Plasma obestatin is lower at fasting and not suppressed by insulin in insulin-resistant humans

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Anderwald-Stadler M, Krebs M, Promintzer M, Mandl M, Bischof MG, Nowotny P, Kästenbauer T, Lugner A, Prager R, Anderwald C. Plasma obestatin is lower at fasting and not suppressed by insulin in insulin-resistant humans. Am J Physiol Endocrinol Metab 293: E1393–E1398, 2007. First published September 4, 2007; doi:10.1152/ajpendo.00330.2007.—Obestatin, a recently discovered 23-amino acid peptide, is involved in the regulation of appetite and body weight in antagonistic fashion to ghrelin, both deriving from a common precursor peptide. Ghrelin was shown to be associated with body weight in antagonistic fashion to ghrelin, both deriving from a common precursor peptide. Ghrelin appears to have actions opposite to ghrelin in the regulation of food intake, emptying of the stomach, and body weight in rodents and could be part of a dual system connecting the gut and the brain to regulate energy homeostasis (38). However, other recent studies (25, 32, 36) did not provide evidence for a crucial role of obestatin in regulating food intake. Intracerebroventricular and intravenous administration of obestatin in rats did not affect food intake and could not antagonize the ghrelin-stimulated increase in food intake.

The administration of ghrelin increased the food intake and body weight in rodents and humans (23, 34, 35) and accelerated gastric emptying (10). In the presence of a negative energy balance, such as starvation, cachexia, or anorexia nervosa, the secretion of ghrelin increases (22), whereas a positive energy balance, such as feeding, hyperglycemia, or obesity, is associated with a fall in ghrelin plasma concentrations (16). Ghrelin was also reported to be linked to insulin resistance, as we previously reported that ghrelin was more suppressed in non-diabetic humans than in type 2 diabetic patients during supra-physiological hyperinsulinemia (3, 8), whereas fasting ghrelin concentrations were comparable in healthy controls and type 2 diabetes mellitus, most likely because of its circadian rhythm (3, 24).

Very little is known about obestatin’s physiological role in humans. In a study in adult humans, decreasing concentrations of obestatin were associated with diabetes and impaired glucose regulation and the insulin sensitivity surrogate homeostasis model assessment (HOMA) of insulin resistance (30). In morbidly obese patients, a rise in fasting serum obestatin was reported (14) after invasive gastric banding surgery and subsequent weight loss. Plasma obestatin levels were found to be higher in children with the Prader-Willi syndrome than in matching controls (6), conflicting with another report that found similar fasting levels that did not change during an oral glucose tolerance test (28).

However, no significant association was found when correlating HOMA with fasting obestatin concentrations (28). A study in obese and lean humans (13) showed a postprandial suppression of both plasma obestatin and ghrelin compared with the fasting state. After the ingestion of a meal, the combined increase in plasma glucose and insulin could account for the decrease in plasma ghrelin and obestatin levels. We (3) previously showed that insulin reduces plasma ghrelin in non-diabetic patients and, to a relatively lesser extent, also in insulin-resistant type 2 diabetic patients at very high supraphysiological insulin concentrations.

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In contrast to the data on ghrelin, information concerning the action of insulin on plasma obestatin is still lacking in humans. To the best of our knowledge, no study in humans has yet evaluated the association between whole body insulin resistance determined by the gold standard, the hyperinsulinemic clamp test, and plasma obestatin concentrations.

Thus, we hypothesized that plasma concentrations of the appetite-regulating hormones obestatin and ghrelin could be affected in the presence of insulin resistance at fasting and during postprandial-like insulin concentrations. We performed precise anthropometrical and metabolic characterizations, including isoglycemic hyperinsulinemic clamp tests, in nondiabetic individuals to study the correlations between circulating obestatin and ghrelin concentrations, insulin (in) sensitivity, and anthropometrical measures. Furthermore, we investigated the effects of prolonged intravenous insulin infusion on plasma concentrations of obestatin and ghrelin in these humans.

**RESEARCH DESIGN AND METHODS**

**Participants**

Thirty-eight nondiabetic humans were recruited with local advertising. The subjects were not on any blood glucose-, blood pressure-, or lipid-lowering agent or any other regular medication known to influence glucose homeostasis. Three days prior to the examinations, the subjects refrained from excessive physical exercise and were instructed to ingest an isocaloric carbohydrate-rich diet. The protocol was approved by the local ethics committee, and all study participants gave their written, informed consent.

**Oral Glucose Tolerance Test**

After a 12-h overnight fast, an oral glucose (75 g) challenge was performed in all participants to confirm their nondiabetic glucose metabolism. Complete medical history taking, routine laboratory tests, and a physical examination were performed to confirm the subjects’ health status. Waist and hip circumferences were measured according to a standardized protocol (15). Body weight and fat mass were assessed using the Tanita bioimpedance balance (Yiewsley, UK) (26).

**Isoglycemic Hyperinsulinemic Clamp Test**

After another 12-h overnight fast, two catheters (Vasofix; Braun, Melsungen, Germany) were inserted into the left and the right antecubital veins for blood sampling and infusions, respectively. The mean of three fasting plasma glucose measurements immediately before the clamp start was calculated as the isoglycemic clamp goal. In case the calculated clamp target was <80 or >100 mg/dl, 80 and 100 mg/dl, respectively, were taken as the clamp goal. The hyperinsulinemic isoglycemic clamp test was performed during a primed, continuous insulin (Actrapid; NovoNordisk, Bagsvaerd, Denmark) infusion (40 mU insulin·min⁻¹·m⁻² body surface area) (2). According to their insulin-stimulated glucose utilization, the subjects were divided into an insulin-resistant (IR) group and an insulin-sensitive (IS) group, with a cutoff value of 5.1 mg·kg⁻¹·min⁻¹, indicating overt insulin resistance (1).

**Laboratory Measurements**

Hb A₁c and serum concentrations of triglycerides, LDL and HDL cholesterol, and creatinine were measured using routine laboratory methods (http://www.kimcl.at/). Blood was rapidly centrifuged, and aliquots were stored at −70°C until further analysis. Plasma concentrations of glucose, insulin, and free fatty acids (FFA) were measured as previously described (1, 2, 19). Plasma obestatin concentrations were measured by a commercially available radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA) (28) with intra- and inter-assay coefficients of variation of <10%. Plasma ghrelin concentrations were assessed with a commercially available radioimmunoassay (Peninsula Laboratories, San Carlos, CA) (4); the intra- and interassay coefficients of variation were <10%. Plasma obestatin and ghrelin were measured at fasting and at stable insulin-stimulated conditions during the steady state of the isoglycemic hyperinsulinemic clamp test (at 120 min after start).

**Calculations and Statistics**

Insulin-stimulated glucose disposal (M), given in mg glucose·min⁻¹·kg⁻¹, was calculated as previously described (1, 2, 9, 18). A relative decrease or increase in plasma obestatin and ghrelin at 120 min in the isoglycemic hyperinsulinemic clamp test was calculated in percentages of fasting plasma concentrations. Before further analysis, normal distribution of the variables was tested by applying the Kolmogorov-Smirnov test. This test showed that all of the continuous variables except for fasting plasma insulin, ghrelin, and FFA suppression at 60 and 120 min were distributed normally. Therefore, insulin and ghrelin data were logarithmically transformed to achieve normal distributions, and statistical tests were applied to the transformed variable. Differences between groups were assessed with the two-sided paired Student’s t-test, and intrapersonal differences were assessed with the two-sided paired Student’s t-test. Linear correlations are based on Pearson’s product moment correlations. Multiple linear regression analysis, based on the data of all participants using fasting plasma obestatin as dependent variable, was applied. Variables correlating with obestatin at a level of P < 0.05 were considered for the first model to identify potential predictors for fasting plasma obestatin concentrations. The final model was verified by backward stepwise linear multiple regression analysis. The general linear modeling function analysis was used to control for potential confounders. Statistical analyses were performed using the SPSS (SPSS, Chicago, IL) computer software. Data are given as means ± SE. Differences between groups and between fasting and clamp values at P = 0.05 were considered to be statistically significant.

**RESULTS**

Characteristics of the study participants are presented in Table 1. The IR and IS groups were matched for age, sex, and body mass index (BMI). The two groups did not differ in waist circumference, fat mass, plasma FFA, serum LDL cholesterol, triglycerides, and blood pressure, but IR had ~15% lower serum HDL cholesterol than did IS.

**Clamp Results**

Plasma glucose was not different in the two groups at fasting (IR: 89.7 ± 2.0 mg/dl vs. IS: 86.4 ± 1.9 mg/dl, P = 0.4). During the clamp test, glucose concentrations differed only between 20 and 50 min during the clamp and were comparable at the start (100 min: IR: 84.1 ± 2.3 mg/dl vs. IS: 91.7 ± 2.5 mg/dl, P = 0.2) and during the entire final 20-min clamp interval (100–120 min). Fasting plasma insulin was higher in IR than in IS (IR: 8.8 ± 1.4 μU/ml vs. IS: 6.1 ± 1.8 μU/ml, P < 0.0001) and was similarly increased in both groups during the clamp test (IR: 73.0 ± 3.9 μU/ml vs. IS: 73.7 ± 3.4 μU/ml at 120 min, P = 0.9). Mean glucose infusion rates were higher in IS than in IR at all time intervals during the clamp from 30 min until the end of the clamp test (each P < 0.001, data not shown). The M value at the final 20-min clamp interval (M₁₀₀−₁₂₀ min) in IR (4.4 ± 0.2 mg·min⁻¹·kg⁻¹) was markedly lower than in IS (10.7 ± 0.7 mg·min⁻¹·kg⁻¹, P < 10⁻⁵). Plasma FFA concentrations at fasting were not different (Table 1), but during the clamp test IR had higher FFA plasma concentrations than did IS at 60 (107 ± 15 vs. 43 ± 5 μmol/l,
Table 1. Clinical characteristics of IR and IS subjects and relative suppression of plasma ghrelin, obestatin, and FFA concentrations during the isoglycemic hyperinsulinemic clamp test

<table>
<thead>
<tr>
<th></th>
<th>IR</th>
<th>IS</th>
<th>P (IR vs. IS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (Females/males)</td>
<td>18 (12/6)</td>
<td>18 (13/5)</td>
<td>0.49</td>
</tr>
<tr>
<td>Age, yr</td>
<td>45±2</td>
<td>47±2</td>
<td>0.13</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>96.0±3.3</td>
<td>89.2±3.2</td>
<td>0.08</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.5±1.1</td>
<td>25.5±0.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>26.0±2.2</td>
<td>21.5±2.0</td>
<td>0.13</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>5.6±0.1</td>
<td>5.5±0.1</td>
<td>0.13</td>
</tr>
<tr>
<td>RR sys/dia, mmHg</td>
<td>120±3/79±2</td>
<td>119±3/78±2</td>
<td>0.78/0.74</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>2.0±0.1</td>
<td>2.0±0.1</td>
<td>0.94</td>
</tr>
<tr>
<td>Serum LDL cholesterol, mg/dl</td>
<td>127±0.9</td>
<td>127±0.9</td>
<td>0.48</td>
</tr>
<tr>
<td>Serum triglycerides, mg/dl</td>
<td>973±14.7</td>
<td>856±7.2</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Data are means ± SE, unpaired Student’s t-test. IR, insulin resistant; IS, insulin sensitive; FFA, free fatty acids; BMI, body mass index; RR sys/dia, systolic/diastolic blood pressure. *P = 1.5 × 10⁻⁵ vs. basal; †P < 0.001 vs. basal.

P < 0.0002, 90 (61 ± 10 vs. 27 ± 5 µmol/l, P = 0.004) and 120 min (48 ± 9 vs. 17 ± 2 µmol/l). Insulin-dependent suppression of FFA during clamp was lower in IR than in IS (Table 1).

Ghrelin. Plasma ghrelin concentrations were not different in the two groups at fasting (Fig. 1A). At the end of the clamp, plasma ghrelin in absolute concentrations was significantly reduced in IS (654 ± 87 pg/ml, P < 0.02) and in IR (556 ± 84 pg/ml, P < 0.01) compared with basal conditions (Fig. 1A). The insulin-mediated reduction of plasma ghrelin relative to basal values was similar in both groups (IS: 72 ± 7%, P < 0.001 vs. basal; IR: 76 ± 9%, P < 0.001; IR vs. IS: P = 0.7; Table 1).

Obestatin. IR had ~19% lower fasting plasma obestatin concentrations compared with IS (383 ± 26 vs. 469 ± 23 pg/ml, P < 0.02; Fig. 1B). The plasma obestatin levels remained significantly different after adjustment for BMI by using the general linear model (IR: 383 ± 26 pg/ml vs. IS: 469 ± 23 pg/ml, P < 0.02). At the end of the isoglycemic hyperinsulinemic clamp test, plasma obestatin in absolute values was lowered in IS (368 ± 14 pg/ml, P < 0.00007 vs. basal), but not in IR. At that time, obestatin levels in percent of basal levels were reduced to ~81% (P < 0.00002 vs. basal; Table 1) in IS (P < 0.001 vs. basal; Table 1), whereas in IR plasma obestatin remained unchanged compared with basal values (Table 1).

Correlation Analyses

Obestatin. Fasting plasma obestatin concentrations were closely and positively correlated with those of ghrelin, with M₁₀₀−₁₂₀ min×clamp plasma FFA suppression at 60, 90, and 120 min, and HDL cholesterol (Table 2). Parameters of adiposity (waist-to-hip ratio, waist circumference, body weight, and BMI), clamp plasma FFA at 90 and 120 min, and systolic blood pressure were negatively correlated with fasting plasma obestatin (Table 2). Relative obestatin alteration in percent of basal values was correlated negatively with M₁₀₀−₁₂₀ min and positively with waist circumference (Table 2).

Ghrelin. Fasting plasma ghrelin was correlated negatively with waist-to-hip ratio, waist circumference, body weight, BMI, and systolic and diastolic blood pressure and positively with HDL (Table 2). Fasting plasma ghrelin was negatively correlated with clamp FFA plasma concentrations at 60, 90, and 120 min and positively correlated with clamp plasma FFA suppression at 90 min (Table 2). Ghrelin plasma concentrations at 120-min clamp time were negatively associated with waist circumference, BMI, and waist-to-hip ratio (Table 2).

Multiple Regression Analysis

The M value, BMI, waist circumference, ghrelin, systolic blood pressure, and HDL cholesterol were correlated with fasting plasma obestatin and were therefore included in the first model. The stepwise backward regression performed with the remaining variables revealed that fasting plasma ghrelin and BMI were the strongest predictors of fasting plasma obestatin concentrations (Table 3). After elimination of the other predictor, the estimates of ghrelin and BMI remained nearly the same as in the first model, suggesting that ghrelin and BMI are independent predictors of fasting plasma obestatin concentrations.

DISCUSSION

The study primarily showed that nondiabetic humans who were markedly insulin resistant, as measured by the hyperin-
Ghrelin plasma concentrations at 120 min clamp and fasting plasma ghrelin and relative obestatin alteration (% basal values) and ghrelin-stimulated increase in food intake (11, 12, 25, 32, 36).

Alterations of fasting plasma obestatin and ghrelin concentrations with parameters of adiposity and hormones in correlation analyses (only significant correlations are shown)

<table>
<thead>
<tr>
<th>Correlation Analyses</th>
<th>r</th>
<th>P</th>
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| Fasting plasma obestatin and ghrelin concentrations than did matching insulin-sensitive subjects, whereas fasting plasma ghrelin was comparable in both groups. Intravenous insulin infusion decreased plasma obestatin in insulin-sensitive but not in insulin-resistant humans, whereas plasma ghrelin was similarly reduced in both groups. Fasting plasma concentrations of obestatin, but not of ghrelin, were positively correlated with whole body insulin sensitivity. Both plasma obestatin and ghrelin were closely correlated with each other, insulin resistance as well as the metabolic syndrome (21). Fasting plasma obestatin and ghrelin were closely correlated with each other, and ghrelin levels were highly predictive of obestatin levels. This appears reasonable because both hormones derive from the same precursor (38).

**Fasting Plasma Obestatin**

The role of obestatin in the balance of energy homeostasis, body weight control, and insulin sensitivity is still unclear and under debate (25, 37, 38). In our study, insulin-resistant participants had lower fasting plasma obestatin concentrations than did insulin-sensitive participants. Furthermore, we found a relationship between fasting plasma obestatin and both BMI and abdominal obesity, which is in line with recent studies in humans (13, 30). Fasting plasma obestatin was directly associated with serum HDL cholesterol levels and indirectly with systolic blood pressure, which, taken together, suggest an association between fasting plasma obestatin and insulin resistance as well as the metabolic syndrome (21). Fasting plasma obestatin and ghrelin were closely correlated with each other, and ghrelin levels were highly predictive of obestatin levels. This appears reasonable because both hormones derive from the same precursor (38).

So far there is no evidence for differential processing and/or secretion by different tissues, and one would expect that the two hormones are secreted in parallel. On the other hand, the two hormones appear to underlie different plasma kinetics (27, 38). In a study in rats, plasma obestatin exhibited an ultradian pulsatility with a frequency and a half-life lower than those of ghrelin (33, 39). Thus, it remains to be determined whether obestatin secretion and/or plasma clearance are altered in insulin resistance, because this would explain the altered circulating levels at fasting and during hyperinsulinemia.

**Insulin-Mediated Modulation of Obestatin and Ghrelin**

We (3) previously showed that insulin reduces plasma ghrelin in nondiabetic subjects, which is now confirmed by the results of the present study. Being also in line with our previous study, ghrelin fasting plasma concentrations were not different in nondiabetic IR and IS. In addition, ghrelin was not detectable in human plasma and tissues (5), which is in contrast with findings from others (13, 14, 30, 38) and ourselves.

**Table 2. Associations of fasting plasma obestatin and ghrelin concentrations with parameters of insulin sensitivity and parameters of adiposity and hormones in correlation analyses (only significant correlations are shown)**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficients</th>
<th>SE</th>
<th>P</th>
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<tbody>
<tr>
<td>Ghrelin, pg/ml</td>
<td>0.061</td>
<td>0.016</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI, kg/m^2</td>
<td>-8.1</td>
<td>3.5</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Factors not included in the model: waist circumference, RR sys, HDL cholesterol, and M value100-120 min (all P > 0.13).

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inversely associated with body weight, visceral obesity, BMI, and blood pressure, which is also in line with previous studies (17, 29, 31).

In contrast to the data on ghrelin, information concerning the action of insulin on plasma obestatin in humans is still scarce. A study in obese and lean humans (13) showed a postprandial suppression of both plasma obestatin and ghrelin compared with the fasting state. After the ingestion of a meal, the combined increase in plasma glucose and insulin could account for the decrease in plasma obestatin and ghrelin levels.

In this study in nondiabetic lean humans, we used the hyperinsulinemic clamp test, which is the gold standard for measuring insulin sensitivity. Using this test we were able to demonstrate a correlation between obestatin and whole body insulin sensitivity. Furthermore, plasma obestatin levels decreased during the hyperinsulinemic clamps only in the IS, but not in the IR, subjects. On the basis of these data, we suggest that insulin and whole body insulin sensitivity could also be regulators of obestatin secretion. As plasma insulin was ~7 times higher at 120-min clamp than at fasting, although the degree of glycaemia remained at the fasting level, the reduction of plasma obestatin in IS is most likely due to either hyperinsulinemia itself or the fall in FFA secondary to insulin.

The mechanism by which insulin might influence obestatin and ghrelin release is unclear. The insulin-signaling cascade has been identified in the gastrointestinal tract, which may be regarded as insulin-sensitive tissue (7). Therefore, the insulin-mediated decrease in plasma obestatin and ghrelin might result from insulin-dependent inhibition of the production of the common precursor peptide preproghrelin or the release of the two hormones from gastric cells.

Alternatively, during the clamp test, insulin also suppressed plasma FFA, which was correlated with obestatin and ghrelin concentrations. Thus, it cannot be ruled out that the reduction of obestatin and ghrelin could, in part, be due to the decrease in plasma FFA in IS subjects.

Conclusions

Fasting plasma concentrations of obestatin, but not ghrelin, are reduced in insulin resistance and are directly associated with whole body insulin sensitivity in nondiabetic humans. Furthermore, plasma obestatin is reduced by insulin in insulin-sensitive but not insulin-resistant persons.

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GRANTS

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