Deranged Secretion of Ghrelin and Obestatin in the Cephalic Phase of Vagal Stimulation in Women with Anorexia Nervosa

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Background: Vagal activation in the cephalic phase response to food ingestion promotes ghrelin secretion. Because underweight individuals with anorexia nervosa (AN) are characterized by increased vagal tone, we hypothesized an enhanced ghrelin production in the cephalic phase of vagal stimulation. Therefore, we investigated the responses of ghrelin and its recently discovered sibling peptide obestatin to modified sham feeding (MSF) in both AN and healthy women.

Methods: Eight AN women and eight age-matched healthy female subjects underwent MSF, with initially seeing and smelling a meal and then chewing the food without swallowing it. Blood samples were drawn before and after MSF for hormone assays.

Results: Circulating ghrelin increased, whereas obestatin decreased after MSF. Compared with healthy women, AN individuals exhibited enhanced ghrelin and obestatin baseline plasma levels and amplified MSF-induced ghrelin increase and obestatin drop. Ghrelin secretion positively correlated with subjects' eating behavior as assessed by the Three-Factor Eating Questionnaire.

Conclusions: Opposite changes in circulating ghrelin and obestatin occur in the cephalic phase of vagal stimulation, and these changes are amplified in symptomatic AN patients. Given the opposite effects of ghrelin and obestatin on food intake, these findings may have pathophysiologic implications for the dysregulated eating behavior of AN individuals.

Key Words: Anorexia nervosa, cephalic phase, ghrelin, obestatin, vagal tone

Gastrointestinal responses to nutrient intake are usually subdivided into cephalic, gastric, and intestinal phases and are regulated by the autonomic nervous system. In humans, the preabsorptive cephalic phase response consists mostly of vagal efferent activation and concomitant release of some gastro-entero-pancreatic hormones (1,2). The cephalic phase of vagal stimulation can be assessed by using a modified sham feeding (MSF) technique (3), which has been shown to stimulate ghrelin secretion (4), supporting the view that the physiologic preprandial increase of plasma ghrelin may be due to vagal stimulation.

In underweight subjects with anorexia nervosa (AN), the physiology of ghrelin has been reported to be deranged (5). Moreover, AN patients are characterized by an increased peripheral vagal tone (6–8). Because of this vagal hyperactivity, underweight AN individuals should exhibit enhanced secretion of ghrelin in the cephalic phase of vagal stimulation.

Obestatin, a sibling of ghrelin derived from the same precursor peptide, has been recently discovered (9). Because obestatin counteracts ghrelin effects on food intake and gastrointestinal motility (9), changes opposite to those of ghrelin should occur in the cephalic phase. To test these hypotheses, we investigated ghrelin and obestatin responses to MSF in underweight AN women and age-matched healthy control subjects and also assessed relationships between hormone responses and subjects' eating behavior.

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Methods and Materials

Sixteen women (eight outpatients and eight healthy control subjects) were recruited for the study. Six patients fulfilled DSM-IV criteria for binge-purging AN (AN-BP) and two for restricting AN, as assessed by the Structured Clinical Interview for DSM-IV (SCID)—Patient Edition (10); three patients had a lifetime comorbid major depression, none had a past history of bulimia nervosa. All patients were drug-free for more than 8 weeks and were tested before entering any specific nutritional or psychotherapeutic treatment.

Control women were within 15% of their ideal body weight; they were mentally healthy as assessed by the SCID—Nonpatient Edition (11) and had no positive family history of mental disorders as assessed by the Family History Research Diagnostic Criteria (12), although underreporting may occur with the family history method. They were regularly menstruating and tested in the follicular phase of their menstrual cycle (day 5–10 from menses) to have an estrogen milieu similar to that of amenorrheic AN patients.

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.

At 8:15 AM, after a 12-hour fasting, each subject received a standard breakfast of 200 Kcal (67% carbohydrates, 13% proteins and 20% fat). At 11:45, an intravenous catheter was inserted into an antecubital vein and kept patent by a slow saline infusion. At 12:00, a test meal of 1220 Kcal (67% carbohydrates, 13% proteins and 20% fat) was served. The test meal consisted of white bread (150 g), smoked ham (100 g), marmalade (85 g), peach in syrup (200 g), and orange juice (200 mL). Subjects underwent MSF with initially seeing and smelling the meal for 5 min; this was followed by chewing and spitting each bite into a napkin. Subjects were instructed to avoid swallowing any of the food during the 15-min time period of MSF, and their behavior was monitored by a nurse. Blood samples were drawn before and after MSF. Blood was collected in tubes with lithium heparin and dipeptidyl

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Table 1. Clinical and Hormone Characteristics of the Study Sample

	Control Women	Women with AN	*F*	*p*
Age, yrs	22.1 ± 1.7	23.7 ± 5.2	.68	.4
Body Weight, Kg	55.6 ± 5.4	44.8 ± 8.9	8.42	.01
BMI, Kg/m^2	21.3 ± 2.4	16.4 ± 2.2	17.67	.0009
Past Minimum BW, Kg	52.2 ± 3.9	42.8 ± 8.8	7.48	.01
Past Maximum BW, Kg	60.5 ± 7.4	59.2 ± 10.1	.08	.7
Duration of the Illness (years)	—	4.1 ± 2.4	—	—
TFEQ Factor 1	4.7 ± 1.9	14.2 ± 4.9	25.65	.0002
TFEQ Factor 2	5.2 ± 2.2	8.7 ± 5.1	2.69	.1
TFEQ Factor 3	2.1 ± 1.8	5.8 ± 3.9	5.98	.02
Baseline Plasma Ghrelin (pg/mL)	273.9 ± 35.4	333.5 ± 64.1	5.29	.03
Baseline Plasma Obestatin (pg/mL)	82.5 ± 29.3	130.3 ± 16.5	12.38	.004

AN, anorexia nervosa; BW, body weight; TFEQ, Three-Factor Eating Questionnaire.

peptidase IV inhibitor. Plasma was separated by centrifugation and stored at −80°C.

In each subject, eating behavior was assessed by the Three-Factor Eating Questionnaire (TFEQ) (13).

Ghrelin was measured by a commercially available radioimmunoassay (Phoenix Pharmaceuticals, Mountain View, California); intra- and interassay coefficients of variation (CV) were below 5.3% and 13.6%, respectively. Obestatin was measured by a commercially available enzyme-linked immunosorbent assay kit (Peninsula Laboratories, San Carlos, California); intra- and interassay CV were < 5% and < 9%, respectively. Glucose was determined by a commercial enzymatic ultraviolet method (Sigma Diagnostics, St. Louis, Missouri).

The Biomedical Data Package statistical software package (14) was used for data analysis. One-way analysis of variance (ANOVA), two-way ANOVA with repeated measures, the post hoc Tukey's Test and the Pearson's Correlation were used as appropriate.

Results

Differences between AN patients and control women in nutritional and clinical variables and pretest levels of ghrelin and obestatin are shown in Table 1.

Plasma Ghrelin

The group-by-time repeated-measures ANOVA yielded significant effects for group [$F(1,14) = 10.47$, $p = .006$] and time [$F(7,98) = 5.41$, $p < .00001$] and a significant group-by-time interaction [$F(7,98) = 2.25$, $p = .03$], indicating that circulating ghrelin changed significantly across sampling times with significant quantitative differences between AN and control women (Figure 1). Mean maximum percent increase in plasma ghrelin after MSF (89.8 ± 50.4% vs. 44.4 ± 25.4%) and ghrelin area under the curve (AUC) (Figure 1) were significantly higher in AN women than in control subjects [$F(1,14) = 5.17$, $p = .03$; $F(1,14) = 9.60$, $p = .007$, respectively].

Plasma Obestatin

Because of technical problems, blood samples for obestatin measurement were available for only six AN patients and seven healthy control subjects.

The group-by-time repeated-measures ANOVA yielded significant effects for group [$F(1,11) = 9.71$, $p = .009$] and time [$F(7,77) = 9.31$, $p < .00001$] and a significant group-by-time interaction [$F(7,77) = 3.46$, $p = .002$], indicating that circulating obestatin changed significantly across sampling times with significant quantitative differences between AN and control women (Figure 2). Mean maximum percent decrease in plasma obestatin after MSF (51.8 ± 13.8% vs. 29.4 ± 13.3%) and obestatin AUC (Figure 2) were significantly higher in AN women than in control subjects [$F(1,11) = 8.74$, $p = .01$; $F(1,11) = 9.46$, $p = .01$, respectively].

Plasma Glucose

The group-by-time repeated-measures ANOVA yielded no significant effects for group [$F(1,14) = 3.95$; $p = .06$] and time [$F(7,98) = .66$, $p = .7$], and no significant group-by-time interaction [$F(7,98) = 1.66$, $p = .1$], indicating that circulating glucose

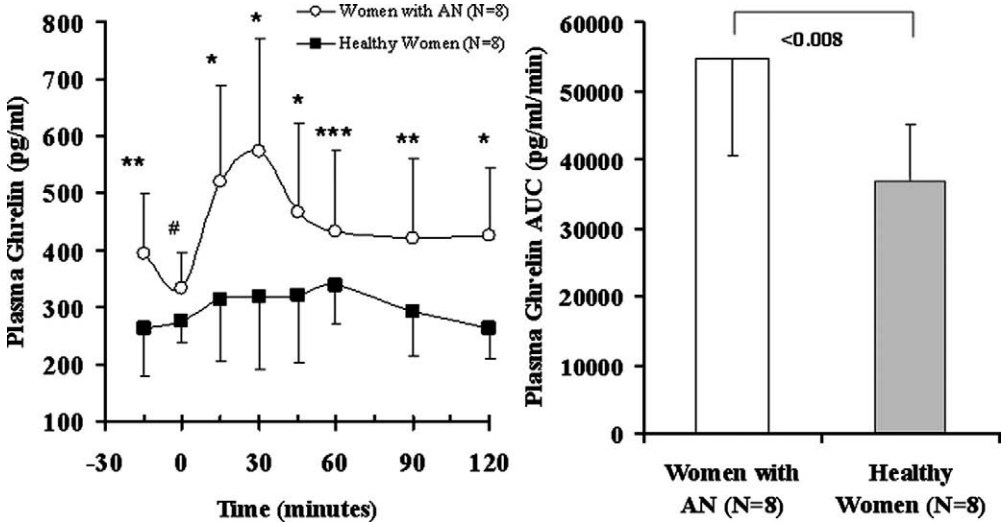

Figure 1. Plasma ghrelin response to modified sham feeding in drug-free patients with anorexia nervosa and healthy control subjects. Mean ± SD. *$p < .001$; ** $p < .02$; *** $p < .005$; # $p < .05$ versus healthy control women (post hoc Tukey's Test). AN, anorexia nervosa; AUC, area under the curve.