Ghrelin secretion is modulated in a nutrient- and gender-specific manner

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Summary

BACKGROUND Ghrelin is a potent GH secretagogue that also plays an important role in appetite and weight regulation. Ghrelin increases hunger and food intake, and its levels decrease after a standard meal or glucose.

OBJECTIVE To examine the effects of standard oral glucose, lipid and protein loads on ghrelin levels, investigating the possibility that these responses may be modulated by several anthropometric and metabolic factors.

SUBJECTS AND METHODS There were 24 adult nondiabetic subjects (13 men/11 women; mean age 55.3 ± 2.9 years, range 26–74 years). Each participant underwent one or more of the following nutrient loads: (i) a standard oral glucose (75 g) load (n = 18); (ii) an oral lipid load (40 g, with 24 g saturated fat; n = 13); (iii) an oral protein load (40 g; n = 11).

RESULTS Fasting ghrelin levels were negatively related to body mass index (BMI; r = −0.47; P = 0.02), waist circumference (r = −0.58; P = 0.0028), waist/hip ratio (r = −0.56; P = 0.0046), fasting insulin (r = −0.44, P = 0.03), and homeostasis model assessment insulin resistance index (HOMA-R; r = −0.43, P = 0.034). Glucose load induced a decrease in ghrelin levels (P < 0.0001), and this response was modulated by sex (P < 0.0001), in that levels were significantly higher in females. The presence of obesity affected ghrelin response to glucose (< 0.0217), in that log-transformed ghrelin levels started to increase back to baseline after its initial decline earlier in obese than in lean subjects. Ghrelin levels after a glucose load were lower over time in subjects with more pronounced insulin resistance (P < 0.0001). Similarly, ghrelin levels decreased significantly following the lipid meal (P = 0.035), and were modulated by HOMA-R (P = 0.027) and gender (P = 0.029). Protein did not affect ghrelin levels.

CONCLUSIONS This study demonstrates that ghrelin levels respond in a different manner to glucose, lipid and protein loads, and are subject to modulation according to gender, obesity and insulin sensitivity.
Hansen et al., 2002) leads to an increase in circulating ghrelin levels. Nevertheless, it appears that factors other than body composition impact on ghrelin levels, as re-feeding significantly decreases plasma ghrelin levels in anorectic patients even before changes in body mass index (BMI) occur (Tolle et al., 2003). Furthermore, healthy lean subjects have lower ghrelin levels than anorectic patients with similar BMI (Tolle et al., 2003), whereas patients with bulimia nervosa have higher ghrelin levels than weight-matched controls (Tanaka et al., 2002). From the aforementioned studies it appears that ghrelin plays an important role in both acute and long-term control of energy balance and is also influenced by behavioral parameters (Tanaka et al., 2003).

The complexities of the effects of food consumption on ghrelin plasma levels have not been well elucidated yet. An oral glucose load causes a 20–30% decrease from basal fasting ghrelin levels in young healthy volunteers (Shiiya et al., 2002). A glucose load given intravenously elicits a similar response, suggesting that gastric exposure to incoming nutrients is not essential for the regulation of ghrelin secretion, stressing the effects of energy balance status in this context. On the other hand, meals with largely different caloric contents were equally effective in suppressing plasma ghrelin in normal women (Nedvidkova et al., 2003), but gastric distension by itself or the presence of nutrients in the stomach are not sufficient to this end, suggesting that the meal-related suppression of plasma ghrelin requires postgastric stimulation (Williams et al., 2003). The effects of diet composition and individual macronutrients on ghrelin levels have not been well characterized. Reports on age (Cummings et al., 2001; Haqq et al., 2003) and sex influences on ghrelin levels have not been consistent (Tschop et al., 2001; Shiiya et al., 2002; Barkan et al., 2003). Although most studies reported a negative correlation between BMI, fasting insulin levels and fasting ghrelin levels, these findings were not universal (Pagotto et al., 2002; Orio et al., 2003). Reports in children (Ikezaki et al., 2002; Haqq et al., 2003) have shown consistent negative interactions between ghrelin and several indices of insulin resistance.

In this study we examined the acute effects of glucose, protein and lipid oral loads on ghrelin levels in lean and obese adults of different ages and of both genders. We also evaluated the possibility that other variables such as blood pressure, waist circumference, waist-to-hip ratio (WHR), presence of hypertension and presence of hyperlipidaemia may impact on circulating ghrelin levels and on its response to different nutrient loads.

**Modulation of ghrelin secretion**

Anthropometric measurements including weight, height, BMI, waist and hip circumferences, as well as heart rate and blood pressure were recorded in all patients. Patients with BMI lower than 27 kg/m² were considered as having normal weight whereas patients with BMI higher than 30 kg/m² were considered obese. Subjects were studied after an overnight fast. An antecubital vein catheter was inserted upon arrival. Each participant underwent one or more of the following customary nutrient loads (Lacroix et al., 1992), with an interval of at least a week between tests:

- **Oral glucose load**: After collecting baseline blood samples, patients ingested 75 g dextrose (300 kcal), diluted in one glass of water. Blood collection was repeated 30, 60, 90, 120 and 150 min after the glucose load.
- **Fat load**: After collecting baseline blood samples, patients ingested 150 g of cream (400 kcal; 91% fat, 5.5% carbohydrates and 4.5% protein). Blood samples were collected 30, 60, 90, 120 and 180 min after the fat load.
- **Protein load**: After collecting baseline blood samples, patients ingested 200 g of low fat chicken breast (240 kcal; 83.5% protein, 5% carbohydrates, 11.5% fat). Blood samples were collected 30, 60, 90, 120 and 180 min after the protein load.

Meals were consumed over a 5- to 10-min period.

Blood samples were collected in prechilled tubes containing EDTA (1 mg/ml) and aprotinin (500 U/ml; Phoenix Pharmaceuticals, Belmont, CA, USA) and immediately centrifuged. Plasma was stored at –80°C until assayed. Human plasma ghrelin was measured with a commercial radioimmunoassay (Phoenix Pharmaceuticals). Blood was also collected for serum measurements of glucose, insulin, triglycerides, blood urea nitrogen (BUN) and TSH. Insulin was measured with a commercial radioimmunoassay (DiaSorin s.r.l., Saluggia, Italy). TSH was measured using a two-site sandwich chemiluminescent immunoassay (ADVIA Centaur assay, Bayer Corporation, Tarrytown, NY, USA). Glucose, triglycerides and BUN were measured on an ADVIA 1650 Chemistry System (Bayer).

Insulin sensitivity was determined by homeostasis model assessment insulin resistance index (HOMA-R; Matthews et al., 1985) and the quantitative insulin sensitivity check index (QUICKI; Katz et al., 2000). HOMA-R was calculated as [fasting blood glucose (FBG) (mg/dl) X fasting insulin (mU/l)]/405, and QUICKI, as 1/(log insulin + log FBG).

**Statistical analysis**

Results are given as mean ± SEM. Comparisons between groups were performed by unpaired Student’s t-test. Relationships between fasting ghrelin concentration and various anthropometric and metabolic variables were examined by Spearman rank analysis, followed by multivariate regression analysis. Ghrelin response curves to macronutrient loads were analysed by ANOVA with repeated measurements of ANOVA according to the mixed

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the subjects had normal weight (BMI < 27 kg/m²). There were 13 male and 11 female subjects. Half of the study population. Although there were no differences in BMI (range 26–39 kg/m²), there was one underweight, seven normal weight (BMI (27–122) to a nadir of 328 ± 38 pg/ml (Ln ghrelin levels were lower in patients with more pronounced age of 57 ± 2 years, range 26–74 years) participated in the study. Seven subjects underwent all three tests, four subjects underwent two tests and the remaining 13 underwent just one test. Ten volunteers had been previously diagnosed and treated for essential hypertension, 11 had hyperlipidaemia, three had osteoporosis, three had treated primary hypothyroidism and one suffered from idiopathic diabetes insipidus. There were 13 male and 11 female subjects. Half of the subjects had normal weight (BMI < 27 kg/m²) and half were obese (BMI > 30 kg/m²). Table 1 depicts characteristics of the study population. Although there were no differences in BMI values between male and female subjects (29·7 ± 2·9 and 30·6 ± 8·9 kg/m², respectively), mean (± SEM) fasting ghrelin levels were twofold higher in female (794 ± 188 pg/ml) than in male (397 ± 72 pg/ml, P = 0·04) subjects.

Correlation analysis

There was an inverse correlation between fasting ghrelin levels and BMI (r = −0·47, P = 0·02), waist circumference (r = −0·58, P = 0·0028), and W/H ratio (r = −0·56, P = 0·0046). Figure 1 depicts these data after Ln transformation. No correlation was found between basal ghrelin levels and age. Systolic and diastolic blood pressure measured at the beginning of each test also did not correlate with ghrelin levels.

Table 1 Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male (n = 13) mean ± SEM (range)</th>
<th>Female (n = 11) mean ± SEM (range)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 3·7 (30–74)</td>
<td>51 ± 4·7 (26–72)</td>
<td>ns</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29·0 ± 1·5 (23–38)</td>
<td>29·4 ± 3 (16–47)</td>
<td>ns</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>104·5 ± 4 (88–122)</td>
<td>89·5 ± 6·6 (60–125)</td>
<td>0·057</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0·95 ± 0·03 (0·82–1·11)</td>
<td>0·79 ± 0·02 (0·69–0·9)</td>
<td>0·0004</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123 ± 4·8 (90–170)</td>
<td>120·5 ± 4 (90–130)</td>
<td>ns</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>78·8 ± 2 (65–90)</td>
<td>77·3 ± 2·5 (60–90)</td>
<td>ns</td>
</tr>
<tr>
<td>Basal plasma ghrelin (pg/ml)</td>
<td>397 ± 72 (126–1054)</td>
<td>794 ± 198 (236–2120)</td>
<td>0·04</td>
</tr>
<tr>
<td>Median: 273</td>
<td>Median: 476</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant.

Model. The influence of parameters such as BMI, sex, age, presence of hypertension, presence of hyperlipidaemia, fasting insulin and insulin resistance indexes, on ghrelin response to nutrient loads was examined in a multivariate analysis of variance (MANOVA) model. For this procedure log-transformation (Ln) of ghrelin levels was performed due to the wide range of basal ghrelin concentrations. The final model included the following parameters: time, sex, presence of obesity, presence of hypertension, HOMA-R index and age. P level < 0·05 was considered statistically significant.

Results

Twenty-four subjects (mean age 55·3 ± 2·9 years, range 26–74 years) participated in the study. Seven subjects underwent all three tests, four subjects underwent two tests and the remaining 13 underwent just one test. Ten volunteers had been previously diagnosed and treated for essential hypertension, 11 had hyperlipidaemia, three had osteoporosis, three had treated primary hypothyroidism and one suffered from idiopathic diabetes insipidus. There were 13 male and 11 female subjects. Half of the subjects had normal weight (BMI < 27 kg/m²) and half were obese (BMI > 30 kg/m²). Table 1 depicts characteristics of the study population. Although there were no differences in BMI values between male and female subjects (29·7 ± 5·1 and 30·6 ± 8·9 kg/m², respectively), mean (± SEM) fasting ghrelin levels were twofold higher in female (794 ± 188 pg/ml) than in male (397 ± 72 pg/ml, P = 0·04) subjects.

Correlation analysis

There was an inverse correlation between fasting ghrelin levels and BMI (r = −0·47, P = 0·02), waist circumference (r = −0·58, P = 0·0028), and W/H ratio (r = −0·56, P = 0·0046). Figure 1 depicts these data after Ln transformation. No correlation was found between basal ghrelin levels and age. Systolic and diastolic blood pressure measured at the beginning of each test also did not correlate with ghrelin levels.

There was a negative correlation between fasting ghrelin levels and insulin (r = −0·44, P = 0·03), and between ghrelin and HOMA-R (r = −0·43, P = 0·034). A positive correlation was found between fasting ghrelin levels and QUICKI (r = 0·53, P = 0·007). These data are presented in Fig. 1 after Ln transformation. No correlation was found between fasting ghrelin and glucose, triglyceride or TSH levels. Age, the presence of hypertension or hyperlipidemia did not have any effect on ghrelin levels in the multivariate model.

Multivariate analysis of variance

Oral glucose load. Eighteen patients underwent an oral glucose load. There were 10 men and eight women, with a mean age of 57 ± 3 years and mean BMI 29·3 ± 1·6 kg/m² (range 16–39 kg/m²). There was one underweight, seven normal weight and 10 obese subjects in this group. Ghrelin levels significantly decreased from basal levels of 538 ± 107 pg/ml (Ln ghrelin 6·07 ± 0·14 pg/ml) to a nadir of 328 ± 38 pg/ml (Ln ghrelin 5·68 ± 0·1 pg/ml), 120 min after the glucose load (P < 0·0001, Fig. 2), and this response was significantly modulated by sex (P < 0·0001), presence of obesity (P = 0·0217) and HOMA-R index (P < 0·0001). Glucose levels increased from 83 ± 3·2 mg/dl to 149 ± 8 mg/dl at 30 min (P = 0·0077 by ANOVA). The influence of gender in ghrelin response to glucose over time was present in all time points (P < 0·0001). The impact of the HOMA-R index on Ln ghrelin response to glucose over time was evident in all time segments (P = 0·018 for the second time segment and P < 0·0001 for the others), such that ghrelin levels were lower in patients with more pronounced insulin resistance. The presence of obesity also affected Ln ghrelin response to glucose over time (< 0·0217, Fig. 3). Significantly, ghrelin levels started to rise back to baseline levels after the initial fall at 30 min in obese patients, whereas in lean subjects nadir ghrelin levels occurred after 120 min (P = 0·021 and P = 0·02, respectively, for the differences between the curves in the defined time points) and only then started to rise back.
Oral lipid load. Thirteen subjects (five men, eight women, mean age 57 ± 3.4 years; mean BMI 31.1 ± 2.2 kg/m², range 20–47 kg/m²) underwent an oral lipid load. There were six normal weight and seven obese subjects in this group. Ghrelin levels significantly declined from a basal level of 728 ± 205 pg/ml (Ln ghrelin 6.23 ± 0.23) to a nadir of 533 ± 114 pg/ml (Ln ghrelin 6.03 ± 0.1), 120 min after the lipid load (*P = 0.035), according to the multivariate analysis. Triglycerides increased from 154 ± 26 to 287 ± 43 mg/dl at 180 min (**P = 0.0003, ANOVA). Gender had an overall effect on ghrelin response to the lipid load in that levels were consistently higher in female subjects (*P = 0.029). HOMA-R had a significant impact on Ln ghrelin levels in the multivariate model (**P = 0.0278) in that nadir Ln ghrelin levels were lower in insulin resistant patients and negatively correlated with the HOMA-R index (r = -0.67, **P = 0.012). The decrease of Ln ghrelin after the lipid load was significant only in the first time period (**P = 0.005), and this response was modulated by gender.

Fig. 1 Correlation between fasting log-transformed (Ln) ghrelin levels and BMI (a); waist circumference (b); fasting insulin (c) and HOMA-R (d).

Fig. 2 Effect of an oral glucose load on log-transformed (Ln) ghrelin levels (*P < 0.0001, after correction for sex, presence of obesity and HOMA-R index). *P < 0.0001, **P = 0.0003.
Furthermore, the decrease of Ln ghrelin during the first 30 min following the lipid meal was significant only in women ($P = 0.013$), whereas in men no effect was observed (Fig. 4).

**Discussion**

Our study adds important new physiological information to the rapidly growing knowledge accumulating since the isolation of ghrelin (Kojima et al., 1999). We confirm the existence of a negative correlation between BMI and ghrelin levels (Tschop et al., 2001). Further, we present data supporting the concept that both basal ghrelin levels and glucose-induced ghrelin suppression are directly related to insulin sensitivity. Ghrelin was inversely related to waist circumference, WHR, fasting insulin levels and the HOMA-R index and directly related to the QUICKI index. Overall, these consistent correlations with a number of inherently related parameters and determinants of insulin sensitivity suggest that ghrelin levels are decreased in obesity in association with insulin resistance. An inverse correlation between ghrelin levels and markers of insulin sensitivity has been reported in women with the polycystic ovary syndrome but not in matched healthy obese women (Pagotto et al., 2002; Schofl et al., 2002). In contrast, we studied both men and women, with a wide range of BMI and age, and insulin resistance markers not only correlated with basal ghrelin levels, but also significantly impacted on ghrelin response to the glucose and to the lipid load in a multivariate analysis of variance model. It has been previously demonstrated that insulin infusion causes a decrease in ghrelin concentration (Saad et al., 2002), independent of concomitant glucose levels (Lucidi et al., 2002). More interestingly, a strong correlation reportedly exists between insulin sensitivity and the percentage of insulin-induced suppression of ghrelin from baseline (Lucidi et al., 2002). Presumably, obese people not only have low fasting ghrelin levels, but also respond less to insulin mediated suppression of ghrelin, perhaps reflecting yet another feature of insulin resistance.

We have also shown that the ghrelin response qualitatively differs according to the macronutrient ingested. Glucose consistently caused a decrease in ghrelin levels in the study group; whereas a protein load had no effect. Furthermore, lipid administration caused a significant decrease in ghrelin levels, but only in women. The secretion of other peptide hormones such as cholecystokinin (Reidelberg et al., 2003) and peptide YY (Batterham et al., 2002), which are involved in short-term regulation of food intake seem also to be differentially regulated by various macronutrients (Onaga et al., 2002). Collectively, this implies that meals rich in fat, protein or carbohydrates could affect appetite via differential...
mechanisms, based on differences in the type of the invoked satiety signal as well as in the magnitude of the changes in the various satiety modulating peptides.

In this respect, our study brings to light an additional aspect of ghrelin regulation in obese subjects: ghrelin suppression by a glucose load is much shorter in this population than in lean individuals. This early rebound in ghrelin levels could lead to earlier re-initiation of signals leading to food craving after a carbohydrate containing meal in obese compared with normal weight subjects. In contrast, the presence of obesity did not alter ghrelin response to the lipid (decrease in ghrelin) or protein (no change in ghrelin) loads. In a previous study, a mixed meal failed to suppress ghrelin levels in obese subjects (English et al., 2002) but this result is difficult to compare with our protocol, in which macronutrients’ effects were analysed individually.

Of additional interest is our finding of the effect of gender on ghrelin levels. Female subjects had significantly higher ghrelin levels than men, both in the fasting state and after glucose and lipid loads. The gender-related dichotomy remained significant after correction for BMI, age and HOMA-R index. This finding is in accordance with higher levels in females of hormones involved in appetite regulation such as leptin (Casabiell et al., 2001). Ghrelin regulated hormones such as GH and prolactin are also higher in women than in men (Maccario et al., 2000).

In summary, this study demonstrates that ghrelin levels respond in a different manner to glucose, lipid and protein loads, and are subject to modulation according to gender, presence of obesity and degree of insulin resistance. Subnormal suppression of ghrelin by a carbohydrate challenge in obese, insulin-resistant individuals may contribute to dysregulation of appetite control in such individuals.

References


