Applied nutritional investigation

Obestatin and ghrelin interplay in hemodialysis patients

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Abstract
Objective: Compounds involved in the regulation of appetite and body composition appear to be of interest in chronic kidney disease. The purpose of this study was to analyze plasma obestatin and acyl and des-acyl ghrelin in patients on hemodialysis (HD).

Methods: Fifty patients on HD (56.0% women, mean age 62.2 ± 15.2 y) were studied. Blood samples were collected during fasting, before a regular HD session. Serum acyl and des-acyl ghrelin levels, leptin, and obestatin were measured using enzyme immunometric assay methods. Anthropometric parameters, appetite score, and food intake were recorded.

Results: Patients showed elevated serum leptin (34.1 ± 30 ng/mL), normal acyl ghrelin (137 ± 116.5 pg/mL), high des-acyl ghrelin (670 ± 479 pg/mL), and low obestatin (2.0 ± 1.4 ng/mL) levels compared with healthy volunteers. According to body mass index (BMI), patients with a BMI > 23 kg/m² had significantly lower plasma obestatin. In contrast, leptin levels were increased and acyl ghrelin tended to be higher in these patients. There was a strong positive correlation between obestatin and des-acyl ghrelin (r = 0.56, P = 0.0001) and inverse correlations between obestatin and BMI (r = −0.40, P = 0.007), waist circumference (r = −0.38, P = 0.024), and C-reactive protein (r = −0.29, P = 0.048). By multivariate analysis, obestatin was independently and positively correlated with des-acyl ghrelin (P = 0.01), but not with C-reactive protein, BMI, or waist circumference.

Conclusion: In summary, patients on HD exhibited increased plasma levels of des-acyl ghrelin, normal acyl ghrelin levels, and low obestatin levels. In lean patients, the obestatin and des-acyl ghrelin levels were increased, suggesting that these hormones may influence appetite and body composition in patients on HD.

Introduction
Recent studies have led to the discovery of ghrelin, a stomach-derived circulating hormone that strongly stimulates food intake [1,2]. Ghrelin must be acylated at the serine-3 level to become active, and this acylated form of ghrelin (acyl ghrelin) is quite unstable and rapidly metabolized into the des-acylated form (des-acyl ghrelin) [3]. In addition to the ghrelin mature peptide, Zhang et al. [4] identified a 23–amino acid peptide with a flanking conserved glycine residue at the C-terminus. This compound was named ghrelin-associated peptide, or obestatin, and it has a negative effect on feeding. However, these anorexigenic properties of obestatin are still controversial [4–6]. It is interesting to note that these two peptides with potentially opposite actions on food intake and weight regulation are derived from the same ghrelin gene and released by the stomach [4].

Studies on ghrelin and obestatin levels in patients with chronic kidney disease (CKD) appear of interest because these compounds may be implicated in reduced appetite, loss of weight, and/or malnutrition. Studies have shown that plasma ghrelin is increased in patients on hemodialysis (HD), without any particular correlation with body composition, suggesting that there might be a resistance to ghrelin action in patients on HD [7–9]. However, virtually no study has independently
examined acyl and des-acyl ghrelin levels in patients with CKD, and, at present, levels of obestatin in patients with CKD are unknown. The purpose of the present report was to describe the plasma obestatin and acyl and des-acyl ghrelin concentrations in patients on maintenance HD and their relations with appetite and body composition.

Materials and methods

Methods

The following anthropometric parameters were measured: body weight, height, waist circumference, and skinfold measurements at four standard sites (biceps, triceps, subscapular, and suprailiac) for determining body fat. Body density was calculated from the sum of the four skinfold measurements, (biceps, triceps, subscapular, and suprailiac) for determining body fat. Body nutritional assessment

Methods

Nutritional assessment

The following anthropometric parameters were measured: body weight, height, waist circumference, and skinfold measurements at four standard sites (biceps, triceps, subscapular, and suprailiac) for determining body fat. Body density was calculated from the sum of the four skinfold measurements, according to Durnin and Womersley [10], and percentage of body fat was calculated by Siri’s equation [11]. Measurements were made after the dialysis session by a trained staff member. Based on these measurements, the following indexes were calculated: BMI, arm muscle area, waist circumference, triceps skinfold, body fat percentage, and free-fat mass. BMI was calculated as weight divided by height squared. Triceps skinfold was measured with a Lange Skinfold Caliper (Cambridge Scientific Products, Cambridge, MA, USA), and the arm muscle area was calculated according to the following formula: ([midarm circumference (cm) – 3.14 x tricipital skinfold thickness (mm)]^2/4π) – n, where n = 10 for men and 6.5 for women [12]. Fat-free mass was calculated by subtracting fat mass from body weight. The corresponding percentiles were determined based on the tables developed by Frisnachho [12], and values between percentiles 15 and 95 defined normality. The waist circumference values were based on the National Cholesterol Education Program’s Adult Treatment Panel III [13].

The average daily intakes of calories, protein, carbohydrate, and fat were estimated using 2-d (dialysis day and non-dialysis day) food records. Average daily ingestion of nutrients was calculated using computerized diet software (Bilout 4; S.C.D.A. NUTRISOFT, Cerelles, France). Appetite was rated by asking the following question: “How would you rate your appetite?” 1) very good, 2) good, 3) fair, 4) poor, or 5) very poor [14].

Biochemical variables

Serum albumin, prealbumin, glucose, C-reactive protein (CRP; by immunoturbidimetry, normal values <5 mg/L), parathormone (PTH), urea and creatinine were measured using standard laboratory methods.

Blood samples were obtained from the arterial line of the HD before the start of the session, after the patients had fasted overnight, and serum was immediately frozen at −20°C until analyzed. Serum acyl and des-acyl ghrelin levels were measured using the enzyme immunoassay method (SPI Bio, Montigny, France). We calculated the ratios acyl ghrelin to obestatin and des-acyl to acyl ghrelin. Serum total leptin concentrations were measured by enzyme immunoassay method (SPI Bio). The reference value in healthy volunteers was 5.5 ± 4.0 ng/mL. We also analyzed these hormone levels according to BMI: group 1, a BMI lower than 23 kg/m² for patients with a low BMI (according to the International Society for Renal Nutrition and Metabolism [15]), and group 2, a BMI higher than 23 kg/m².

Statistical analysis

Results were expressed as mean ± standard deviation or percentage change, as needed. Student’s t test was used to test the difference between means, and the Kruskal-Wallis test was used for non-parametric data. Spearman’s correlation coefficient was searched to examine the relation between variables. Statistical significance was accepted as P < 0.05. Statistical analyses were performed with SPSS 16.0 (SPSS, Inc., Chicago, IL, USA).

Results

Clinical and biochemical characteristics of the subjects are listed in Table 1. Of the patients, 30% presented BMI values below 22.9 kg/m², 20% presented values from 23 to 25, and 50% above 25 kg/m². Body fat percentages ranged from 11.2% to 35.3% for men (mean 24.7 ± 6.2%) and from 16.0% to 42.8% for women (mean 33.0 ± 7.3%), and 88% had values above normal [16]. Of the patients, 28.0% and 15% presented triceps skinfold and arm muscle area values below the 15th percentile, and 55.6% of patients presented a large waist circumference. Daily protein intake was lower than 1.2 g/kg in 73.0% of patients, and mean was 1.03 ± 0.36 g·kg⁻¹·d⁻¹. Mean energy intake was 26.0 ± 9.3 kcal·kg⁻¹·d⁻¹; 82% had an energy intake lower than 35 kcal·kg⁻¹·d⁻¹ (26–60 y old), and 72.7% lower than 30 kcal·kg⁻¹·d⁻¹ (61–86 y old). Among 50 records, patient appetite scores were very good (9.8%), good (46.3%), satisfactory (29.3%), and poor (14.6%).

Serum albumin was 39.5 ± 2.5 g/L, and 24.0% patients had a serum albumin level lower than 38 g/L; 24.0% had a prealbumin level lower than 0.3 g/L (mean 0.35 ± 0.08 g/L). The mean CRP was 6.3 ± 8.6 mg/L, and 38% of patients presented a CRP above 5 mg/L. CRP was increased in patients with a BMI higher than 23 kg/m² (Table 1), but there was no significance. Leptin levels were above normal in 64.5% of patients, and des-acyl ghrelin levels were above 385 pg/mL in 74.5% of patients. The acyl ghrelin levels were below 63 pg/mL in 37.5% of patients, and 97% of the patients presented obestatin levels below 4.6 ng/dL. The des-acyl ghrelin moiety was strongly positively correlated with serum obestatin (Fig. 1; r = 0.56, P < 0.0001). According to BMI

![](image)

**Table 1**

<table>
<thead>
<tr>
<th>Patient characteristics and serum parameters according to BMI</th>
<th>Mean ± SD (range) (n = 50)</th>
<th>BMI &lt;23 kg/m² (n = 15)</th>
<th>BMI &gt;23 kg/m² (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>62.0 ± 15.2 (26–86)</td>
<td>58.5 ± 19.2</td>
<td>63.4 ± 13.8</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.4 ± 4.4 (16.7–37.4)</td>
<td>20.6 ± 1.8</td>
<td>27.4 ± 3.4</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>94.6 ± 14.0 (63–122.5)</td>
<td>81.6 ± 10.3</td>
<td>100.7 ± 11.4 b</td>
</tr>
<tr>
<td><strong>Albumin (g/L)</strong></td>
<td>39.5 ± 2.5 (30.0–46.2)</td>
<td>39.3 ± 2.3</td>
<td>39.5 ± 2.7</td>
</tr>
<tr>
<td><strong>Prealbumin (mg/dL)</strong></td>
<td>0.35 ± 0.08 (0.12–0.57)</td>
<td>0.39 ± 0.07</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td><strong>Glucose (mmol/L)</strong></td>
<td>6.3 ± 2.7 (3.01–17)</td>
<td>4.2 ± 1.4 a</td>
<td>7.0 ± 2.8 a</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td>6.3 ± 8.6 (0.2–43.6)</td>
<td>2.9 ± 2.7</td>
<td>7.2 ± 9.4</td>
</tr>
<tr>
<td><strong>PTH (pg/mL)</strong></td>
<td>404 ± 410.6 (2.5–1223)</td>
<td>449 ± 404</td>
<td>383.7 ± 404</td>
</tr>
<tr>
<td><strong>Urea (mmol/L)</strong></td>
<td>19.7 ± 4.7 (12–33.5)</td>
<td>18.8 ± 4.2</td>
<td>20.0 ± 4.9</td>
</tr>
<tr>
<td><strong>Creatinine (μmol/L)</strong></td>
<td>695 ± 135.6 (412–1026)</td>
<td>705.8 ± 118</td>
<td>695 ± 143</td>
</tr>
</tbody>
</table>

BMI, body mass index; CRP, C-reactive protein; Kn/V, dialyzer clearance of urea, t, dialysis time, V, patient’s total body water; PTH, parathormone

Different superscript letters in the same line = P < 0.05.
effects on appetite regulation, we have assessed whether CKD is
longer with acyl ghrelin, CRP, age, BMI, or waist circumference
(Table 3).

We did not find any direct relation among ghrelin moieties,
leptin, and obestatin levels in 50 patients on HD. The patients
presented normal acyl ghrelin levels, a compound shown to
initiate food intake, and high leptin and des-acyl ghrelin values,
two compounds associated with satiety. They also had low serum
obestatin levels as compared with healthy subjects. Obestatin
levels were lower in patients with a BMI higher than 23 kg/m²,
and there was no relation with appetite score or food intake.
Obestatin possesses well-established anorexigenic properties in
rodents. Zizzari et al. [17] showed that obestatin partly inhibited
ghrelin-induced food intake. Lagaud et al. [18] reported that
a single 300-nmol/kg intraperitoneal dose of obestatin reduced
food intake and opposed the action of ghrelin in mice and rats. In
addition, chronic administration of obestatin (three times a day
for 7 d) reduced food consumption and body weight gain.

The clear negative influence of BMI on circulating obestatin in
patients on HD (Fig. 2) suggests a down regulation of its stomach
release by as yet unidentified adipokines. It is interesting to note
that, as shown by Huda et al. [19] in obese patients, a reduced
expression of the ghrelin gene leads to decreases of plasma
ghrelin and obestatin levels in obesity. Nevertheless, the reduc-
tion of obestatin in obese humans is probably maladaptive and
may further propagate the obese state. Exactly as for leptin, the
patients who become insensitive to these anorectic factors will in
fact continue to gain weight and become obese, whereas the
sensitive patients will reduce their nutrient intake and stop
gaining weight, thus delineating two phenotypes of patients, an
adaptive one and the other maladaptive. The same observation
may apply to our patients because obestatin was, in general, low
and even lower in overweight patients (Table 2).

Serum obestatin was strongly and negatively correlated with
CRP in the present study. This observation has not been reported
to date and appears of interest in the context of kidney disease,
during which a permanent mild chronic inflammation is present
in about 40% to 50% of patients. Thus, in contrast to healthy
adults, the persistent inflammation, directly or indirectly related
to obesity (Table 1), could be a cause for the reduced obestatin
values observed in patients with a high BMI (Fig. 2). This relation
may be obscured by other metabolic abnormalities because, by
multivariate analysis, we did not observe an independent role of
CRP in obestatin (Table 3). However, this preliminary observation
probably deserves further research.

We did not find any direct relation among ghrelin moieties,
obestatin, and patients’ food intake or appetite (Table 3). This is
not unexpected because several independent factors that posi-
tively or negatively affect food regulation are increased during
CKD, and the final biological effect may mainly depend on the
ratios of positive over negative stimulants rather than on crude
serum values of a given factor. In this regard, we found that the
ratio of des-acyl ghrelin to acyl ghrelin increased when BMI
decreased. The des-acyl/acyl ghrelin ratio is thought to be an

Discussion

In light of the recently discovered obestatin peptide and its
effects on appetite regulation, we have assessed whether CKD is
associated with an altered regulation of obestatin. For this

Table 2
Serum hormones concentrations in patients on maintenance hemodialysis

<table>
<thead>
<tr>
<th>Patients (n = 50)</th>
<th>Healthy controls (n = 50)</th>
<th>BMI &lt;23 kg/m² (n = 15)</th>
<th>BMI &gt;23 kg/m² (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obestatin (ng/mL)</td>
<td>2.0 ± 1.4a (1.0–9.1)</td>
<td>4.6 ± 1.6 (1.2–10.4)</td>
<td>2.3 ± 1.3</td>
</tr>
<tr>
<td>Acyl ghrelin (pg/mL)</td>
<td>137 ± 116.5b (15–418)</td>
<td>77.6 ± 121.6 (4.7–797)</td>
<td>129 ± 147</td>
</tr>
<tr>
<td>Des-acyl ghrelin (pg/mL)</td>
<td>670 ± 479b (154–2400)</td>
<td>385 ± 252 (63–1037)</td>
<td>900 ± 737</td>
</tr>
<tr>
<td>Des-acyl/acyl ghrelin</td>
<td>10.5 ± 12.2 (0.65–59.2)</td>
<td>11.2 ± 11.2 (0.21–59.6)</td>
<td>22.0 ± 21.0b</td>
</tr>
<tr>
<td>Acyl ghrelin/obestatin</td>
<td>82.8 ± 79.0b (2.9–328)</td>
<td>20.4 ± 44.5 (0.45–315)</td>
<td>53.0 ± 57.0b</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>34.1 ± 30.0 (0.3–90.4)</td>
<td>—</td>
<td>101.3 ± 10.0b</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>15.0 ± 18.9 (1.6–119.1)</td>
<td>—</td>
<td>8.2 ± 6.2</td>
</tr>
</tbody>
</table>

BMI, body mass index

* P < 0.05 between patients and healthy controls. Different superscript letters in the same line = P < 0.05.
important parameter of energy homeostasis in humans and was significantly increased in patients with a BMI lower than 23 kg/m². An increased des-acyl/acyl ghrelin ratio has also been reported in cachectic patients [20]. The less pronounced increase of acyl ghrelin compared with des-acyl ghrelin could be due to a decrease in acylation or an increase in des-acylation in these cachectic patients [21]. Thus, an overall increase of des-acyl ghrelin in CKD (Table 2) may be responsible for additional anorexia. Reasons for these altered ghrelin moieties in CKD are presently unknown. Similarly, the ratio acyl ghrelin/obestatin decreased when BMI decreased (Table 2), suggesting lost appetite stimulation in lean patients; however, we did not find a direct relation with food intake based on patient’s spontaneous declaration.

Yoshimoto et al. [22] suggested that the kidney can modulate the ratio of the different ghrelin gene-derived peptides. Des-acyl ghrelin plasma levels, but not total ghrelin levels, were significantly correlated with serum creatinine and were increased 2.8-fold in patients with end-stage renal disease compared with subjects with normal renal function. Bilateral nephrectomy in mice caused a marked increase in des-acyl ghrelin without significant changes in the total plasma ghrelin and ghrelin mRNA in the stomach, the main site of ghrelin production. These findings suggest that the kidney is an important site for clearance and/or degradation of des-acyl ghrelin.

The interaction between ghrelin and obestatin is still under debate. Much of the current research is focused on the ghrelin/obestatin ratio in obesity and related metabolic disorders [23].

There is one limitation of our study; the study would have been stronger had we analyzed the patients with age- and gender-matched healthy individuals.

In summary, we found that patients on HD exhibited increased plasma levels of des-acyl ghrelin, normal levels of acyl ghrelin, and low obestatin levels. However, obestatin and des-acyl ghrelin levels, two compounds associated with reduced appetite, were increased in lean patients. In addition, the ratio des-acyl/acyl ghrelin, a potential index of satiety, was increased in lean patients. The strong positive association between serum des-acyl ghrelin and obestatin, compounds released by the gastric cells, suggests a common post-translational regulation and a potent appetite control system. Because anorexia is common in maintenance dialysis and is associated with protein-energy malnutrition [15], it appears of interest to further clarify the effects and regulation of obestatin in CKD.

References


