). Oligomenorrhea was defined as irregular menstrual bleeding occurring less than six times a year. Each PCOS subject had enlarged polycystic ovaries according to ultrasound. The antral follicle count per ovary was >12 in all subjects. None of the follicles exceeded 9 mm in diameter, and the vast majority were 2–5 mm in size. Normal women did not exhibit enlarged ovaries, had antral follicle counts of 7–10 per ovary, and no follicles >10 mm in diameter. PCOS and normal women had similar mean (±SE) ages of 27.5 ± 0.9 and 27.9 ± 1.4 years, respectively. Mean body mass index (BMI) was higher in PCOS subjects (34.7 ± 16 vs. 29.3 ± 2.2 kg/m^2, respectively; $P<.05$). Late-onset congenital adrenal hyperplasia was excluded by serum 17OH-P <2 ng/mL. Circulating TSH and PRL were normal among all subjects. No subject had received hormone medication for 2 months before the study. The study was approved by the Human Research Protection Program at the University of California, San Diego (UCSD), and written informed consent was obtained from each participant.

Procedures

Subjects were admitted to the General Clinical Research Center at UCSD on the day of testing. Each subject received 25 μg recombinant hCG (r-hCG) as an intravenous bolus. In normal subjects, r-hCG was given during the midfollicular phase of the menstrual cycle. In PCOS women, r-hCG was administered on a random day. Blood samples were obtained at 0 and 24 hours after r-hCG administration. None of the PCOS subjects had experienced recent ovulation, as evidenced by absence of recent menstrual bleeding for 2 months before the study and serum 17OH-P <2.0 ng/mL.

Assays

Serum Inh B levels were measured with the use of a commercially available Gen II ELISA (Beckman Coulter), with a sensitivity of 2.6 pg/mL, intraassay coefficient of variation (CV) of 2.2%–3.8%. Serum E_2, A, and T were measured by well established RIA with intra-assay CV <7% (18, 19). Briefly, radioimmunoassay for E_2, A, and T were developed in house. The labeled antigen is commercially available. The antibodies were raised in rabbits and checked for cross-reactivity to other steroid hormones. Standards were made from reagents which are also commercially available. To ensure a specific assay, the samples were purified in a two-step process. Initially, to separate hydrophilic from hydrophobic hormones, 7.0 mL solvent (hexane:ethyl acetate) was added to 0.8 mL serum and vortexed. The solvent was decanted and chromatographed on a microcelite column. Chromatography columns used ethylene glycol:propylene glycol as the stationary phase. The chromatography system was checked for separation by comparing radioactive peaks with immunoreactive peaks. Each sample was chromatographed on a celite column separating the steroids based on their polarity. Individual purified steroid fractions were then used in their respective radioimmunoassay. Serum 17OH-P was measured by RIA with intra-assay CV <7% (Diagnostic Systems Laboratories). Serum concentrations of LH and FSH were measured by RIA with intra- and interassay CV of 5.4% and 8.0%, respectively, for LH and 3.0% and 4.6%, respectively, for FSH (Diagnostic Products Corp.).