Leptin secretory dynamics and associated disordered eating psychopathology across the weight spectrum

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Abstract

Objective: Leptin secretory dynamics across the weight spectrum and their relationship with disordered eating psychopathology have not been studied. Our objective was to compare leptin secretory dynamics in 13 anorexia nervosa (AN), 12 overweight/obese (OB) and 12 normal-weight women using deconvolution analysis.

Methods: In this cross-sectional study conducted at a tertiary referral center, serum leptin levels were obtained every 20 min from 2000 to 0800 h. Dual energy X-ray absorptiometry was used to measure percent body fat. Disordered eating psychopathology was assessed by the Eating Disorders Examination-Questionnaire (EDE-Q) and the Eating Disorders Inventory-2 (EDI-2).

Results: The groups differed for basal leptin secretion (BASAL) ($P=0.02$). Mean leptin pulse amplitude, pulse mass, total pulsatile secretion (TPS) and area under the curve (AUC) were significantly different between groups before and after adjustment for BASAL ($P<0.0001$ for all). Leptin AUC correlated strongly with TPS ($r=0.97$, $P<0.0001$) and less with BASAL ($r=0.35$, $P=0.03$). On multivariate analysis, only TPS was a significant predictor of leptin AUC ($P<0.0001$). TPS was inversely associated with most EDE-Q and EDI-2 parameters and the associations remained significant for EDE-Q eating concern ($P=0.01$), and EDI-2 asceticism, ineffectiveness and social insecurity ($P<0.05$) after adjusting for BASAL. These relationships were not significant when controlled for percent body fat.

Conclusion: Secretory dynamics of leptin differ across weight spectrum, with mean pulse amplitude, mean pulse mass and TPS being low in AN and high in OB. Pulsatile, rather than basal secretion, is the major contributor to leptin AUC. Decreased pulsatile leptin is associated with disordered eating psychopathology, possibly reflecting low percent body fat in AN.

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Introduction

Leptin, an anorexigenic hormone that is primarily produced by adipocytes, plays a major role in the regulation of food intake and energy homeostasis. Leptin is secreted in a pulsatile fashion (1) and levels of leptin are strongly associated with percent body fat (2), such that leptin levels are decreased in patients with low weight conditions such as anorexia nervosa (AN) (3) and elevated in high weight states such as obesity (4). Nutritional and hormonal factors are known to regulate leptin secretion;

however, it is unclear how these mechanisms impact basal vs pulsatile leptin secretion. Glucose and amino acid containing foods are the primary stimuli for leptin secretion and may account for its pulsatility (5, 6). In contrast, fatty acids inhibit leptin secretion (7). Additionally, endogenous hormones such as basal insulin and glucocorticoids are strong stimuli for leptin secretion. However, very little is known regarding the pattern of secretion of this hormone across the weight spectrum.

Leptin acts in conjunction with other appetite regulating hormones such as ghrelin, peptide YY (PYY) and cortisol to regulate food intake. Of these, ghrelin and cortisol stimulate appetite while leptin and PYY are anorexigenic. Although relationships between levels of leptin and these hormones have been described (8, 9, 10), data evaluating the associations between leptin secretory patterns and appetite regulating hormones are limited to our study in adolescents (11), and have not been reported in adults. Leptin levels are known to vary with pubertal status due to the combined influence of the divergent effects of fat and lean mass accumulation and the varying testosterone levels during puberty, resulting in girls having an increase and boys having a decrease in leptin levels as puberty progresses (12). However, there are no data regarding secretory patterns of leptin in adult women with AN in whom the impact of pubertal status no longer exists. Additionally, although one study examined leptin secretory patterns in overweight/obese (OB) women with polycystic ovarian syndrome (13), a condition in which variable leptin levels have been reported (14, 15), data on leptin secretory patterns in healthy OB women with regular menses are limited.

Disordered eating behavior is often seen across the weight spectrum and has been shown in one earlier study to be associated with leptin levels regardless of body weight (16) suggesting that eating behaviors may affect leptin secretion. Our group previously reported an inverse association of leptin levels with several measures of disordered eating assessed by eating disorder questionnaires, however these relationships were not significant when controlling for body weight (17). Assessment of leptin secretory dynamics would allow for a more in depth examination of the relationship between leptin dynamics, including basal and pulsatile secretion as well as integrated leptin levels, and disordered eating measures, and may improve our understanding of these associations with respect to weight status.

We therefore used state-of-the-art deconvolution analysis to examine leptin secretory dynamics and its relationship to disordered eating psychopathology in AN, healthy normally menstruating OB and healthy normal-weight women. We hypothesized that leptin secretory parameters would differ between groups, demonstrating the mechanism for low leptin levels in AN and high levels in OB women compared with normal-weight controls. We also hypothesized that secretory parameters of leptin would be inversely associated with severity of eating disorder thoughts and behaviors.

Subjects and methods

We evaluated the secretory patterns of leptin in 37 adult women; 13 with AN, 12 OB women and 12 normal weight women (C) using deconvolution analysis. Subject characteristics, hormone levels, and Eating Disorders Examination-Questionnaire (EDE-Q) and Eating Disorders Inventory-2 (EDI-2) scores have been previously reported (17, 18, 19, 20, 21). Analysis of leptin secretory parameters and their relationship with measures of disordered eating psychopathology and other appetite-regulating hormones have not been published and are presented in this report.

All participants were between ages 18 and 45 years. Inclusion criteria for AN participants included a DSM IV criteria of a weight <85% of ideal body weight (IBW) associated with an intense fear of gaining weight, an impaired body image and amenorrhea for at least 3 consecutive months preceding study participation. OB controls were required to have a BMI between 25 and 40 kg/m², and normal weight controls were at least 90% of IBW with a BMI <25 kg/m². Both control groups were otherwise healthy with normal menstrual cycles and no significant medical or psychiatric disease. Subjects with abnormal thyroid function tests, diabetes mellitus, active drug or alcohol abuse, and those who were pregnant or breastfeeding were excluded from the study.

The study was approved by Partners Human Research Committee. Informed consent was obtained prior to any procedures. At a screening visit, height and weight were obtained, medical history and physical exam were performed, and labs were drawn. Eligible participants were admitted to the Clinical Research Center overnight for the study procedures. %IBW and BMI were reevaluated at this visit. Dual energy X-ray absorptiometry was performed to assess body composition. Disordered eating psychopathology was assessed by administering EDE-Q and EDI-2 questionnaires. An IV catheter was placed by 1800 h and subjects were allowed to acclimate to the environment. Serum was drawn every 20 min from 2000 h to 0800 h. Subjects were asked to start fasting at 2000 h. Serum was pooled for integrated measures of 12 h cortisol. Fasting ghrelin, PYY, insulin, insulin-like growth factor 1 (IGF1) and estradiol (E₂) levels were obtained at 0745 h.

Assay and biochemical analysis

All blood samples were frozen and stored at –80 °C until analysis. Serum leptin was measured using a radio-immunoassay by Linco Research, St Charles, MS. The intra-assay coefficient of variation (CV) ranged from 5.2 to

7.5% and inter-assay variation from 3.2 to 8.9%. The minimum detectable concentration was 0.1 ng/ml. Serum cortisol was measured by a chemiluminescent micro particle immunoassay (Architect System, Abbot Diagnostics, Abbott Park, IL, USA) with an intra-assay CV of 2.1–4.8% and sensitivity of 0.8 mcg/dl. An RIA kit from Linco Research, a division of Millipore Inc., was used to assess plasma ghrelin levels (intra-assay CV 10.0–14.4% and inter-assay CV 14.7–16.7%, sensitivity 93 pg/ml) and serum PYY (intra-assay CV 1.5–2.7% and inter-assay CV 6.1–6.9%, sensitivity 1.4 pg/ml). Serum insulin was measured using an ultrasensitive assay (Access Immunoassay Systems, Beckman Coulter, Brea, CA, USA) which had an intra-assay CV of 2.0–4.2%, inter-assay CV of 3.1–5.6% and a sensitivity of 0.03 μ U/ml. Fasting serum IGF1 levels were measured by chemiluminescent immuno-metric assay (Immulite 2000; Diagnostics Products Corp., Los Angeles, CA, USA) (intra-assay CV 2.3–3.9%, sensitivity 20 ng/ml). E₂ was measured by chemiluminescent immunoassay (Access Immunoassay Systems, Beckman Coulter) (intra-assay CV 2.4–4.2%, inter-assay CV 4.4–8.2% and sensitivity 20 pg/ml).

Deconvolution analysis ► Leptin levels obtained by frequent sampling, every 20 min, were analyzed using Deconv feature of the Autodecon software. The software can be downloaded online from: 'https://www.researchgate.net/publication/262566415_AutoDecon_and_Pulse_XP_Software?ev=prf_pub.' Deconvolution analysis is a mathematical algorithm based software that provides information regarding basal secretory rate, half-life, number of pulses, pulse interval, mean pulse amplitude, mean pulse mass and total area under the curve (AUC) based on levels obtained from frequent sampling (22). As in our study in adolescent AN, we applied a half life of (24.9 ± 4.4 min) to the model as determined by Klein *et al.* (23). Total basal secretion (BASAL) was then calculated by multiplying the basal secretory rate (ng/ml per min) with the total duration of blood draw (720 min). Total pulsatile secretion (TPS) was calculated by multiplying mean pulse mass by the total number of pulses over the duration of frequent sampling. The sum of BASAL and TPS provided the total leptin secretion over 12 h.

Disordered eating questionnaires ► EDE-Q is a self-reported measure assessing the severity of eating disorder psychopathology in four categories: i) dietary restraint, ii) eating concern, iii) shape concern and iv) weight concern. A global score can be calculated to render a dimensional assessment of eating disorder

psychopathology (24). Normative data (mean $\pm$ s.d.) for EDE-Q measures were presented in our earlier report (17) based on prior studies in adult women (25, 26) and are as follows: dietary restraint, 1.3 ± 1.4 (25), 1.62 ± 1.54 (26); eating concern, 0.76 ± 1.06 , 1.11 ± 1.11 ; weight concern, 1.79 ± 1.51 , 1.97 ± 1.56 ; shape concern, 2.23 ± 1.65 , 2.27 ± 1.54 ; and global concern, 1.52 ± 1.25 , 1.74 ± 1.30 .

The EDI-2 is a self report questionnaire with 91 items and 11 subscales (27). It is a validated measure used to assess drive for thinness, bulimia, body dissatisfaction, ineffectiveness, perfectionism, interpersonal distrust, interoception, maturity fears, asceticism, impulse regulation and social insecurity. The normative data (mean $\pm$ s.e.m. of measurement) for EDI-2 are based on the EDI manual norms (27) and are as follows: drive for thinness, 5.5 ± 2.2 ; bulimia, 1.2 ± 0.9 ; body dissatisfaction, 12.2 ± 3.0 ; ineffectiveness, 2.3 ± 1.6 ; perfectionism, 6.2 ± 2.5 ; interpersonal distrust, 2.0 ± 1.3 ; interoceptive awareness, 3.0 ± 2.1 ; maturity fears, 2.7 ± 1.3 ; asceticism, 3.4 ± 1.6 ; impulse regulation, 2.3 ± 1.6 ; and social insecurity, 3.3 ± 1.5 .

Statistical analysis

JMP Pro 11 was used for statistical analysis (SAS Institute, Cary, NC, USA). Descriptive data are presented as mean $\pm$ s.d. The Shapiro–Wilk test was used to determine whether or not data were normally distributed. Continuous variables were assessed using ANOVA. The Tukey Kramer test was used to compare differences between any two groups while accounting for multiple comparisons. For non-parametric data, the Kruskal–Wallis test was used to compare group differences, followed by the Steel Dwass test to control for multiple comparisons. As leptin secretory parameters were not normally distributed, we used non-parametric tests (Spearman correlation) to evaluate associations of leptin secretion with other hormones and with disordered eating psychopathology. Multivariate analysis was performed to assess associations of leptin TPS with other hormones and disordered eating psychopathology after adjusting for total BASAL and percent body fat obtained by DEXA. A *P* value of <0.05 was considered statistically significant.

Results

Clinical characteristics

The participant characteristics and hormonal parameters are summarized in Table 1. As per study design, the BMI

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Table 1 Participant characteristics and hormonal parameters across the groups.

	AN (n=13)	C (n=12)	OB (n=12)	P value
Age (years)	26.6±6.5	28.4±6.3	28.5±8.2	NS
Baseline BMI (kg/m ²) ^a	18.5±0.9	22.0±1.5	31.3±4.1	<0.0001
Percent body fat (%) ^b	18±4	26±5	37±4	<0.0001
Fasting ghrelin (pg/ml) ^c	1126±372	1006±324	539±195	0.0002
12 h pooled cortisol (μg/ml) ^d	11.3±3.3	7.8±1.4	5.9±1.8	<0.0001
Fasting PYY (pg/ml) ^e	97±27	80±24	66±8	0.006
*Fasting insulin (μU/ml) ^f	2.6±1.4	4.6±1.7	7.7±3.4	<0.0001
Fasting IGF1 (ng/ml)	174±73	234±62	243±112	0.11
*Estradiol (pg/ml) ^g	36±18	53±25	68±53	0.02

*P value obtained by Wilcoxon and Steel Dwass used for multiple comparisons.

^aOB vs AN and C $P<0.0001$, AN vs C $P=0.004$.

^bOB vs AN and C $P<0.0001$, AN vs C $P=0.0001$.

^cOB vs AN and C $P<0.01$.

^dOB vs AN $P<0.0001$ AN vs C $P=0.003$.

^eOB vs AN $P=0.004$ ^{a-e} P value obtained by ANOVA and Tukey Kramer used for multiple comparisons.

^fOB vs AN and C $P\leq 0.02$, AN vs C $P=0.02$.

^gOB vs AN $P=0.04$.

and percent body fat of the subjects were significantly different among the three groups. Fasting ghrelin, 12 h pooled cortisol and PYY values were highest in the AN group and lowest in the OB group ($P<0.01$).

Deconvolution analysis

Leptin secretory parameters obtained from deconvolution analysis are shown in Table 2. Figure 1 depicts leptin secretory patterns obtained using deconvolution analysis in one representative subject from each group. The number of peaks, half-life and pulse interval were similar in the AN, OB and control groups. Leptin AUC, mean pulse amplitude, mean pulse mass, TPS and total secretion were lowest in AN and highest in OB groups ($P<0.0001$). Leptin BASAL was significantly higher in the OB group compared with the AN group ($P=0.04$), but did not differ when AN or OB groups were compared with normal-weight controls. Because there was a wide range of age for

study participants (18–45 years), we divided subjects into two age groups based on the median age of 26 years. There were no significant differences in leptin secretory parameters between those who were 26 years old or younger compared with those over 26 years old.

Leptin AUC correlated strongly with TPS ($r=0.97$, $P<0.0001$) and less so with BASAL ($r=0.35$, $P=0.03$). On multivariate analysis, only TPS was a significant predictor of leptin AUC ($P<0.0001$) indicating that TPS was the major contributor to leptin AUC. In order to assess the impact of BASAL vs TPS on other hormones and disordered eating psychopathology measures, we performed multivariate analysis controlling for BASAL.

Leptin secretory dynamics and body fat

Both leptin TPS ($r=0.94$, $P<0.0001$) and AUC ($r=0.95$, $P<0.0001$) were strongly associated with percent body fat. In contrast, the correlation between BASAL and percent

Table 2 Leptin secretory dynamics across the groups. Overall P value obtained using the Kruskal–Wallis test. P values for differences between any two groups after adjusting for multiple comparisons were obtained using the Steel–Dwass test.

	AN (n=13)	C (n=12)	OB (n=12)	P value	AN vs C	AN vs OB	OB vs C
Number of peaks in 12 h	18.2±0.4	18.2±0.9	18.3±0.7	NS	NS	NS	NS
Half-life (minutes)	24.56±4.42	24.87±4.03	24.90±3.81	NS	NS	NS	NS
Pulse interval (minutes)	39.89±0.80	39.33±0.89	39.93±1.44	NS	NS	NS	NS
AUC (ng/ml per 12 h)	3011±1986	8897±3385	24062±10359	<0.0001	0.0009	<0.0001	0.0002
Mean pulse amplitude (ng/ml)	0.18±0.12	0.58±0.29	1.40±0.72	<0.0001	0.002	<0.0001	0.0006
Mean pulse mass (ng/ml)	4.8±3.1	14.3±5.6	38.2±18.5	<0.0001	0.001	<0.0001	0.0001
Total pulsatile secretion (ng/ml per 12 h)	87.9±56.0	263.6±110.7	709.8±382.4	<0.0001	0.001	<0.0001	0.0002
Total basal secretion (ng/ml per 12 h)	0.08±0.09	0.07±0.03	0.13±0.08	0.02	NS	0.04	NS
Total leptin secretion (ng/ml per 12 h)	87.9±56.0	263.7±110.7	709.9±382.4	<0.0001	0.001	<0.0001	0.0002

Figure 1

Deconvolution analysis. (Top panel) Depicts leptin concentration in a subject with anorexia nervosa (AN), a normal weight healthy control and an overweight/obese control (OB). (Bottom panel) Depicts the individual secretory pattern for the same individuals. Patients with AN had low pulse amplitude and

body fat was less robust ($r=0.33$, $P=0.05$). Leptin TPS ($r=0.71$, $P<0.0001$) but not BASAL was strongly correlated with trunk to extremity fat ratio (a surrogate for visceral fat) (28). However, both leptin TPS ($r=0.94$, $P<0.0001$) and BASAL ($r=0.36$, $P=0.03$) correlated with total fat mass (a surrogate for subcutaneous fat mass) (29).

Leptin secretory dynamics and other hormones including appetite-regulating hormones

Leptin TPS and AUC were inversely associated with fasting ghrelin ($r=-0.67$ for both), 12-h pooled cortisol ($r=-0.77$ and -0.75) and PYY ($r=-0.63$ and -0.59) ($P<0.001$ for all). BASAL was inversely associated with PYY ($r=-0.35$, $P=0.04$), but was unrelated to fasting ghrelin or 12 h pooled cortisol. After controlling for BASAL in multivariate models, TPS retained its negative associations with fasting ghrelin ($P<0.0001$), 12 h pooled cortisol ($P<0.0001$) and PYY ($P=0.002$).

We performed additional analyses examining the relationship between leptin secretory parameters and hormones that could potentially impact leptin levels, such as insulin (6, 30), and hormones that could impact eating disorder psychopathology, such as IGF1 and E_2 (31).

pulse mass compared with normal weight controls ($P<0.0001$). OB controls had higher pulse amplitude and pulse mass compared with normal weight controls ($P<0.0001$). The unit of time for the x axis is minutes.

Leptin TPS and AUC were strongly correlated with fasting insulin ($r=0.83$ and 0.82 respectively, $P<0.0001$) and there were less robust associations between BASAL and insulin ($r=0.35$, $P=0.03$) levels. Leptin TPS was also associated with fasting IGF1 ($r=0.35$, $P=0.04$) and E_2 ($r=0.53$, $P=0.0008$) levels. These associations of TPS with insulin ($P<0.0001$) and E_2 ($P=0.01$) persisted after controlling for BASAL. However, associations of these hormones with TPS were no longer significant after controlling for percent body fat.

Leptin secretory dynamics and disordered eating measures

Participant scores for EDI-2 and EDE-Q subscales are presented in Table 3. Most EDI-2 measures and all EDE-Q measures were elevated in AN compared with C and OB. Further, TPS was negatively associated with most scales for EDI-2 including drive for thinness, ineffectiveness, interpersonal distrust, interoceptive awareness, asceticism, impulse regulation and social insecurity, and all EDE-Q subscales and global score (Table 4). BASAL was negatively associated with scores for EDI-2 bulimia, ineffectiveness, asceticism and social insecurity. Among the EDE-Q

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Table 3 EDI-2 and EDE-Q scores across the groups. Overall *P* value was obtained using the Kruskal–Wallis test. *P* values for differences between any two groups after adjusting for multiple comparisons were obtained using the Steel–Dwass test.

	AN	C	OB	<i>P</i> value	AN vs C	AN vs OB	OB vs C
EDI-2							
Drive for thinness	10.8±7.4	0.9±1.3	1.7±2.0	0.001	0.005	0.01	NS
Bulimia	0.9±1.3	0.3±0.7	0.3±0.8	NS	NS	NS	NS
Body dissatisfaction	13.4±8.2	3.9±4.3	9.2±7.2	0.009	0.02	NS	NS
Ineffectiveness	7.5±6.2	0.8±1.1	0.8±1.1	0.003	0.02	0.01	NS
Perfectionism	7.0±5.3	3.5±2.5	4.1±3.7	NS	NS	NS	NS
Interpersonal distrust	6.3±4.0	1.1±2.0	2.2±1.9	0.002	0.006	0.02	NS
Interceptive awareness	7.2±7.4	0.6±1.3	0.8±1.3	0.01	0.03	NS	NS
Maturity fears	6.2±3.4	3.9±1.7	4.2±1.4	NS	NS	NS	NS
Asceticism	6.7±3.1	3.2±2.6	2.3±1.4	0.002	0.03	0.002	NS
Impulse regulation	2.0±2.6	0.4±1.3	0.2±0.6	0.01	NS	0.03	NS
Social insecurity	8.4±4.7	0.7±1.1	1.6±1.4	0.0002	0.002	0.004	NS
EDE-Q							
Dietary restraint	2.8±1.5	0.6±0.7	0.9±1.0	0.0009	0.001	0.02	NS
Eating concern	2.8±1.5	0.2±0.3	0.2±0.2	<.0001	0.0001	0.0003	NS
Shape concern	3.7±1.8	0.8±0.7	1.4±1.2	0.0007	0.002	0.01	NS
Weight concern	3.3±1.7	0.5±0.6	1.4±0.9	0.0003	0.002	0.02	NS
Global concern	3.1±1.5	0.5±0.5	0.9±0.7	0.0002	0.0009	0.005	NS

measures, only dietary restraint had an association with BASAL. In multivariate analysis, TPS continued to be significantly associated with EDI-2 measures of asceticism, ineffectiveness and social insecurity ($P<0.05$) and the EDE-Q measure of eating concern ($P=0.01$) after adjusting for BASAL. However, all associations were lost when percent body fat was included in the model.

Discussion

To our knowledge, this is the first study comparing secretory dynamics of leptin in women across the weight spectrum, and examining the relationship between leptin secretion and i) appetite regulating hormones, as well as ii) disordered eating psychopathology. We show that leptin basal and pulsatile secretory parameters are low in AN and high in OB women. Next, we demonstrate that pulsatile leptin secretion is the major contributor to leptin AUC across the weight spectrum and is negatively associated with levels of appetite-regulating hormones cortisol, ghrelin and PYY. Finally, our findings demonstrate that lower pulsatile leptin secretion is associated with greater severity of disordered eating thoughts and behaviors as assessed by the EDI-2 and EDE-Q.

Although leptin secretory dynamics have been previously described in adolescent girls with AN, healthy and OB men, and OB women with PCOS, leptin secretory patterns have not been previously explored across adult women with AN, normal-weight women and OB women with regular menses. Examining the differences in

secretory patterns across the weight spectrum in a sex specific manner is all the more important given that women in general have 40% higher leptin levels compared with men (32). Here we report that adult women with AN have lower leptin pulse amplitude, pulse mass, TPS and AUC compared with healthy normal weight controls. Interestingly, these findings in adult women are similar to

Table 4 Relationship between leptin secretion and eating disorder psychopathology across groups. The correlation of EDI-2 and EDE-Q scores with leptin pulsatile and basal secretion is presented. Spearman's correlation was used given the non-parametric distribution of leptin pulsatile and basal secretion.

	Total pulsatile secretion		Total basal secretion	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Drive for thinness	−0.39	0.02	−0.32	0.06
Bulimia	−0.31	0.08	−0.35	0.04
Body dissatisfaction	−0.23	0.20	−0.13	0.46
Ineffectiveness	−0.48	0.004	−0.41	0.02
Perfectionism	−0.28	0.12	−0.18	0.32
Interpersonal distrust	−0.37	0.03	−0.20	0.25
Interceptive awareness	−0.41	0.02	−0.34	0.05
Maturity fears	−0.34	0.06	−0.13	0.47
Asceticism	−0.52	0.002	−0.39	0.02
Impulse regulation	−0.34	0.05	−0.25	0.15
Social insecurity	−0.45	0.008	−0.36	0.03
EDE-Q				
Dietary restraint	−0.43	0.01	−0.47	0.005
Eating concern	−0.64	<0.0001	−0.23	0.19
Shape concern	−0.43	0.01	−0.22	0.20
Weight concern	−0.38	0.03	−0.30	0.09
Global score	−0.46	0.006	−0.32	0.06

our prior findings in adolescent girls with AN in whom one would expect the impact of pubertal status on the endocrine milieu (11). BASAL did not differ in AN women compared with normal weight controls, which suggests that lower leptin levels in AN women are a consequence of decreased TPS. Further, leptin half life and other specific leptin pulse characteristics that might impact TPS such as pulse frequency and pulse interval did not differ between groups, while the groups did differ for leptin pulse mass and amplitude. This indicates that decreased TPS and therefore total leptin secretion and AUC in the AN group is likely a result of reduced leptin pulse mass and pulse amplitude.

In contrast to the findings in AN women, OB women demonstrated increased leptin pulse amplitude, pulse mass, TPS and AUC compared with healthy normal weight controls. Similar to the AN group, OB women did not differ from controls in the number of pulses, interval between pulses or the half life of leptin. Our findings are consistent with those of Yildiz *et al.* (33), who compared the leptin secretory dynamics in lean and OB healthy men using cluster analysis and found no differences in pulse frequency or inter-peak interval between the two groups. In addition, in this study obese men had a significantly higher pulse amplitude compared with lean controls, similar to our findings. These data lead us to propose that leptin pulsatility is preserved in OB subjects, as was also seen in an early pilot study comparing leptin dynamics in one obese woman compared with a normal weight woman (34). Another study comparing women with PCOS with normally menstruating BMI matched controls using deconvolution analysis did not demonstrate any difference in the pulse frequency or amplitude. This study also had a low secretory burst mass (5.37 ± 1.47 (controls) vs 8.31 ± 2.90 ng/ml (PCOS)) compared with ours. However, this study yielded a calculated half life (262 ± 34.5 (controls) vs 313 ± 64.5 min (PCOS)) that was much higher than most studies, which might have impacted the findings (13). Additionally androgens are known to suppress leptin secretion (12), and therefore PCOS-associated hyperandrogenemia could account for the relatively low amplitude pulses in the PCOS women in this study. Although, it is well known that levels of leptin are elevated in OB subjects secondary to leptin resistance, our mechanistic data demonstrate that increased pulse amplitude and mass underlie the increased leptin secretion in overweight/obesity. Another important finding our study highlights is that leptin pulsatile secretion contributes to AUC, a measure of integrated leptin concentration. When we performed multivariate analysis

controlling for BASAL, TPS emerged as the major predictor for leptin AUC.

When we examined associations of leptin secretory parameters with appetite regulating hormones, we found that TPS (but not BASAL) was inversely associated with ghrelin and cortisol. Ghrelin, produced in the stomach, and leptin have reciprocal effects on hypothalamic appetite pathways to produce orexigenic and anorexigenic effects respectively (35). Our earlier study in adolescents with AN and others have reported an inverse relationship between fasting levels of ghrelin and leptin (8, 10, 11). Consistent with this, we found that TPS was also inversely correlated with fasting ghrelin levels. The association between leptin and cortisol, however, is less clear. Patients with Cushing's disease have high leptin levels (36) and glucocorticoids induce leptin secretion *in vivo* (37), suggesting a positive association between these hormones. In contrast, leptin has been shown to inhibit cortisol secretion *in vitro* (38) and in our earlier study in adolescents we reported a negative association between leptin pulsatile secretion and cortisol AUC and total cortisol secretion (11), consistent with the current findings. However, as seen in our earlier study, when percent body fat was included in the multivariate model, there were no significant associations between leptin characteristics and cortisol (11).

PYY is secreted peripherally in the intestine in response to food intake and acts on neuropeptide Y in the hypothalamus to induce satiety (39, 40). Although both leptin and PYY are anorexigenic in nature, patients with AN have high PYY and low leptin levels (17). Likewise, obese subjects also share an inverse relationship between PYY and leptin levels (41). TPS showed a similar inverse relationship to fasting PYY levels in our study subjects, which persisted after controlling for BASAL.

We found strong associations between both TPS and BASAL leptin and fasting insulin levels, which may reflect the established role of insulin in inducing leptin secretion (6, 30). We also report positive associations between leptin TPS and IGF1, which is consistent with prior studies showing relationships between levels of these nutritionally regulated hormones in both low and high weight states (42, 43). In our study, leptin TPS was positively associated with levels of E_2 , as previously shown in adolescents with AN (11) and healthy women (44), likely secondary to the positive influence of leptin on GnRH secretion. However, associations between leptin and other hormones were no longer significant after controlling for percent body fat, suggesting that underlying nutritional status may drive these correlations. The strong

interrelationship between percent body fat and leptin as well as other endocrine factors makes it difficult to differentiate between the impact of body fat vs other hormonal factors on regulation of leptin secretion. Future interventional studies evaluating leptin secretory dynamics following nutritional and hormonal stimuli are warranted.

Low fasting leptin levels were associated with severity of disordered eating psychopathology in studies conducted in patients with AN (17, 45), while one other study showed contrasting findings (46). In our earlier study, we reported negative associations of fasting leptin levels with ineffectiveness, interoceptive awareness, interpersonal distrust and ascetism (17). The association of low fasting leptin levels with avoidance of sexual relationship measured by ascetism in EDI-2 has also been demonstrated (45). However, leptin secretory dynamics were not evaluated in these studies. We now show that the leptin TPS is negatively associated with EDI-2 measures of asceticism, ineffectiveness and social insecurity and EDE-Q eating concern, and these relationships remain significant after controlling for BASAL. However, as seen with hormonal parameters, the associations of leptin TPS with eating disorder measures were no longer significant after controlling for percent body fat, suggesting that these associations may be consequent to underlying nutritional status and body fat.

Because leptin is secreted by adipocytes, percent body fat is undoubtedly the single major determinant of leptin concentrations. However, our findings go a step further and show that pulsatile rather than basal leptin secretion impacts leptin concentrations (AUC). At this time, our understanding of factors that drive basal vs pulsatile secretion of leptin is limited. Further studies exploring the impact of pulsatile vs basal secretion on disordered eating behavior are necessary.

It is difficult to attribute a causal relationship to the findings we demonstrate here given the cross sectional nature of this study. Although there is a relatively small number of subjects in each of the three groups, our sample size compares well with other studies that have used deconvolution analysis for evaluating leptin secretory dynamics (33, 34) as well as those reporting psychopathology measures (47, 48). Additionally, the ability to detect significant differences in this sample size indicates the strength of our findings. All women with AN in our study were low weight. A future direction would be to examine changes in leptin secretory dynamics with weight recovery.

Conclusion

Secretory dynamics of leptin vary notably across the weight spectrum. Pulsatile leptin secretion makes a significant contribution to total circulating leptin levels and is associated with levels of other appetite regulating hormones and disordered eating psychopathology, possibly reflecting nutritional status and proportion of body fat. To better understand these associations, further investigations exploring the role of pulsatile vs basal leptin secretion in regulation of disordered eating are warranted.

Declaration of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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