

Evidence for Insulin Suppression of Baseline Luteinizing Hormone in Women with Polycystic Ovarian Syndrome and Normal Women

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Context: In women with polycystic ovarian syndrome (PCOS), the relationship of insulin to LH secretion and responses to GnRH remains unresolved. A rigorous analytical examination of this relationship has not been performed.

Objective: Our objective was to determine the relationship of basal LH secretion and responses to GnRH, insulin, and other endocrine variables in normal and PCOS women.

Design: In PCOS and normal women, mean composite 12-h LH secretion was analyzed for correlating factors. LH responses to varying doses of GnRH during a fixed rate of insulin infusion and LH responses to a fixed dose of GnRH during varying doses of insulin infusion were analyzed for contributing factors.

Patients and Setting: Eighteen PCOS and 21 normal women underwent studies of frequent blood sampling and GnRH stimulation before and during insulin infusion at the General Clinical Research Center, University of California, San Diego.

Main Outcome Measures: Group mean composite 12-h LH levels were assessed with respect to other endocrine variables. In addition, LH responses to GnRH with or without insulin infusion were assessed.

Results: In normal women, insulin negatively predicted mean LH. In PCOS, the combined effect of body mass index (negative) and testosterone (positive) predicted LH. The best predictor of LH was body mass index and insulin combined. Basal LH and LH responses to GnRH were unaltered by insulin infusion in normal women. These measures were reduced during insulin infusion in PCOS women.

Conclusions: In PCOS, insulin infusion suppresses pituitary response to GnRH. In normal women, insulin negatively correlates with mean LH and suppresses GnRH response at a high infusion rate. (*J Clin Endocrinol Metab* 93: 2089–2096, 2008)

In women with polycystic ovary syndrome (PCOS) inappropriate pituitary gonadotropin secretion is generally characterized by increased mean LH serum concentrations, greater LH pulse frequency, and enhanced LH responses to GnRH compared with that of normal women (1–3). The basis for altered LH secretion has been attributed to increased activity of the GnRH pulse generator that may result from disruption of steroid feed-

back or the influence of other endocrine factors (4–6). In particular, previous studies demonstrated that in cultured rat pituitary cells, insulin was shown to amplify LH responses to GnRH (7). Additionally, results of recent studies in a gonadotrope cell line suggested that insulin can increase LH β -subunit promoter activity or increase expression of another GnRH-responsive gene, early growth response gene-1 (8, 9). These findings are

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

relevant to women with PCOS in whom insulin resistance and compensatory hyperinsulinemia are common.

The precise role of insulin on pulsatile gonadotropin secretion has not been clearly established (10, 11). In PCOS women, administration of insulin by infusion has not demonstrated consistent effects on gonadotropin release (11–13). Similarly, restoration of normal LH secretion has been observed in PCOS women after restricted caloric intake or treatment with insulin-lowering drugs in some but not in all studies (14–22). In contrast, other studies have suggested that insulin has a negative effect on gonadotropin secretion. Treatment of normal and obese men with diazoxide resulted in lowered insulin levels that was associated with increased circulating LH levels (23). However, in PCOS women, diazoxide treatment failed to alter basal or GnRH-stimulated LH or FSH levels (24).

An effect of insulin infusion on GnRH-induced LH release in PCOS women was shown to be similar to that observed in normal women based on percent change from baseline (25, 26). LH responses were largely dependent upon basal concentrations, which suggested that in PCOS women, increased LH secretion was likely a reflection of enhanced baseline LH rather than greater pituitary sensitivity to GnRH. Thus, factors that influence basal LH output would appear to contribute to inappropriate gonadotropin secretion in PCOS women. The factors influencing baseline LH levels have not been thoroughly examined. We have performed a detailed analysis of both new and previous studies using improved statistical methods to identify factors that may contribute to inappropriate gonadotropin secretion in PCOS women. Our findings suggest that insulin may reduce spontaneous gonadotropin release and responsiveness to GnRH.

Subjects and Methods

Subjects

A total of 18 women with PCOS and 21 normal women with regular menstrual cycles were included in this study. The diagnosis of PCOS was based on clinical or biochemical evidence of hyperandrogenism and oligo- or amenorrhea as described by the 1990 National Institutes of Health conference on PCOS. Most of the PCOS subjects had ultrasound evidence of bilaterally enlarged polycystic ovaries. Congenital adrenal hyperplasia was excluded by determination of a serum 17-hydroxyprogesterone (17-OHP) level less than 3 ng/ml. Serum prolactin and TSH levels were normal in all subjects. Normal subjects were monitored by menstrual calendar for 3 months and by urinary LH testing for 1 month before establishing the regularity of cycling. No subjects received any hormone medication for at least 3 months before the study. This study was approved by the Human Research Protection Program at the University of California, San Diego, and written informed consent was obtained from each subject before participation.

Procedure

In one subset of subjects, 13 PCOS women and 13 normal women underwent assessment of episodic LH secretion. Of these individuals, 11 PCOS and nine normal women were further examined for LH responses to increasing GnRH dose before and during a fixed dose of insulin infusion as previously described (12, 25). A second set of five PCOS women and eight normal women participated in studies to assess the effect of low-dose and high-dose insulin infusion on basal LH secretion and LH responses to a fixed dose of GnRH. Details of the procedure for insulin

and GnRH treatment and sample collection are provided in the supplemental material (published as supplemental data on The Endocrine Society's Journals Online web site at <http://jcem.endojournals.org>).

Assays

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

Statistical analysis

Analyses were performed using the statistical software packages JMP version 6.03 (SAS, Cary, NC) and R (<http://www.r-project.org/>) version 2.4.1. Details of the analysis are provided in supplemental material. Briefly, descriptive statistics for baseline endocrine values were computed, and data were analyzed for normality and variance homogeneity. If necessary, values were corrected by appropriate transformation before analysis in Tables 1–3 and Fig. 1. Similar methodology was used for analysis of baseline and area under the curve analysis of LH values for insulin infusion at low and high doses in Fig. 4. For analysis of multiple GnRH doses at a single insulin infusion rate as presented in Table 4 and Figs. 2 and 3, linear mixed-effects modeling was employed to accommodate repeated GnRH doses in the presence and absence of insulin infusion. Interaction variables were considered in the model-building process and eliminated due to insignificance.

Results

Comparison of mean hormone levels between PCOS and normal women

In the first subset of subjects, composite 12-h LH mean values, basal serum insulin, body mass index (BMI), and glucose levels and other endocrine variables were pooled among each group of PCOS subjects and normal women to determine whether group differences existed (Table 1). Although groups showed considerable overlap, in PCOS subjects, mean composite LH ($P = 0.002$), insulin ($P = 0.003$), BMI ($P = 0.001$), and T ($P < 0.0001$) were significantly greater compared with corresponding values in normal controls. In addition, mean levels of A4 ($P = 0.0004$), E1 ($P < 0.0001$), and E2 ($P = 0.015$) in PCOS women were significantly higher than those observed in normal women, consistent with increased ovarian steroid output, defined as a component of diagnosis of PCOS. Serum DHEA-S, 17-OHP, P4, and glucose levels were similar between groups. There was no significant difference in age between groups, and 30% of the normal control subjects had a BMI over 30 in this study.

Multivariable analysis of baseline LH levels in PCOS and normal women

It has been demonstrated previously that LH pulse amplitude, 24-h mean LH levels, and peak pituitary responsiveness to GnRH were inversely correlated to BMI in PCOS, but the rela-

TABLE 1. Baseline endocrine and metabolic values of PCOS and normal subjects (\pm SEM)

Factor	NC	PCOS	P
Age (yr)	24.93 \pm 1.69	29.15 \pm 1.62	NS
LH (IU/liter)	3.4 \pm 0.3	6.5 \pm 0.9	0.002
Insulin (μ U/ml)	11.9 \pm 2.2	25.4 \pm 3.5	0.003
BMI (kg/m ²)	26.4 \pm 1.4	35.0 \pm 2.0	0.001
Glucose (mM)	4.4 \pm 0.1	4.5 \pm 0.2	NS
17-OHP (ng/ml)	1.0 \pm 0.1	1.2 \pm 0.2	NS
P4 (ng/ml)	0.8 \pm 0.1	0.9 \pm 0.1	NS
DHEA-S (ng/ml)	1447 \pm 198	1592 \pm 243	NS
A4 (nM)	3.2 \pm 0.4	6.0 \pm 0.6	0.0004
T (nM)	1.3 \pm 0.1	2.6 \pm 0.1	<0.0001
E1 (pM)	176 \pm 17	415 \pm 34	<0.0001
E2 (pM)	199 \pm 16	301 \pm 48 ^a	0.015

Values reflect untransformed scale of variable. *P* values show significance of group differences of transformed variables as determined by two-sided *t* test. NS, Not significant.

relationship of insulin to LH was not investigated directly (27, 28). We sought to assess whether BMI or another factor may be used to generally predict pituitary output of LH as measured by composite mean circulating LH levels. The significant differences between normal control and PCOS identified in the group comparisons indicated that the contribution of insulin, BMI, and steroids to the overall determination of covariance was not equivalent between groups. Therefore, regression analysis was conducted with each diagnostic group separately. In this analysis, normal and PCOS groups showed distinct differences in covariance of LH with T, BMI, and insulin (Table 2). In the normal control group, all steroid measurements and BMI failed to show significant covariance with mean LH. However, insulin did show significant negative correlation with mean LH ($r = -0.674$; $P = 0.016$) in normal women as determined by Pearson product moment. In the PCOS group, significant positive correlation with T was identified ($r = 0.567$; $P = 0.043$) compared with an inverse correlation with BMI ($r = -0.656$; $P = 0.015$). Thus, in the control group, insulin alone was predictive, but within PCOS women, both T and BMI were predictive of mean LH levels. Further regression modeling of both normal and PCOS groups against BMI, T, and insulin (Fig. 1) illustrate the differences between diagnostic groups with respect to each of these variables individually. In normal subjects, bivariate regression modeling between LH and insulin confirmed this relationship ($R^2 = 0.455$;

$P = 0.016$). In PCOS subjects, regression of LH vs. BMI ($R^2 = 0.430$; $P = 0.015$) and T ($R^2 = 0.322$; $P = 0.043$) was also confirmed.

Although the univariate correlation and bivariate regression models demonstrate a significant relationship of T, BMI, and insulin to LH levels, it is important to evaluate the contribution of individual variables in the context of others. In particular, BMI may be a reflection of other endocrine disorders not well described or understood and therefore should be accounted for in the models comparing other factors. To develop a more satisfactory model of the relationship between mean LH and insulin, BMI, and T values, multivariable modeling was undertaken to identify any interaction and to develop a stronger model predicting mean LH levels (Table 3). In the normal control group, multifactor models including T and BMI did not greatly improve fitting or reveal significant interactions between variables. Insulin alone provided the only significant regression of mean LH ($LH = I$). Controlling for BMI by inclusion in the model only modestly improved the fitting ($LH = I + BMI$). Inclusion of testosterone ($LH = I + T$) in the model degraded the significance of the fitting. Overall, these results suggest that in the normal group, 12-h mean LH levels were best predicted by insulin alone, and no interaction with BMI or T was detected. In PCOS, BMI was the strongest predictor of composite LH levels in the model ($LH = BMI$). Inclusion of T as a second variable in a more complex model ($LH = BMI + T$) modestly improved the overall fitting of the data, but each individual variable in the model lost significance, indicating colinearity or a lack of independence. Further testing for interaction or synergy between BMI and T with the model $LH = BMI + T + (BMI \times T)$ did not improve the fitting. Most interestingly, inclusion of insulin with BMI in the model and testing for interaction or synergy [$LH = I + BMI + (I \times BMI)$] provided the strongest prediction of mean LH in PCOS women. This suggests that insulin may contribute negatively to the level of LH in PCOS through interaction with BMI. Larger sample populations would be required to firmly establish any synergistic effect. Although these models predict an inverse relationship between LH and that of insulin or BMI, the reason for the initially high levels of LH in lean PCOS subjects is not clear.

TABLE 2. Correlation of endocrine values with 12-h composite LH

Factor	Normal		PCOS	
	R	P	R	P
17-OHP	-0.027	NS	0.231	NS
P4	-0.350	NS	0.397	NS
DHEA-S	-0.072	NS	0.092	NS
A4	0.203	NS	0.239	NS
T	0.249	NS	0.567	0.043
E1	-0.181	NS	-0.138	NS
E2	0.170	NS	0.493	NS
BMI	-0.252	NS	-0.656	0.015
Insulin	-0.674	0.016	-0.201	NS

NS, Not significant; R, Pearson product moment for pairwise correlation with mean LH.

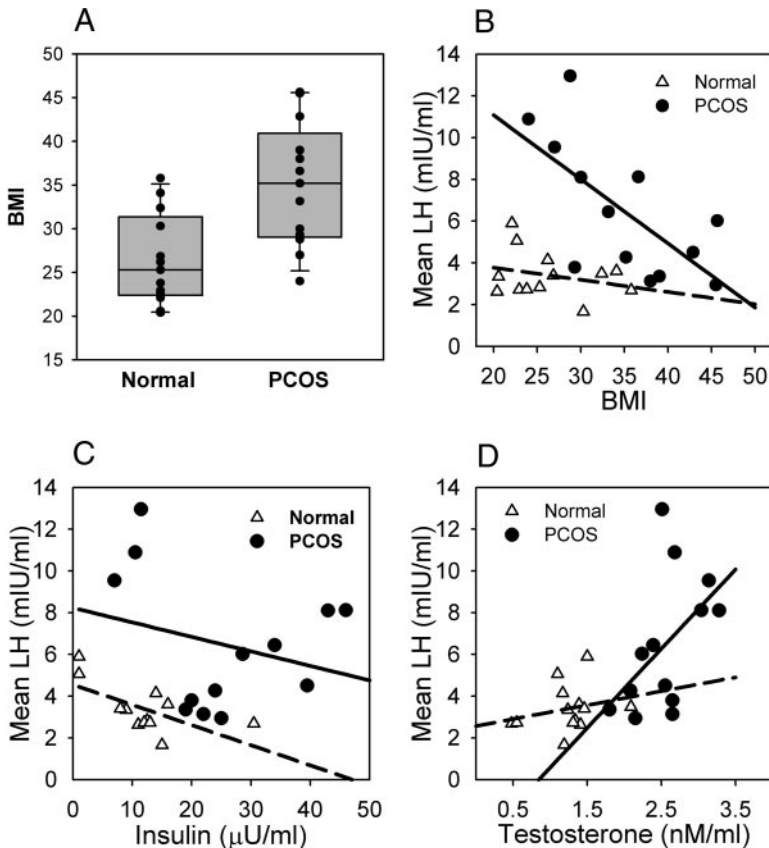


FIG. 1. Linear modeling of mean 12-h LH levels of normal and PCOS women vs. serum insulin and BMI. Untransformed mean LH, insulin, and BMI values were used in single-variable linear models to determine significance of fit. A, Box plot of BMI values between normal and PCOS subjects showing range of values. Differences are noted in Table 1. B, Regression of mean LH vs. BMI for normal ($R^2 = 0.07$; $P = 0.37$) and PCOS ($R^2 = 0.45$; $P = 0.01$) subjects contrasting the relationship of LH levels to BMI. C, Linear regressions of mean LH values vs. serum insulin of normal ($R^2 = 0.40$; $P = 0.027$) and PCOS ($R^2 = 0.07$; $P = 0.38$) subjects contrasting difference in relationship of LH levels to insulin between populations. D, Regression of mean LH to T for normal and PCOS subjects. Neither population displayed a significant relationship of mean LH to testosterone when modeled using untransformed values.

Effect of a fixed rate of insulin infusion on basal LH secretion

A second study was conducted assessing the effect of a fixed rate of insulin infusion on basal LH secretion. Subjects were administered a euglycemic, hyperinsulinemic clamp to maintain

TABLE 3. Linear modeling of 12-h composite LH with identified covariates

Model	R ²	P
Normal group		
LH = I	0.455	0.016
LH = I + BMI	0.489	0.048
PCOS group		
LH = T	0.322	0.043
LH = BMI	0.430	0.015
LH = I + BMI	0.455	0.048
LH = BMI + T	0.498	0.032
LH = I + BMI + (I × BMI)	0.614	0.030

Variables were optimally transformed to correct nonhomogeneous variance before modeling. Models not shown did not display significance in the fitting. R² is reported for the whole model. I, Insulin.

a steady-state serum glucose level between 85 and 90 mg/dl in both groups as described (25). Serum insulin levels were raised in both groups, although circulating values were higher in PCOS women ($235 \pm 25.5 \mu\text{U/ml}$) compared with that of normal women ($173 \pm 19.3 \mu\text{U/ml}$) ($P = 0.02$). In normal women, the composite 12-h mean LH level before infusion ($3.4 \pm 0.4 \text{ mIU/ml}$) was not significantly different from that observed during insulin administration ($3.3 \pm 0.3 \text{ mIU/ml}$). In PCOS women, serum LH was reduced by insulin infusion from 6.6 to 4.9 mIU/ml, which approximated statistical significance ($P = 0.051$).

Effect of a fixed rate of insulin infusion on baseline LH after varying doses of GnRH

Baseline LH levels before each dose of GnRH were assessed in the presence and absence of insulin infusion in both groups. Analysis of baseline preinjection LH levels at each GnRH dose revealed that in normal women, LH values were unaltered by the effect of insulin (Fig. 2A). However, in PCOS women, mean baseline LH levels before each dose of GnRH were consistently lower during insulin administration compared with values observed in the absence of insulin (Fig. 2B). The insulin infusion data were further examined using a mixed-effects analysis to model the interaction of insulin, diagnosis, GnRH dose, BMI, and T on the outcome of preinjection LH levels in the insulin infusion study (Table 4). Mixed-effects modeling of preinjection baseline LH across doses showed that neither insulin infusion nor diagnosis group alone were significant in predicting baseline LH outcome. However, dosage ($P < 0.0001$) and BMI ($P < 0.008$) were significant. Most notably, the interaction between insulin and diagnosis was also significant ($P < 0.0002$), which is consistent with the individual paired *t* test results showing that insulin infusion affects baseline LH in PCOS women.

Effect of a fixed rate of insulin infusion on LH responses to varying doses of GnRH

Cross-sectional comparisons of GnRH dose effect on maximal LH increment vs. baseline level after each GnRH challenge in normal women showed no statistically significant difference due to insulin infusion (Fig. 3A). However, for PCOS women, peak LH excursions from baseline values were significantly lower or trended lower ($P = 0.061$ for dose = 2 μg ; $P = 0.049$ for dose = 10 μg ; $P = 0.009$ for dose = 20 μg) during insulin infusion compared with responses observed in the absence of insulin (Fig. 3B), suggesting that insulin infusion suppressed baseline LH increases. Mixed-effects modeling for change in LH across doses showed that only baseline LH is significant ($P < 0.0001$, Table 4) and likely accounts for lowered incremental changes in LH after each dose of GnRH.

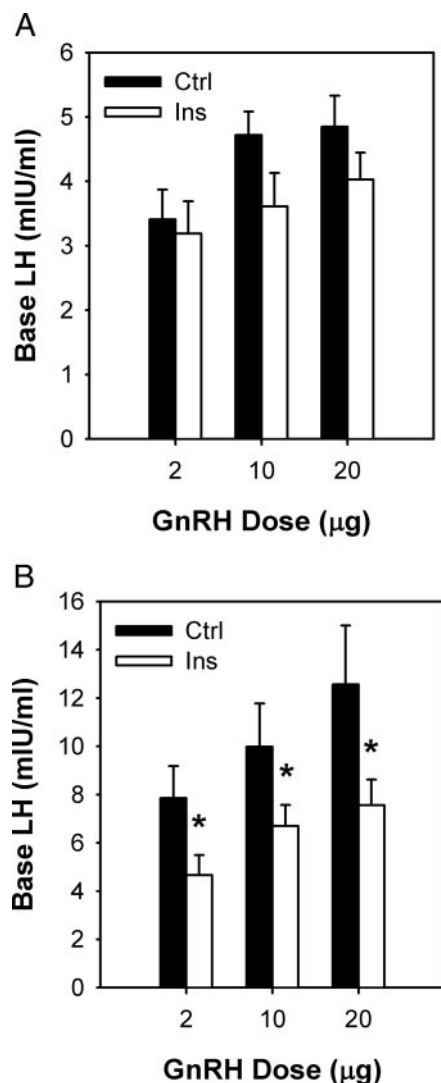


FIG. 2. Effect of increasing doses of GnRH on baseline LH levels in normal and PCOS women with and without insulin infusion. Mean baseline LH values before GnRH dosing are compared without (*black bars*) and with (*white bars*) insulin infusion under a hyperinsulinemic-euglycemic clamp. *, Significant differences between control (Ctrl) and insulin-infused (Ins) condition at each GnRH dose as determined by paired *t* test. A, Comparison between vehicle and insulin infusion in normal subjects; B, comparison between vehicle and insulin infusion in PCOS subjects. In PCOS subjects, GnRH doses of 2 µg ($P = 0.004$), 10 µg ($P = 0.024$), and 20 µg ($P = 0.011$) all show significant differences in preinjection base LH between vehicle and insulin infusion treatments.

LH response to a fixed dose of GnRH during low- and high-dose insulin infusion

To address the dose effect of insulin on LH secretion, a third study was conducted assessing LH responses to a fixed dose of GnRH under conditions of physiological and pharmacological concentrations of serum insulin. After 3 h of infusion at 60 µIU/ml (physiological), and 1600 µIU/ml (pharmacological) insulin, GnRH was administered as a single 10-µg bolus. Baseline LH levels before GnRH administration were not altered in normal women by either low- or high-dose insulin infusion. In contrast, PCOS subjects exhibited significant reductions in preinjection LH levels at both doses of insulin infusion (Fig. 4A). Persistent decreases of serum LH were observed for up to 4 h after

TABLE 4. Mixed-effects modeling of baseline and change in LH

Factor	Effect	SE	P
Baseline LH as outcome			
Insulin	-0.719	0.0589	NS
PCOS	4.328	2.616	NS
GnRH Dose	0.143	0.027	<0.0001
BMI	-0.268	0.089	0.008
T	2.757	1.364	0.06
Insulin × PCOS	-3.106	0.795	0.0002
Peak vs. Baseline LH as Outcome			
Insulin	-1.566	1.109	NS
PCOS	-10.550	8.046	NS
GnRH Dose	0.013	0.073	NS
BMI	0.329	0.282	NS
T	6.122	4.276	NS
Baseline LH	1.958	0.222	<0.0001

Factors testing significant in multivariable models above were utilized in mixed effects models testing significance under insulin infusion and GnRH dosing. Factors not shown did not show significance in either outcome tested. NS = Not significant.

GnRH administration. Assessment of LH area under the curve showed insulin infusion significantly decreased total LH output after GnRH challenge at high dose in normal and at both doses in PCOS women (Fig. 4B).

Discussion

We employed multivariable, regression and mixed-effects linear modeling to examine the relationship of insulin, BMI, and T to composite LH in normal and PCOS women. Using this approach, we have identified distinct relationships between composite LH to insulin, BMI, and T that are not readily evident using group comparisons or nonparametric methods of analysis. We find that in normal women, insulin is negatively correlated to mean LH levels. In PCOS women, T (positive correlation) and BMI (negative correlation) are the most significant variables related to 12-h mean LH. Interestingly, T and BMI appear to be colinear and not independent. The dominance of BMI and T as parallel covariates in PCOS provides evidence that this population is distinct from normal cycling women. We also demonstrate with multivariable modeling that insulin influences basal LH secretion in PCOS women. Overall, we identify an effect of insulin on LH secretion. Insulin is implicated in regression analysis of normal and PCOS (in conjunction with BMI) subjects in 12-h composite LH levels (Tables 2 and 3 and Fig. 1), in baseline levels of LH after insulin infusion in comparison to vehicle (Table 4 and Fig. 3), and in area under the curve of GnRH-elicited LH release compared with vehicle infusion (Fig. 4). The latter observations directly demonstrate a change in LH levels as a direct result of insulin infusion.

The linear mixed-effects approach to modeling endocrine variables in PCOS has revealed a relationship between insulin infusion and pituitary sensitivity to GnRH that has also not been appreciated. Although BMI and T overwhelmingly distinguish

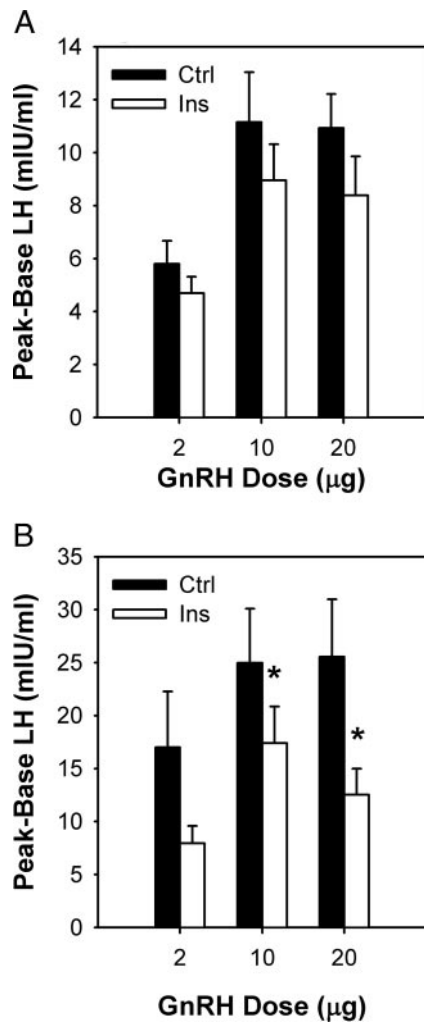


FIG. 3. Effect of increasing doses of GnRH on peak minus baseline change in LH in normal (A) and PCOS (B) women with and without insulin infusion. Mean change in LH after GnRH dosing are compared without (Ctrl, black bars) and with (Ins, white bars) insulin infusion under a hyperinsulinemic-euglycemic clamp. *, Significant differences between control and insulin infused condition at each GnRH dose as determined by paired *t* test. In PCOS subjects, the GnRH doses of 10 μ g ($P = 0.049$) and 20 μ g ($P = 0.009$) show significant differences in peak minus base LH between vehicle and insulin infusion treatments.

the PCOS population with respect to 12-h mean LH, both normal and PCOS groups exhibit some relationship between insulin and LH. In PCOS, the pituitary response to GnRH is suppressed under a euglycemic, hyperinsulinemic clamp. The direct effect of insulin on GnRH sensitivity provides confirmation of the direct effect observed in cultured primary pituitocytes or gonadotrope-derived cell lines *in vitro*. Although *in vitro* studies have demonstrated that insulin promotes GnRH action, it is not clear that this occurs *in vivo* because some animal models also show that LH levels are suppressed by insulin (29).

A number of studies have attempted to identify a direct effect of insulin on gonadotropin secretion. Although group comparisons between PCOS and normal women have not demonstrated a significant relationship between insulin and LH levels, such a relationship is suggested by previous examination of BMI and LH in both normal and PCOS women (27). However, the relationship between insulin and LH was not directly examined.

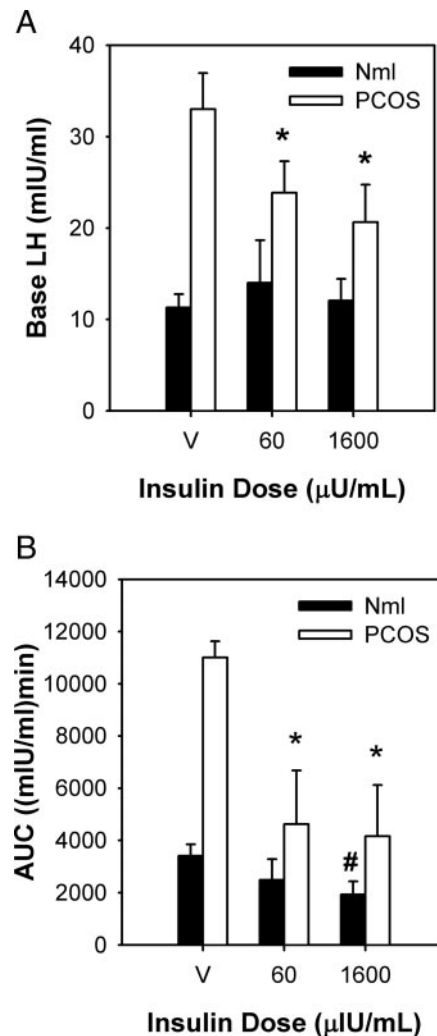


FIG. 4. Effect of increasing insulin concentrations on baseline LH (A), and area under the curve (B) after a single 10- μ g GnRH treatment in normal and PCOS women. Normal (Nml, black bars) and PCOS (white bars) women infused with vehicle or 60 or 1600 mIU/ml insulin for 3 h before and 1 h after a single 10- μ g GnRH dose were assessed for LH levels by sampling at 10-min intervals. Group comparisons of baseline LH before GnRH administration and area under the curve LH levels were compared with respective vehicle-treated controls. #, Significant difference ($P < 0.05$) between normal subject vehicle and treatment groups; *, significant difference ($P < 0.05$) between PCOS subject vehicle and treatment groups.

Studies attempting to normalize insulin levels by treatment with thiazolidinediones or metformin to reduce hepatic glucose output have not consistently shown effects on LH (15, 18, 30). Generally, these drugs improve insulin sensitivity and increase ovulation. The effectiveness of these drugs supports the role of insulin or insulin sensitivity in modulating reproductive function, but it is not clear that the site of action is at the level of the hypothalamus, pituitary, or ovary. Based on the prediction that in PCOS, BMI is inversely correlated with LH levels and the recent demonstration that the effect of BMI on LH is manifested at least in part at the level of the pituitary (28), lowered BMI may not be accompanied by lowered LH. It is also possible that systemic modulation of insulin sensitivity may alter the relationship between insulin, BMI, and LH. In this study, direct correlation of insulin with composite LH is detected only in normal control

women, indicating that direct effects of high BMI and T or other associated effects on steroid biosynthesis or metabolism may dominate in PCOS (31, 32).

Although mean BMI differs between groups, substantial overlap in BMI between diagnostic groups exists, as is seen in Fig. 1. Ideally, both diagnostic groups would match in terms of BMI. However, our analysis controls for BMI by inclusion as a covariate in the regression models. It is almost certain that factors other than insulin affect gonadotropin secretion in both normal control and PCOS populations. Past results show colinearity between insulin and leptin as well as BMI and leptin. However, this did not differ between diagnostic groups (33). More recent studies of the effects of leptin showed that although trends were detected, leptin was not significantly different between groups (34), that leptin differences may be caused by insulin resistance (35), and that the leptin to adiponectin ratio, rather than leptin alone, may be a more useful indicator of metabolic syndrome and PCOS (36). Our use of a GnRH stimulatory model bypasses hypothalamic action of leptin, opioids, or other unknown factors but does not rule out unknown variables responding to insulin infusion acting on the pituitary.

A consistent observation in our examination of insulin effects on LH is the repression of baseline values. The LH response to GnRH stimulation in terms of fold or percent change from baseline was similar between groups and generally unaffected by endogenous or clamped insulin values, the exception being PCOS and normal subjects under the 3-h 1600-mIU and PCOS subjects 60-mIU clamp before the single GnRH challenge (Fig. 4B). The observed differences in maximum LH increment and area under the curve can be largely attributed to lowered baseline values, as shown in mixed-effects modeling of parameters affecting LH (Table 4). Baseline spontaneous secretion of LH may be a reflection of overall LH biosynthesis, and this may in turn be affected by insulin. The division of secretory *vs.* synthetic response to GnRH is possible. GnRH signaling proceeds through two major signaling arms: a calcium-dependent arm that stimulates secretion and a MAPK signaling arm that elicits a biosynthetic response that ultimately increases mRNA and protein synthesis. A common point of signaling interaction is the MAPK signaling arm, which is activated by both GnRH and insulin. Although insulin may act cooperatively with GnRH, chronic hyperstimulation *in vivo* may induce a refractory state ultimately leading to a decreased biosynthetic capacity. Such a mechanism would explain both the short-term cooperative effect observed *in vitro* (7–9) and the long-term repressive effect observed *in vivo* (29) as well as some observations in humans (23). Further studies of the action of insulin on gonadotropes will elucidate the mechanisms of GnRH and insulin interaction.

In summary, we have used a novel approach to model the relationships of multiple endocrine characteristics of normal and PCOS women and have identified distinct relationships of LH to insulin, BMI, and T. We have also used this technique to evaluate the effect of insulin on pituitary sensitivity to GnRH and have found that in PCOS, insulin exhibits a suppressive effect on baseline, peak minus baseline, and area under the curve LH response to GnRH dosing. Our observations provide evidence for a role for insulin in modulating LH levels and provide further support

for the dominance of abnormalities in BMI and T in determining the unique endocrine characteristics of PCOS.

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