CLINICAL STUDY

Visceral fat mass is a strong predictor of circulating ghrelin levels in premenopausal women

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Abstract

Objective: A well known inverse relationship exists between obesity and circulating ghrelin concentrations. However, obesity is a heterogeneous disease entity and upper-body obesity (UBO) is associated with more profound metabolic disturbances than lower-body obesity (LBO). We therefore aimed to investigate the impact of body composition on circulating ghrelin levels in women spanning a wide range of body composition phenotypes.

Subjects and methods: Ten (UBO; waist-to-hip ratio (WHR) > 0.85, body mass index (BMI) > 28 kg/m²), ten LBO (WHR < 0.80, BMI > 28 kg/m²) and ten lean women (BMI < 25 kg/m²) were studied. Total ghrelin levels were measured under basal and hyperinsulinemic (0.6 mU/kg per min) conditions. Body fat distribution was determined by dual X-ray absorptiometry in combination with computed tomography at the L2-L3 level.

Results: As expected, an inverse correlation existed between basal ghrelin concentration and BMI (r = -0.40, P = 0.03) and total fat mass (r = -0.39, P = 0.04). Visceral fat mass was a strong predictor (r = -0.56, P = 0.003) of circulating ghrelin levels, even when adjusted for BMI (P = 0.02) or body composition group (P = 0.04). The suppressive effect of insulin on ghrelin concentration was significantly diminished in the UBO compared with the lean controls (P = 0.012) and a highly significant inverse correlation existed with visceral fat mass (r = -0.52, P = 0.004).

Conclusions: Visceral fat mass is a strong predictor of basal ghrelin concentrations and also attenuates the suppressive effect of insulin on ghrelin concentrations. These data provide further evidence that the UBO phenotype is associated with more profound metabolic abnormalities than obesity per se.

Introduction

Ghrelin, an endogenous ligand for the GH secretagogue-receptor, is involved in the regulation of appetite and stimulates food intake (1). Circulating ghrelin levels are inversely correlated with degree of obesity assessed by body mass index (BMI) (2, 3). It is not clear which factor regulates this negative feedback mechanism, but various hormones and metabolites that are altered in obesity such as insulin (4) and free fatty acids (FFAs) (5) have been suggested. However, obesity is a heterogeneous disease entity, where upper-body obesity (UBO) is characterized by a greater extent of hyperglycemia, type 2 diabetes, and elevated FFAs (6, 7) than lower-body obesity (LBO) (8). It is therefore possible that circulating ghrelin levels may be influenced by body fat distribution.

Previous studies assessing the impact of body fat distribution on ghrelin levels report conflicting results. A recent study in postmenopausal women reports an inverse relationship with abdominally located fat (9), whereas others report a positive correlation with visceral fat (10). Highlighting some of the difficulties when comparing body composition studies, the former study used dual X-ray absorptiometry (DXA) to measure abdominal fat, a technique unsuited to differentiate between abdominal s.c. and visceral fat. In the latter study, magnetic resonance imaging (MRI) provided estimates of visceral fat area rendering comparisons between the two studies difficult. To our knowledge, only one study (11) designed to assess the impact of body fat location on ghrelin levels have measured visceral fat by combined DXA and computed tomography (CT). This technique is considered by many the best to measure absolute visceral fat mass (12). However, both men and women were included in the study by Purnell et al. (11) and correct interpretation of their results may therefore be difficult.

The aim of the present study was therefore to investigate the impact of body composition in healthy premenopausal women on circulating levels of ghrelin. In these women, body fat distribution was thoroughly characterized by DXA, CT, and waist-to-hip ratio (WHR).

A second purpose was to assess the suppressive effect of insulin on circulating ghrelin levels. It has previously been demonstrated that insulin suppresses ghrelin
levels (4) and that the suppression is blunted in obesity (13). But whether the inhibitory effect of insulin on ghrelin secretion is modified by body fat distribution has to our knowledge not previously been investigated.

Methods

Thirty healthy, premenopausal women with varying body fat distribution (10 UBO (WHR > 0.85, BMI > 28 kg/m²), 10 LBO (WHR < 0.80, BMI > 28 kg/m²), and 10 lean (BMI < 25 kg/m²) were recruited in a study designed to investigate triglyceride metabolism in women with different obesity phenotypes (unpublished observations). All women were studied in the luteal phase. The two groups of obese women were matched according to BMI to ensure comparability. All participants were normotensive, non-smokers, used no medication except oral contraceptives, and had a normal blood count and chemistry panel documented before participation.

The study protocol was approved by the local Ethics Committee (The Central Denmark Region Committees on Biomedical Research Ethics) and written informed consent was obtained from all participants.

Subjects were studied after an overnight fast. The study day included a 5-h basal study period followed by a 2 h hyperinsulinemic-euglycemic clamp (Insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) 0.6 mU/kg per min). During the clamp, plasma glucose concentrations were maintained at 5.0 mmol/l by infusion of 20% glucose solution.

Serum ghrelin was determined by an in-house RIA (RIA). The assay measures immunoreactive levels of ghrelin using 125I-labeled bioactive ghrelin as tracer and rabbit polyclonal antibodies raised against octanoylated human ghrelin. The assay recognizes the COOH-terminal of ghrelin and determines acylated as well as des-acylated ghrelin. The intra-assay coefficient of variation is less than 3.9%. Values presented are averages from hourly measurements.

Total body fat, leg fat, fat percent, and fat-free mass were examined by DXA (QDR-2000). Upper-body and visceral fat mass were assessed using the CT measures of intra-abdominal and s.c. adipose tissue combined with abdominal fat mass measured by DXA as previously described (12). Upper body s.c. fat was estimated as the difference between upper body fat (DXA) and visceral fat.

Statistical analysis

Data are expressed as the mean ± s.d. Between groups, comparisons were done by one-way ANOVA with Tukey’s test for multiple comparisons applied when appropriate. Univariate and multivariate regression analysis were performed to identify potential determinants of circulating ghrelin levels as well as determinants of the suppressive effect of insulin on ghrelin levels. Correlations were evaluated by Spearman’s rank correlation coefficient. To compare the strength of correlations, the approach of Choi was used (14). It involves regression of the ranked dependent variable versus the difference in rank between the two independent variables. All statistical analyses were performed using the SPSS 15.0 software program. A level of P < 0.05 was accepted as statistically significant.

Results

Characteristics of the three study groups are presented in Table 1. By design, both groups of obese subjects had comparable BMI’s and a significantly larger fat mass than the lean group. However, even though we had anticipated that visceral fat mass would be greater in the UBO group than in the LBO; the difference was not statistically significant. The excess abdominal fat mass of UBO subjects was therefore both s.c. and visceral. Both obese groups were insulin resistant in terms of greater levels of fasting insulin and impaired glucose infusion rate (GIR) during the hyperinsulinemic-euglycemic clamp. There were no differences in GIR between the obese groups (P = 0.54).

Table 1 Basal characteristics and ghrelin levels of the upper-body obese (UBO), the lower-body obese (LBO) and the lean groups.

<table>
<thead>
<tr>
<th></th>
<th>UBO (n=10)</th>
<th>LBO (n=10)</th>
<th>Lean (n=10)</th>
<th>ANOVA (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>41.9 ± 7.9</td>
<td>39.0 ± 8.1</td>
<td>39.0 ± 10.2</td>
<td>0.669</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>91.4 ± 10.0*</td>
<td>86.3 ± 6.9*</td>
<td>65.5 ± 6.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>32.3 ± 2.3*</td>
<td>30.4 ± 1.9*</td>
<td>22.9 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Waist-to-hip ratio</strong></td>
<td>0.91 ± 0.05</td>
<td>0.77 ± 0.04**</td>
<td>0.79 ± 0.07**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Total fat mass (kg)</strong></td>
<td>36.4 ± 4.2*</td>
<td>35.7 ± 3.8*</td>
<td>18.2 ± 4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Visceral fat (kg)</strong></td>
<td>4.8 ± 1.5*</td>
<td>4.0 ± 1.5*</td>
<td>2.3 ± 1.0</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>S.c. abdominal fat (kg)</strong></td>
<td>13.5 ± 2.6*</td>
<td>11.8 ± 2.4*</td>
<td>5.3 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Fasting insulin (pmol/l)</strong></td>
<td>60.3 ± 16.2*</td>
<td>53.7 ± 16.4*</td>
<td>23.4 ± 10.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>GIR (clamp; mg/kg per min)</strong></td>
<td>3.9 ± 1.6*</td>
<td>4.5 ± 2.6</td>
<td>7.0 ± 2.6</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Ghrelin basal (μg/l)</strong></td>
<td>0.60 ± 0.16*</td>
<td>0.69 ± 0.22</td>
<td>0.85 ± 0.22</td>
<td>0.033</td>
</tr>
<tr>
<td><strong>Ghrelin clamp (μg/l)</strong></td>
<td>0.56 ± 0.17</td>
<td>0.62 ± 0.18</td>
<td>0.72 ± 0.20</td>
<td>0.158</td>
</tr>
</tbody>
</table>

All values are means ± s.d. *P < 0.05 compared with lean group, **P < 0.05 compared with UBO group.
**Basal ghrelin levels**

The basal level of ghrelin was significantly lower in the UBO (29%) and LBO (19%) group compared with the lean group (ANOVA, \( P=0.033 \)), but there was no significant difference between the UBO and the LBO groups (Fig. 1).

As expected, an inverse correlation existed between basal ghrelin concentration and BMI (\( r=-0.40, P=0.03 \)), total fat mass (\( r=-0.39, P=0.04 \)), and fasting insulin (\( r=-0.47, P<0.01 \)). Additionally, there was also a positive correlation between high density lipoprotein (HDL) cholesterol and basal ghrelin (\( r=0.51, P<0.01 \)). Since abdominal s.c. fat has been proposed as a determinant of ghrelin levels, we also tested whether this was the case in our study, but no significant correlation was found (\( P=0.10 \)).

In line with our preliminary hypothesis, visceral fat mass was a strong predictor (\( r=-0.56, P=0.002 \)) of circulating ghrelin levels (Fig. 2). We used the approach of Choi to determine if visceral fat mass was a better predictor of basal ghrelin levels than BMI, but there were no significant differences (\( P=0.074 \)).

Since visceral fat accumulation predisposes to insulin resistance and elevated fasting insulin, a multivariate regression analysis with basal ghrelin levels as dependent variable and visceral fat mass and insulin as independent variables was performed to elucidate the independent effect of visceral fat. The regression analysis revealed that visceral fat was an independent significant predictor of ghrelin levels even when adjusted for insulin (\( P=0.03 \)), GIR (\( P=0.01 \)), BMI (\( P=0.02 \)), body composition group (LBO, UBO or lean; \( P=0.04 \)) or HDL cholesterol (\( P=0.01 \)), a known ghrelin carrier molecule (15).

**The suppressive effect of insulin on ghrelin concentrations**

As anticipated, ghrelin was inversely correlated with fasting insulin. The suppressive effect of insulin on ghrelin concentration (ghrelin decrease during the clamp (\( \Delta \text{ghrelin}_{(\text{BASAL-CLAMP})} \))) was significantly attenuated in the UBO group when compared with the lean controls (\( P=0.012 \)), whereas this was not the case in the LBO group (\( P=0.084 \); \( \Delta \text{ghrelin}_{(\text{BASAL-CLAMP})} \)) (\( \mu g/l \)): UBO: 0.03 ± 0.03 vs LBO: 0.07 ± 0.09 versus lean: 0.13 ± 0.05, ANOVA \( P=0.003 \); Fig. 3). The insulin stimulated ghrelin decrease did not significantly correlate to BMI or total fat mass, whereas a highly significant inverse correlation existed with visceral fat mass (\( r=-0.52, P=0.004 \); Fig. 4).

**Discussion**

In the present study, we demonstrate that the visceral fat mass is a strong predictor of basal ghrelin concentrations in a well characterized group of premenopausal women. The inverse correlation between visceral fat mass and ghrelin levels persisted even when adjusted for known predictors of ghrelin levels such as BMI (2), insulin sensitivity (3), insulin levels (3) or HDL cholesterol (11). In addition, we observed that the suppressive effect of insulin on ghrelin levels was blunted in UBO women compared with lean, whereas this was not the case in LBO women. Our findings provide further evidence that upper-body obesity impacts more profoundly on hormonal and metabolic parameters than LBO.

Previous reports on the impact of body composition on ghrelin levels have been contradictory, which may partly be attributable to methodological differences in quantification of abdominal obesity. For instance,
Lindeman et al. observed a positive relationship between visceral fat area and ghrelin levels in 11 obese women (assessed by MRI) (10), whereas Katsuki et al. found a negative correlation between visceral fat area (assessed by CT) and ghrelin in 18 patients with type 2 diabetes (3). Both studies assessed visceral fat using a 2D technique that does not allow precise quantification of fat mass. Other studies have assessed the impact of abdominal fat on ghrelin levels by measuring waist circumference (WC) (16), DXA (2) or by a combination of WC and DXA (9). Although these techniques are useful when studying large cohorts and expose trial subjects to minimal radiation, they do not accurately assess the amount of visceral fat. To quantify visceral fat mass it is necessary to use CT in combination with DXA. Using that technique, Purnell et al. found no significant correlation between the visceral fat mass and ghrelin levels. However, both men and women were included in that study, which may have caused interference by gender-related differences in ghrelin levels and other hormones.

Although ghrelin secreting cells have been demonstrated to exist in the mucosa lining the small intestine (17) and therefore may be in close contact with visceral adipocytes, only ~30% of total ghrelin is estimated to be secreted from these cells (18). It is therefore more likely that the impact of visceral fat accumulation on ghrelin levels is indirect, i.e. via altered levels of metabolites or hormones. Visceral adipocytes are more lipolytically active than s.c. (19) thus releasing relatively larger quantities of FFAs into the circulation. We have previously shown that elevated FFA concentrations suppress ghrelin levels (5). Since the mild hyperglycemia observed in UBO subjects also elicits a hormonal response in the form of increased levels of insulin, another known inhibitor of gastric ghrelin secretion (20), we tested whether including insulin in the regression analysis would cancel out the effect of visceral fat on ghrelin levels. That this was not the case strengthens the notion that visceral adipocytes may secrete ghrelin inhibiting substances. Potential candidates include TNFα (21) and leptin (20).

In addition to lowering total levels of ghrelin, obesity also affects the normal oscillating pattern of the hormone. Thus, the suppressive effect of a test meal on ghrelin secretion (13) as well as the nocturnal increase in ghrelin (7) is blunted in obese persons. In this study, we demonstrate that not only the size but also the location of excess fat depots impacts on the ability of insulin to suppress ghrelin concentrations under hyperinsulinemic-euglycemic clamp conditions. To our knowledge, this loss of hormonal flexibility in UBO subjects has not previously been reported. However, since our obese volunteers were comparable in terms of the absolute amount of visceral fat, this effect cannot be attributed to visceral fat alone.

Some limitations to the study should be acknowledged. First, the measurement of visceral fat by both CT and DXA was laborious and prevented us from investigating a large cohort. That the LBO group did not differ from the UBO or lean in terms of the suppressive effect of insulin on ghrelin levels may therefore be a statistical type 2 error. Second, we used an in-house ghrelin assay that measures the total ghrelin as opposed to other assays specifically aimed at determining the bioactive acylated ghrelin. Total ghrelin levels were assumed to serve as a marker of bioactive ghrelin because total and acylated ghrelin levels usually change in parallel (22). Whether upper-body obesity impacts the ratio between acylated and des-acylated ghrelin remains to be elucidated. Third, due to the cross-sectional nature of this investigation, the exact nature of the relationship between upper-body adiposity and ghrelin concentrations can obviously only be speculated upon. Whether selective loss of visceral fat induces a more potent change in ghrelin concentrations than loss of s.c. fat could be an interesting field for future studies.

In summary, visceral fat mass is a strong predictor of circulating ghrelin levels. In addition, the suppressive effect of insulin on ghrelin levels is attenuated in UBO subjects but not in LBO subjects when compared with lean controls. These findings underscore the unfavorable metabolic and hormonal profile of upper-body obesity.

Declaration of interest
E Sondergaard, L C Gormsen, B Nellemann, E T Vestergaard, and S Nielsen have no disclosures. J S Christiansen has served as a consultant for Novo Nordisk, Pfizer and Ipsen. There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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