Constitutional thinness and lean anorexia nervosa display opposite concentrations of peptide YY, glucagon-like peptide 1, ghrelin, and leptin¹²

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ABSTRACT

Background: Food intake is controlled by the arcuate nucleus through integration of peripheral hormonal signals such as leptin, ghrelin, peptide YY (PYY), and glucagon-like peptide 1 (GLP-1). The most common condition resulting in underweight young women in the developed world is restrictive anorexia nervosa (AN). However, constitutional thinness (CT) is also known to exist in the same low-weight range. Women with CT have normal menstrual periods and do not have the psychological or hormonal features of AN. Little is currently known about regulation of food intake in subjects with CT.

Objective: We tested the hypothesis that concentrations of leptin, ghrelin, PYY, and GLP-1 in persons with AN are significantly different from those in persons with CT.

Design: Concentrations of PYY, GLP-1, ghrelin, and leptin were measured in 3 groups of young women: normal weight (n = 7), CT (n = 10), and AN (n = 12). Samples were collected every 4 h for 24 h.

Results: PYY concentrations were significantly higher in CT subjects than in AN or control subjects. GLP-1 concentrations were significantly higher in AN than in CT subjects, whereas ghrelin was significantly higher in AN subjects than in control and CT subjects. CT subjects had the lowest ghrelin concentrations. Leptin concentrations were significantly lower in AN subjects. PYY and leptin circadian variations were not significantly different between CT and control subjects, whereas these profiles were blunted in AN subjects.

Conclusions: Orexigenic and anorexigenic hormones in CT contrast with an adaptive profile characterizing AN. The hormones appear to be valuable biomarkers for distinguishing these 2 categories of severely underweight subjects.

KEY WORDS Constitutional thinness, anorexia nervosa, peptide YY, ghrelin, glucagon-like peptide 1, leptin

INTRODUCTION

Appetite is controlled by the arcuate nucleus through integration of peripheral hormonal and metabolic signals. Ghrelin, the only orexigenic hormone, is secreted from the stomach (1) with concentrations peaking preprandially. Ghrelin plays a role in meal initiation by direct activation of medial arcuate nucleus neurons (2). Peptide YY (PYY) is released from the L cells in the distal gut (3). PYY has an anorexigenic effect, as evidenced by a reduction in caloric intake in humans and other mammals after the exogenous administration of PYY3-36 (4). Glucagon-like peptide 1 (GLP-1), which is also released from the endocrine L cells in the distal gut, acts as an incretin (5) and also reduces food intake (6). Leptin is a adipose tissue–derived hormone that suppresses appetite by inhibiting neuropeptide Y gene expression in the hypothalamic arcuate nucleus (7).

Fasting concentrations of ghrelin are decreased in obesity (8) and increased in anorexia nervosa (AN) (8, 9). Ghrelin concentrations decrease after weight gain in patients with AN (9, 10) and increase after weight loss in obese subjects (11). PYY is low in obese subjects (12), but conflicting results have been reported for PYY in AN (13–15). Circulating GLP-1 was found to be low both in obesity and in AN (16). Leptin concentrations correlate with adipocyte mass and are low in starvation and elevated in obesity (17).

The World Health Organization has suggested 3 underweight categories: grade 1 [mild underweight; body mass index (BMI) in kg/m²]: 17–18.5], grade 2 (moderate underweight; BMI: 16–16.9), and grade 3 (severe underweight; BMI: <16) (18). The most common reason for young women in the developed world to be underweight is restrictive AN (19). We have previously defined a group of women with constitutional thinness (CT) who satisfied the World Health Organization’s definition for moderate-to-severe underweight (BMI: 13–16.9) (10). This group of CT women does not have psychological or hormonal features of AN and displays normal menstruation (20), thyroid function (21), and insulin sensitivity (22). The body weight of persons with CT has always been in the lower centiles for age, sex, and ethnicity. Thus, patients with CT are a variant of normal (23).

We hypothesized that concentrations of appetite-regulating hormones in CT subjects would be comparable with those of normal-weight subjects, but different from those of patients with AN. In this study, we measured PYY, GLP-1, ghrelin, and leptin in 3 groups of young women, respectively, with CT, AN, and normal BMI as control subjects.

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SUBJECTS AND METHODS

The local research and ethics committee of Saint Etienne, France, approved the study. All subjects gave written informed consent.

Subjects

The study included 3 age-matched groups of white women: CT, AN, and control subjects. The CT and AN subjects were matched for BMI.

Ten CT subjects were recruited from our outpatient clinic. We used the following criteria: BMI between 14.5 and 16.5, stable weight throughout the postpubertal period, presence of menstrual periods without progesterone treatment, and the desire to gain weight as the main reason for medical consultation.

Twelve AN subjects were recruited during initial hospitalization before any therapeutic intervention. All patients met the criteria for AN in the Diagnostic and Statistical Manual of Mental Disorders (24) and had a BMI between 14.5 and 16.5. None of the patients used oral contraceptives, and all presented with secondary amenorrhea for >6 mo. Seven normal-weight control subjects (mean BMI: 20.4 ± 0.9) were matched by age (18–27 y) with the AN and CT subjects.

In the CT and control subjects, all data were collected during the follicular phase of the menstrual cycle. None of the subjects had documented chronic or congenital disease, and none of them were taking any medication.

Sampling

Venous blood samples were collected in dry glass tubes containing EDTA and centrifuged (1500 × g for 10 min at 4 °C), and plasma was divided into aliquots and kept frozen at −80 °C before the assay. After an overnight fast, blood was obtained at 0800 for the measurement of serum insulin-like growth factor I (IGF-I), 17β-estradiol, free triiodothyronine (T3), follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, sex hormone–binding globulin (SHBG), and dehydroepiandrosterone sulfate (DHEAS). Two-factor (group and time) repeated-measures ANOVA was performed to evaluate the circadian variations of appetite-regulating hormones within each group.

Assays

All samples were assayed simultaneously. PYY-like immunoreactivity was determined with the use of a specific and sensitive radioimmunoassay as previously described (4). The assay recognizes 2 biologically active forms of PYY (PYY3–36 and PYY1–36). There was no cross-reactivity with pancreatic polypeptide, neuropeptide Y, or other known gastrointestinal peptides. GLP-1 immunoreactivity was measured with a specific and sensitive radioimmunoassay. The intraassay CV was <10% (5). Plasma total (acylated and deacylated) ghrelin immunoreactivity was determined by radioimmunoassay (RK-031-30; Phoenix Pharmaceuticals, Belmont, CA). Intraassay CV was <5.3% (14).

Radioimmunoassay was used to measure plasma cortisol (intraassay CV: 7%; normal range: 107–310 nmol/L; Immunootech, Prague, Czech Republic), DHEAS (normal range: 85–225 μg/dL; Beckman-Coulter, San Diego, CA), 17β-estradiol (manufacturer’s reference range during the follicular phase: 30–50 ng/L; Dia Sorin, France), leptin (manufacturer’s reference range for a normal BMI (18–25): 3.7–11.1 μg/L; Nichols Institute Diagnostics, San Juan Capistrano, CA), and T3 (manufacturer’s reference range: 2.5–5.8 pmol/L; Beckman-Coulter). Immuno-radiometric assay was used to measure GH (manufacturer’s reference concentration: <5 mU/L; Beckman-Coulter), IGF-I (intraassay CV: 7%; manufacturer’s reference range: 107–310 μg/L; Beckman-Coulter), LH (manufacturer’s reference range during the follicular phase: 0.5–5 UI/L; Beckman-Coulter), FSH (manufacturer’s reference range during the follicular phase: 1.8–10.5 UI/L; Beckman-Coulter), and SHBG (manufacturer’s reference range: 20–85 nmol/L; BioMérieux, Lyon, France). Radioimmunoassay (Beckman-Coulter) extraction and chromatography were used to measure testosterone (manufacturer’s reference range: 7–65 ng/dL). Free testosterone index was calculated as testosterone divided by SHBG.

Energy intake

The study estimated the food intake during a period of 4 d, including 2 weekdays and a weekend. Dietary records were performed with the use of a photographic reference book, which was previously validated for the Supplementation en Vitamines et Minéraux Antioxydants study (25, 26). A nutritionist met the subjects twice, once to explain the collection of the data and the second time to ensure the accuracy of the collected data.

Body-composition measurements

Dual-energy X-ray absorptiometry (Lunar DPX-L; Lunar Corporation, Madison, WI; CV: <1%) measured the percentage of total body fat mass (FM) and fat-free mass expressed in kilograms (27, 28).

Statistical analysis

All values are presented as means ± SEMs. Analysis of variance (ANOVA) was first used to perform a three-group analysis for variables with a single assessment (BMI, FM, IGF-I, 17β-estradiol, free T3, FSH, LH, total testosterone, SHBG, and DHEAS). Two-factor (group and time) repeated-measures ANOVA was used for PYY, ghrelin, GLP-1, leptin, GH, and cortisol because 6 determinations within 24 h were performed to evaluate the significance of the main effects of interactions between group and group-by-time interactions. When ANOVA was significant (P < 0.05), we performed post hoc ANOVA tests for comparisons within all groups. One-factor (time) repeated-measures ANOVA was performed to evaluate the circadian variation of appetite-regulating hormones within each group. Adapted post hoc analysis (Tukey’s test) was performed with P < 0.05. Pearson’s correlation index was calculated to evaluate the relation between appetite-regulating peptides and FM in the overall group: CT + AN + control subjects. All statistical analyses were performed with STATVIEW 4.5 software (Abacus Concepts Inc, Palo Alto, CA).

RESULTS

Estimates of food intake, body weight composition, and baseline hormone characteristics for the 3 groups are shown in Table 1. Despite the significant differences in BMI (P < 0.001) and percentage of FM (P < 0.05) between the CT and control groups, no differences were observed in cortisol, GH, IGF-I, free
Table 1

<table>
<thead>
<tr>
<th>Subjects</th>
<th>AN (n = 12)</th>
<th>CT (n = 10)</th>
<th>Control (n = 7)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>20.7±1.2</td>
<td>20.2±1.2</td>
<td>23±0.8</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.2±0.4a</td>
<td>15.7±0.2a</td>
<td>20.4±0.3b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>9.4±1.1a</td>
<td>19.9±1.9b</td>
<td>25.4±1.5c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Food intake (kJ/d)</td>
<td>4304±625a</td>
<td>7821±850b</td>
<td>8150±958b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PYY (pmol/L)</td>
<td>24.5±1.0a</td>
<td>31.1±1.3b</td>
<td>26.8±1.2a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GLP-1 (pmol/L)</td>
<td>92.9±3.6a</td>
<td>73.2±4.8b</td>
<td>82.6±5.5a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ghrelin (pmol/L)</td>
<td>701±50a</td>
<td>324±24b</td>
<td>499±23c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (µg/L)</td>
<td>1.9±0.1a</td>
<td>7.7±0.7b</td>
<td>9.3±0.5b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>364±21a</td>
<td>212±13b</td>
<td>258±26b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GH (mUI/L)</td>
<td>6.9±0.7a</td>
<td>5.3±1.3b</td>
<td>5.1±1.3b</td>
<td>0.004</td>
</tr>
<tr>
<td>IGF-I (µg/L)</td>
<td>163±16a</td>
<td>295±34b</td>
<td>283±20b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free T3 (pmol/L)</td>
<td>2.7±0.1a</td>
<td>4.0±0.1b</td>
<td>3.6±0.1b</td>
<td>0.01</td>
</tr>
<tr>
<td>17β-Estradiol (ng/L)</td>
<td>11.3±1.4a</td>
<td>73.1±8.6b</td>
<td>47.6±11.4b</td>
<td>0.02</td>
</tr>
<tr>
<td>FSH (U/L)</td>
<td>3.0±0.6a</td>
<td>7.3±0.9b</td>
<td>5.3±1.0b</td>
<td>0.04</td>
</tr>
<tr>
<td>LH (U/L)</td>
<td>1.0±0.2a</td>
<td>13.4±3.0b</td>
<td>7.1±1.7b</td>
<td>0.002</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>75±21.5</td>
<td>71±11.7</td>
<td>110±23.2</td>
<td>0.32</td>
</tr>
<tr>
<td>FTO</td>
<td>0.8±0.1</td>
<td>0.6±0.1</td>
<td>0.8±0.1</td>
<td>0.98</td>
</tr>
</tbody>
</table>

1 All values are ± SEM. PYY, peptide YY; GLP-1, glucagon-like peptide 1; GH, growth hormone; IGF-I, insulin-like growth factor I; T₃, triiodothyronine; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; FTO, free testosterone index. Values in a row with different superscript letters are significantly different, P < 0.05 (ANOVA and Tukey’s test).

2 Calculated with the use of ANOVA.

DISCUSSION

CT is less prevalent among young underweight women in the developed world because restrictive AN is a more common condition, and the 2 diagnoses can be confused. Indeed, despite a low BMI (14.5–16.5), CT females do not exhibit any abnormal clinical features such as amenorrhea, fear of weight gain, or deranged hormonal values, characteristic of AN patients. The current study provides evidence on the differences of appetite-regulating hormones between CT and AN. The CT subjects were characterized by high PYY concentrations, low ghrelin concentrations, and low-to-normal concentrations of GLP-1 and leptin.

These biological differences could be related to some behavioral and physiologic aspects that contrast CT and AN, with specific reference to their different food intake and psychological profile. We recently showed that, although AN has a negative energy balance, CT subjects display an equilibrated energy metabolism similar to that of control subjects. CT subjects attempt to gain energy, whereas AN patients have a fear of gaining weight (29).

In this context we show for the first time an anorexigenic ratio between PYY and ghrelin despite a normal caloric intake in subjects with CT. Because the plasma ratio of orexigenic to anorexigenic hormones and food intake display a reciprocal regulation (2, 4, 30), 2 possible scenarios could account for this unusual combination. First, there may be a primary disturbance in PYY and ghrelin secretion. In this regard, recent reports suggest that PYY deficiency may contribute to the pathogenesis of obesity (31, 32). By analogy we postulate that high PYY concentrations may contribute to CT steadiness. Low ghrelin could also be considered pathophysiologic in CT as suggested by the ghrelin knock-out mouse model, which is protected from rapid

T₃, and 17β-estradiol. The caloric intake for the CT subjects (7821 ± 850 kJ/d) was not different from the control subjects (8150 ± 958 kJ/d) but was significantly higher than that of the AN patients (4304 ± 625 kJ/d; P < 0.05). The circadian profiles of appetite-regulating hormones are shown in Figure 1. The main effect of group within a 2-factor repeated-measures ANOVA was significant (P < 0.05) for PYY, GLP-1, ghrelin, and leptin. Post hoc analysis showed that PYY concentrations were significantly higher in the CT subjects than in the control and AN subjects. GLP-1 was significantly higher in the patients with AN than in the patients with CT, whereas ghrelin was significantly higher in the AN patients than in the control and CT subjects. The CT subjects had the lowest ghrelin concentrations. The leptin circadian cycle was significantly lower in the AN patients.

The CT and control subjects displayed significant intragroup circadian variations for PYY and leptin concentrations (P < 0.05). Thus, in both groups leptin concentrations peaked at 0400 (P < 0.05 compared with values at 0800, 1200, 1600, and 2000) and reached a nadir at 1200 (P < 0.05 compared with values at 0400, 2000, and 2400). PYY reached the lowest value at 0400 (P < 0.05 compared with values at 0800, 1600, and 2000) and the highest at 1600 (P < 0.05 compared with values at 0400, 1200, and 2400). Within the AN group, the circadian profiles for plasma leptin and PYY were blunted. No significant circadian variation was found for ghrelin or GLP-1 in any of the groups. When relations between appetite-regulating peptides and FM (expressed in kg or %) were evaluated in the 3 groups combined (CT + AN + control), only leptin (r = 0.70, P < 0.001) and ghrelin (r = −0.40, P < 0.001) displayed a significant correlation.
weight gain induced by early exposure to a high-fat diet (33). This pathophysiologic mechanism should lead to a low-food intake which is not the case in CT subjects. CT subjects often overeat because of their desire to gain weight. Therefore, this anorexigenic ratio could be interpreted as an appropriate response to food intake considered normal when compared with control subjects but abnormally high when related to body weight. The anorexigenic profile of hormones involved in regulation of appetite in CT is biological evidence that can be used to differentiate CT subjects from control subjects.

In the AN subjects, we found an association of low PYY, high ghrelin, and low leptin concentrations that could represent an orexigenic adaptive response of the appetite regulation network to a low-food intake. Despite the orexigenic profile, food intake is not increased, leading us to conclude that psychological determinism is predominant in AN. In these subjects the association between elevated ghrelin and low leptin concentrations was reported by us (10) and others (8, 9, 17), whereas recent data on the fasting PYY concentrations of AN patients are conflicting (14, 15). Thus, Stock et al (14) found PYY to be higher in AN (17.5 pg/mL) than in control (4.3 pg/mL) subjects. Because both studies assessed total PYY, the discrepancies found between these studies were probably due to differences in technique sensitivity. With the use of the same assessment technique, our PYY concentrations in control subjects were similar to those described by Batterham et al (4) and Stock et al (14). PYY concentrations in AN tend to be lower than those of control subjects in 6 determinations made throughout the day. These data need further confirmation. Only one study assessed GLP-1 in young AN patients and found fasting GLP-1 concentrations to be low (16). In the present study, circadian GLP-1 concentrations in the AN group were significantly higher than in the CT group and tended to be higher than in the control subjects. To better characterize the role of GLP-1 in these groups of lean subjects, it may be of interest to study oxyntomodulin, another peptide co-secreted with GLP-1, which is known to inhibit food intake in rodents and humans, exclusively through the GLP-1 receptor (34).

Physiologic gonadal activity is absent in AN and is undamaged in CT. Thus, high ghrelin concentrations and low leptin concentrations, in association with abnormal corticotropin-releasing hormone activity, mediate the suppression of the reproductive system in AN, or in female athletes (35–37). The relatively low concentrations of ghrelin and normal concentrations of leptin and cortisol in CT are in concordance with normal menstruation and normal estradiol concentrations. The variant of AN without amenorrhea recently proposed (38) should be considered as a diagnosis only after excluding CT.

Our study had several limitations. First, total (acylated and deacylated) ghrelin and total (1–36 and 3–36) PYY concentrations were measured. Acylated ghrelin and PYY3-36, the isoforms responsible for appetite regulation, should be assessed to

FIGURE 1. Mean (±SEM) concentrations of plasma peptide YY (PYY), glucagon-like peptide 1 (GLP-1), ghrelin, and leptin at 6 time points in subjects with constitutional thinness (CT) or anorexia nervosa (AN) and in control subjects. Similar cyclic circadian shapes of leptin and PYY were noticed in the CT and control subjects, whereas the profiles were blunted in the AN subjects. Intergroup differences and intragroup circadian variations were evaluated by using a 2-factor repeated-measures ANOVA followed by adequate Tukey’s test when the interaction was significant (P < 0.05). Groups with different lowercase letters are significantly different at nadir (*) and peak (**) point values of circadian profiles.
confirm our hypothesis on appetite control. Meanwhile, the relation between PYY or ghrelin variations and those of hunger and fullness remains to be confirmed (39). Conversely, the measurement of total hormones appears sufficient to distinguish CT from AN. Second, 4-h sampling intervals may be too infrequent to precisely identify the peaks and nadirs in the 24-h ghrelin profile (11). However, this method allowed us to detect circadian variations of leptin and PYY in the CT and control subjects and to calculate 24-h averages of these hormones.

We conclude that all of the anorexigenic and orexigenic peptides evaluated in our study (PYY, GLP-1, ghrelin, and leptin) are useful tools for differentiating 2 categories of severe underweight, namely CT, an almost unknown entity, and AN. However, the observed abnormalities in hormonal profiles were insufficient to explain the appetite regulation profile of CT. Further dynamic studies, including food supplementation or PYY neutralization or both, ghrelin enhancement, and consecutive hunger and fullness evaluation, are required.

NG, BG, and BE designed the study. NG, CWLR, and MAG performed the experiment. BG, CWLR, FL, and CB collected the data. NG, BG, MAG, and SRB wrote the manuscript. None of the authors had any financial support from or any personal interest in any company or organization sponsoring the research, including advisory board affiliations.

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