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Enhanced ghrelin secretion in the cephalic phase of food ingestion in women with bulimia nervosa

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Summary In humans, the cephalic phase response to food ingestion consists mostly of vagal efferent activation, which promotes the secretion of entero-pancreatic hormones, including ghrelin. Since symptomatic patients with bulimia nervosa (BN) are characterized by increased vagal tone, we hypothesized an enhanced ghrelin secretion in the cephalic phase of vagal stimulation. Therefore, we investigated ghrelin response to modified sham feeding (MSF) in both BN and healthy women.

Six drug-free BN women and 7 age-matched healthy females underwent MSF with initially seeing and smelling a meal, and then chewing the food without swallowing it. Blood samples were drawn immediately before and after MSF for hormone assay.

Circulating ghrelin increased after MSF in both groups with BN individuals exhibiting a greater ghrelin increase, which positively correlated with the patients' weekly frequency of binge-purging.

These results show for the first time an increased ghrelin secretion in the cephalic phase of vagal stimulation in symptomatic BN patients, likely resulting in a potentiation of the peripheral hunger signal, which might contribute to their aberrant binge-purging behavior.

1. Introduction

Bulimia nervosa (BN) is an eating disorder characterized by eating-related psychopathology resulting in episodes of massive food ingestion, followed by vomiting, prolonged periods of starvation, and other compensatory behaviors aiming to reduce energy intake or to increase its expenditure, generally with no pathological change in BW. Recent studies reported a derangement in the secretion of some gastro-entero-pancreatic hormones in response to food intake in symptomatic BN women and such alterations have been hypothesized to play a role in promoting and/or maintaining the patients' aberrant eating behaviors (Monteleone et al., 2008a). For instance, the food-induced increase of plasma levels of peptide YY and cholecystokinin have been found to be reduced and/or delayed in symptomatic bulimic patients (Monteleone et al., 2005; Kojima et al., 2005; Keel et al., 2007) while the post-prandial ghrelin secretion has been reported to be blunted (Monteleone et al., 2005; Kojima et al., 2005). The physiological increase in circulating ghrelin occurring before the consumption of a meal, that is in the preabsorptive cephalic phase of food ingestion, has never been investigated in BN.

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In humans, the preabsorptive cephalic phase response consists mostly of vagal efferent activation and concomitant release of some gastro-entero-pancreatic hormones, including the orexigenic ghrelin (Feldman and Richardson, 1986). BN patients have been shown to be characterized by an increased peripheral vagal activity resulting mainly in decreased hearth rate variability, bradycardia and increased somatic pain threshold (de Zwaan et al., 1996; Rissanen et al., 1998; Murialdo et al., 2007). Based on this background, we hypothesized that, because of their vagal hyperactivity symptomatic BN individuals should exhibit an enhanced secretion of ghrelin in the cephalic phase of vagal stimulation.

The cephalic phase of vagal stimulation may be assessed by using a modified sham feeding (MSF) technique, where a subject thinks about a meal, smells a meal and chews a meal without actually swallowing the meal (Robertson et al., 2001). Therefore, we investigated the ghrelin response to a sham feeding condition in symptomatic women with BN as compared to age-matched healthy controls, and assessed relationships between hormone responses and subjects' eating behavior.

2. Methods

Thirteen women were recruited for the study. They were 6 outpatients enrolled among those consecutively attending the Eating Disorder Center of our Department and 7 healthy controls. According to DSM-IV criteria, patients fulfilled the diagnosis of BN purging subtype with self-induced vomiting as the sole purging behavior. Diagnostic assessment was made by a trained interviewer using the Structured Clinical Interview for DSM-IV (SCID)-patient edition (First et al., 1995). At the time of the study, 2 patients had a lifetime comorbid major depression; none had a past history of anorexia nervosa; all of them were drug-free for more than 8 weeks; none had taken fluoxetine in the past or underwent any specific treatment program for her eating disorder. The 2 patients with lifetime comorbid depression had been treated with paroxetine in the past.

Control women were within 15% of their ideal BW; they were mentally healthy as assessed by the SCID-non-patient edition (First et al., 1996). They were regularly menstruating and had normal diets.

Both patients and healthy volunteers had normal physical examinations, normal values of routine blood and urine tests. None of the subjects was taking oral contraceptives or had a past history of alcohol or drug abuse.

The experimental protocol was approved by the local ethics committee and all subjects gave their written consent after being fully informed of the nature and procedures of the study.

All of the subjects were tested in the follicular phase of their menstrual cycle (day 5–10 from menses). On the test day, subjects arrived to our Clinical Investigation Unit at 0800 h, after a 12-h fasting. Patients were carefully instructed not to binge or purge at home at least from 1200 h of the day before testing, and their eating behavior was checked by a relative who made impossible the patient's access to food and personally reported to the investigators. At 0815 h, each subject received a standard breakfast of 200 kcal with 67% carbohydrates, 13% proteins and 20% fat. At 1145 h, an intravenous catheter was inserted into an antecubital vein and connected to a saline solution that was slowly infused to keep the catheter patent. At 1200 h, a test meal of 1220 kcal (with 67% carbohydrates, 13% proteins and 20% fat) was served. Subjects underwent modified sham feeding with initially seeing and smelling the meal; this was followed by chewing and spitting each bite into a napkin over the 15 min time period for the sham feeding. Subjects were instructed to avoid swallowing any of the food during the sham feeding. After spitting the food into a napkin, it was immediately removed from the place of testing. Blood samples were drawn immediately before ($T = -15$ and 0 min) and 15, 30, 45, 90, and 120 min after the sham feeding test. Blood was collected in tubes with lithium heparin as anticoagulant and DPP-IV inhibitor. Plasma was separated by centrifugation and stored at $-80\ ^{\circ}C$.

In each subject, current BW and height were measured, and the body mass index (BMI) was calculated; lifetime minimum and maximum BW were recorded historically.

Plasma ghrelin was measured by a commercially available RIA (Phoenix Pharmaceuticals, Mountain View, CA, USA); intra- and inter-assay CV were below 5.3% and 13.6%, respectively. Plasma glucose was determined by a commercial enzymatic UV method (Sigma Diagnostics, St. Louis, MO, USA).

The BMDP statistical software package (Dixon, 1985) was used for data analysis. One-way analysis of variance (ANOVA) was employed to test differences between patients and controls in pre-prandial hormone levels, nutritional and demographic characteristics. Differences in the hormone and glucose responses to MSF between the two groups were analyzed by two-way analysis of variance (ANOVA) with repeated measures, followed by the post hoc Tukey's test. Correlations between MSF-induced response in circulating ghrelin and the patients' clinical characteristics were assessed by the Pearson's correlation test.

3. Results

No significant differences emerged between BN patients and control women in age, BW, BMI, past minimum and maximum BW, but the delta maximum BW-minimum BW of patients was significantly higher than that of controls (17.0 ± 62 kg vs 8.5 ± 4.2 kg; $F_{1,11} = 8.26$, $P = 0.01$).

3.1. Plasma ghrelin

The group $\times$ time repeated measures ANOVA yielded a significant main effect for time ($F_{5,55} = 4.15$, $p < 0.002$) and a significant group $\times$ time interaction ($F_{5,55} = 2.825$, $p = 0.02$), indicating that circulating ghrelin changed significantly across sampling times and that BN patients and control subjects displayed quantitative differences over the samplings. Indeed, the post hoc Tukey's test of group differences indicated that, as compared to control women, BN patients had significantly higher ghrelin levels at 30, 90 and 120 min time point of the MSF test (Fig. 1).

When post-prandial changes in circulating ghrelin were expressed as percent of the time 0 values, two-way ANOVA with repeated measures showed significant effects for group ($F_{1,11} = 10.88$, $p < 0.007$) and time ($F_{5,55} = 6.57$, $p < 0.0001$)