Insulin Has a Biphasic Effect on the Ability of Human Chorionic Gonadotropin to Induce Ovarian Cysts in the Rat

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Hyperinsulinemia enhances the ability of subovulatory doses of human chorionic gonadotropin (hCG) to induce ovarian follicular cysts in the rat. To determine the relative contribution of these hormones to the development of ovarian cysts, adult female rats were treated with either (1) vehicle alone (controls), (2) a high-fat diet (HFD) to control for the effects of weight gain, (3) 1.5 to 6 IU hCG twice daily plus 6 U insulin (Ins)/d, or (4) 1.5 to 9 U Ins/d plus 3 IU hCG twice daily. On day 23 of the in vivo treatments, all groups that received at least 6 U Ins/d displayed increased body weight compared with control and HFD rats ($P \leq .05$). No control rats and only one HFD rat displayed ovarian cysts on this day. Plasma estrone (E1) and androstenedione (A4) were elevated in HFD rats with noncystic follicles compared with control rats ($P \leq .05$). Between 64% and 80% of rats on 6 U Ins/d plus twice-daily injections of 1.5 to 6 IU hCG displayed ovarian cysts on day 23. Plasma estradiol (E2) concentrations for these treatment groups were similar to those of control rats. Of the hormonally treated animals, only those that had ovarian cysts in response to twice-daily injections of 4.5 or 6 IU hCG plus 6 U Ins/d displayed elevated plasma A4 and/or testosterone compared with controls. In contrast, plasma E1 concentrations were elevated on day 23 for animals bearing ovarian cysts in response to increasing doses of hCG plus the fixed dose of 6 U Ins/d. Between 70% and 80% of rats treated twice daily with 3 IU hCG plus a daily dose of 1.5 to 6 U Ins displayed ovarian cysts on day 23. In marked contrast, only 25% of rats treated with this dose of hCG plus 9 U Ins/d developed cystic follicles. Of the plasma steroids tested, only E1 and A4 were elevated in these treatment groups compared with controls. However, these increases in plasma steroid concentrations did not correlate with the dose of insulin. We conclude from these data that, although the mechanisms remain to be elucidated, extreme hyperinsulinemia has the paradoxical ability to attenuate the induction of ovarian cysts by hCG in some animals.

POLYCYSTIC OVARY SYNDROME (PCOS) is the leading cause of infertility in women.[1-5] It has been suggested that hyperinsulinemia may play a role in the pathogenesis of PCOS either directly at the level of the ovary or indirectly by contributing to the hyperandrogenic state observed in this syndrome.[1-15] However, unambiguous evidence of a mechanistic link between hyperandrogenism, insulin resistance, and the induction of PCOS remains to be demonstrated in women.

We established previously that although hyperinsulinemia cannot induce ovarian cysts by itself,[16] it can enhance the induction of large ovarian cysts in rats by subovulatory doses of luteinizing hormone (LH)-like activity (human chorionic gonadotropin [hCG]).[17-19] The present study was undertaken to determine the extent to which correlations arise between the dose of insulin (Ins) or hCG administered in vivo and morphological changes that occur in the ovary with the development of ovarian cysts. Suppression of the neuroendocrine axis by either progesterone or a gonadotropin-releasing hormone antagonist greatly enhances the ability of low doses of hCG to induce large ovarian cysts in the absence of hyperinsulinemia.[17-19] Therefore, to avoid the risk of masking the potential contribution of hyperinsulinemia to ovarian cyst development in this model, non–neuroendocrinologically suppressed rats were used in the present studies.

MATERIALS AND METHODS

Experimental Animals

In vivo procedures were performed in the Beth Israel Medical Center Animal Facility using a protocol approved by the Committee for Scientific Activities of Beth Israel Medical Center. A total of 90 female Sprague-Dawley rats aged 54 ± 3 days (Taconic Laboratories, Germantown, NY) were housed in cages in rooms with 12-hour light/dark cycles. Initially, all animals were given rat chow and water ad libitum. The animals underwent daily vaginal smears for 31 days to document consecutive 4- to 5-day estrous cycles and were randomly assigned to nine in vivo treatment groups on day 85 ± 3. Vehicle (phosphate-buffered saline [PBS], pH 7.0, containing 0.09% pig skin gelatin [gel-PBS]) was administered subcutaneously (SC) twice daily to control rats throughout the in vivo treatment period. Insulin (NPH-Humulin U-100; Lilly, Indianapolis, IN) was administered as previously described[16,19] to obtain the desired dosage of insulin (Ins) per day without inducing hypoglycemia. Briefly, the daily insulin dosage was increased from 0.5 U on day 1 to the final desired dosage by day 10 of treatment. The maximal doses of insulin were subsequently administered along with twice-daily SC injections of the desired doses of hCG (Sigma Chemical, St Louis, MO) on days 11 to 22 of treatment. To simplify the text, doses of hCG in both the text and figures will be referred to in terms of the total daily dose given to the animals. Thus, a total daily dose of 6 IU hCG is the result of twice-daily treatments with 3 IU hCG per injection.

All animals were decapitated on day 23 of treatment. Trunk blood was collected in individual heparinized tubes for measurement of plasma aromatizable androgen and estrogen concentrations, and the ovaries were excised, cleaned of adhering tissue, and fixed in Formalin. Sections were prepared and stained with hematoxylin-eosin by the Histology Core Facility at the University of South Carolina School of Medicine. Histological data were obtained using a Zeiss (Thornwood,

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NY) 35 IM microscope with planachromat objectives and an ocular micrometer.

Preparation of Plasma for Radioimmunoassays

Blood samples were centrifuged at 1,000 × *g*, and the resulting supernatants were transferred to individual polypropylene tubes. Plasma steroids were extracted by column chromatography prior to analysis for steroid content by radioimmunoassay (RIA). Briefly, a known volume of plasma from each experimental sample received a known amount of ^{3}H-steroid (approximately 2,000 cpm) to account for procedural losses. Serum proteins were precipitated with 3 mL Nylon-66 (Millipore, Bedford, MA)–filtered, high-performance liquid chromatography (HPLC)-grade methanol and pelleted by centrifugation at 1,000 × *g* for 15 minutes. The resulting supernatants were transferred to new polypropylene tubes and evaporated to the point at which primarily aqueous phase remained. The samples then were brought to a total volume of 3 mL with Nylon-66–filtered Milli-Q water (HPLC-grade water; Millipore) and warmed in a 37°C water bath with vortexing. Validation studies of this technique indicated that greater than 98% of the radiolabeled form of the authentic steroids tested (androstenedione [A4], testosterone, estradiol [E2], and estrone [E1]) are recovered at this point in the extraction procedure.

After the experimental samples were allowed to return to room temperature, they were chromatographed individually on 3-mL C_{18} extraction columns. Briefly, the columns were primed with 6 mL HPLC-grade methanol followed by 6 mL HPLC-grade water. Samples were applied to the C_{18} columns, which then were washed with 6 mL HPLC-grade water followed by 6 mL HPLC-grade methanol. Only the methanol washes, which contained the radiolabeled authentic steroid, were evaporated to dryness in a Savant centrifuge evaporator (Savant Instruments, Farmingdale, NY). The resulting residues were reconstituted individually with 1 mL gel-PBS, heated for 45 minutes at 37°C, vortexed, and stored at −20°C until analysis for A4, E1, E2, and testosterone content by RIA procedures used routinely in one of our laboratories.[17-20] During validation of the extraction and column chromatography procedure (n = 11 or 12), the mean recovery values for A4, E1, E2, and testosterone in gel-PBS–reconstituted samples were 64.8% ± 1.9%, 67.8% ± 1.5%, 66.1% ± 2.8%, and 69.8% ± 3.1%, respectively. There was no significant difference among these values ($P > .05$). Therefore, equal amounts of each of these radiolabeled steroids were used to obtain an individual "recovery" value for each extracted and chromatographed plasma sample. The overall mean ± SEM of individual recovery values for the experimental samples described in this report is 63.5% ± 1.6%.

RIA kits for Ins and hCG were obtained from Diagnostic Systems Laboratories ([DSL] Webster, TX). Plasma samples were analyzed for these peptide hormones according to the procedures outlined in the kits. Antisera against E2 and A4 were generous gifts from Dr Gordon Niswender (Fort Collins, CO) and Dr Barry England (Ann Arbor, MI), respectively. Antisera for E1 and the iodinated ligand were obtained from Pantex (Santa Monica, CA). Antisera and iodinated ligand for the testosterone assay and iodinated ligand for E2 and A4 RIAs were obtained from DSL. Due to the limited amount of plasma collected from some animals, the number of samples that could be assayed for A4 was limited to four for controls and five to eight for the other in vivo treatment groups.

Antisera for testosterone displayed approximately 10% cross-reactivity with dihydrotestosterone, and less than 1% cross-reactivity with A4. Antisera against E1 and E2 displayed less than 2% cross-reactivity with E2 and E1, respectively. The respective intraassay and interassay coefficients of variation were as follows: A4, 6% and 18%; E2, 6% and 10%; E1, 7% and 12%; and testosterone, 4% and 10%.

Statistics

The largest ovarian cross-sectional areas (LOCAs) were calculated for each rat using the formula $\pi \times r_1 \times r_2$, where r_1 and r_2 equal half the average of each ovary pair's longest length and width (millimeters), respectively. Mean LOCAs were calculated for each in vivo group (n = 8 to 10).

The SigmaStat Statistical Program (Jandel Scientific, San Rafael, CA) was used to calculate correlation coefficients and to determine if differences in ovarian morphology or serum steroid values were statistically significant by ANOVA (dose-response comparisons) and *T* test (comparisons between control and high-fat diet [HFD] rats). A *P* value of .05 or less was considered to indicate a statistical difference within or among groups.

RESULTS

Plasma Peptide Hormone Concentrations

Figure 1A and B depicts the respective concentrations of hCG and Ins observed in the plasma of each in vivo treatment group on day 23. As expected, hCG was not detected in the plasma of control and HFD rats. Mean plasma hCG concentrations increased with the increasing dose of hCG. In addition, plasma hCG concentrations were similar within a specific

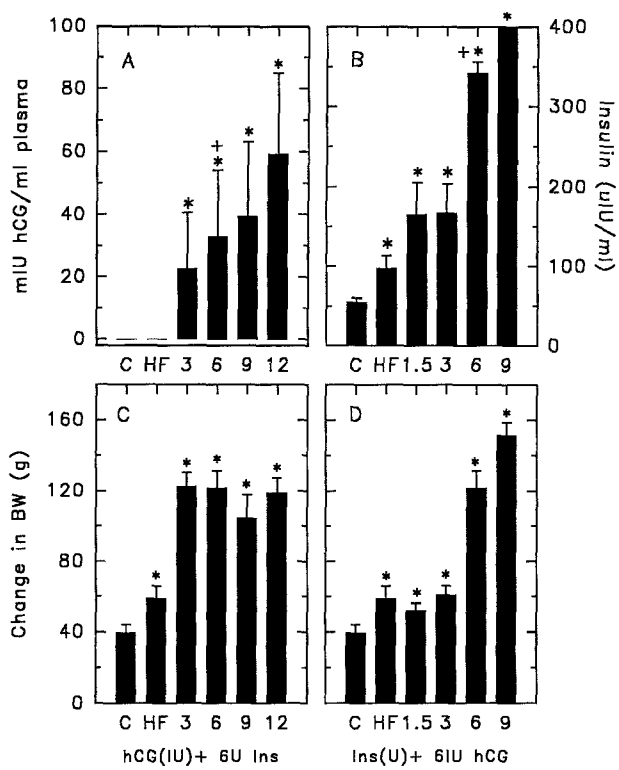

Fig 1. Plasma hCG and Ins concentrations and changes in body weight (BW) in response to hormonal treatments. (A) Plasma hCG: + Includes hCG values for all animals that received a total dose of 6 IU hCG/d (ie, all animals receiving increasing amounts of Ins also received 6 IU hCG/d, n = 37); (B) plasma Ins: +includes values for all animals that received 6 U Ins/d (ie, all animals receiving increasing amounts of hCG also received 6 U Ins/d, n = 36); (C) changes in BW in response to increasing doses of hCG in the presence of 6 U Ins/d; (D) changes in BW in response to increasing doses of Ins in the presence of twice-daily treatments with 3 IU hCG. Data are the mean ± SEM for 8-10 rats. Abbreviations for this and all subsequent figures: C, control; HF, HFD. *Significantly different *v* control values ($P \leq .05$).

treatment group regardless of whether or not the animals in the group displayed ovarian cysts. Plasma Ins values were significantly greater for rats fed a HFD and for animals treated with Ins versus control rats. Ins concentrations for animals treated with 6 IU hCG plus 1.5 or 3 U Ins per day were similar, whereas rats receiving 6 and 9 U Ins/d displayed dose-related increases in plasma Ins values. Indeed, insulin concentrations were elevated beyond the range of assay detection (400 μIU) in all rats treated with 9 U Ins/d.

Animal Weight

Figure 1C and D illustrates that HFD rats and all groups that received Ins treatment displayed significantly greater increases in mean body weight during the in vivo treatment period (52.2 ± 4.1 to 151.8 ± 7.2 g) than control animals (39.6 ± 4.3 g, $P \leq .05$). All treatment groups that received less than 6 U Ins/d displayed a change in body weight similar to that observed for HFD rats (Fig 1B), whereas groups that received 6 or 9 U Ins/d displayed significantly greater increases in body weight than HFD rats. There was a significant correlation between the change in body weight and the dose of insulin ($r = .985$, $P \leq .05$), whereas the increasing doses of hCG in vivo had no effect on changes in body weight (Fig 1A; $r = -.849$, $P > .05$).

Ovarian Morphology

In this series of experiments, the term "cyst" refers to follicles generally greater than 0.8 mm in diameter with well-developed thecal shells and just a remnant of granulosa cells. Ovaries possessing cystic follicles also displayed stimulated stromal-interstitial tissue. Representative histological sections for the in vivo treatment groups are presented in Fig 2. In addition, Fig 3A and B illustrates the fraction of animals that displayed ovarian cysts by the morning of day 23 of treatment. Control rats (Fig 2A) and eight of nine HFD rats (Fig 2B) did not display ovarian cysts by the end of the in vivo treatments (Fig 3). However, greater than 60% of each group of rats treated with hCG plus a maximal 6 U Ins/d displayed ovarian cysts and stimulated interstitial tissue by day 23 (Figs 2C and 3A and B). In contrast, only two of eight rats treated with 6 IU hCG plus 9 U Ins per day (the highest dose of Ins used in these experiments) displayed large cystic follicles by the end of the treatment protocol (Fig 3B). That is, the incidence of ovarian cysts in this group decreased 65% compared with the mean incidence in the other groups that received increasing doses of Ins plus a total of 6 IU hCG per day. Figure 2D illustrates a representative ovarian section from one of six animals in this group that did not develop ovarian cysts.

Neither increasing doses of hCG (Fig 3C: $r = .930$, $P > .05$) nor increasing doses of Ins (Fig 3D; $r = -.583$, $P > .05$) had a significant effect on the mean number of ovarian cystic follicles per cyst-bearing rat. The diameters of ovarian cysts in the groups that received increasing doses of hCG were significantly greater than the diameters of the largest antral follicles in control and noncystic HFD rats (Fig 4E). However, the diameters of ovarian cysts in response to twice-daily treatments with hCG were maximal with the lowest dose of hCG used in these experiments and did not increase in a dose-related manner ($r = .749$, $P > .05$).

In contrast, compared with control and HFD rats, the LOCAs of animals with cystic follicles increased in a dose-related manner in response to stimulation by hCG (Fig 3E; $r = .995$, $P \leq .05$). This increase in LOCAs in response to increasing doses of hCG appears to be related, at least in part, to an increase in the number of corpora lutea (CL) in the ovaries of these animals (Fig 4A; 4.8 ± 0.9 and 5.7 ± 1.6 CL for animals

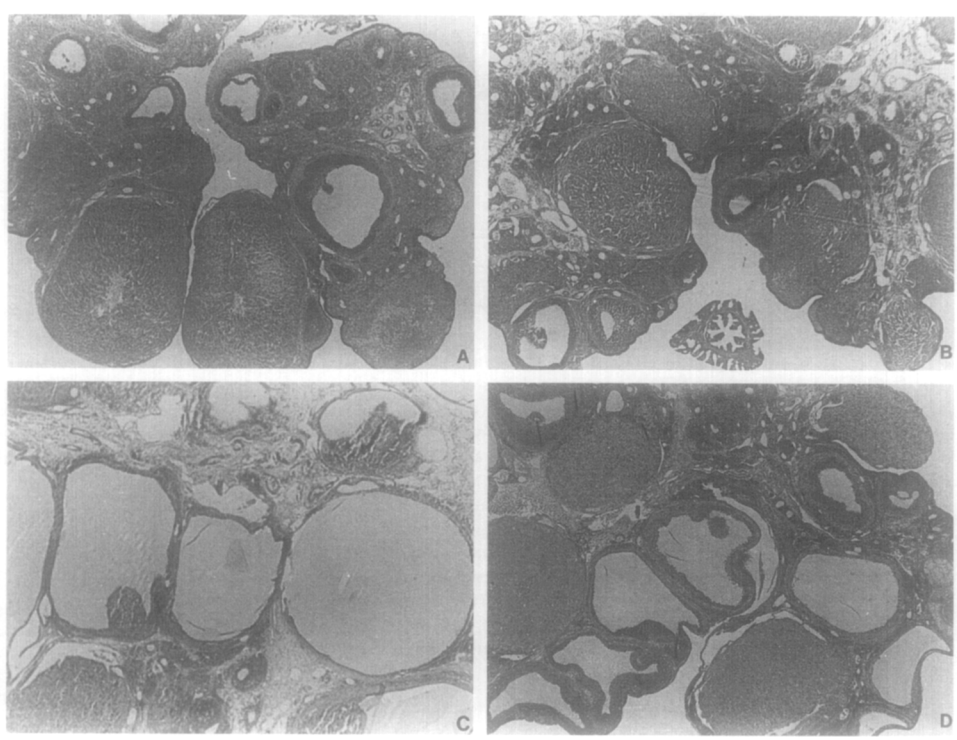

Fig 2. Photomicrographs of representative ovaries from rats treated with (A) vehicle only (controls), (B) HFD, (C) 12 IU hCG plus 6 U Ins per day, and (D) 6 IU hCG plus 9 U Ins per day.

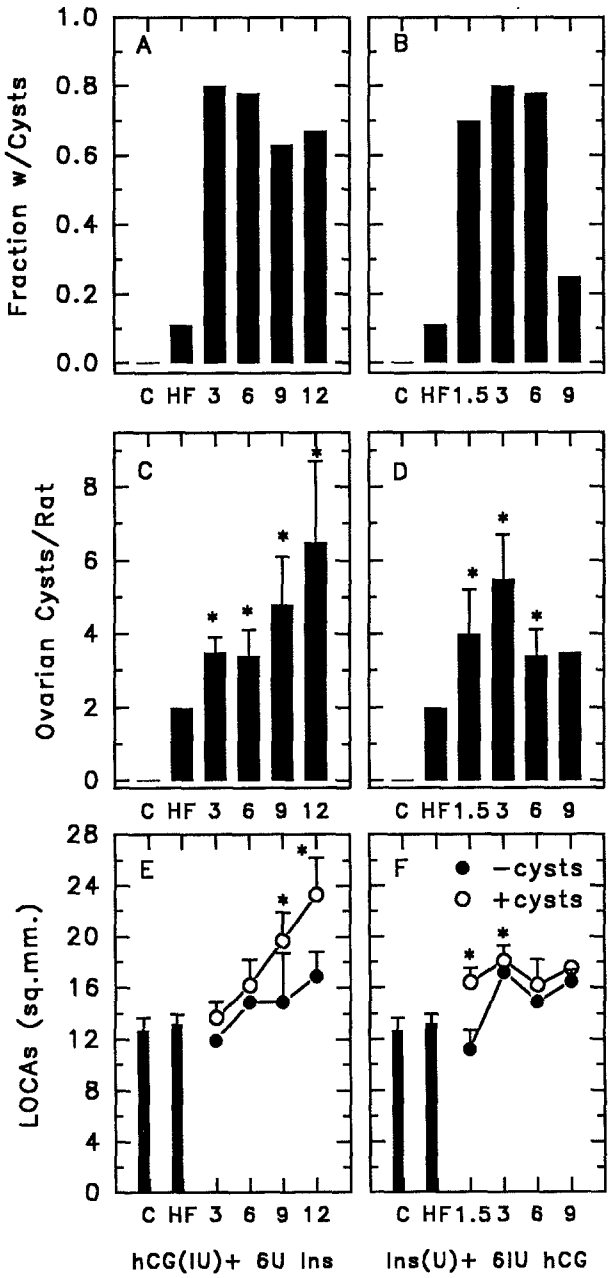

Fig 3. Effect of prolonged stimulation by increasing doses of hCG plus 6 U Ins per day (A, C, and E) or increasing doses of Ins plus 6 IU hCG per day (B, D, and F) on the fraction of animals in each treatment group bearing ovarian cysts (A and B), the mean number of ovarian cysts per rat with cysts (C and D), and the LOCAs (E and F). Data are the mean ± SEM for 8-10 rats, except as follows: In this and all subsequent figures, ■ and ● represent data from animals without ovarian cysts, and the lack of error bars in these groups indicates n < 3. □ and ○ represent data from animals with ovarian cysts. As previously described, only 1 HF rat and only 2 rats treated with 6 IU hCG plus 9 U Ins per day developed cystic follicles. Therefore, these data do not have error bars. Missing error bars for data from any other groups indicate that the SEMs were too small to depict. *Significantly different *v* control values ($P \leq .05$).

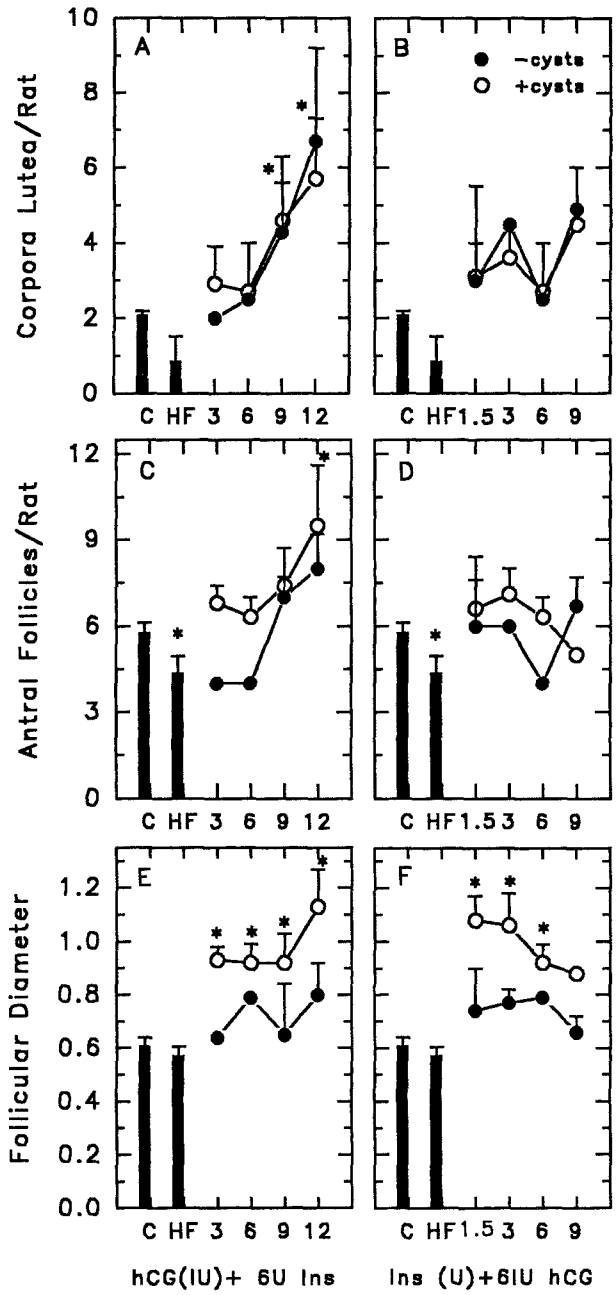

Fig 4. Effect of prolonged stimulation by increasing doses of hCG plus 6 U Ins per day (A, C, and E) or increasing doses of Ins plus 6 IU hCG per day (B, D, and F) on the number of CL (A and B) and noncystic antral follicles (C and D) per rat, as well as the diameter of the largest follicles/cysts present in these ovaries (E and F). Data were obtained from the same histological sections used for Fig 3 and are the mean ± SEM for 8-10 rats. *Significantly different *v* control values ($P \leq .05$).

treated with the two highest doses of hCG and 2.1 ± 0.9 CL for controls, $r = .957$, $P \leq .05$).

In animals bearing cysts, LOCAs failed to increase in a dose-related manner in response to increasing doses of Ins plus 6 IU hCG per day (Fig 3F; $r = .125$, $P > .05$). In fact, there was an inverse correlation between the diameter of the ovarian cysts and the dose of Ins administered each day in the presence of a daily total dose of 6 IU hCG ($r = -.971$, $P \leq .05$).

Plasma Steroid Concentrations

Plasma steroid concentrations for the single HFD rat that developed cystic follicles are not presented in either Figs 5 or 6, since no statistical analysis or inferences can be made with such limited data. Plasma concentrations of A4, testosterone, E1, and E2 for this animal were 1.72, undetectable, 0.196, and 0.137 ng/mL, respectively.

Figure 5 illustrates the effects of the in vivo treatments on plasma aromatizable androgens. A4 concentrations for HFD rats without ovarian cysts (4.83 ± 0.90 ng/mL) were slightly but significantly greater than the values observed for control rats (3.36 ± 0.37 ng/mL, $P \leq .05$). Further, plasma A4 values for HFD rats were similar to those observed for hyperinsulinemic animals treated with increasing doses of hCG (Fig 5A). In addition, the increases observed in plasma A4 for these animals correlated with the observed increases in CL for these groups (Fig 4A; $r = .984$, $P \leq .05$) but did not correlate with increasing doses of hCG, changes in LOCAs, or with the number of antral follicles. The two animals that developed ovarian cysts in response to treatment with 6 IU hCG plus 9 U Ins per day (Fig 5B) displayed the most marked increase in plasma A4 of all in vivo treatment groups.

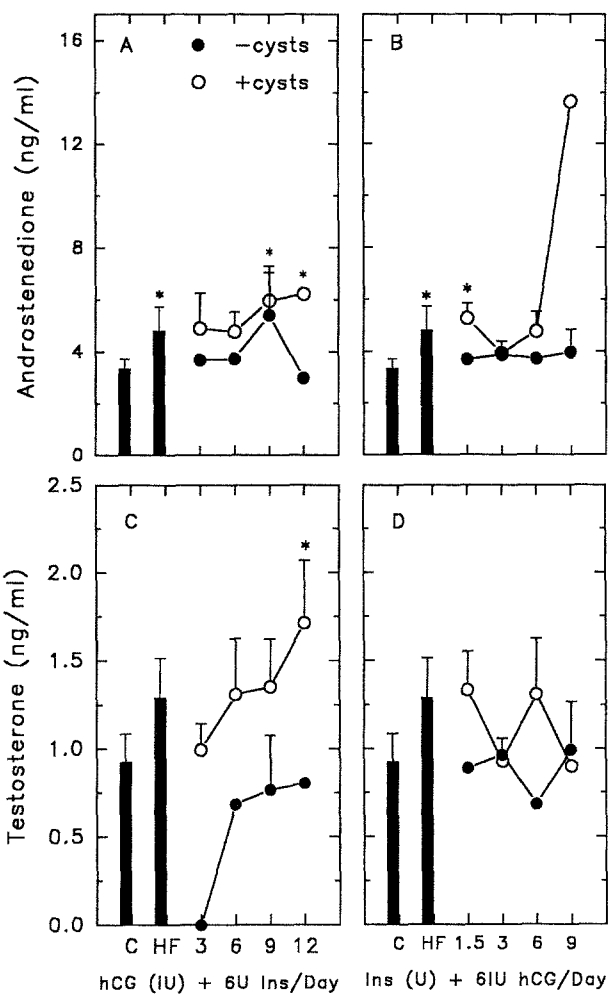

Fig 5. Effect of prolonged stimulation by increasing doses of hCG plus 6 U Ins per day (A and C) or increasing doses of Ins plus 6 IU hCG per day (B and D) on plasma A4 (A and B) and plasma testosterone (C and D) by day 23 of treatment. For this figure and Fig 6, plasma steroid values were obtained by RIA and are the mean ± SEM. *Significantly different *v* control values ($P < .05$).

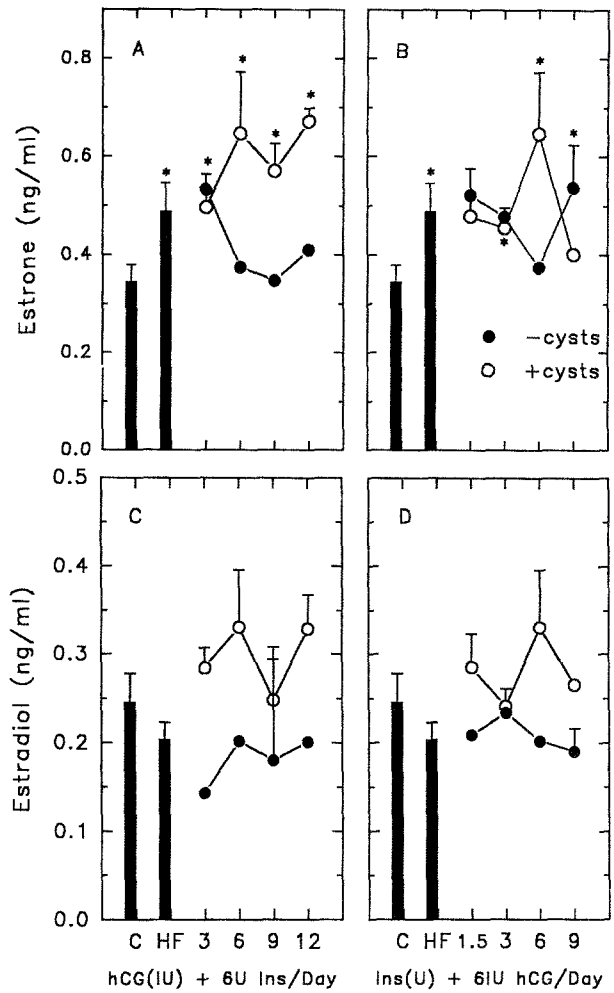

Fig 6. Effect of prolonged stimulation by increasing doses of hCG plus 6 U Ins per day (A and C) or increasing doses of Ins plus 6 IU hCG per day (B and D) on plasma E1 (A and B) and E2 (C and D) by day 23 of treatment. *Significantly different *v* control values ($P < .05$).

Compared with control animals, plasma testosterone concentrations were elevated only in hyperinsulinemic rats treated with 12 IU hCG/d (Fig 5C and D). Still, the changes observed in these testosterone values correlated with both the increasing doses of hCG ($r = .964$, $P \leq 0.05$) and the increasing LOCAs observed in response to increasing doses of hCG (Fig 3E; $r = .957$, $P \leq .05$). No correlation was observed between plasma testosterone values and either the number of CL or number of antral follicles present in the ovaries of these animals on day 23.

Plasma E1 concentrations were greater in HFD rats without ovarian cysts and in hyperinsulinemic rats treated with 3 to 12 IU hCG/d and bearing ovarian cysts versus control rats (Fig 6A: 0.35 ± 0.03 ng/mL, $P \leq .05$). Maximal E1 values were observed in response to 6 IU hCG plus 6 U Ins per day. No correlation was observed between plasma E1 values and the

doses of either hormone administered (Fig 6A and B; $r = .734$ and .089, respectively) or between plasma E1 and any ovarian morphological parameter that was measured. Plasma E2 concentrations for HFD rats and hormone-treated rats were similar to those observed for control animals (Fig 6C and D; 0.25 ± 0.03 ng/mL).

DISCUSSION

Although many hypotheses have been formulated in an attempt to explain the pathogenesis of PCOS,[1-5] the fundamental mechanisms underlying this syndrome remain to be determined. One currently popular hypothesis regarding PCOS is that the PCO state develops as a result of a synergy between the effects of elevated serum LH and Ins in the majority of these patients.[6-16,19,21] Although these purported interactions remain to be demonstrated in women, our group has demonstrated a synergy between prolonged stimulation with a single subovulatory dose of hCG (LH-like activity) and a fixed-dose hyperinsulinemic state in intact adult rats.[19]

The present series of experiments were undertaken in an attempt to determine the relative contributions of Ins and hCG to ovarian growth and cyst development using two treatment patterns: (1) increasing doses of hCG in the presence of a fixed dose of insulin and (2) increasing doses of Ins in the presence of a fixed dose of hCG. We hypothesized that the observation of a change in a specific morphological measure or plasma hormone concentration in response to an increasing dose of one or the other hormone would indicate the degree to which the physiological characteristic is influenced by the individual hormonal treatments. For example, body weight increased profoundly in response to increasing doses of Ins but was not affected by hCG. In contrast, ovarian size (as indicated by LOCAs) and the number of CL increased in response to increasing doses of hCG, but did not increase in response to increasing doses of Ins.

The observations that neither hCG nor Ins had a dose-related effect on the observed number of fully developed ovarian cysts are new and of interest. These observations in freely cycling adult rats confirm observations obtained with similar doses of hCG in neuroendocrinologically synchronized immature and adult rats.[17-20] The present series of experiments extend these previous observations by demonstrating that the number of ovarian cysts that develop in response to LH-like stimulation does not increase even in response to a near-ovulatory dose of hCG (12 IU hCG/d) in the presence of a hyperinsulinemic state. In contrast, this combination of hormonal treatments does increase the number of small antral follicles and CL observed in the ovaries of these animals. Together, these observations support the concept that a factor other than LH-like activity or Ins is responsible for the development of the polyfollicular cystic ovaries observed in classic PCOS.[1,3] In this regard, it is interesting to note that tonic stimulation of hypophysectomized rats by follicle-stimulating hormone (FSH) in combination with subovulatory doses of hCG is sufficient for induction of large polyfollicular ovarian cysts.[22] Therefore, the data appear to indicate that an inhibitor of polyfollicular cyst development is absent in hypophysectomized rats, but remains effective in intact animals even in the presence of a hyperinsulinemic state. Further studies are needed to determine what this putative "inhibitor" may be.

The observation that the size of the ovarian cysts did not increase in response to increasing doses of hCG or Ins is also of interest. This observation and the relatively limited size of the ovarian cysts observed in the present experiments are in contrast to the observed development of extremely large ovarian cysts in hypothyroid rats in response to single daily injections of 10 to 20 IU hCG.[23,24] Until the results of the present series of experiments were obtained, it was possible that administration of progressively higher doses of hCG might result in progressively larger ovarian cysts, approaching the size observed in hCG-treated hypothyroid rats.[23,24] The present results exclude this possibility. Together, these observations underscore the importance of understanding the intricate impact of peripheral endocrinopathies such as hyperinsulinemia, hypothyroidism, and hyperprolactinemia on the effects of unabated stimulation by subovulatory doses of LH-like activity on ovarian follicular development.

The observation that hCG-induced ovarian cysts have a limited ability or capacity to metabolize A4 to testosterone and 5α-reduced androgens in vitro[20] directly supports the concept that it is unlikely that ovarian cysts were the source of the observed increase in plasma testosterone in this series of experiments. Plasma testosterone values correlated with the dose of hCG and with the increase in LOCAs, but did not correlate with changes in the number of CL or antral follicles. In contrast to plasma testosterone concentrations, plasma A4 values correlated only with the observed number of CL. Further, there was no correlation between plasma concentrations of A4 and testosterone in this series of experiments. Together, these observations indirectly support the proposition that the source of the plasma testosterone and A4 values observed in hormone-treated animals may have been separate ovarian tissues such as the stromal-interstitial tissue and the CL, respectively. However, in the absence of direct evidence, the cause and source of the elevated plasma testosterone concentrations in these animals remain to be determined.

Although plasma A4 concentrations did not correlate with increasing doses of Ins, the two animals that developed ovarian cysts in response to 9 U Ins plus hCG displayed the highest plasma A4 concentrations of any hormone treatment group. The relationship between plasma concentrations of A4 and E1 and the dose of Ins administered in vivo is complex and cannot be explained at this time. Nevertheless, it is tempting to speculate, from the observation of increased plasma E1 in the present experiments and of increased serum E2 during induction of ovarian cysts by FSH plus hCG in hypophysectomized rats,[22] that estrogens may play a direct role in the induction of cystic follicles in the rat.

Of great interest are the two unexpected observations: (1) fewer animals in the group treated with 6 IU hCG/d plus the highest dose of Ins (9 U/d) developed ovarian cysts than in any other hormonally treated group, and (2) there was an inverse correlation between the diameter of induced ovarian cysts and the dose of Ins used in vivo. There are several potential explanations for this seemingly paradoxical phenomenon. For example, the animals may have been chronically or repeatedly

hypoglycemic. Such a state would cause multiple hormonal changes such as an increase in peripheral concentrations of growth hormone, corticotropin, glucagon, cortisol, and catecholamines, and it is not yet known what effect such changes would have on ovarian cyst formation. However, it is unlikely that these animals were hypoglycemic, since they were able to feed ad libitum.

It is more likely that the inverse correlation between ovarian cyst diameter and of Ins dose, and the decreased incidence of ovarian cysts in animals treated with 9 U Ins and hCG, reflect a "biphasic" hormonal effect. Thus, ovarian follicles may respond positively to low-level hyperinsulinemic states, as demonstrated by our previous observation of a synergistic effect between hCG and Ins with regard to the size of the induced ovarian cysts.[19] However, once peripheral Ins concentrations are attained that cause the tissue to become less responsive to this hormone as a result of downregulation of its receptors, one might expect the size and number of ovarian cysts observed to begin to decrease as illustrated in the data presented here. Biphasic effects also have been observed between Ins and LH with regard to androgen production in cultures of human ovarian stromal tissue[25] and between insulin-like growth factor-I and thyrotropin with regard to ^{3}H-thymidine incorporation in cultures of FRTL5 cells.[26] The mechanisms underlying the loss of synergy between the hormones in these in vitro studies[25,26] and the potential loss of synergy in the present in vivo study remain to be determined. It is tempting to speculate that these observations may provide a partial explanation for the absence of cystic follicles in some women with biochemical features of PCOS and hyperinsulinemia.

In summary, the present series of experiments demonstrate that (1) Ins is the primary driving force for weight gain in these protocols, but marked increases in body weight are not required for the induction of ovarian follicular cysts; (2) LH-like activity (hCG) is the primary driving force for increasing the ovarian cross-sectional area even in the presence of a hyperinsulinemic state; (3) this increase in LOCAs is related primarily to the increased number of CL in these ovaries, and is not related to either the number or the size of ovarian cysts that develop in response to these treatments; (4) hyperinsulinemia and hCG appear to contribute to the development of ovarian cysts in intact non–neuroendocrinologically suppressed rats in distinct ways; and (5) extreme hyperinsulinemia appears to inhibit the development of ovarian cysts in at least some animals. Together, these observations indicate that the effects of Ins on the ovary and the role(s) of Ins in the development of ovarian cysts in vivo may be more complex than previously thought.

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