PLASMA OBESTATIN, GHRELIN AND GHRELIN/OBESTATIN RATIO ARE INCREASED IN UNDERWEIGHT PATIENTS WITH ANOREXIA NERVOSA BUT NOT IN SYMPTOMATIC PATIENTS WITH BULIMIA NERVOSA

Short title: Obestatin and ghrelin in eating disorders

Palmiero MONTELEONE, Cristina SERRITELLA, Vassilis MARTIADIS, Pasquale SCOGNAMIGLIO, and Mario MAJ

Department of Psychiatry, University of Naples SUN, Largo Madonna delle Grazie, 80138 Naples, Italy

Key words: anorexia nervosa, bulimia nervosa, eating disorders, ghrelin, leptin, obestatin.

Correspondence and Reprint Request to: Palmiero Monteleone, M.D.
Department of Psychiatry, University of Naples SUN
Largo Madonna delle Grazie, 80138 Naples, Italy
Phone: +39-081-5666517; Fax: +39-081-5666523
e-mail: monteri@tin.it

Disclosure statement: The authors have nothing to disclose.

Number of words (text): 1787, number of words (abstract):238; number of tables: 1; number of figures:1
Abstract

Introduction: Peptides of the gut-brain axis have a pivotal role in the regulation of energy homeostasis. Obestatin, a sibling of ghrelin derived from preproghrelin, is thought to oppose ghrelin effects on food intake. Since changes in ghrelin levels have been associated with anorexia nervosa (AN) and bulimia nervosa (BN), the investigation of obestatin production may further contribute to understanding the role of peripheral peptides in patients with eating disorders.

Methods: In the present study, we measured circulating blood levels of obestatin and ghrelin and assessed their relationships with anthropometric and clinical measures in 20 AN patients, 21 BN patients and 20 appropriate healthy controls.

Results: As compared to healthy women, patients with BN showed no significant differences in plasma obestatin and ghrelin concentrations and in the ghrelin/obestatin ratio whereas underweight AN patients displayed significantly increased circulating levels of both obestatin (P <0.009) and ghrelin (P <0.002) and an increased ghrelin/obestatin ratio (P <0.04). Moreover, in AN women, positive correlations emerged between the ghrelin/obestatin ratio and current body weight and body mass index.

Conclusions: Underweight AN patients are characterized by increased concentrations of ghrelin and obestatin and a higher ghrelin to obestatin ratio. No changes in circulating ghrelin or obestatin as well as in ghrelin to obestatin ratio seem to occur in acutely ill patients with BN. Although those changes likely reflect the physiological state of symptomatic AN individuals, they may also contribute to the pathophysiology of the disorder.
1 Introduction

Ghrelin is a 28-amino acid peptide secreted by the stomach, which increases food intake and down-regulates energy expenditure. The ghrelin gene encodes a 7 polypeptide containing 117 residues, called preproghrelin, which undergoes stepwise processing to form ghrelin [1]. Recently, it has been shown that preproghrelin undergoes additional proteolytic cleavage, generating a 23-amino acid peptide, which has been named obestatin [2]. In contrast to ghrelin, obestatin has anorexigenic effects, reduces gastric emptying, inhibits jejunal contractions and suppresses body weight (BW) gain in the animal [2]. Therefore, obestatin has been postulated to antagonize ghrelin actions on energy homeostasis and gastrointestinal functions, although this has been questioned by other authors [3-4].

Ghrelin has been suggested to be involved in the pathophysiology of anorexia nervosa (AN) and bulimia nervosa (BN). Indeed, baseline plasma ghrelin concentrations and plasma ghrelin responses to calorie ingestion have been found to be deranged in both underweight AN subjects and symptomatic BN patients [5]. Based on the above reported connection between ghrelin and obestatin, it seems plausible that obestatin may be a further candidate to the pathophysiology of eating disorders. Therefore, to shed light on the putative role of obestatin in AN and BN, we measured circulating blood levels of obestatin and ghrelin and assessed their relationships with anthropometric and clinical measures in patients with AN or BN and in appropriate healthy controls.

2 Patients and Methods

A total of 61 women were recruited for the study. They were 41 outpatients attending the Eating Disorder Center of our Department and 20 healthy controls. Twenty patients fulfilled the Diagnostic and Statistical Manual for Mental Disorders-IV edition (DSM-IV) diagnosis of AN and 21 that of BN as assessed by the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) [6]. Seven AN women were exclusively food restrictors (AN-R) and 13 binged and/or purged (AN-BP) with a frequency no greater than twice a week. Patients with BN were all of the purging subtype, with self-induced vomiting as the main compensatory behaviour. The mean frequencies of bingeing 61 and vomiting were $6.1 \pm 7.7$ and $7.7 \pm 9.4$ episodes/week, respectively. All AN and 46 BN women were amenorrheic; the remaining patients had normal regular menses. At the time of the study, 13 AN and 15 BN women had never taken psychotropic medications; the remaining patients had received paroxetine, citalopram or escitalopram in the past and were free for more than 6 weeks. All of them were studied before entering specific treatment programs.

Control women were recruited among 73 medical students; they had no current or past DSM-IV Axis I disorder, as assessed by the SCID-I non-patient edition [7], were regularly menstruating and had normal eating habits; all of them were drug-free for more than 8 weeks. Female controls and patients who were normally menstruating were tested in the follicular phase of their menstrual cycle (day 5-10 from menses).

The local ethic committee approved the study and to collect blood samples from healthy controls; subjects gave written informed consent prior to study participation. All subjects underwent a blood sample collection by venipuncture between 8.00 and 9.00 a.m., after an overnight fast; blood was collected in tubes containing ethylenediamine tetraacetic acid disodium salt (EDTA 2Na) and aprotinin (500 U/ml) and was immediately centrifuged. Plasma was separated and stored at -80 °C until assayed. Ghrelin was measured by a commercial ELISA kit (Phoenix Pharmaceuticals Inc; Burlingame, CA, USA); intra- and inter-assay coefficients of variation (CV) were $<5\%$ and $<9\%$, respectively. Obestatin was measured by a commercial ELISA kit (Peninsula...
Laboratories Inc., San Carlos, CA, USA); 1
intra- and inter-assay CV were <5% and <9%,
respectively. Leptin was determined by a
commercial ELISA kit (Alexis Biochemicals;
Laufelfingen, Switzerland); intra- and inter-
assay CV were 6.1% and 8.5%, respectively. 7
The ghrelin to obestatin ratio was calculated
8 [8].

The BMDP statistical software package
was used for data analysis. There was no
significant deviation from normality in
obestatin, ghrelin and ghrelin/obestatin ratio
values in each group as assessed by means of
the Shapiro Wilk normality test. One-way
analysis of variance (ANOVA) and the
Pearson’s correlation test were used where
appropriate.

Results
Clinical and demographic
characteristics and plasma leptin
concentrations of the study sample are shown
in table 1. One-way ANOVA showed statistically
significant inter-group differences in plasma
obestatin (F2,58=3.70, P=0.03), ghrelin
(F2,58=9.57, P<0.0004) and leptin levels
(F2,58=18.68, P<0.0001) as well as in the
ghrelin/obestatin ratios (F2,58=4.18, P<0.025).
As compared to healthy women, patients with
AN had significantly higher plasma obestatin
levels (86.2 ± 24.4 vs 68.3 ± 14.8 pg/ml; F1,38=7.82,
P=0.009) and ghrelin concentrations (370.6 ±
3613.8 vs 221.1 ± 95.6 pg/ml; F1,38=12.41,
P=0.0016) (Fig. 1), but significantly lower
leptin levels (F1,38=47.22, P<0.0001) (Table 1),
whereas BN women had similar circulating
levels of leptin (74.9 ± 22.4 vs 68.3 ± 14.8
pg/ml; F1,38=1.15, P=0.2), ghrelin (217.4 ±
34111.8 vs 221.1 ± 95.6 pg/ml; F1,38=1.01,
P=0.9) (Fig. 1) and leptin (F1,38=0.68, P=0.4)
(Table 1). The ghrelin/obestatin ratio was
significantly higher in AN women (F1,38=4.31,
P<0.045) but not in BN patients (F1,39=0.42,
P=0.5) (Fig. 1). No significant difference in
plasma obestatin, ghrelin and leptin levels as
well as in the ghrelin/obestatin ratio emerged
between AN-R and AN-BP patients.

In the AN group, statistically significant
positive correlations emerged between the
ghrelin/obestatin ratio and current BW
(r=0.53, P=0.01) and BMI (r=0.48, P=0.03).
No other significant correlation emerged
between hormone and clinical variables in
each diagnostic group.

Discussion
We found that, as compared to healthy
women, underweight AN patients displayed
significantly increased circulating levels of
obestatin and ghrelin and an increased
ghrelin/obestatin ratio as well as reduced
leptin concentrations; no significant changes
in these variables were detected in BN
individuals. Moreover, in AN women, the
ghrelin/obestatin ratio resulted significantly
and positively correlated with BW measures.

Literature studies are consistent with
present findings on ghrelin and leptin changes
in AN and BN patients [5], although
decreased leptin concentrations in BN were
detected in a previous study of ours [10].
Differences in the severity and/or length of
the patients’ illness have been suggested to
explain this discrepancy [10]. To the best of
our knowledge, no study reported on obestatin
levels in symptomatic BN patients while,
according to our results, Harada et al. [11]
and Nakahara et al. [12] showed increased
plasma obestatin and ghrelin concentrations in
small groups of AN patients as compared to
age-matched healthy women. None of these
two studies, however, calculated the
ghrelin/obestatin ratio and assessed its
relationships with nutritional parameters.

Zhang et al. [2] initially suggested a role
of obestatin as an antagonist of the ghrelin
actions in energy homeostasis. These
observations have been confirmed by some
94 authors but questioned by others [3-4], and
controversies still exist on the definite effects
of obestatin on food intake/energy balance as
well as on the measurements of the hormone
elevations in the human blood [13]. Nonetheless,
if one assumes the suggested role of obestatin
as an antagonist of ghrelin actions, one would
expect opposite changes of these peptides in
1 pathological conditions characterized by 2 alterations in energy balance. Therefore, in 3 underweight AN patients decreased levels of 4 obestatin would be expected. Present findings 5 and literature data show that this is not the 6 case. On the other hand, in obese patients who 7 show decreased fasting plasma ghrelin levels 8 [8, 14] and were expected to have enhanced 9 obestatin, circulating obestatin concentrations 10 have been found to be reduced by most 11 although not all of the authors [8, 15]. 12 Therefore, present findings and literature data 13 show parallel changes in ghrelin and obestatin 14 secretion in pathological conditions 15 characterized by energy imbalance, 16 suggesting that dysregulated metabolic states 17 may potentially affect the preproghrelin gene 18 expression and/or the splicing of its products 19 and that the conclusive effect on food intake 20 and energy homeostasis could depend upon 21 the ratio between ghrelin and obestatin 22 peptides. 23 Therefore, it might be hypothesized that, 24 in underweight AN subjects, an enhanced 25 expression of the preproghrelin gene leads to 26 an enhanced production of ghrelin and 27 obestatin, which likely does not occur on a 28 one to one ratio, with the consequent increase 29 in the ghrelin/obestatin ratio. Mechanisms 30 responsible for such an imbalance of ghrelin 31 to obestatin production are not clear, since 32 little is known about the post-translational 33 cleavage of the preproghrelin peptide. 34 Nonetheless, the increased ghrelin to 35 obestatin ratio would result in a potentiation 36 of the peripheral orexigenic signal, which 37 aims to oppose the patient’s over-control on 38 her food intake. This hypothesis seems to be 39 corroborated by the finding of significant 40 positive correlations between the 41 ghrelin/obestatin ratio and BW measures in 42 our AN group, which may suggest that when 43 the hunger signal of ghrelin is stronger than 44 the satiety signal of obestatin, as expressed by 45 higher values of the ghrelin/obestatin ratio, 46 then AN patients are able to reach higher BW 47 likely because of an increased drive to eat. 48 Recently, in a family trios study assessing the 49 transmission of two single nucleotide 50 polymorphisms (SNP) of the preproghrelin 51 gene in AN probands, it has been found that 52 patients carrying the obestatin Gln90Leu SNP 53 had a lower lifetime BMI as compared with 54 the homozygotes for the wild allele [16]. 55 Although the functional significance of this 56 SNP is not known, one could speculate that, if 57 it was responsible for enhanced obestatin 58 secretion/activity, the association with a lower 59 lifetime BMI might strengthen our hypothesis 60 that the balance between ghrelin and obestatin 61 activities is crucial for BW regulation in AN. 62 A potential limitation of our study is 63 that we measured “total” obestatin, that is 64 both amidated and non-amidated peptide, and 65 “total” ghrelin, that is both acyl- and desacyl- 66 ghrelin. Amidation of obestatin is likely 67 essential for its biological activity as well as 68 acylation of ghrelin. Since desacyl-ghrelin, 69 similarly to obestatin, is provided of opposing 70 effects to acyl-ghrelin on appetite and 71 gastrointestinal motility [17], different results 72 might emerge if the two forms of peptides are 73 measured. A second limitation of the present 74 study is represented by the low number of 75 AN-R patients with respect to AN-BP 76 individuals. Since in AN-BP subjects ghrelin 77 concentrations have been reported to be 78 higher than in AN-R ones [18], we cannot 79 exclude that the prevalence of binge-purging 80 subjects in our sample might cause higher 81 ghrelin/obestatin ratio. However, other studies 82 failed to find increased levels of ghrelin in 83 binge-purging anorexics as compared to 84 restricted ones [19, 20], and we could not 85 detect any significant difference in hormone 86 levels between the two subtypes of AN 87 individuals. 88 In conclusion, this study shows that 89 underweight AN patients are characterized by 90 increased concentrations of ghrelin and 91 obestatin and a higher ghrelin to obestatin 92 ratio, which was positively correlated with 93 BW measures. Although these changes likely 94 reflect the physiological state of symptomatic 95 AN individuals, they may also contribute to 96 the pathophysiology of the disorder. No 97 changes in circulating ghrelin or obestatin as 98 well as in ghrelin to obestatin ratio seem to 99 occur in acutely ill patients with BN.
References


Fig. 1 Plasma levels of obestatin (top panel) and ghrelin (middle panel), and ghrelin to obestatin ratio (bottom panel) in women with anorexia nervosa or bulimia nervosa and in healthy women. Data are expressed as mean ± SD.
<table>
<thead>
<tr>
<th></th>
<th>Control Women</th>
<th>Women with AN</th>
<th>Women with BN</th>
<th>F_{2, 58}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>23.6 ± 5.5</td>
<td>23.4 ± 7.5</td>
<td>26.2 ± 7.1</td>
<td>1.11</td>
<td>0.3</td>
</tr>
<tr>
<td>Body Weight, Kg</td>
<td>55.3 ± 6.0</td>
<td>45.0 ± 6.8 *</td>
<td>57.5 ± 8.7</td>
<td>16.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI, Kg/m$^2$</td>
<td>21.1 ± 2.2</td>
<td>16.6 ± 1.6 *</td>
<td>21.4 ± 3.3</td>
<td>23.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Past Minimum BW, Kg</td>
<td>52.1 ± 6.0</td>
<td>43.0 ± 6.3 *</td>
<td>50.3 ± 9.6</td>
<td>8.10</td>
<td>&lt;0.0009</td>
</tr>
<tr>
<td>Past Maximum BW, Kg</td>
<td>59.4 ± 7.8</td>
<td>58.9 ± 11.6</td>
<td>68.0 ± 10.0 **</td>
<td>5.53</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>Plasma Leptin (ng/ml)</td>
<td>12.2 ± 5.6</td>
<td>3.2 ± 1.5 *</td>
<td>10.6 ± 6.3</td>
<td>18.68</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* P <0.0001; ** P <0.005 as compared to control women (one-way ANOVA)
Healthy women

Anorexia Nervosa

Plasma Obestatin Levels (pg/ml)

P < 0.009

Bulimia Nervosa

Healthy women

Anorexia Nervosa

Plasma Ghrelin Levels (pg/ml)

P < 0.002

Bulimia Nervosa

Healthy women

Anorexia Nervosa

Plasma Ghrelin/Obestatin Ratio

P < 0.04

Bulimia Nervosa