0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 11
Printed in U.S.A.

Inappropriate Gonadotropin Secretion in Polycystic Ovary Syndrome: Influence of Adiposity*

A. ARROYO, G. A. LAUGHLIN, A. J. MORALES, AND S. S. C. YEN†

Department of Reproductive Medicine, School of Medicine 0633, University of California, San Diego, La Jolla, California 92093-0633

ABSTRACT

In recent years, there has been uncertainty concerning the association of inappropriate gonadotropin secretion (high LH and normal FSH) and the polycystic ovary syndrome (PCOS). In the present study, we ascertained the influence of body composition on LH pulsatile parameters in 33 PCOS and 32 normal cycling (NC) women across a wide range of body mass index (BMI, 19–42 kg/m^2). Twenty four-hour pulsatile parameters for serum LH (10-min sampling) and pituitary gonadotropin responses to iv bolus GnRH (10 μg) were evaluated. Fasting (0800 h) FSH and steroid hormone concentrations and 24-h mean insulin levels were determined. Insulin sensitivity (S_I) was assessed by rapid iv glucose tolerance test in a subset of 28 PCOS and 29 NC subjects.

Our results showed that BMI, an indicator of relative adiposity, had a significant negative impact on 24-h mean LH pulse amplitude ($r = -0.63$, $P < 0.001$) and the peak increment of LH in response to GnRH stimulation ($r = -0.41$; $P = 0.02$) for PCOS but not NC women. In contrast, 24-h LH pulse frequency was uniformly increased (40%) in PCOS as compared with NC women independent of BMI. In PCOS women, the blunting of pulse amplitude with increasing BMI resulted in a decline in 24-h mean LH levels ($r = -0.63$, $P < 0.001$) and the ratio of LH/FSH ($r = -0.44$, $P = 0.02$) not seen in NC. With BMI <30 kg/m^2, 24-h mean LH values for PCOS women were greater than the normal range for NC in 95% (18/19) of cases, whereas 24-h LH levels failed to discriminate PCOS from NC women in 43% (6/14) of obese (BMI >30 kg/m^2) PCOS women. Thus, the diagnostic value of LH determinations is retained for PCOS women with BMI <30 kg/m^2. For screening purposes, the mean of two LH values in samples collected at 30-min intervals was found to have a discriminatory power equal to that of the 24-h mean.

These findings suggest that 1) BMI negatively influences LH pulse amplitude in PCOS women principally by an effect at the pituitary level; 2) accelerated LH pulse frequency in PCOS women is not influenced by BMI and represents a basic component of hypothalamic dysfunction in PCOS women; and 3) BMI does not influence gonadotropin secretion in normal cycling women. Thus assessments of basal LH levels and the LH/FSH ratio in hyperandrogenic anovulatory women are clinically meaningful when BMI is taken into account. Investigations to define the factor(s) that link adiposity and the attenuation of LH pulse amplitude in PCOS women would add further understanding of this complex neuroendocrine-metabolic disorder. (*J Clin Endocrinol Metab* **82:** 3728–3733, 1997)

IN 1970 we reported that inappropriate gonadotropin secretion is associated with the classical form of polycystic ovary syndrome (PCOS) (1). When compared with the follicular phase of the normal menstrual cycle, women with PCOS exhibit a disproportionately high LH secretion with relatively constant low FSH secretion. Further, high levels of LH, but not FSH, respond appropriately to the negative feedback action of an infusion of 17β-estradiol (1). In a subsequent study (15-min sampling/6 h), we demonstrated that LH pulse amplitude was elevated, and pituitary LH sensitivity to GnRH stimulation was remarkably heightened in PCOS women (2). Based on these observations, we proposed that a disturbance of hypothalamic regulation of pituitary gonadotropin secretion may be causally related to the chronic anovulation and abnormal ovarian steroidogenesis in patients with PCOS (1–3). Numerous studies that followed using a more frequent sampling interval (10 min) of varying duration (6–12 h) generally confirmed the augmented LH pulse amplitude in PCOS women as compared with the early or mid follicular phase of the cycle (4–9). When the duration of sampling was extended to a 24-h period, several studies (10–13) disclosed an increased LH pulse frequency, suggesting that an accelerated hypothalamic GnRH pulse generator exists in PCOS women. Recently, Apter *et al.* showed that adolescent girls with ovarian hyperandrogenism have increased 24-h LH pulse frequency and amplitude (14) and hyperinsulinemia (15). These observations collectively establish the association of PCOS with LH hypersecretion and its peripubertal onset (16–18).

However, when basal LH measurements were used as clinical markers of PCOS, a significant number of patients failed to exhibit an elevated LH and hence LH/FSH ratio (19–21). This issue prompted an NICHHD-sponsored consensus conference on diagnostic criteria for PCOS in 1990, and the recommendation that LH and the LH/FSH ratio are not required for the diagnosis of PCOS (21). However, several recent studies (22–26) confirmed and extended earlier observations (27–30) that in women with PCOS there is a negative influence of obesity on LH values. An important question then arises: does adiposity suppress LH pulsatile secretion in women with PCOS? Because these studies lack sufficient intensity and/or duration of sampling to assess LH pulse amplitude and frequency, we recently addressed this issue by examining 24-h pulse parameters in obese and lean PCOS women and body mass index (BMI)-matched controls

Received June 4, 1997. Revised July 16, 1997. Accepted August 1, 1997.
Address all correspondence to (no reprints available): Samuel S. C. Yen, Department of Reproductive Medicine, School of Medicine 0633, University of California, 9500 Gilman Drive, BSB5046A, La Jolla, California 92093-8856.
* This work was supported by the National Institute of Child Health and Human Development (NICHHD) Center Grant HD-12303–19 and the General Clinical Research Center, NIH Grant MO1-RR00827, and NIH Grant T32-HD07203-19.
† Clayton Foundation investigator.

(13). Although an unequivocal attenuation of LH pulse amplitude occurs in obese PCOS women, the relationship between LH pulsatility and adiposity across a spectrum of BMI in PCOS women remains to be defined. We report here the results of such an assessment with the aim of providing both a clinical reference and a pathophysiological basis for the heterogeneity of inappropriate gonadotropin secretion in PCOS women.

Subjects and Methods

Subjects

Thirty three women with the diagnosis of PCOS and 32 women of similar age and BMI with regular menstrual cyclicity (NC) were studied. Data on these subjects has been presented in a previous study of leptin levels in PCOS women (31). The age range of the subjects was 17–36 yr; BMI ranged from 19–42 kg/m^2. The diagnosis of PCOS was based on perimenarcheal onset of oligomenorrhea, elevated serum levels of androstenedione (A) and/or testosterone (T), and ultrasound evidence of bilateral enlarged polycystic ovaries. All subjects were euglycemic, euthyroid, and had normal PRL levels. They were nonsmokers and had not been on any medications for at least 3 months before the study. In PCOS subjects, late-onset congenital adrenal hyperplasia was excluded by a normal 17-hydroxyprogesterone (17-OHP) level 60 min after an ACTH stimulation test. The protocol for this study was approved by the Committee on Investigations Involving Human Subjects of the University of California, San Diego (UCSD), and written informed consent was obtained from each participant.

Procedures

Studies were conducted in regularly cycling controls during the early follicular phase (days 2–5) of their menstrual cycle and in PCOS women on a random day. In no case had recent ovulation occurred in PCOS women as evidenced by retrospective measurement of serum progesterone levels on the day of the study. Subjects were admitted to the General Clinical Research Center (GCRC) of the UCSD Medical Center at 0700 h after an overnight fast. Blood samples were drawn through an indwelling iv catheter every 10 min for 24-h beginning at 0800 h. Subjects refrained from napping and drinking caffeinated beverages during the study and received standard meals at 0800 h, 1200 h, and 1700 h and a 200-kcal snack at 2200 h. The total caloric content of meals was adjusted to 30 kcal/kg body weight with a nutrient composition of 15% protein, 55% carbohydrate, and 30% fat and a caloric division of 1/5, 2/5, and 2/5 for breakfast, lunch, and dinner, respectively. Subjects were allowed to sleep from 2300–0700 h. Serum LH concentrations were determined at 10-min intervals, and concentrations of FSH, sex hormone binding globulin (SHBG), and steroid hormones on 0800-h fasting samples. Serum insulin and plasma glucose levels were measured hourly and at 30 min after each meal. Each individual's samples were analyzed in the same assay in duplicate. Pituitary responses to iv bolus GnRH (10 μg) were determined at 1800 h after completion of the 24-h study. Blood samples were obtained before (−20, −10, and 0 min) and after (10, 20, 30, 40, 60, 90, 120, 150, and 180 min) GnRH administration. Serum LH concentrations were determined on each sample.

Insulin sensitivity (S_I) was assessed in a subset of 28 PCOS and 29 control subjects by a modified rapid iv glucose tolerance test (32). After an overnight fast of 10 h, an iv line was established in each forearm, and baseline samples were drawn at −10 and 0 min before administration of an iv bolus of glucose (0.3 g/kg 50% dextrose) over 1 min in the opposite arm. At 20 min after the glucose injection, an iv bolus of regular insulin (0.03 units/kg for women with a BMI <28 kg/m^2 and 0.05 units/kg for those with a BMI >28 kg/m^2) was injected over 20 sec, and the line immediately flushed with saline. Blood samples were then drawn at 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 min. Plasma glucose and serum insulin concentrations were determined for each sample.

Data analysis

S_I was analyzed by the MINMOD computer program (copyright RN Bergman) (32). LH pulsatile activity was analyzed using the Cluster pulse detection algorithm (33). A cluster configuration of 2 × 1 and t statistics of 2.1 × 2.1 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 a function of hormone concentration. Pulse number/24-h, mean pulse amplitude (the difference in concentration between the preceding nadir and the pulse peak), and 24-h mean concentration were determined for each subject.

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. Serum insulin levels were measured by a double-antibody RIA with an assay sensitivity of 15 pmol/L, and intra- and interassay CVs of 7% and 9%, respectively. Plasma glucose concentrations 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%. SHBG was measured by a time-resolved immunofluorometric assay (Delfia SHBG kit; Wallac, Gaithersburg, MD) with intra- and interassay CVs of 7% and 9%, respectively. Serum concentrations of estrone (E_1), estradiol (E_2), A, T, and 17-OHP were measured by established RIAs with intraassay CVs less than 7%.

Statistical analyses

Non-Gaussian–distributed variables were $\log_{10}$ transformed to achieve normality. This applied to insulin sensitivity, 24-h mean insulin and LH levels, and 24-h mean LH pulse amplitude. Results for PCOS and NC women were compared by group t tests. Relationships between variables were sought by Pearson product-moment correlations and stepwise multivariate linear regression analysis with forward selection. When more than five correlations were performed, a protected P value of 0.01 was used to reduce false positive assignment of significance to no more than 1/100. Results are expressed as the mean ± SE. $P < 0.05$ was considered significant.

Results

Clinical and endocrine-metabolic characteristics

When compared with euandrogenic NC of similar age and BMI, PCOS women displayed elevated ($P < 0.0001$) serum 17-OHP, A, T, and E_1 levels (Table 1). Serum concentrations of SHBG were decreased ($P < 0.01$), and the ratios of E_1/SHBG, E_2/SHBG, and T/SHBG, reflecting nonprotein bound

TABLE 1. Clinical and endocrine-metabolic characteristics for NC and PCOS women

	NC (n = 32)	PCOS (n = 33)
Age (yr)	28.2 ± 0.9	25.9 ± 1.0
BMI (kg/m^2)	27.6 ± 1.1	31.1 ± 1.5
Body fat mass (%)[a]	35.8 ± 2.7	40.0 ± 2.8
17-OHP (nmol/L)	0.60 ± 0.06	1.58 ± 0.12[b]
A (nmol/L)	1.96 ± 0.10	4.90 ± 0.30[b]
T (nmol/L)	0.63 ± 0.04	1.72 ± 0.13[b]
E_1 (pmol/L)	103 ± 11	185 ± 12[b]
E_2 (pmol/L)	147 ± 19	152 ± 8
SHBG (nmol/L)	40 ± 3	27 ± 2[c]
E_1/SHBG ratio (×10^3)	3.07 ± 0.38	8.95 ± 1.13[b]
E_2/SHBG ratio (×10^3)	4.4 ± 0.7	7.1 ± 0.7[c]
T/SHBG ratio (×10^2)	2.0 ± 0.2	7.7 ± 0.7[c]
Glucose (mmol/L)[d]	5.40 ± 0.05	5.73 ± 0.13[c]
Insulin (pmol/L)[d]	196 ± 19	394 ± 55[b]
Insulin sensitivity[e]	3.33 ± 0.51	1.660 ± 0.32[c]

Values are the mean ± SE.
[a] Subset (PCOS: n = 14, NC: n = 17).
[b] $P < 0.0001$ *vs.* NC.
[c] $P < 0.01$ *vs.* NC.
[d] Values are 24-h means.
[e] Units: ×10^{-4}/min μU/ml; subset (PCOS: n = 28, NC: n = 29).

steroid levels, were increased ($P < 0.01$) in PCOS women. PCOS women displayed 2-fold elevations ($P < 0.0001$) of 24-h mean insulin levels, increased 24-h mean glucose levels ($P < 0.02$), and a 50% reduction in S_I ($P < 0.01$).

Gonadotropin levels and 24-h LH pulsatility parameters

Twenty four-hour LH pulse frequency was accelerated by ≈40% ($P < 0.0001$) in the PCOS group as compared with the NC group (Table 2). This was accompanied by an 80% elevation ($P < 0.001$) of 24-h mean LH pulse amplitude and 2-fold higher ($P < 0.0001$) 24-h mean LH levels. FSH levels for PCOS women did not differ from NC. Thus, the augmentation of LH levels alone accounted for a 2.6-fold higher ($P < 0.001$) LH/FSH ratio in the PCOS group. Pituitary LH sensitivity was enhanced in PCOS women as evidenced by a 3-fold greater peak increment ($P < 0.0001$) of LH in PCOS than in NC women in response to GnRH (10 μg bolus) stimulation.

Influence of BMI on LH pulsatility parameters

The relationships of LH pulsatility features with BMI for PCOS and NC women are shown in Fig. 1. Twenty four-hour LH pulse frequency was uniformly elevated in PCOS as compared with NC women independent of BMI, with minimal overlap between the two groups. In contrast, mean LH pulse amplitude for PCOS but not NC women was inversely dependent on BMI ($r = -0.63$, $P < 0.001$). At a BMI of 20 kg/m^2, mean LH pulse amplitude was elevated ≈2.5-fold in PCOS women. Thereafter, LH pulse amplitude decreased with increasing BMI. This relationship was continuous until BMI reached 40 kg/m^2, when LH pulse amplitude for PCOS women became indistinguishable from that of NC women.

Accelerated pulse frequency and elevated pulse amplitude resulted in 3-fold higher 24-h mean LH levels in PCOS women with low BMI. The BMI-dependent blunting of pulse amplitude resulted in a decline in 24-h mean LH levels for PCOS women with increasing BMI ($r = -0.63$, $P < 0.001$), not seen in NC women (Fig. 1). Using a BMI of 30 kg/m^2 as a reference point, 24-h mean LH levels for PCOS women with BMI ≤30 kg/m^2 overlapped with the 95% confidence interval for NC women in only 5% (1/19) of cases, whereas 43% (6/14) of PCOS women with BMI >30 kg/m^2 were within the 95% confidence interval. The ability to discriminate PCOS from NC women on the basis of LH measurements using single 0800-h values and the mean of the two values at 0800 h and 0830 h was also evaluated. As shown in Table 3, although a single measurement of LH was not as effective as the 24-h mean in distinguishing PCOS from NC women, the mean of two samples drawn at 30-min intervals had a discriminatory power equivalent to that of the 24-h mean.

The peak increment of LH in response to GnRH (10 μg bolus) stimulation was negatively correlated to BMI ($r = -0.41$; $P = 0.02$) for PCOS, but not NC women (Fig. 2). FSH levels did not relate to BMI for either PCOS or NC women, whereas the ratio of LH/FSH was negatively correlated with BMI for PCOS women only ($r = -0.44$; $P = 0.02$) (Fig. 3).

TABLE 2. Twenty four-hour mean (±SE) serum LH levels and pulsatility characteristics in PCOS and NC women

	NC (n = 32)	PCOS (n = 33)
LH 24-h mean (IU/L)	11.1 ± 0.6	24.3 ± 1.9[a]
Pulse frequency (#/24-h)	16.5 ± 0.5	22.8 ± 0.6[a]
Mean amplitude (IU/L)	5.11 ± 0.3	9.12 ± 1.08[b]
LH peak response to GnRH	44.4 ± 3.2	117.8 ± 12.3[a]
FSH (IU/L)	11.2 ± 0.5	10.3 ± 0.6
LH/FSH ratio	1.1 ± 0.1	2.6 ± 0.3[b]

[a] $P < 0.0001$ *vs.* NC.
[b] $P < 0.001$ *vs.* NC.

Relationship of LH pulsatility parameters with steroid hormone and insulin levels and S_I

Levels of 17-OHP for PCOS but not NC women were positively related to 24-h mean LH levels ($r = 0.52$, $P = 0.006$) and pulse amplitude ($r = 0.52$, $P = 0.007$), independent of BMI. Twenty four-hour mean LH levels, mean pulse amplitude, and pulse frequency were not related to serum concentrations of A, T, E_1, E_2 or the ratios of T/SHBG, E_1/SHBG, or E_2/SHBG for either PCOS or NC women.

Both 24-h mean LH levels and pulse amplitude for PCOS but not NC women were related positively with S_I ($r = 0.49$, $P = 0.009$ and $r = 0.52$, $P = 0.005$, respectively) and inversely with 24-h mean insulin levels ($r = -0.56$, $P = 0.001$ and r =

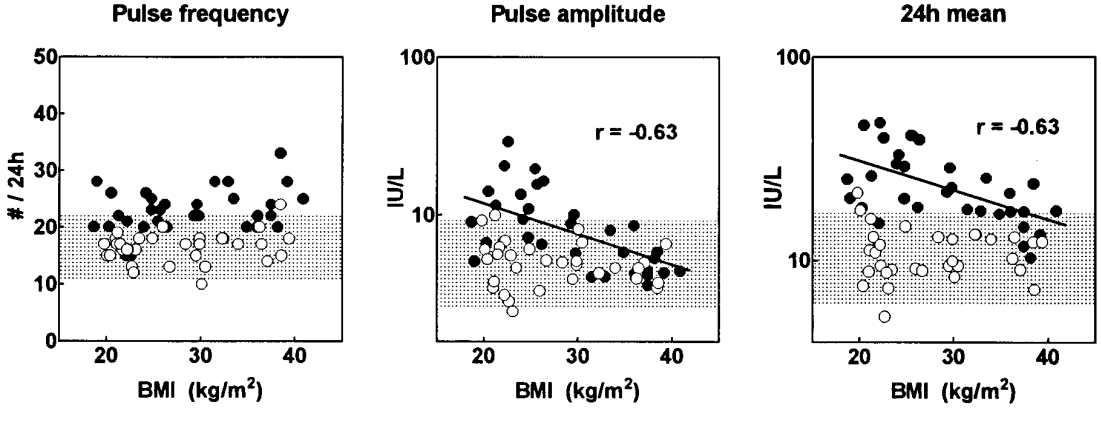

FIG. 1. Regression of BMI for PCOS (●) and NC (○) women against LH pulse frequency (PCOS and NC: P = NS), LH pulse amplitude (PCOS: $r = -0.63$; $P < 0.001$; NC: P = NS), and 24-h mean LH levels (PCOS: $r = -0.63$; $P < 0.001$; NC: P = NS). Values for LH pulse amplitude and 24-h mean are $\log_{10}$ transformed. *Shaded area* represents 95% confidence interval for NC (frequency: 11–22 pulses/24 h; amplitude: 2.6–9.2 IU/L; 24-h mean: 6.1–18.2 IU/L).

TABLE 3. Power of LH values to discriminate PCOS from NC using single samples, mean of two samples collected at 30-min intervals, and 24-h mean (10-min sampling interval)

Sample frequency	BMI ≤ 30 kg/m^2 (19/33) (%)[a]	BMI > 30 kg/m^2 (14/33) (%)
Single value (0800 h)	84	50
Mean of two samples (0800 and 0830 h)	95	64
24-h mean (10-min interval)	95	57

[a] Percent of PCOS subjects with LH values greater than the 95% confidence interval for NC.

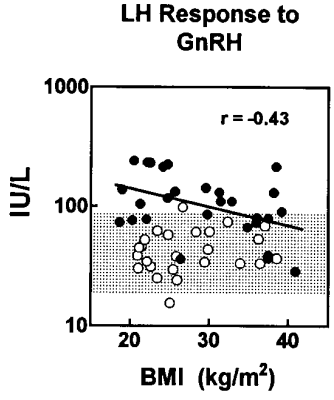

FIG. 2. Regression of BMI against LH peak levels in response to GnRH for PCOS (●) and NC (○) women (PCOS: r = −0.41, P = 0.03; NC: P = NS). *Shaded area* represents 95% confidence interval for NC women (9–80 IU/L).

−0.52, P = 0.003, respectively). Not unexpectedly, stepwise regression analyses indicated these relationships were dependent on the influence of BMI on both S_I (r = −0.70, P < 0.0001) and insulin levels (r = 0.80, P < 0.0001) (Fig. 4).

Discussion

In the present cross-sectional study, we evaluated the issue of inappropriate gonadotropin secretion and its heterogeneity among patients with PCOS. Assessment of the impact of BMI on 24-h LH pulse parameters, 24-h mean LH levels, pituitary responses to GnRH, and LH/FSH ratios were made in hyperandrogenic anovulatory women with PCOS and euandrogenic women with normal menstrual cyclicity (NC). The major findings of this study were: 1) LH pulse amplitude, 24-h mean LH levels, and LH secretion in response to GnRH varied negatively with BMI in PCOS women; 2) LH pulse frequency for PCOS women was uniformly increased independent of BMI; 3) the mean of two LH values in samples collected at a 30-min interval has a discriminatory power for PCOS and NC women equal to that of the 24-h mean (95% of cases with BMI<30 kg/m^2); and 4) the BMI-related changes seen in PCOS women were not found in euandrogenic cycling women, a finding consistent with previous reports (for review, see Ref. 34).

The blunting effect of BMI on LH pulse amplitude in PCOS women (r = −0.63, P < 0.001) may explain the heterogeneity of inappropriate gonadotropin secretion observed in previous studies (19–21), in which the effect of BMI was not accounted for. The present study showed that at a BMI of 20 kg/m^2 mean LH pulse amplitude was elevated ≈2.5-fold in

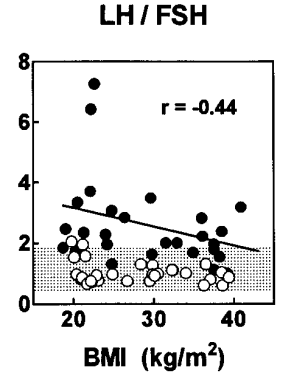

FIG. 3. Regression of BMI for PCOS (●) and NC (○) women against ratio of LH/FSH (PCOS: r = −0.44, P = 0.02; NC: P = NS). *Shaded area* represents 95% confidence interval for NC women (0.4–1.85).

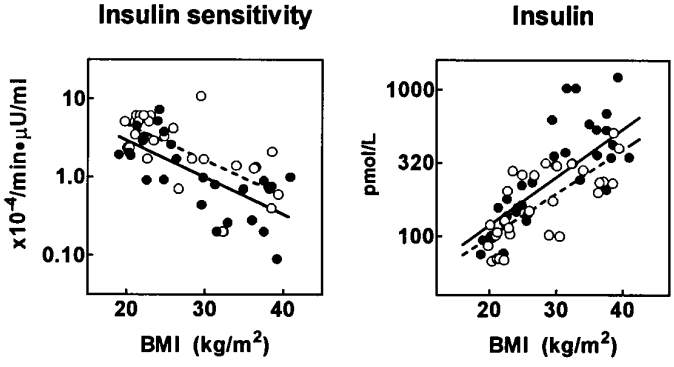

FIG. 4. Regression of BMI for PCOS (●) and NC (○) women against insulin sensitivity (PCOS: r = −0.63, P < 0.0002; NC: r = −0.65, P = 0.0002) and 24-h mean insulin levels (PCOS: r = 0.80, P < 0.0001; NC: r = 0.73, P < 0.0001). Values for insulin sensitivity and 24-h mean insulin levels are $\log_{10}$ transformed.

PCOS women from that of NC women. Thereafter, LH pulse amplitude decreased progressively and became indistinguishable from that of NC women when BMI reached 40 kg/m^2 (Fig. 1). This BMI-dependent blunting of LH pulse amplitude in PCOS women was accompanied by a parallel attenuation of pituitary LH sensitivity to GnRH stimulation (Fig. 2). These findings suggest that factors associated with obesity exert an inhibitory effect on endogenous GnRH action at the level of the pituitary and are operative selectively in PCOS women, but not in normal cycling women.

The link between adiposity and blunting of pituitary LH responses to GnRH in PCOS women is unknown. In this study, LH levels and pulse amplitude for PCOS women were inversely related to both 24-h insulin levels and the degree of insulin resistance, however these relationships were not independent of the strong influence of BMI based on regression analyses. It is recognized that multiple interdependent interactions may not be accurately dissected by regression analyses. Thus, a negative influence of hyperinsulinemia and insulin resistance on LH levels in PCOS women cannot be categorically excluded. However, LH levels in PCOS women were not altered during insulin infusion (35), after suppression of insulin levels by diazoxide (36), or lowering of insulin after weight-loss (37, 38). Moreover, most (39–42), but not all (43), studies using metformin or troglitazone have shown

reduced LH levels in conjunction with suppression of insulin levels in PCOS women, suggesting an enhancing influence of insulin on LH secretion. None of the cited studies identified an inverse relationship between insulin and LH levels. Thus, it is unlikely that insulin serves as a link between adiposity and blunting of LH pulse amplitude and pituitary LH responses to GnRH.

The increase (40%) in 24-h LH pulse frequency was relatively uniform in PCOS women independent of BMI (Fig. 1). Because LH pulse frequency in NC women was also not influenced by increasing BMI, these observations suggest that factors associated with adiposity, including leptin (31, 44–46), have no discernible effect on hypothalamic control of GnRH pulse frequency. Thus, as previously proposed (3, 10–14), our present data indicate that the accelerated GnRH pulse generator represents a basic neuroendocrine aberration in PCOS women. Slowing of LH pulse frequency with lowering of LH levels were found in PCOS women when studies were performed following spontaneous ovulation, and in response to exogenous progestin-induced withdrawal bleeding in PCOS women (47–49). Under these circumstances, hypothalamic opioidergic tone is increased by the feedback action of progesterone resulting in a decreased frequency of the GnRH pulse generator, which can be reversed by naloxone infusion (50, 51). Consequently, the endogenous neuroendocrine milieu is desynchronized, and LH levels are reduced contributing, in part, to the artifact of disparate reports of inappropriate gonadotropin secretion in PCOS women (19–21).

Because 24-h mean LH levels for both PCOS and NC women are highly dependent on the combined effects of pulse frequency and amplitude (52), accelerated pulse frequency together with elevated LH pulse amplitude resulted in 3-fold higher 24-h mean LH levels in PCOS women with low BMI. The blunting of LH pulse amplitude with increasing BMI resulted in a parallel decline in 24-h mean LH levels in PCOS women not observed in NC women. In this study, 95% of PCOS women with BMI ≤ 30 kg/m^2 had 24-h mean LH levels greater than the 95% confidence interval for NC women, whereas LH levels failed to discriminate PCOS from NC women in 43% of obese subjects (BMI >30 kg/m^2) (Fig. 1). Of clinical significance, we found that the mean of two LH values in samples collected at a 30-min interval, but not a single determination, had a discriminatory power equal to that of the 24-h mean LH value (53). FSH levels for PCOS women were not influenced by BMI, thus the decline of LH/FSH ratio with increasing adiposity ($r = -0.44$, $P = 0.02$) was expected (Fig. 3). Taken together, these findings suggest that elevated LH levels will not be consistently disclosed in PCOS women with BMI greater than 30 kg/m^2. However, we propose that measurements of LH and FSH remain clinically meaningful if the negative influence of BMI/adiposity is taken into account. Confirmation of our findings by studying a larger number of subjects may generate a specific reference point for BMI or adiposity to further define the usefulness of LH levels and LH/FSH ratios as markers for PCOS women.

We conclude that 1) BMI/adiposity negatively influences LH pulse amplitude in PCOS women principally by an effect at the pituitary level; 2) accelerated LH pulse frequency in PCOS women is not influenced by BMI and is a defining pathophysiology of hypothalamic dysfunction in PCOS women; and 3) BMI does not influence gonadotropin secretion in normal cycling women. Thus assessments of basal LH levels and the LH/FSH ratio in hyperandrogenic anovulatory women are clinically meaningful when BMI is taken into account. Investigations to define the factor(s) that link adiposity and the attenuation of LH pulse amplitude in PCOS women would add further understanding of this complex neuroendocrine-metabolic disorder.

Addendum

During the revision process of our manuscript Taylor *et al.* (J Clin Endocrinol Metab. 82:2248–2256, 1997) reported on gonadotropin secretion across a wide spectrum of BMI in PCOS women with similar results; elevated LH pulse frequency in PCOS women independent of BMI and a blunting of LH pulse amplitude and integrated levels with increasing adiposity. In contrast to our results, a similar effect of adiposity on LH secretion was seen in normal cycling women.

Acknowledgments

We are grateful to Ms. Pam Malcom, Mr. Jeff Wong, Ms. Shannon Petze, and the nurses and staff of the Clinical Research Center for their dedicated care, and Dawn Nye for assistance in the preparation of this manuscript.

References

1. **Yen SSC, Vela P, Rankin J.** 1970 Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. J Clin Endocrinol Metab. 30:435–442.
2. **Rebar R, Judd HL, Yen SSC, Rakoff J, VandenBerg G, Naftolin F.** 1976 Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest. 57:1320–1329.
3. **Yen SSC.** 1980 Polycystic ovary syndrome. Clin Endocrinol (Oxf). 12:177–208.
4. **Burger CW, Schoemaker J.** 1985 Pulsatile luteinizing hormone patterns in the follicular phase of the menstrual cycle, polycystic ovarian disease (PCOD) and non-PCOD secondary amenorrhea. J Clin Endocrinol Metab. 61:1126–1132.
5. **Kazer R, Yen SCC.** 1987 Circulating luteinizing hormone pulse frequency in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 65:233–236.
6. **Filicori M, Campaniello E, Michelacci L, et al.** 1988 Gonadotropin-releasing hormone (GnRH) analog suppression renders polycystic ovarian disease patients more susceptible to ovulation induction with pulsatile GnRH. J Clin Endocrinol Metab. 66:327–333.
7. **Venturoli S, Porcu E, Fabbri R, et al.** 1988 Episodic pulsatile secretion of FSH, LH, prolactin, oestradiol, oestrone, and LH circadian variations in polycystic ovary syndrome. Clin Endocrinol (Oxf). 28:93–107.
8. **Couzinet B, Thomas G, Thalabard JC, Brailly S, Schaison G.** 1989 Effects of a pure antiandrogen on gonadotropin secretion in normal women and in polycystic ovarian disease. Fertil Steril. 52:42–50.
9. **Graf MA, Bielfeld P, Distter W, Weiers C, Kuhn-Velten W.** 1993 Pulsatile luteinizing hormone secretion pattern in hyperandrogenic women. Fertil Steril. 59:761–767.
10. **Waldstreicher J, Santoro NF, Hall JE, Filicori M, Crowley, Jr, WF.** 1988 Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotroph desensitization. J Clin Endocrinol Metab. 66:165–172.
11. **Christman GM, Randolph JF, Kelch RP, Marshall JC.** 1991 Reduction of gonadotropin-releasing hormone pulse frequency is associated with subsequent selective follicle-stimulating hormone secretion in women with polycystic ovarian disease. J Clin Endocrinol Metab. 72:1278–1285.
12. **Berga SL, Guzick DS, Winters SJ.** 1993 Increased luteinizing hormone and alpha-subunit secretion in women with hyperandrogenic anovulation. J Clin Endocrinol Metab. 77:895–901.
13. **Morales AJ, Laughlin GA, Bützow T, Maheshwari H, Baumann G, Yen SSC.** 1996 Insulin, somatotropic, and luteinizing hormone axes in lean and obese women with polycystic ovary syndrome: common and distinct features. J Clin Endocrinol Metab. 81:2854–2864.
14. **Apter D, Bützow T, Laughlin GA, Yen SSC.** 1994 Accelerated 24-h LH pulsatile activity in adolescent girls with ovarian hyperandrogenism: relevance to the developmental phase of polycystic ovarian syndrome. J Clin Endocrinol Metab. 79:119–125.

15. **Apter D, Bützow T, Laughlin GA, Yen SSC.** 1995 Metabolic features of polycystic ovary syndrome are found in adolescent girls with hyperandrogenism. J Clin Endocrinol Metab. 80:2966–2973.
16. **Yen SSC, Chaney C, Judd HL.** 1976 Functional aberrations of the hypothalamic-pituitary system in polycystic ovary syndrome: a consideration of the pathogenesis. In: Serio M, ed. The endocrine function of the human ovary. New York: Academic Press; 373–385.
17. **Huffman JW.** 1976 Polycystic ovaries in young girls. Proc of the 3rd Int Symp on Pediatric and Adolescent Gynecol. pp 193–206.
18. **Venturoli S, Porcu E, Fabbri R, et al.** 1986 Ovarian multifollicularity, high LH and androgen plasma levels and anovulation are frequent and strongly linked in adolescent irregular cycles. Acta Endocrinol (Copenh). 111:368–373.
19. **Franks S.** 1989 Polycystic ovary syndrome: a changing perspective. Clin Endocrinol (Oxf). 31:87–120.
20. **Conway GS, Honour JW, Jacobs H.** 1989 Heterogeneity of the polycystic ovary syndrome: clinical, endocrine and ultrasound features in 556 patients. Clin Endocrinol (Oxf). 30:459–470.
21. **Zawadzki JK, Dunaif A.** 1992 Diagnostic criteria: towards a rational approach. In: Hershmann JM, ed. Current issues in endocrinology and metabolism. Boston: Blackwell Scientific Publications; 377–384.
22. **Conway GS, Jacobs H, Holly JM, Wass JA.** 1990 Effects of luteinizing hormone, insulin, insulin-like growth factor-I and insulin like growth factor small binding protein 1 in the polycystic ovary syndrome. Clin Endocrinol (Oxf). 33:593–603.
23. **Anttila L, Ding YQ, Ruutiainen K, Erkkola R, Irjala K, Huhtaniemi I.** 1991 Clinical features and circulating gonadotropin, insulin, and androgen interactions in women with polycystic ovarian disease. Fertil Steril. 55:1057–1061.
24. **Dale PO, Tanbo T, Vaaler S, Abyholm T.** 1992 Body weight, hyperinsulinemia, and gonadotropin levels in the polycystic ovarian syndrome: evidence of two distinct populations. Fertil Steril. 58:487–491.
25. **Grulet H, Hecart AC, Delemar B, et al.** 1993 Roles of LH and insulin resistance in lean and obese polycystic ovary syndrome. Clin Endocrinol (Oxf). 38:621–626.
26. **Holte J, Bergh T, Gennarelli G, Wide L.** 1994 The independent effects of polycystic ovary syndrome and obesity on serum concentrations of gonadotropins and sex steroids in premenopausal women. Clin Endocrinol (Oxf). 41:473–481.
27. **Givens JR, Andersen RA, Umstot ES, Wiser WL.** 1976 Clinical findings and hormonal responses in patients with polycystic ovarian disease with normal *versus* elevated LH levels. Obstet Gynecol. 47:388–394.
28. **Laatikainen A, Tulenheimo A, Andersson B, Karkkaninen J.** 1983 Obesity, serum steroid levels, and pulsatile gonadotropin secretion in polycystic ovarian disease. Eur J Obstet Gynecol Reprod Biol. 15:45–53.
29. **Paradisi R, Venturoli S, Pasquali R, et al.** 1986 Effects of obesity on gonadotropin secretion in patients with polycystic ovarian disease. J Endocrinol Invest. 9:139–144.
30. **Smith S, Ravnikar VA, Barbieri RL.** 1987 Androgen and insulin response to an oral glucose challenge in hyperandrogenic women. Fertil. Steril. 48:72–77.
31. **Laughlin GA, Morales AJ, Yen SSC.** 1997 Serum leptin levels in women with polycystic ovary syndrome: the role of insulin resistance/hyperinsulinemia. J Clin Endocrinol Metab. 82:1692–1696.
32. **Bergman RN, Prager R, Volund A, Olefsky JM.** 1987 Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. J Clin Invest. 79:790–800.
33. **Veldhuis JD, Johnson ML.** 1986 Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection. Am J Physiol. 250:E486–E493.
34. **Kopelman PG.** 1994 Hormones and obesity. Balliere's Clin Endocrinol Metab. 8:549–575.
35. **Dunaif A, Graf M.** 1989 Insulin administration alters gonadal steroid metabolism independent of changes in gonadotropin secretion in insulin-resistant women with the polycystic ovary syndrome. J Clin Invest. 83:23–29.
36. **Nestler JE, Cornelius OB, Mat DW, et al.** 1989 Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 68:1027–1032.
37. **Kiddy DS, Hamilton-Fairley D, Bush A, et al.** 1992 Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 36:105–111.
38. **Holte J, Bergh T, Berne C, Wide L, Hans L.** 1995 Restored insulin sensitivity but persistently increased early insulin secretion after weight loss in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 80:2586–2593.
39. **Velasquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ.** 1994 Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism. 53:647–654.
40. **Nestler JE, Jakubowicz D.** 1996 Decreases in ovarian cytochrome P450c17alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med. 335:617–622.
41. **Velasquez EM, Mendoza SG, Wang P, Glueck CJ.** 1997 Metformin therapy is associated with a decrease in plasma plasminogen activator inhibitor-1, lipoprotein (a), and immunoreactive insulin levels in patients with the polycystic ovary syndrome. Metabolism. 46:454–457.
42. **Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb F.** 1996 The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab. 81:3299–3306.
43. **Ehrmann DA, Schneider DJ, Sobel BE, et al.** 1997 Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 82:2108–2116.
44. **Chapman IM, Wittert GA, Norman RG.** 1997 Circulating leptin in polycystic ovary syndrome: relation to anthropometric and metabolic parameters. Clin Endocrinol (Oxf). 46:175–181.
45. **Mantzoros CS, Dunaif A, Flier JS.** 1997 Leptin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metab. 82:1687–1691.
46. **Rouru J, Antilla A, Koskinen P, et al.** 1997 Serum leptin concentrations in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 82:1697–1700.
47. **Antilla L, Ding Y-Q, Ruutianen K, Erkkola R, Irjala K, Huhtaniemi I.** 1991 Clinical features and circulating gonadotropin, insulin, and androgen interactions in women with polycystic ovarian disease. Fertil Steril. 55:1057–1061.
48. **Robinson S, Rodin DA, Deacon A, Wheeler MJ, Clayton RN.** 1992 Which hormone tests for the diagnosis of polycystic ovary syndrome? Br J Obstet Gynecol. 99:232–238.
49. **Obhrai M, Lynch SS, Holder G, Jackson R, Tang L, Butt WR.** 1990 Hormonal studies on women with polycystic ovaries diagnosed by ultrasound. Clin Endocrinol (Oxf). 32:467–474.
50. **Quigley ME, Yen SSC.** 1980 The role of endogenous opiates on LH secretion during the menstrual cycle. J Clin Endocrinol Metab. 51:179–181.
51. **Berga SL, Yen SSC.** 1989 Opioidergic regulation of LH pulsatility in women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 30:177–184.
52. **Kazer RR, Liu CH, Yen SSC.** 1987 Dependence of mean levels of circulating luteinizing hormone upon pulsatile amplitude and frequency. J Clin Endocrinol Metab. 65:796–800.
53. **Apter D, Bützow T, Laughlin GA, Yen SSC.** 1994 Increasing the clinical value of serum LH measurements by reducing short term variation. Presented at the 20th Spring Meeting of the Finnish Endocrine Society. Tallin, Estonia, 1994.