Fasting Plasma Ghrelin Levels Are Negatively Correlated With Insulin Resistance and PAI-1, but Not With Leptin, in Obese Children and Adolescents

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Ghrelin is a novel growth hormone—releasing peptide isolated from human and rat stomach that induces weight gain by increasing food intake and reducing fat utilization. Although recent data indicate that ghrelin is downregulated in human adult obesity, the characteristics of human obesity are heterogeneous, especially in children and adolescents, and depend on the distribution of subcutaneous and visceral fat tissue. We measured fasting plasma ghrelin concentrations by radioimmunoassay in 49 obese Japanese children and adolescents (38 boys and 11 girls; mean age 10.2 ± 2.8 years; BMI 28.0 ± 4.5 kg/m², percent overweight 56.0 ± 20.7%), and analyzed associations of their ghrelin concentrations with their body composition, insulin resistance, and adipocytokine concentrations. Fasting plasma ghrelin levels were negatively correlated with BMI and waist circumference, but not with percent overweight or percent body fat, whereas fasting leptin levels were positively correlated with all of the following parameters: BMI, waist circumference, percent overweight, and percent body fat. Plasma ghrelin levels were negatively correlated with fasting immunoreactive insulin, homeostasis model assessment insulin resistance index, and quantitative insulin sensitivity check index values. There was no correlation between plasma ghrelin and leptin, but ghrelin was negatively correlated with the PAI-1 concentrations. The results suggest that the downregulation of ghrelin secretion may be a consequence of higher insulin resistance associated with visceral fat accumulation and elevated PAI-1 concentrations, and not a consequence of total body fat accumulation associated with elevated leptin concentrations.

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RESEARCH DESIGN AND METHODS

For this study, 49 Japanese children and adolescents (38 boys, 11 girls) with simple obesity were recruited. Their ages ranged from 5 to 19 years (mean age 10.2 ± 2.8 years). Standing height and weight were measured, and BMI and the percent overweight were calculated. The percent overweight was determined on the basis of the Japanese standard body weights for height by age and sex using the following formula: [(actual body weight − standard weight)/standard weight] × 100%. The percent overweight values of the subjects ranged from 22.0 to 127.2% (mean value 56.0 ± 20.7%). The mean BMI was 28.0 ± 4.5. Waist circumference was also determined. The percent body fat was estimated by hand-to-foot impedance measured with a bioelectrical

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FBG, fasting blood glucose; GH, growth hormone; HOMA-R, homeostasis model assessment insulin resistance index; HII, immunoreactive insulin; PAI-1, plasminogen activator inhibitor-1; QUICKI, quantitative insulin sensitivity check index; RIA, radioimmunoassay.
TABLE 1
Correlation between the body composition and blood concentrations of ghrelin, leptin, and PAI-1

<table>
<thead>
<tr>
<th></th>
<th>Ghrelin</th>
<th>Leptin</th>
<th>PAI-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent overweight</td>
<td>-0.224*</td>
<td>0.587‡</td>
<td>0.287*</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.317*</td>
<td>0.548†</td>
<td>0.382*</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>-0.231</td>
<td>0.604‡</td>
<td>0.128</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>-0.301*</td>
<td>0.542‡</td>
<td>0.385*</td>
</tr>
<tr>
<td>Height SD score</td>
<td>0.013</td>
<td>-0.048</td>
<td>-0.067</td>
</tr>
</tbody>
</table>

Data are  values by Spearman rank correlation test. *P < 0.05; †P < 0.005; ‡P < 0.0005.

TABLE 2
Correlation between insulin resistance indexes and blood concentrations of ghrelin, leptin, and PAI-1

<table>
<thead>
<tr>
<th></th>
<th>Ghrelin</th>
<th>Leptin</th>
<th>PAI-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin</td>
<td>-0.317*</td>
<td>0.324*</td>
<td>0.481†</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>-0.300†</td>
<td>0.257</td>
<td>0.450†</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.302*</td>
<td>-0.251</td>
<td>-0.451†</td>
</tr>
<tr>
<td>FBG</td>
<td>0.015</td>
<td>-0.332*</td>
<td>-0.009</td>
</tr>
</tbody>
</table>

Data are  values by Spearman rank correlation test. HOMA-R = [FBG (mg/dl) x IRI (mU/l)]/405. QUICKI = 1/(log IRI + log FBG). *P < 0.05; †P < 0.005.

RESULTS

The fasting plasma ghrelin levels were 69.1–369.8 fmol/ml. There was no significant difference between the boys (range 69.1–369.8, median 157.8 fmol/ml) and girls (range 77.8–202.2, median 137.7 fmol/ml). The circulating ghrelin levels tended to correlate negatively with age, but the correlation was not significant (r = -0.268, P = 0.054).

The fasting leptin levels were positively correlated with percent overweight (r = 0.587, P < 0.0005), BMI (r = 0.548, P < 0.005), percent body fat (r = 0.604, P < 0.0005), and waist circumference (r = 0.542, P < 0.0005) (Table 1, Fig. 1). The PAI-1 levels were positively correlated with the indexes of body composition, except percent body fat, but less strongly than leptin. By contrast, the fasting plasma ghrelin levels were negatively correlated with BMI (r = -0.317, P < 0.05) and waist circumference (r = -0.301, P < 0.05), but not with percent overweight or percent body fat (Table 1, Fig. 1). The fasting plasma ghrelin concentrations were not correlated with the height SD scores of the obese children and adolescents.

Table 2 shows the correlations between the indexes for insulin resistance and the fasting concentrations of ghrelin, leptin, and PAI-1. Leptin and PAI-1 were positively correlated with fasting IRI, and ghrelin was negatively correlated with fasting IRI (Table 2, Fig. 2). Fasting plasma ghrelin was negatively correlated with HOMA-R (r = -0.300, P < 0.05) and positively correlated with QUICKI (r = 0.302, P < 0.05), but the leptin levels were not correlated with either HOMA-R or QUICKI. PAI-1 was positively correlated with HOMA-R (r = 0.450, P < 0.005) and negatively correlated with QUICKI (r = -0.451, P < 0.005). It was noteworthy that there was no correlation between the fasting plasma ghrelin and fasting blood glucose concentrations (Table 2, Fig. 2).

There was also a negative correlation between the fasting plasma ghrelin and PAI-1 concentrations (r = -0.322, P < 0.05), but not between the ghrelin and leptin concentrations (r = -0.297, P = 0.40) (Fig. 3).

FIG. 1. Correlations of fasting plasma ghrelin and leptin levels with percent body fat. A: Fasting plasma ghrelin levels were not correlated with percent body fat as assessed by bioelectrical impedance (r = -0.231; P = 0.11). B: Fasting plasma leptin levels were positively correlated with percent body fat (r = 0.604, P < 0.0005). •, boys (n = 38); ○, girls (n = 11).
The plasma ghrelin levels in our study tended to correlate negatively with age, but the correlation was not significant ($r = -0.268, P = 0.054$). It may be important and interesting to clarify the effect of growth and maturation on fasting plasma ghrelin levels; however, in this study, we measured the fasting plasma ghrelin levels of only obese children and adolescents, not of nonobese subjects, and thus further study is needed to address the issue of changes in plasma ghrelin levels in association with growth and maturation.

We did not perform the glucose clamp test as the gold standard for quantifying insulin sensitivity/resistance in vivo, because it requires intravenous infusion of insulin and glucose and frequent blood samples over a 3-h period, and we could not justify applying a glucose clamp to apparently healthy children and adolescents with simple obesity. Instead we used fasting IRI, HOMA-R (16), and QUICKI (17), which are well-accepted alternatives for estimating insulin sensitivity/resistance, with QUICKI values in particular having been reported to be well correlated with indexes of insulin sensitivity in glucose clamp studies (17).

Tschöp et al. (7) has demonstrated negative correlations of fasting plasma ghrelin with percent body fat, fasting insulin concentrations, and leptin concentrations of obese adult Caucasians and Pima Indians. Furthermore, studies in normal-weight and obese humans have confirmed that serum leptin concentrations accurately reflect BMI and percent body fat (18, 19). Our study showed that leptin

**FIG. 2.** Correlations of fasting plasma ghrelin with fasting IRI concentrations and FBG levels. A: There was a negative correlation between fasting plasma ghrelin and fasting IRI ($r = -0.317; P < 0.05$). B: There was no correlation between the fasting plasma ghrelin and fasting blood glucose concentrations ($r = 0.015; P = 0.93$). ▲, 20–40% overweight boys ($n = 9$); ●, 40–70% overweight boys ($n = 20$); ■, >70% (max. 127.2%) overweight boys ($n = 9$); ⊙, 20–40% overweight girls ($n = 2$); ⊛, 40–70% overweight girls ($n = 7$); ▼, >70% (max. 90.5%) overweight girls ($n = 2$).

**FIG. 3.** Correlations of fasting plasma concentrations of ghrelin and leptin with PAI-1. A: There was no correlation between the fasting plasma ghrelin and leptin concentrations ($r = -0.297; P = 0.40$). B: There was a negative correlation between the fasting plasma ghrelin and PAI-1 concentrations ($r = -0.322; P < 0.05$). ▲, 20–40% overweight boys ($n = 9$); ●, 40–70% overweight boys ($n = 20$); ■, >70% (max. 127.2%) overweight boys ($n = 9$); ⊙, 20–40% overweight girls ($n = 2$); ⊛, 40–70% overweight girls ($n = 7$); ▼, >70% (max. 90.5%) overweight girls ($n = 2$).
levels were positively correlated with BMI, percent body fat, and percent overweight (Table 1, Fig. 1), but our observations of Japanese obese children and adolescents showed no correlation between fasting plasma ghrelin and percent body fat or leptin concentrations (Table 1, Figs. 1 and 3). Fasting plasma ghrelin was significantly correlated with BMI, waist circumference, fasting IRI, HOMA-R, QUICKI, and plasma PAI-1. Plasma PAI-1 levels in humans have been demonstrated to be closely correlated with visceral fat area, but not with subcutaneous fat area, and to be clearly related to insulin resistance (11,12). The plasma PAI-1 levels of our obese children and adolescents were also significantly correlated with the IRI, HOMA-R, and QUICKI values (Table 2). The characteristics of the obesity in our study population were heterogeneous in terms of insulin resistance and blood leptin and PAI-1 levels, and the differences in our results from those in the previous report on adult obese Caucasians and Pima Indians (7) may be attributable to this heterogeneity. The results of our study suggest that the downregulation of ghrelin secretion may be a consequence of a higher insulin resistance associated with visceral fat accumulation and elevated PAI-1 concentrations, and not a consequence of total body (mainly subcutaneous) fat accumulation associated with elevated leptin concentrations.

Cummings et al. (20) recently reported that plasma ghrelin levels nearly doubled immediately before each meal, and fell to trough levels within 1 h after eating, a pattern reciprocal to that of insulin levels. Those researchers suggested that single measurements of ghrelin levels during the troughs before breakfast might serve as surrogates suggested that single measurements of ghrelin levels during the troughs before breakfast might serve as surrogates for 24-h profiles in estimating overall ghrelin levels, as they correlated strongly with the 24-h integrated area-under-the-curve values (20). The fasting plasma ghrelin concentrations in our study were not correlated with FBG concentrations and were negatively correlated with insulin resistance. These results warrant further studies on the association between insulin resistance and higher PAI-1 concentrations and the mechanisms influencing total daily secretion of ghrelin.

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