

Increased Luteinizing Hormone Secretion in Women with Polycystic Ovary Syndrome Is Unaltered by Prolonged Insulin Infusion

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In PCOS women with insulin resistance, hyperinsulinemia may contribute to inappropriate gonadotropin secretion. To determine whether insulin influences gonadotropin release in PCOS, pulsatile LH secretion and gonadotropin responses to GnRH were evaluated before (phase 1) and during (phase 2) insulin infusion. In phase 1, 11 PCOS and 9 normal women on separate days underwent 1) frequent blood sampling (q 10 min) for 12 h and 2) gonadotropin stimulation by successive doses of GnRH, 2 μ g, 10 μ g, and 20 μ g, administered iv at 4 h intervals over a continuous 12 h. In phase 2, studies were repeated 2 h after initiation of a 12-h hyperinsulinemic-euglycemic clamp (80 mU/m²·min). Administration of insulin to both groups failed to alter mean serum gonadotropin concentrations, LH pulse frequency, or LH pulse amplitude. More-

over, gonadotropin responses to GnRH were unchanged by insulin infusion. In PCOS and normal women, a significant reduction of serum androstenedione was associated with insulin administration, whereas no differences were noted for the remaining androgens and estrogens measured.

These findings demonstrated that in PCOS women, LH secretion and gonadotropin responses to GnRH were not influenced by insulin administration. Insulin infusion had little effect on steroid hormone production with the possible exception of androstenedione. These results suggest that inappropriate LH secretion in PCOS is not a direct consequence of insulin resistance and compensatory hyperinsulinemia. (*J Clin Endocrinol Metab* 88: 5456–5461, 2003)

IT HAS BEEN well recognized that women with polycystic ovary syndrome (PCOS) exhibit inappropriate LH secretion characterized by elevated serum concentrations and increases in pulse frequency and amplitude (1, 2). This abnormal pattern of gonadotropin release has been attributed largely to increased activity of the hypothalamic GnRH pulse generator as well as to the feedback effects of ovarian steroid hormones. Insulin has also been implicated in the regulation of LH secretion as evidenced by both *in vitro* and *in vivo* studies (3–18). It has been previously demonstrated *in vitro* that rat pituitary cells preincubated with insulin exhibit increased LH responsiveness after administration of GnRH in a dose-dependent manner compared with those of untreated cells, which suggests a facilitative role for insulin on GnRH stimulated LH release (3–5). However, efforts to determine an effect of insulin infusion in PCOS women have not documented consistent alterations in LH secretion or LH release after GnRH stimulation (6, 7). Reduction of serum LH levels in PCOS has been associated with lowered circulating insulin concentrations induced either pharmacologically or by dietary restriction (8–18). In these studies, because the rate of ovulation improved during treatment, it was unclear whether decreased serum LH was a direct result of lowered circulating insulin levels or secondary to the feedback effect of increased ovarian estrogen production. In contrast to the above findings, several reports have been unable to show an

effect of decreased serum insulin levels on serum LH or LH responses to GnRH after drug treatment or significant loss of body weight in women with PCOS (18–26). Interestingly, the failure to document a decline in LH levels despite improved insulin sensitivity was independent of whether ovulatory activity has resumed in these studies. To address the issue of whether hyperinsulinemia influences gonadotropin secretion in PCOS, serum LH and FSH responses to GnRH and pulsatile LH secretion were evaluated before and during insulin infusion using the hyperinsulinemic-euglycemic clamp technique.

Subjects and Methods

Subjects

Eleven women with PCOS and nine normal women with regular menstrual cycles were recruited for study. All PCOS subjects exhibited excessive facial hair growth and had irregular menstrual bleeding ranging from three to six bleeding episodes per year. Serum androgen levels were elevated in each PCOS subject (Table 1). In addition, all women had ultrasound evidence of bilateral polycystic ovaries (27). Late onset congenital adrenal hyperplasia was excluded by a serum 17-hydroxyprogesterone level (17-OHP₄) of less than 3 ng/ml (<9.1 nmol/liter). The normal subjects were monitored by menstrual calendar for 3 months and by urinary LH testing for 1 month before study to establish the regularity of their cycles. None of the subjects had received any hormone medication for at least 3 months before study. In PCOS women, the mean (\pm SE) age was 28.6 \pm 0.6 yr and not significantly greater than that of the normal control group, 26.3 \pm 0.8 yr. The mean body mass index (BMI) was significantly greater in PCOS subjects compared with that of normal women (35.3 \pm 0.8 vs. 27.4 \pm 0.7, $P < 0.02$), whereas the difference in mean waist-to-hip ratio (0.88 \pm 0.03 vs. 0.80 \pm 0.06) failed to achieve statistical significance. Circulating TSH and PRL levels were normal and not significantly different between groups. The study had been approved by the Institutional Review Board at the University of California,

Abbreviations: A₄, Androstenedione; BMI, body mass index; CV, coefficient of variation; DHEA-S, dehydroepiandrosterone sulfate; E₁, estrone; E₂, estradiol; 17-OHP₄, 17-hydroxyprogesterone; P₄, progesterone; PCOS, polycystic ovary syndrome; T, testosterone.

TABLE 1. Mean (\pm SE) endocrine-metabolic values of PCOS and normal subjects

	Normal (n = 9)	PCOS (n = 11)	Significance P value
LH (mIU/ml)	3.4 \pm 0.5	7.9 \pm 1.5	<0.01
FSH (mIU/ml)	4.8 \pm 0.5	4 \pm 0.4	ns
T (nmol/ml)	1.15 \pm 0.1	2.57 \pm 0.1	<0.001
A (nmol/ml)	2.8 \pm 0.4	5.7 \pm 0.7	<0.001
17-OHP (pmol/ml)	2.1 \pm 0.3	3.3 \pm 0.4	<0.05
DHEA-S (nmol/ml)	3.9 \pm 0.6	4.3 \pm 0.6	ns
P ₄ (pmol/ml)	2.2 \pm 0.3	2.5 \pm 0.2	ns
E ₁ (pmol/ml)	199 \pm 27	390 \pm 37	<0.001
E ₂ (pmol/ml)	189 \pm 22	248 \pm 185	<0.04
Insulin (μ U/ml) ^a	13.1 \pm 3.1	38.2 \pm 10.0	<0.03
Glucose (mmol/ml)	4.3 \pm 0.1	4.4 \pm 0.2	ns

^a Conversion to pmol/liter: multiply by 7.18. ns, Not significant.

San Diego, and written informed consent was obtained from each participant before study.

Procedure

Each subject was admitted to the General Clinical Research Center at the University of California, San Diego, for 2 d of study. The PCOS subjects were tested at random, whereas normal control subjects were studied during the mid-follicular phase defined as d 6–8. On d 1 of study an iv cannula was inserted and after 30 min baseline blood samples were drawn. At 0800, blood samples were obtained at 10-min intervals for 12 h. On d 2 of study, after an overnight rest, beginning at 0800, three successive doses of GnRH (2, 10, and 20 μ g) were administered iv at 4-h intervals over a continuous 12-h period. The sequence of GnRH dosing was intentional and not randomized to minimize potential residual increases in serum LH before administration of the next dose of GnRH. The GnRH (Factrel) was kindly provided by Wyeth Pharmaceuticals. None of the PCOS subjects had experienced recent ovulation as evidenced by changes in their menstrual patterns or scant bleeding episodes unaccompanied by premenstrual molimina. In addition, at the time of study each PCOS woman had serum progesterone (P₄) levels of less than 1 ng/ml (<3.0 nmol/liter) at the baseline sample. Frequent blood samples were obtained before and for up to 120 min after each dose of GnRH. Subsequently, each subject was readmitted to the General Clinical Research Center and the 2-d study protocol was repeated during a euglycemic hyperinsulinemic clamp. In PCOS and normal subjects, the repeat protocol was administered at a minimum interval of 1 month.

Hyperinsulinemic-euglycemic clamp

Studies were performed in the morning after a 12-h overnight fast. At 2100 h, an 18-gauge iv catheter was inserted into an antecubital vein and an infusion of normal saline was started. At 0700 h, another iv catheter was inserted in a retrograde fashion in a hand vein, with the hand placed in a hand warmer for sampling of arterialized blood. An iv infusion of insulin (Humulin; Eli Lilly, Indianapolis, IN) diluted in 0.15 mol/liter saline containing 1% wt/vol human albumin was then begun at a rate of 80 mU/m²·min, which was started 2 h before the first GnRH dose and continued for 12 h. Potassium and phosphate were given iv to compensate for the intracellular movement of these ions and to maintain normal blood levels. A variable infusion of 20% glucose was delivered to maintain a plasma glucose concentration of 4.72 mol/liter (85 ng/dl). Blood samples were obtained every 5 min for measurement of plasma glucose with a glucose analyzer (YSI 2700 analyzer; Yellow Springs Instrument Co., Yellow Springs, OH). During the last 30 min of insulin infusion, blood samples were obtained at 10-min intervals for determination of plasma glucose concentrations.

The glucose infusion rate in each patient was calculated as the amount of glucose (milligram) infused per kilogram body weight during the last 30 min of the clamp study. The mean steady-state insulin level achieved at the end of the clamp is known to suppress hepatic glucose output and, therefore, the glucose infusion rate was equivalent to the glucose disposal rate.

Assays

Serum LH and FSH concentrations were measured by RIA with intra- and interassay coefficients of variation (CVs), respectively, of 5.4% and 8.0% for LH and 3.0% and 4.6% for FSH (Diagnostic Products Corp., Los Angeles, CA). Serum concentrations of estrone (E₁), estradiol (E₂), androstenedione (A₄), and testosterone (T) were measured by well-established RIA with intraassay CVs less than 7%. Serum P₄, 17-OHP₄, and dehydroepiandrosterone sulfate (DHEA-S) were measured by RIA with intraassay CVs less than 7% (Diagnostic Systems Laboratories, Webster, TX). Serum insulin levels were measured by a double antibody RIA with an assay sensitivity of 2 μ U/ml and intra- and interassay CVs of 7% and 9%, respectively. Plasma glucose levels were determined by the glucose oxidase method (Yellow Springs Instrument Co.) with an intraassay CV less than 2% and an intraassay CV of 3%.

Pulse analysis

LH pulse activity was analyzed using the Cluster pulse detection algorithm (Veldhuis '86). A cluster configuration of 2 \times 2 and *t* statistics of 2.45 \times 2.45 were chosen to minimize false positive and false negative errors. Dose-dependent intrasample variance was assessed by employing a second-degree polynomial regression of SD as function of hormone concentration. Pulse number per 12 h and mean pulse amplitude (difference in serum concentration between the preceding nadir and the pulse peak) were determined for each subject.

Statistics

Depending on the analysis, LH responses were measured as the difference between the maximal and baseline levels (maximal increment), and the maximal percent change from baseline. A log-transformation was applied when appropriate, and square root transformation was used for percent change in LH response. To determine interaction between group and dose as well as main effects, two-group repeated measures ANOVA and analysis of covariance were used. *Post hoc* testing was done with a Bonferroni correction. Comparisons of mean baseline values between PCOS and normal women were performed using independent Student's *t* tests (SPSS 11.0 software, SPSS Inc., Chicago, IL). Correlations among variables were analyzed using the Pearson correlation coefficient method.

Results

Baseline studies

Baseline hormone values are shown in Table 1. In PCOS, mean (\pm SE) circulating levels of LH, T, A, E₁, E₂, 17-OHP₄, and fasting insulin were significantly greater than those of normal controls. Serum FSH, DHEA-S, P₄, and glucose levels were similar in both groups.

Hyperinsulinemic-euglycemic clamp

In the PCOS group, mean steady-state plasma insulin levels, 235 \pm 25.5 μ U/ml, resulting from the hyperinsulinemic clamp were significantly (*P* = 0.02) higher than those achieved in normal women, 173 \pm 19.3 μ U/ml, despite equivalent infusion rates and similar serum glucose concentrations (Fig. 1). Steady-state serum glucose levels were maintained between 85 and 90 mg/dl in both groups. The mean glucose disposal rate in PCOS subjects was significantly less (*P* < 0.02) than that found in normal women and indicative of insulin resistance.

Effect of insulin infusion on pulsatile LH secretion

The composite mean 12-h serum LH concentration in PCOS was significantly higher than that of normal women as expected (Table 2). Assessment of pulsatile LH secretion in

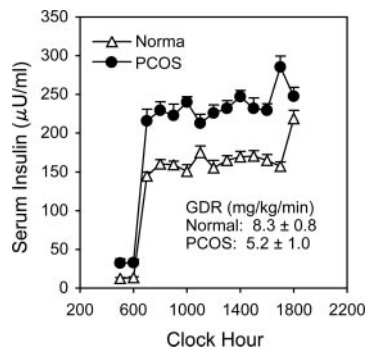


FIG. 1. Mean (\pm SE) serum insulin levels during the hyperinsulinemic-euglycemic clamp ($80 \text{ mU/m}^2\text{-min}$) in normal and PCOS women. The mean (\pm SE) glucose disposal rate (GDR) for each group is also shown. In PCOS women, the mean composite serum insulin level and GDR were significantly ($P < 0.05$) higher and lower, respectively, than those found for normal women.

TABLE 2. Effect of insulin infusion on mean (\pm SE) 12-h composite mean LH, pulse frequency, and LH pulse amplitude in normal and PCOS women

	Normal	PCOS
12-h composite LH (mIU/ml)		
No insulin	3.5 ± 0.4	6.7 ± 0.1^a
Insulin infusion	3.2 ± 0.3	4.9 ± 0.9
LH pulse frequency (no./12 h)		
No insulin	8.8 ± 0.8	10.2 ± 0.4^b
Insulin infusion	8.1 ± 0.8	10.3 ± 0.5^b
LH pulse amplitude (mIU/ml)		
No insulin	1.6 ± 0.2	1.8 ± 0.3
Insulin infusion	1.6 ± 0.2	1.4 ± 0.2

Normal vs. PCOS; ^a $P < 0.01$; ^b $P < 0.006$.

PCOS women revealed that the mean pulse frequency value, pulses/12 h, was significantly greater than that found in the normal women. The corresponding mean pulse amplitude in PCOS women was higher, but not statistically different from that of the normal group.

Administration of insulin to women with PCOS was not associated with a significant alteration in serum LH as the mean 12-h composite concentration was comparable to that observed without insulin infusion (Table 2). In addition, throughout the duration of the hyperinsulinemic clamp, LH pulse frequency and LH pulse amplitude were unchanged from those values measured before insulin infusion. In normal women, changes in the mean composite LH level, LH pulse frequency, and LH pulse amplitude were not detected during the hyperinsulinemic clamp.

Effect of insulin infusion on gonadotropin responses to GnRH

In the absence of insulin administration, PCOS subjects exhibited significantly higher ($P < 0.02$) mean maximal serum LH responses to multidose GnRH compared with those of normal women (Fig. 2), which is consistent with previous results from our group (1). In PCOS women, there were subtle increases in mean baseline LH levels before each successive dose of GnRH, which likely represented carryover effect as a result of prior GnRH stimulation. These increases were accompanied by corresponding increments of LH after GnRH, in a dose-dependent manner. In normal women a

similar pattern of GnRH-stimulated LH release was found. Notably, in PCOS and normal women, the mean maximal rise of LH was related to the respective baseline LH concentration as the percent incremental change with each dose of GnRH was equivalent within and between groups.

Baseline serum FSH levels before each dose of GnRH in PCOS women were not significantly different from those observed in normal women. In response to GnRH, FSH release in PCOS women was not significantly different from those of normal women.

At the time of insulin administration in PCOS women, the mean preinfusion concentration of serum LH was $4.0 \pm 1.2 \text{ mIU/ml}$. An effect of insulin was not apparent 2 h after initiation of the clamp, as the mean level of circulating LH was $4.7 \pm 0.9 \text{ mIU/ml}$ (Fig. 3). In response to multidose GnRH, significant increases in serum LH responses in PCOS women were noted, the pattern of which was essentially the same as that demonstrated without insulin infusion. The progressive mean maximal rise of LH in response to $2\text{-}\mu\text{g}$ and

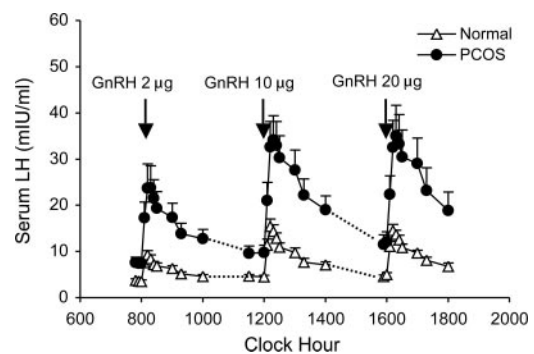


FIG. 2. Time-course of mean (\pm SE) serum LH concentrations after iv administration of three successive doses of GnRH given at 4-h intervals over 12 h to normal and PCOS women. In PCOS, significant increases in LH increment ($P < 0.02$) at each dose as well as the integrated (area under the curve) LH response ($P < 0.01$) over the entire course of study were detected compared with those of normal women.

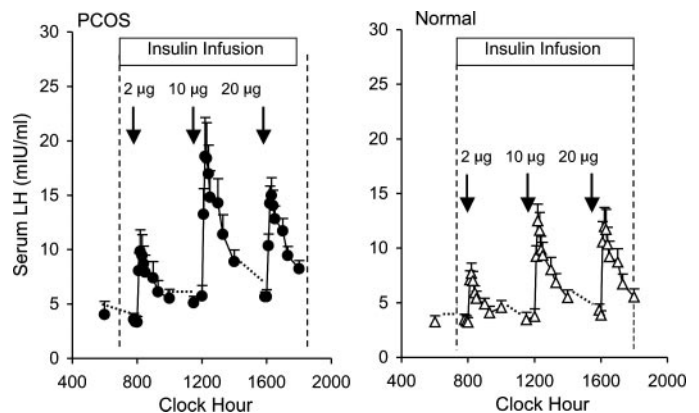


FIG. 3. Time-course of mean (\pm SE) serum LH concentrations after iv administration of three successive doses of GnRH given at 4-h intervals during the hyperinsulinemic-euglycemic clamp ($80 \text{ mU/m}^2\text{-min}$) in PCOS and normal women. In PCOS, significant increases in LH increment ($P < 0.02$) were detected compared with those of normal women. Note that the preinfusion mean concentration of serum LH was similar to the initial preinjection mean baseline level of LH in both PCOS and normal women.

10- μ g doses of GnRH corresponded to their respective baseline levels, in contrast to the response to 20 μ g, which was of lesser absolute magnitude and, therefore, lower relative to baseline. In PCOS women, insulin infusion was not associated with differences in relative LH responsiveness to GnRH as the percent incremental change at each dose was equivalent to that found without insulin administration (Fig. 4). The similarity of responsiveness in PCOS women before and during insulin infusion was reflective of the significant positive correlation between maximal GnRH-stimulated LH levels and preinjection baseline serum LH as depicted in Fig. 5. A lack of insulin effect on LH responsiveness to GnRH was also evident in normal women. Serum LH levels before, and 2 h after beginning the infusion of insulin, did not vary and baseline preinjection LH levels and mean maximal LH responses to multidose GnRH were comparable in the presence and absence of insulin infusion (Fig. 3). In PCOS and normal women, baseline serum FSH and GnRH-stimulated FSH release were not altered by insulin administration, as responses before and during the hyperinsulinemic clamp were similar.

None of these patients ovulated nor did they experience any uterine bleeding after GnRH administration indicating the lack of clinical consequences. Normal ovulatory women did not notice any alteration in their menstrual patterns.

Effect of insulin infusion on steroid hormone levels

Determination of mean circulating levels of serum androgens before and at the end (pooled samples during the last hour) of the hyperinsulinemic clamp, conducted during frequent sampling (without GnRH stimulation), showed that in PCOS, the mean baseline serum androstenedione level, was decreased significantly (Fig. 6). The time-course response during insulin infusion revealed that serum A₄ declined significantly within 2 h of commencing insulin infusion and remained suppressed throughout the interval of insulin administration (Fig. 6). Normal women also sustained a significant reduction of mean serum A₄ after insulin infusion. By comparison, in both PCOS and normal women, serum testosterone levels before and at the end of the insulin clamp were unaltered (Table 3). In addition, circulating levels of E₁, E₂, 17-OHP₄, and DHEA-S in both groups before and at the end of insulin infusion failed to demonstrate any significant differences.

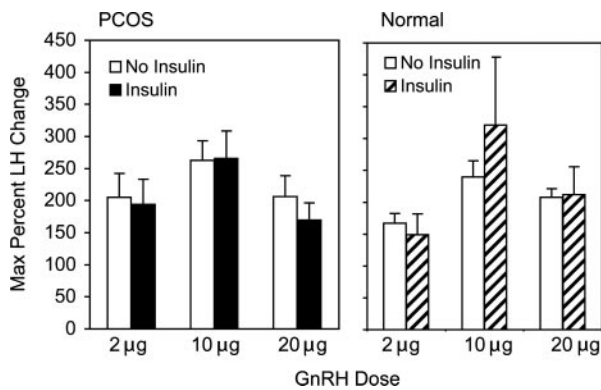


FIG. 4. Mean (\pm SE) maximal percent LH change after administration of GnRH, at the indicated doses, in the absence and presence of insulin infusion in PCOS and normal women.

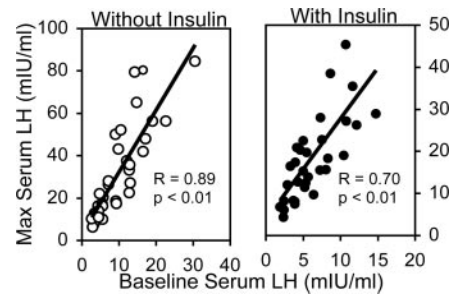


FIG. 5. Relationship of maximal serum LH levels after each dose of GnRH to respective preinjection baseline LH levels in PCOS women before ($r = 0.84$, $P < 0.01$) and during ($r = 0.70$, $P < 0.01$) insulin administration. The correlation between maximal response and baseline values were not altered by insulin infusion.

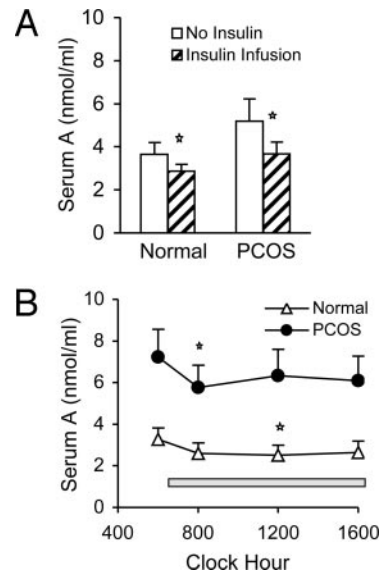


FIG. 6. A, Mean (\pm SE) serum A levels measured before and at the end (pooled samples during the last hour) of the hyperinsulinemic-euglycemic clamp during frequent sampling in normal and PCOS women. Serum A levels were significantly ($P < 0.05$) lower at the end of insulin infusion in both groups. B, Mean (\pm SE) serum A levels measured before insulin infusion and at baseline before each injection of GnRH during insulin administration, represented by the solid bar, in normal and PCOS women. *, $P < 0.03$ for PCOS and $P < 0.05$ for normal.

TABLE 3. Mean (\pm SE) steroid hormone levels before and at the end of the 12-h hyperinsulinemic-euglycemic clamp conducted during frequent sampling in normal and PCOS subjects

Steroid hormone	Normal		PCOS	
	Before	After	Before	After
A (nmol/ml)	3.7 \pm 0.5	2.9 \pm 0.3 ^a	5.2 \pm 1.0	3.7 \pm 0.5 ^b
T (nmol/ml)	1.0 \pm 0.2	1.0 \pm 0.1	1.8 \pm 0.3	1.7 \pm 0.2
17-OHP (pmol/ml)	2.4 \pm 0.3	2.7 \pm 0.6	3.3 \pm 0.6	2.7 \pm 0.3
DHEA-S (nmol/ml)	4.0 \pm 1.0	4.1 \pm 1.1	3.8 \pm 1.0	3.7 \pm 0.9
E ₁ (pmol/ml)	175 \pm 13	181 \pm 23	274 \pm 41	248 \pm 47
E ₂ (pmol/ml)	207 \pm 30	275 \pm 55	232 \pm 74	215 \pm 24

^a $P < 0.04$.
^b $P = 0.004$.

Discussion

The results of this study have demonstrated that in PCOS and normal women pulsatile LH secretion and gonadotropin responses to GnRH were unaltered during administration of

insulin by the hyperinsulinemic-euglycemic clamp technique. In addition, an effect of insulin infusion on ovarian steroid production in both groups was characterized by a significant decrease in serum androstenedione concentrations, whereas the levels of other steroid hormones measured were unchanged.

These findings clarify the results of previous preliminary studies, which showed that GnRH-stimulated LH responses in both normal and PCOS women were significantly decreased during a 4-h hyperinsulinemic-euglycemic clamp compared with those found with saline infusion (28). In that study, lowered LH responsiveness during insulin infusion was associated with reduced preinfusion basal levels of circulating LH, which likely accounted for the apparent insulin-related LH suppression. Our results are consistent with previous studies, which have not been able to establish an effect of insulin on LH or FSH release. In normal women undergoing long-term insulin administration by a 16-h hyperinsulinemic-euglycemic clamp, mean serum LH levels, measured every hour, remained unchanged during the entire course of infusion (6). In women with PCOS, an attempt to identify whether insulin infusion influenced gonadotropin secretion failed to demonstrate conclusive results (7). During 6-h insulin infusions randomized to either one of a consecutive 2 d in PCOS and normal women, consistent alterations in mean serum LH, LH pulse frequency or pulse amplitude, and LH release after GnRH could not be documented. Sequence effects of study d 1 *vs.* study d 2 were readily apparent, which may have obscured any influence of insulin on gonadotropin release. These sequence effects were attributed to spontaneous changes in basal gonadotropin secretion.

The inability of insulin to induce alterations in LH secretion in PCOS women is relevant to studies that have shown an inverse correlation between 24-h insulin levels and both serum LH and LH pulse amplitude (29). In that study, it was also demonstrated that hyperinsulinemia was positively correlated to BMI, whereas insulin sensitivity had an inverse linear relationship to BMI. The parallel relationship between hyperinsulinemia and BMI precluded determination of a possible independent inhibitory effect of insulin on LH secretion. Considering the data of the current study, it seems as if obesity would be more likely to exert a restrictive influence on the level of circulating LH compared with an effect related to hyperinsulinemia.

Our *in vivo* findings are in contrast to previous results obtained from *in vitro* animal studies, which demonstrated that insulin enhanced GnRH-mediated LH release from rat pituitary cells by increasing gonadotrope sensitivity to GnRH in a dose-dependent manner (3–5). The concentration of insulin used to pretreat cells was physiologically relevant compared with that achieved during the hyperinsulinemic-euglycemic clamp employed in the current *in vivo* study. Similar studies conducted in the same rodent model have shown that high physiological doses of insulin enhanced LH responses to GnRH, whereas progressive increases in concentration inhibited LH release in a bimodal manner (30). In our study, the mean steady-state level of insulin achieved during infusion was about 275 $\mu\text{U}/\text{ml}$, which is greater than levels generally encountered in PCOS. Whether lower levels of hyperinsulinemia may have altered gonadotropin secre-

tion or responsiveness to GnRH is unknown. Alternatively, the *in vivo* environment of our study may have precluded expression of an insulin effect, as insulin-mediated increases in LH responsiveness, *in vitro*, have not been observed using serum supplemented media (3).

We observed a significant difference in steady-state insulin levels between PCOS and normal women despite similar rates of insulin infusion and equivalent glucose concentrations. The cause for this discrepancy may have been due to decreased clearance of insulin in the PCOS group in whom the mean BMI was significantly higher. A similar disparity of circulating insulin levels during insulin infusion has been observed previously in obese, weight-matched PCOS and normal women undergoing hyperinsulinemic-euglycemic clamp studies (7). In both studies, assessment of insulin metabolic clearance was not performed.

The mean serum A_4 level was significantly decreased during insulin infusion in both PCOS and normal women whereas serum 17-OH P_4 , DHEA-S, and T levels were unchanged. Previously, it has been demonstrated in normal men and women that short-term insulin infusions at physiological doses of less than 100 $\mu\text{U}/\text{ml}$ were associated with an acute rise of circulating A_4 levels (31). At insulin levels beyond 100 $\mu\text{U}/\text{ml}$, the serum A_4 increment was not significant from baseline values. Similar results were found in women with PCOS who were randomly studied on the first of two consecutive study days (7). In those PCOS subjects studied on the second day of study, insulin administration failed to induce a significant rise in circulating A_4 levels. The lack of serum A_4 response on d 2 may have represented a carry over effect of the first day of study or, possibly, attributed to the relatively short length of infusion of 6 h. The latter consideration was not corroborated by our findings, as the length of insulin administration was 10 h. In addition, an acute effect of insulin was indicated by the significant decline of serum A_4 within 2 h of infusion, as indicated by the time-course pattern of response. That the serum A_4 response may have reflected alteration in clearance is unlikely because corresponding changes in serum E_1 were not observed during insulin infusion. Serum T levels did not vary throughout 10 h of insulin administration, which is consistent with some, but not all studies (6, 7, 31). Dunaif (7) found significant reductions in serum T during insulin infusion, which was unlikely due to increased 17-oxido-reductase, as suggested by concomitant increases in serum estradiol. Studies to determine differences in the metabolic clearance of testosterone were not performed. We were also unable to detect alterations in 17-OHP₄ and DHEA-S. However, Nestler *et al.* (6) demonstrated a significant decline in serum DHEA-S levels in normal women and a woman with hyperandrogenism at the end of a 16-h hyperinsulinemic clamp. The length of insulin administration or resultant steady-state concentration during the present experiments may have been insufficient to expose an effect of insulin on DHEA-S. Summarily, our findings strongly suggest that insulin administration by the hyperinsulinemic-euglycemic clamp method exerts little effect on basal steroid hormone levels in women with PCOS with the possible exception of serum A_4 . The significant decrease of circulating A_4 must be considered in light of inconsistent findings from other studies, which have demonstrated

either an increase or no change as a result of insulin infusion. Further investigation of insulin action on ovarian steroidogenesis is necessary to fully elucidate the role of insulin.

In summary, our findings have clearly shown that in PCOS women, LH secretion and gonadotropin responses to GnRH were not influenced by insulin administration. Moreover, insulin infusion appeared to have little effect on ovarian steroid or adrenal androgen production with the possible exception of androstenedione. These results suggest that inappropriate LH secretion in PCOS is not a consequence of insulin resistance and compensatory hyperinsulinemia. Alternatively, additional studies are necessary in PCOS to determine whether gonadotropin secretion is susceptible to hyperinsulinemia after reversal of insulin resistance.

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