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Leptin secretory burst mass correlates with body mass index and insulin in normal women but not in women with polycystic ovary syndrome

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Abstract

Leptin secretion exhibits a pulsatile, circadian pattern and may play a role in reproduction. No previous studies have compared leptin secretory burst characteristics in normal eumenorrheic women and women with polycystic ovary syndrome (PCOS) who are appropriately matched for body mass index (BMI). To determine if leptin secretory burst characteristics and/or the relationships of BMI, insulin, or testosterone to these characteristics differ between PCOS and normal women, we studied 9 normal eumenorrheic women and 9 women with PCOS. Each woman underwent blood sampling every 10 minutes for 24 hours to measure leptin and insulin under controlled conditions. Leptin secretory bursts were identified and characterized using multiparameter deconvolution procedures (Deconv), and the 24-hour periodicity of leptin was characterized with cosinor analysis. Relationships between BMI, area under the curve (AUC) insulin, and testosterone and leptin secretory burst characteristics in PCOS and normal women were sought using linear regression. There were no significant differences in mean serum leptin concentrations or in secretory burst characteristics between PCOS and normal women. Although the 24-hour serum leptin concentration correlated with BMI in both normal and PCOS women, leptin secretory burst mass correlated with BMI only in normal women. Similarly, the 24-hour serum leptin concentration correlated with serum insulin AUC in both normal and PCOS women; but insulin AUC correlated with leptin burst mass only in normal women. Although there was a strong trend toward a correlation between both mean 24-hour serum leptin concentration and leptin secretory burst mass with the serum testosterone concentration in normal women, such trends were not seen in PCOS women. Both normal and PCOS women exhibited a diurnal rhythm of leptin secretion with the peak occurring at night. However, neither the peak amplitude nor the timing of the peak amplitude differed between normal and PCOS women. The presence of strong relationships between BMI and insulin with both mean serum leptin and leptin secretory burst mass in normal women as opposed to PCOS women suggests that the mechanisms subserving leptin secretion differ in these 2 groups.

1. Introduction

Leptin is an adipocyte cytokine whose primary function has been associated with the regulation of metabolism and appetite [1]. Leptin may well play a role in the regulation of reproductive function in humans and other mammals; that is, animals and humans deficient in leptin are obese and hyperphagic and display a form of hypogonadotrophic hypogonadism [2,3]. Leptin administration to models of leptin deficiency, such as the *ob/ob* mouse, restores normal pubertal development and fertility [2]. In a manner similar to the *ob/ob* mouse, up to 60% of women with the polycystic ovary syndrome (PCOS) are either obese or overweight and are anovulatory [4]. To date, studies have failed to disclose a significant difference in fasting leptin concentrations between normal women and women with PCOS when matched for body mass index (BMI) or adiposity [5-7]. However, leptin appears to be secreted in a pulsatile fashion with a diurnal rhythmicity similar to other hormones [8]. To

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our knowledge, neither the characteristics of pulsatile leptin secretion in normal and PCOS women nor the relationships between such pulsatile secretory characteristics and other important metabolic and hormonal parameters such as BMI, insulin, and testosterone have been reported.

Within this context, we hypothesized that, because women with PCOS frequently are obese; have chronic anovulation, hyperandrogenism, and hyperinsulinism; and display abnormalities of luteinizing hormone (LH) pulsatility [9-11], (1) women with PCOS may manifest altered patterns of pulsatile leptin secretion as compared with normal women and (2) these alterations may relate to BMI and/or to ambient insulin or testosterone concentrations.

2. Methods

2.1. Study protocol

Ten women with PCOS and 10 normal control women were recruited from the Richmond, VA, community and from the Virginia Commonwealth University campus by advertisements. One normal control and one PCOS woman were excluded from the study because of scheduling difficulties. Written informed consent approved by the Virginia Commonwealth University Institutional Review Board and the University of Virginia Human Investigation Committee was obtained for all subjects. *Polycystic ovary syndrome* was defined as chronic oligomenorrhea (8 or fewer menstrual periods annually before pregnancy) and clinical and/or biochemical hyperandrogenism (elevated serum total testosterone concentration >55 ng/dL or hirsutism as determined by Ferriman-Galwey score >10) [12]. All PCOS women had normal thyroid function tests, normal serum prolactin, serum 17α-hydroxyprogesterone <200 ng/dL, dehydroepiandrosterone sulfate <700 ng/dL, and 24-hour urinary free cortisol levels <50 mg/dL. The control subjects had a documented history of regular monthly menses before pregnancy and normal health based on interview, medical history, physical examination, and standard laboratory tests. In addition, none of the subjects had diabetes mellitus as determined by a 2-hour oral glucose tolerance test using a fasting plasma value >125 mg/dL or a 2-hour value ≥200 mg/dL to define diabetes; and none was taking insulin-sensitizing agents or hormonal therapies such as oral contraceptives within 3 months of participation.

Nine control women were evaluated during the early follicular phase of the menstrual cycle and 9 PCOS women on a day when serum estradiol concentrations were statistically indistinguishable from those measured in the control women (within 24 hours of leptin sampling). All subjects were admitted to the General Clinical Research Center for 48 hours. On both days 1 and 2, all women ate meals at 8 AM, noon, and 5 PM and a snack at 8 PM, with meals composed of 50% carbohydrate, 35% fat, and 15% protein at a uniform caloric intake of 30 cal/kg body weight a day. Additional snacks, helpings, and meals were not permitted. Lights remained off from 10 PM to 7 AM the next day. Subjects were not allowed to nap during the day. At 8 AM on day 2, all subjects had an intravenous catheter placed from which serum samples for leptin were drawn every 10 minutes and those for insulin every 30 minutes until 8 AM the next day. Serum testosterone was measured in the 8-AM sample.

2.2. Assays

Serum samples were immediately centrifuged and stored at −70°C until assayed. Serum total testosterone was measured by using commercial radioimmunoassay kits as previously described [13]. Human leptin was measured by enzyme-linked immunosorbent assay (Diagnostics Systems Laboratories, Webster, TX). The interassay coefficient of variation for this assay is 4.4%, and the intraassay coefficient of variation is 3.8%. Serum insulin was measured by a highly specific radioimmunoassay (Diagnostic Products, Los Angeles, CA) that had a cross-reactivity with proinsulin of approximately 40% at midcurve.

2.3. Secretory burst analysis

The deconvolution procedure Deconv [14] was used to identify and characterize the leptin secretory bursts, including burst frequency (number of secretory bursts in 24 hours) and mass (total area under the burst secretion curve). Leptin secretory events were approximated algebraically by a Gaussian distribution of instantaneous molecular secretory rates centered around a particular point in time and dispersed with a finite SD. Deconv also provided an estimate of leptin half-life and of the basal secretory rate. The mathematical relationship of endogenous clearance kinetics to half-life ($T_{1/2}$) is represented by MCR = (Ln2/$T_{1/2}$) × V_d, where MCR is the metabolic clearance rate and V_d is the volume of distribution. Basal hormone secretion was calculated by simultaneous maximum likelihood parameter estimation.

2.4. Cosinor analysis

A least squares mean fit of the leptin concentrations at all time points used a cosine curve (known as *cosinor analysis*) to characterize the 24-hour periodicity of leptin [15]. Cosine amplitude (mean peak-to-nadir concentration of leptin in 24 hours) and cosine phase (time-to-peak amplitude expressed in minutes from start of sampling) were calculated with this technique to express diurnal change in the hormone.

2.5. Statistical analysis

Comparison of secretory burst characteristics was made between the 2 subject groups using Student *t* test in which $P < .05$ was considered statistically significant. A Pearson correlation coefficient was calculated for all linear correlations, and significance was again assumed for the linear model at $P < .05$. For the latter analyses, natural logarithmic transformation was performed for variables where the data

Table 1
Mean (±SEM) age, BMI, and hormonal characteristics of normal and PCOS subjects

Variable	Normal women (n = 9)	PCOS women (n = 9)	P [a]
Age (y)	32.1 ± 2.2	27.3 ± 2.1	.14
BMI (kg/m^2)	28.7 ± 2.3	28.3 ± 2.4	.92
Insulin AUC (U/[mL min])	271 272 ± 59 552	914 617 ± 382 950	.06
Estradiol (pg/mL)	33.1 ± 3.2	37.8 ± 7.2	.54
Leptin (ng/mL)	25.6 ± 4.7	23.2 ± 4.1	.58
Total testosterone (ng/dL)	42.6 ± 4.4	78.1 ± 20.5	<.001

[a] Means analyzed by Student t test. $P < .05$ = significant.

were not normally distributed. All statistical analyses were performed using SAS version 9 (SAS Institute, Cary, NC).

3. Results

Table 1 summarizes the descriptive and hormonal characteristics of the 9 control and 9 PCOS subjects. The groups did not differ significantly with regard to age, BMI, or mean serum fasting leptin. The mean serum total testosterone among women with PCOS was increased in comparison with the normal subjects ($P < .001$). Although not statistically significant, there was a strong trend toward increased area-under-the-curve (AUC) insulin among the PCOS women as compared with the control women ($P = .06$).

3.1. Leptin secretory burst analysis

The number of leptin secretory bursts as resolved by deconvolution analysis did not differ in the normal vs PCOS women (10.7 ± 1.86 vs 10.1 ± 1.87 per 24 hours, $P = .84$). Similarly, there were no differences in the normal vs the PCOS women with regard to leptin secretory burst mass (5.37 ± 1.47 vs 8.31 ± 2.90 ng/mL, $P = .38$), leptin half-life (262 ± 34.5 vs 313 ± 64.5 minutes, $P = .50$), or leptin basal secretion (0.04 ± 0.01 vs 0.12 ± 0.09 ng/[mL min], $P = .43$).

3.2. Regression analysis

Relationships were sought between BMI, serum insulin, and serum testosterone concentrations and both serum leptin and leptin secretory burst mass. As can be seen in Table 2, the mean 24-hour serum leptin concentration correlated strongly and positively with BMI in both PCOS and normal women; leptin burst mass correlated positively with BMI in normal women but not in women with PCOS; the mean 24-hour serum leptin concentration correlated strongly and positively with the AUC insulin concentration in both PCOS and normal women; and leptin burst mass correlated positively with the AUC insulin concentration in normal but not PCOS women. The mean 24-hour leptin concentration failed to correlate with serum testosterone in either PCOS or normal women, although there was a strong trend toward a significant relationship in the latter. Similarly, although leptin burst mass failed to correlate with serum testosterone in either PCOS or normal women, there again was a strong trend toward a significant relationship in the latter.

Table 2
Relationships between BMI, serum insulin, and serum testosterone concentrations and serum leptin concentrations and leptin burst mass in normal women and women with PCOS

	Mean serum leptin (log; ng/mL)		Mean leptin burst mass (log; ng/mL)	
	Normal women	PCOS women	Normal women	PCOS women
BMI (log; kg/m^2)	$r^2 = 0.59$, $P = .016$	$r^2 = 0.87$, $P = .0002$	$r^2 = 0.75$, $P = .003$	$r^2 = 0.24$, $P = .18$
Serum insulin AUC (log; U/[mL min])	$r^2 = 0.48$, $P = .04$	$r^2 = 0.84$, $P = .0005$	$r^2 = 0.53$, $P = .03$	$r^2 = 0.10$, $P = .41$
Serum testosterone (log; ng/dL)	$r^2 = 0.41$, $P = .064$	$r^2 = 0.27$, $P = .15$	$r^2 = 0.37$, $P = .08$	$r^2 = 0.15$, $P = .31$

3.3. Leptin cosinor analysis

Both normal and PCOS women demonstrated a diurnal rhythmicity in leptin concentrations with peak leptin secretion beginning at night. The mean peak-to-nadir concentrations of leptin (cosine amplitude) did not differ in the normal and PCOS women (4.34 ± 1.0 vs 4.83 ± 0.87 ng/mL, $P = .87$). Similarly, there were no differences in the peak amplitude of leptin secretion (cosine phase) in the normal and PCOS women (733 ± 106 vs 704 ± 84 minutes, $P = .83$). As shown in Table 3, linear regression was used to explore relationships between BMI, serum insulin, and serum testosterone concentrations and leptin cosine amplitude and phase. Peak-to-nadir concentrations of leptin (cosine amplitude) correlated strongly with BMI in both women with PCOS and normal women. However, BMI did not correlate with time-to-peak amplitude (cosine phase) in either PCOS or normal women. Cosine amplitude was found to correlate with insulin AUC in PCOS but not in normal women, but cosine phase failed to correlate with insulin AUC in either group. No correlations were found between serum testosterone and cosine amplitude or phase in either the normal or PCOS women.

Table 3
Relationships between BMI, serum insulin, and serum testosterone concentrations and leptin cosine amplitude and phase in normal women and women with PCOS

	Leptin cosine amplitude (log; ng/mL)		Leptin cosine phase (log; min)	
	Normal women	PCOS women	Normal women	PCOS women
BMI (log; kg/m^2)	$r^2 = 0.48$, $P = .04$	$r^2 = 0.58$, $P = .02$	$r^2 = 0.26$, $P = .16$	$r^2 = 0.03$, $P = .65$
Serum insulin AUC (log; U/[mL min])	$r^2 = 0.01$, $P = .77$	$r^2 = 0.69$, $P = .006$	$r^2 = 0.16$, $P = .29$	$r^2 = 0.21$, $P = .21$
Serum testosterone (log; ng/dL)	$r^2 = 0.04$, $P = .62$	$r^2 = 0.01$, $P = .85$	$r^2 = 0.20$, $P = .23$	$r^2 = 0.14$, $P = .32$

4. Discussion

Adiposity and metabolism have long been shown to regulate reproductive function, and leptin is believed to be a key hormone subserving this physiologic relationship. Under conditions of starvation and severe weight loss, when circulating leptin is low, animals and humans develop hypogonadotrophic hypogonadism [16]. Humans with congenital leptin deficiency are prepubertal and exhibit very low serum concentrations of LH and follicle-stimulating hormone (FSH) [3]. Subcutaneous administration of recombinant human leptin to humans with congenital leptin deficiency is associated with significant increases in both the frequency and amplitude of pulses of LH and FSH, consistent with early puberty [3]. In a similar fashion, administration of recombinant human leptin to women with congenital lipodystrophy also restores menstrual cyclicity [17,18]. Administration of recombinant human leptin to women with low adiposity secondary to strenuous exercise has been shown to restore LH and FSH secretion and to increase circulating estradiol and progesterone secretion without significant increases in adiposity [19]. Similarly, intraperitoneal administration of leptin to the female *ob/ob* mouse increases serum LH [20]. Taken as a group, these studies suggest a role for leptin in modulating reproductive function. However, the role of leptin's pulsatile secretory characteristics in modulating LH and FSH has yet to be elucidated.

Given that women with PCOS are insulin resistant and are frequently obese and infertile, we hypothesized that such women would display altered characteristics of pulsatile leptin secretion in comparison with normal subjects and that the relationships of leptin burst secretion to BMI, insulin, and/or testosterone would be altered. Our data are consistent with those obtained in previous studies that found no significant differences in mean fasting leptin concentrations between normal women and women with PCOS when the subjects were matched for BMI or adiposity [5-7]. To our knowledge, however, our study is the first to apply deconvolution procedures to serum leptin concentration-time series obtained in normal and PCOS women. This approach allows for the separation of secretory bursts from the confounding effects of clearance and subsequently characterizes both the secretory event and clearance functions. Our results suggest that leptin is secreted in a burst-like fashion but that neither the frequency with which leptin secretory events occur nor the characteristics of these events differ in normal vs PCOS women. Significant relationships were found between both BMI and insulin and the 24-hour mean concentration of leptin in both normal and PCOS women. Moreover, strong relationships were found in normal women between both BMI and insulin and leptin secretory burst mass. No such relationships were found between these factors in women with PCOS. Of interest, although there were strong trends for positive relationships between testosterone and mean leptin concentration and leptin secretory mass in normal women, no such trends were found in PCOS women. Taken as a group, these observations suggest that there are significant differences in the regulation of leptin secretion in normal vs PCOS women in terms of obesity, insulin status, and possibly androgen concentrations. However, although there are certain differences between normal and PCOS women with regard to these latter characteristics, there are also striking similarities in the diurnal rhythmicity of leptin secretion between women with PCOS and normal women and in many aspects of secretory burst characteristics determined by deconvolution. These similarities imply that factors aside from testosterone and insulin play a significant role in modulating leptin pulsatility in both groups of women.

The fact that both BMI and insulin status correlate strongly with the mean serum leptin concentration raises the possibility that both are working through the same mechanism. Moreover, both BMI and insulin correlate with leptin secretory burst mass in normal women, observations that are again consistent with a mechanism that primarily involves insulin. However, it is intriguing that the relationships between both BMI and insulin and leptin secretory burst mass are not evident in women with PCOS. These data suggest that PCOS women have independent factors that mitigate these relationships that appear so strongly in normal women. The nature of such factors remains to be clarified.

The role of androgens in the regulation of leptin secretion is also controversial. Using the rat as a model, Castrogiovanni and colleagues [21] demonstrated that the administration of testosterone results in an increase in both leptin turnover and clearance. In contrast to our findings, Brzechffa et al [22] reported that women who are relatively more insulin resistant and who exhibit high serum androgen levels have higher serum leptin concentrations than do normal women. However, Krotkiewski and colleagues [23] demonstrated that neither the acute reduction of serum androgen with a gonadotropin-releasing hormone agonist nor the administration of antiandrogens in women with PCOS alters serum leptin concentrations. Moreover, Remsburg and colleagues [24] have shown that lean women with PCOS who manifest higher androgen but lower insulin levels have lower serum leptin concentrations when compared with BMI-matched normal women. These observations suggest that insulin rather than androgen is the primary factor regulating leptin secretion, a conclusion that is consistent with our current findings.

We believe this to be the first study to examine the relationship of diurnal leptin rhythmicity to BMI in women with PCOS as compared with normal women. The results strongly suggest that peak amplitude secretion of leptin correlates with BMI in both normal and PCOS women, whereas the phase shift in leptin secretion over 24 hours is not strongly related to BMI. The observation that leptin cosine amplitude correlates strongly with insulin in PCOS but not normal women is again consistent with the notion

that certain characteristics of leptin secretion are differentially regulated in normal and PCOS women.

In summary, some leptin secretory burst characteristics differ in their relationship to BMI in women with PCOS as compared with normal women. Hormonal factors such as insulin may play a major role in modulating the secretion of leptin in these women that may in turn affect reproductive function axis in women with PCOS. At the same time, this study also suggests that many more features of pulsatile leptin secretion are similar between PCOS and normal women. The latter suggests that factors apart from testosterone and insulin are intrinsic to modulating leptin pulsatility in women in general.

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