Women with bulimia nervosa exhibit attenuated secretion of glucagon-like peptide 1, pancreatic polypeptide, and insulin in response to a meal

Sabine Naessén, Kjell Carlström, Jens J Holst, Per M Hellström, and Angelica L Hirschberg

ABSTRACT

Background: The eating disorder bulimia nervosa (BN) is characterized by frequent episodes of binge eating, followed regularly by inappropriate compensatory behavior, such as self-induced vomiting. Objective: The current investigation was designed to examine possible alterations in the secretion of the gastrointestinal satiety peptides glucagon-like peptide 1 (GLP-1) and pancreatic polypeptide (PP) in women with BN. Design: Twenty-one women with BN and 17 healthy control subjects of comparable age and BMI were recruited. After fasting overnight, the subjects provided blood samples during ingestion of a standardized meal and self-rated their appetite on a visual analog scale. Fasting and meal-related secretion of the incretin GLP-1 and the meal-related feedback signal PP and insulin and glucose as indicators of the metabolic homeostasis were analyzed. Results: Women with BN had significantly lower fasting and postprandial serum concentrations of GLP-1 ($P < 0.01$) and PP ($P < 0.05$) than did the control subjects. Furthermore, both the basal ($P < 0.001$) and peak ($P < 0.05$) concentrations of insulin were significantly attenuated in the bulimic subjects, whereas glucose concentrations were normal. As a consequence, the bulimic homeostasis model assessment of insulin index values were also lower ($P < 0.001$). Conclusions: Women with BN secrete abnormally low amounts of GLP-1 and PP, possibly because of the adaption to large meals in the form of enlarged gastric capacity and reduced muscle tone in the gastric wall. Attenuated secretion of these gastrointestinal satiety peptides may play a role in the maintenance of bulimic behavior. Am J Clin Nutr 2011;94:967–72.
thought to be mediated by specific receptors in the brainstem and hypothalamus (20) and involves signaling via the vagus nerve that is designed to reduce food intake (20). Both basal and meal-stimulated secretion of PP are augmented in patients with AN (13, 23, 24). However, less is known about PP as a biomarker of vagal feedback activity in patients with BN (23, 25).

The current investigation was designed to examine possible aberrations in the biology of gastrointestinal peptides associated with bulimia. Specifically, basal and meal-related secretion of GLP-1 (as an endocrine signal) and PP (as a neural signal) were monitored. As a metabolic component, insulin-glucose homeostasis in women with BN and healthy control subjects was evaluated in relation to subjective ratings of appetite.

SUBJECTS AND METHODS

Subjects

This study was part of a larger investigation described previously (26) and thus included the same subjects. The initial recruitment started January 2005. Twenty-one women with active BN of the purging subtype and 17 healthy subjects of comparable age and BMI were recruited by advertising in the mass media and among students or hospital staff (26). All participants were evaluated by a trained research psychologist using the Diagnostic Interview for Anorexia and Bulimia (27)—a semistructured interview allowing a diagnosis based on the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders, compiled by the American Psychiatric Association in 1994 (28). All of the 17 control subjects and 15 of the 21 bulimic women had regular menstruation cycles, whereas 4 in the BN group were amenorrheic. Three of the bulimic women had a history of AN.

None of the subjects took any medication, including psychotropic drugs or hormone-containing contraceptives, for ≥3 mo before the experiments were conducted. All subjects were non-smokers. This study was preapproved by the local Ethics Committee at the Karolinska University Hospital (Sweden), and written consent was obtained from all of the women before participation.

Experimental design

After fasting overnight, the subjects arrived at the Women’s Health Clinical Research Unit, Department of Obstetrics and Gynecology, Karolinska University Hospital at 0730. Menstruating women were examined during the early follicular phase of the menstrual cycle (cycle days 1–5), whereas those with amenorrhea (no bleeding for the previous 3 mo) were evaluated on arbitrary days. Body weight, height, and systemic blood pressure were measured.

An indwelling catheter was inserted into a vein on the forearm to allow repeated collection of blood samples during the 3-h experiment. After the subjects rested for 30 min, 2 fasting blood samples (at time points designated as −15 and 0 min) were taken. Thereafter, the subjects ate a standardized breakfast consisting of 2 cheese sandwiches, a cup of tea or coffee without sugar, and a bowl of sour milk with a cup of cornflakes; the breakfast provided 500 kcal and contained 18% protein, 54% carbohydrates, and 28% fat (29). The meal was ingested within 15 min under the supervision of a research nurse, who ensured that all of the food was consumed.

Blood samples were drawn 15, 30, 45, 60, 90, 120, and 150 min after the subjects began eating, and serum was separated by centrifugation and stored at −70°C until analyzed. After collection of each blood sample, each woman rated her hunger, feeling of satiety, gastric distension, degree of nausea, and craving for sweets and fat on a visual analog scale ranging from 0 (not at all) to 10 (extremely) for each item.

Analytic procedures

Serum samples were extracted with 75% ethanol before quantifying GLP-1 by radioimmunoassay as described previously by Örskov et al (30). The detection limit was 7.8 pmol/L, and the CV was 7%.

To measure PP, this peptide was first absorbed from the serum onto Sep Pak C18 cartridges (Waters, Millipore Co) and was then eluted; the elute was evaporated to dryness at 45°C under nitrogen, the residue was redissolved in phosphate buffer (0.05 pmol/L, pH 7.4), and an ELISA was performed (Phoenix Europe GmbH). The detection limit was 40 pmol/L and the CV was 9%.

There was no cross-reactivity with insulin, glucagon, calcitonin gene-related peptide, somastostatin, or amylin amide.

Insulin was analyzed with a sandwich ELISA (Insulin Kit K6219; Dako) with a detection limit of 3 mU/L (20 pmol/L) and a CV of 10%. This assay cross-reacts with proinsulin to an extent of 3%, but not with C-peptide. Serum glucose was measured with an enzyme assay (mutarotase and glucose dehydrogenase) (Boehringer Mannheim GmbH) on a Hitachi 917 automated analyzer (Hitachi). Insulin resistance was assessed according to the homeostasis model of assessment, ie, fasting serum concentration of insulin (mU/L) × the corresponding concentration of glucose (mmol/L)/22.5. In addition, the insulinogenic index in response to the meal was calculated as the incremental AUC, depicting the concentration of insulin/the corresponding incremental AUC for glucose from the time point when the subject began to eat until 30 min later.

Statistical analyses

Continuous data are presented as means ± SDs or as medians and interquartile ranges (25th–75th percentile). Baseline values are defined as the mean of the 2 values obtained before consumption of the test meal. Serum concentrations of GLP-1, PP, insulin, and glucose for the bulimic and control groups and for each individual group from the beginning of the meal until 150 min later were compared by using a 2-factor mixed model. In cases of significant interactions, simple main effects were tested, ie, the effect of varying one factor while holding the others fixed. Because the time elapsed was quantitative, this variable was also modeled as a polynomial function. With the mixed procedure of SAS (version 8.2), random coefficient models were constructed to compare the linear and quadratic trends of the response profiles of the 2 different groups.

In addition, the AUCs for the hormones, glucose, and ratings of appetite were calculated, and the Mann-Whitney U test was then used to compare the groups. The distributions of some of the variables were positively skewed and therefore transformed logarithmically before the statistical analyses were performed. Correlations were assessed with the Spearman rank correlation test. A P value <0.05 was considered to be significant.
RESULTS

The bulimic and healthy control subjects had mean (±SD) ages of 26.0 ± 4.4 and 26.8 ± 3.6 y and mean (±SD) BMIs (in kg/m²) of 23.1 ± 2.7 and 22.6 ± 2.1, respectively. The average weight of the individuals in both groups remained stable for ≥3 mo before the start of the study. The members of the bulimic group reported binge eating and purging 6 (3–6) times/wk and scored their weight phobia as 9 (9, 9) out of a maximum of 10. The mean duration of BN was 8.0 ± 4.3 y.

Serum concentrations of GLP-1 increased significantly after the meal (P<0.001), with the bulimic and control groups having similar response profiles (Figure 1). However, the total GLP-1 response (AUC) of the women with BN was significantly lower (P<0.01) (Table 1).

Both at baseline and after the meal, serum concentrations of PP in the bulimic women were significantly lower than those in the control group (P<0.05) (Figure 2). Moreover, the response profiles for this peptide differed (linear trend, P<0.01; quadratic trend, P<0.05). There was a significant meal-related elevation in PP in the bulimic subjects (P<0.001), but only a tendency toward such an increase in the control group (linear trend, P=0.09; quadratic trend, P=0.05). The total response (AUC) was significantly lower in the women with BN (P<0.05; Table 1).

Although the serum concentrations of insulin in our bulimic subjects were significantly lower than those in the healthy control subjects, both at baseline (P<0.001) and 30 min after the meal began (P<0.05; Figure 3), the overall response (AUC) of these

TABLE 1

<table>
<thead>
<tr>
<th>Hormonal and glucose responses (AUC from the beginning of the meal until 150 min later) of bulimic women and healthy control subjects to a test meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1 (pmol/L)</td>
</tr>
<tr>
<td>PP (pmol/L)</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
</tr>
</tbody>
</table>

*All values are medians; interquartile ranges (quartile 25–quartile 75) in parentheses. GLP-1, glucagon-like peptide 1; PP, pancreatic polypeptide. **Significantly different from control subjects (Mann-Whitney U test): *P<0.05, **P<0.01.
correlated with gastric distension (increment in serum glucose (AUC 0–30 min) was positively correlated between the control group, the insulin response (total AUC) correlated positively with subjective ratings of appetite or responses in peptides and glucose in the previous AN, duration and severity of bulimic disease and P0.38. The correlation negatively with the craving for sweets (corresponding increment in self-reported feelings of hunger (GLP-1 (AUC 0–30 min) was negatively correlated with the feeling of satiety (R = 0.65, P < 0.01) and the increment in serum glucose (AUC 0–30 min) was positively correlated with gastric distension (R = 0.57, P < 0.05).

No associations were observed between menstrual pattern, previous AN, duration and severity of bulimic disease and subjective ratings of appetite or responses in peptides and glucose (data not shown).

**DISCUSSION**

Here, we documented significantly lower fasting and postprandial serum concentrations of GLP-1 and PP in women with BN than in healthy control subjects. Furthermore, both baseline and peak concentrations of insulin were also significantly lower in the bulimic women, whereas serum concentrations of glucose were similar in the 2 groups. These findings indicate that aberrant appetite-regulation and behavioral control are associated with BN.

In the only previous report concerning GLP-1 secretion in women with BN (15), Brambilla et al (15) observed higher basal serum concentrations of this peptide in bulimic subjects at 1600 only, with no difference in the postprandial concentrations in the bulimic and control subjects. The reason for this discrepancy in our finding of a decreased serum concentration of GLP-1 in women with BN before and after food intake is currently unclear. One reason for this discrepancy might be the different lengths of the fasting period before the study or the size of the foregoing meal intake, where a prolonged fasting period and small meal size would tend to reduce basal serum concentrations of the gut hormones studied.

One major factor that influences the release of regulatory peptides from the gut is body weight. Overweight has been shown repeatedly to be associated with attenuated release of these peptides into the bloodstream, which leads to conclusions concerning the effect of feedback signaling from the gut on behavior and obesity (10, 21). However, the BMIs of our bulimic and control subjects were similar and only slightly higher than those of the subjects studied by Brambilla et al (15); thus, this factor does not explain the discrepancy in findings.

Instead, a multitude of physiologic factors, including nutrient stimulation, gastric emptying, and gastrointestinal motility, could influence GLP-1 secretion, so that the type of test meal used for peptide release may be of significance. The subjects examined by Brambilla et al (15) consumed a test lunch rich in fat and containing a total of ~1300 kcal, whereas our mixed meal of 500 kcal represents a standard European breakfast. Furthermore, the choice of different analytic procedures for determining GLP-1 might have influenced the results obtained (15), as would any bias in the selection of subjects with BN.

In the current investigation, serum concentrations of PP were clearly lower in the women with BN, in contrast with the report by Fujimoto et al (23) of similar concentrations of this peptide in bulimic subjects and healthy control subjects both before and after consumption of a mixed meal. However, their study involved only 6 patients, had a high-fat test meal (23), and used a different procedure for PP determination. At the same time, Casper et al (25) demonstrated similar PP responses to an oral glucose tolerance test by 13 women with bulimia and 14 control subjects. Thus, the findings to date suggest that energy- or macronutrient-dependent release of PP is likely to give rise to a certain amount of variation among bulimic women.

What mechanisms might underlie the attenuated basal and meal-related secretion of GLP-1 and PP in bulimic women? These women have an enlarged gastric capacity (31), most likely as a consequence of repeated binge eating, and distension of the stomach is involved in evoking meal-related satiety through activation of gastric mechanoreceptors that signal via vagal afferents to central systems (7, 32). An enhanced gastric capacity may require ingestion of larger amounts of food to activate these mechanoreceptors, and, moreover, reduced vagal stimulation may result in less release of gastrointestinal satiety peptides in bulimic than in healthy individuals.

The lack of a normal correlation between blood concentrations of glucose and feelings of gastric distension in bulimic women indicates malfunctioning of the gastric mechanisms that produce
satiety, in agreement with the delay in gastric emptying observed in women with BN (33). Such a delay may also lead to a slower and attenuated release of intestinal signals of satiety, such as cholecystokinin (33), and to reduced secretion of GLP-1 from the distal gut. Thus, the reduced secretion of GLP-1 and PP may be secondary effects of delayed gastric emptying, although a primary secretory disturbance cannot be ruled out. The normalization of both impaired cholecystokinin secretion in bulimic women (33), and the elevated secretion of this same peptide in women with AN (34) after therapy, indicates that these altered responses are consequences to the eating disorders themselves.

We observed here, in all of our subjects, negative correlations between serum concentrations of GLP-1 and subjective hunger and between PP concentrations and the craving for sweets. Even though these correlations were not specific for the bulimic group, it appears possible that attenuated secretion of GLP-1 and PP may reduce the feeling of satiety and thereby promote bulimic behavior. GLP-1 also regulates blood concentrations of glucose concentrations through its insulinotropic and glucagonostatic actions (3), which may affect hunger sensations. Indeed, attenuated GLP-1 concentrations were associated with low concentrations of insulin, but normal glucose concentrations in our bulimic subjects. Thus, BN may be associated with enhanced sensitivity to insulin. It cannot be excluded that the lower insulin concentrations could be related to purging before the study or periods of fasting in the bulimic subjects.

The current investigation reflects the possible alterations of GLP-1 and PP concentrations in a well-characterized population of women with BN of the purging type and the potential consequences of such changes. One limitation of the current study was the lack of direct assessment of gastric emptying. Furthermore, it would have been preferable to admit the subjects to the research ward the day before the study to secure adequate fasting. In conclusion, we documented a low fasting and postprandial secretion of GLP-1 and PP in women with BN, which may be a consequence of the enlarged gastric capacity and adaptation to larger meals previously observed in bulimics. However, the mechanisms underlying these disturbances in endocrine secretion remain to be elucidated in detail. Abnormal secretion of these satiety peptides may play a role in the development and maintenance of bulimic behavior.

We thank Vilhelmina Rommel for her psychiatric evaluations of our bulimic subjects, Lotta Blomberg for technical assistance, and Elisabeth Berg for help with the statistical analyses.

The authors’ responsibilities were as follows—SN and ALH: designed the study; SN, KC, JJH, PMH, and ALH: analyzed the data; ALH, SN, PMH, and KC: wrote the manuscript; and ALH: assumed primary responsibility for the final content. All of the authors read and approved the final version of this manuscript. None of the authors had any conflicts of interest to disclose.

REFERENCES


