GH-binding protein in obese men with varying glucose tolerance: relationship to body fat distribution, insulin secretion and the GH–IGF-I axis

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Abstract
Bioelectrical impedance for measurement of total body fat and computed tomography for visceral and subcutaneous fat at umbilicus levels were performed in 34 obese and 10 lean men. Insulin secretion in response to an oral glucose tolerance test (OGTT) and a GH stimulation test by L-dopa, growth hormone-binding protein (GHBP) and IGF-I were measured. Obese subjects were divided into three groups according to the OGTT. The obese type II diabetes mellitus group had the highest GHBP levels and the most visceral fat. GHBP levels were most strongly correlated with the ratio of visceral fat area to body weight (VWR) above any other parameters \( (r = 0.725, P < 0.001) \). The insulin and free fatty acid (FFA) areas under curves (AUC) during the OGTT, and the IGF-I level, were also positively correlated with GHBP levels \( (r = 0.474, P < 0.005; r = 0.572, P < 0.005; r = 0.453, P < 0.005) \). GH-AUC to the L-dopa stimulation test was negatively correlated with GHBP levels \( (r = -0.432, P < 0.005) \). Stepwise multiple linear regression analysis showed that VWR, FFA-AUC and insulin-AUC significantly contributed to the variability of GHBP \( (r^2 = 0.58) \). In conclusion, we demonstrated that:
(i) visceral fat amount mainly determined GHBP levels in obese men with varying glucose tolerance;
(ii) hyperglycemia per se did not influence the GHBP level, whereas insulin and FFA could play a role in regulation of GHBP; and (iii) although GH was not the main regulator of GHBP, the unchanged IGF-I level despite GH hyposecretion suggests that increased GHBP levels reflect GH hypersensitivity in order to compensate for decreased GH secretion in obesity.

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Introduction
Visceral fat obesity is known to be a major risk factor for glucose intolerance, dyslipidemia, hypertension and atherosclerosis (1, 2). Visceral fat characterized by high lipolytic activity increases the plasma free fatty acid (FFA) level, which causes hyperinsulinemia associated with insulin resistance (3, 4). Furthermore, increased deposition of visceral fat is an antecedent to the pathogenesis of type II diabetes mellitus (DM) (5). Obesity is associated with impaired secretion of growth hormone (GH) and blunted responses to all stimuli (6, 7). High circulating levels of FFA from visceral fat tissue are responsible for GH secretory dysfunction (8, 9). However, the insulin-like growth factor-I (IGF-I) level is not decreased in obesity, despite GH hyposecretion. As GH-binding protein (GHBP) is believed to be derived from proteolytic cleavage of the extracellular domain of the GH receptor and may be regarded as an intrinsic part of the GH–IGF-I axis, an effect of body composition on circulating GHBP levels may be expected (10, 11). Some studies have shown that GH and insulin might participate in regulation of GHBP levels (12, 13). Little is known, however, about GH secretion and GHBP variations in obesity according to the degree of glucose tolerance. Although obese diabetic patients have more visceral fat than similar obese subjects with normal glucose tolerance (NGT), they have lower insulin levels because they also have an impairment of insulin secretion (14). These conditions can cause differences in the regulation of GHBP and GH secretion between obese subjects with NGT and obese diabetic patients. In this study, we investigated GHBP variations in obese men with varying glucose tolerance and its relationship to body fat distribution, insulin secretion and the GH–IGF-I axis.

Subjects and methods

Subjects and protocols
This study included 34 obese men, aged 38–53 years (mean ± S.E.M., 43.8 ± 6.9) and 10 lean men aged 37–49
years (42.3 ± 5.0). The lean control subjects were within 100% of their ideal body weight (IBW), and the obese subjects weighed more than 120% of IBW, as determined by the Fogarty Center Conference on Obesity (15). All subjects were recruited after attending the healthcare center of Yong Dong Severance Hospital for a medical checkup. None of them had used any hormonal preparations or drugs within 60 days prior to the study.

Height and weight were measured unclad without shoes. The waist circumference was measured midway between the lower rib margin and the iliac crest. The hip girth was measured at the widest circumference over the trochanter. Both circumferences were measured in standing subjects after normal expiration. All subjects underwent a 75 g oral glucose tolerance test (OGTT) after a 10–12 h overnight fast. Blood was collected at 0, 30, 60 and 120 min for determination of plasma glucose, insulin, c-peptide and FFA concentrations.

According to WHO criteria, all 10 lean subjects had NGT (lean NGT), while 12 obese subjects had NGT (obese NGT), 10 had impaired glucose tolerance (IGT) and 12 had type II DM. All diabetic subjects were newly diagnosed in this study.

For determination of GH secretion, all subjects underwent an L-dopa stimulation test. L-Dopa (500 mg) was administered orally at time 0 and blood samples were obtained at −60, 0, 60 and 90 min for measurement of GH.

Serum was separated by centrifugation and stored at −20°C until analysis. The study was approved by the Hospital Ethics Committee and informed consent was obtained from each subject.

**Body composition**

Body composition was determined by measurement of total body fat using bioelectrical impedance (Biodynamics Model 400, Bellevue, WA, USA — 12.0 V DC, 0.5 A). A computed tomography scan was performed (Philips, Tomoscan 350, Mahway, NJ, USA) to measure visceral and subcutaneous fat areas at the level of the umbilicus. The adipose tissue (AT) was defined with a density of −150 to −50 Hounsfield units (16). VWR was calculated as the ratio of visceral fat area to body weight.

**Biochemical analytical methods**

GHBP levels were determined by RIA for the exon 3-retaining GHBP as recently described by Kratzsch et al. (17). Sensitivity of the assay was 0.32 ng/ml. In the range between 2.08 and 15.60 ng/ml (n = 12), intra- and inter-assay coefficients of variations (CVs) were below 8.5 and 12.0% respectively.

Serum glucose was measured by the glucose-oxidase method and insulin by ELISA. This ELISA uses two monoclonal antibodies (Novo, Nordisk A/S, Denmark) directed against human insulin and does not cross-react with human proinsulin.

Serum FFA concentrations were determined by colorimetry. Serum GH was measured with an IRMA kit (Daiichi, Tokyo, Japan); the sensitivity was 0.1 µg/l. Its intra- and inter-assay CVs were 1.3 and 1.4% respectively.

Glycated hemoglobin (HbA1c) level was measured by an ion capture assay (Abbott Imx, Abbott Park, IL, USA). The normal range was 3.7–5.7%.

IGF-I was measured with an IRMA kit (Diagnostic System Laboratories, Webster, TX, USA); the sensitivity was 0.3 µg/l. Its intra- and inter-assay CVs were less than 10%.

**Statistical analysis**

The total area under the curve (AUC) was calculated by the trapezoidal method. The results were expressed as the mean ± S.E.M. The significance of differences among four groups was assessed by ANOVA. Comparison between the obese NGT group and the obese DM group was performed by Student’s unpaired t-test. Relationships between variables were analyzed by simple correlation and multiple linear regression analysis. P < 0.05 was accepted as the significance level.

**Results**

Table 1 shows characteristics of the subjects in this study. Age, body mass index, percentage of IBW and waist to hip ratio (WHR) of three obese glucose tolerance categories (obese NGT, IGT and DM) were similar. Total body fat determined by impedance showed a rising orderly trend in obese NGT, IGT and DM groups. The DM group had the highest VWR among the three obese glucose tolerance categories. Insulin-AUC during OGTT was highest in the obese NGT group (Table 2). FFA-AUC was highest in the obese DM group. GH responses to the L-dopa stimulation test were impaired in all obese subjects but serum IGF-I levels were no different between the groups (Table 2). Mean GHBP levels were significantly higher in obese subjects than in lean controls (Fig. 1). The value of GHBP in the DM group was significantly higher than in the obese NGT group (P < 0.05). Associations between GHBP levels and IBW, total body fat, fat distribution and some metabolic parameters are listed in Table 3. Significant positive correlations were found between GHBP levels and variables reflecting total body fat as well as abdominal fat distribution in each subgroup. GHBP levels showed the highest positive correlation with VWR. Insulin-AUC, FFA-AUC and IGF-I showed a positive correlation with GHBP, whereas GH-AUC was negatively correlated with GHBP level. However, glucose-AUC and Hba1c levels were not correlated with GHBP level. Stepwise multiple linear regression analysis of all parameters showed that VWR, FFA-AUC
Serum GHBP levels (means ± S.E.M.) of the lean NGT group and three obese groups (NGT, IGT and type II DM). *P < 0.05 among four groups; +P < 0.05 obese NGT vs DM.

Table 1 Basal anthropometric data, total body fat, and visceral and subcutaneous fat area (means ± S.E.M.) of subjects.

<table>
<thead>
<tr>
<th></th>
<th>Lean NGT</th>
<th>Obese NGT</th>
<th>Obese IGT</th>
<th>Obese DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.3 ± 5.0</td>
<td>41.6 ± 4.3</td>
<td>40.6 ± 2.5</td>
<td>45.0 ± 5.2</td>
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<tr>
<td>IBW (%)</td>
<td>94.5 ± 14.9</td>
<td>135.4 ± 9.6*</td>
<td>142.4 ± 25.6*</td>
<td>130.8 ± 4.0*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.07</td>
<td>0.95 ± 0.02*</td>
<td>0.98 ± 0.04*</td>
<td>0.98 ± 0.03*</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>13.6 ± 3.4</td>
<td>27.3 ± 2.7*</td>
<td>29.5 ± 7.0*</td>
<td>31.1 ± 4.2*</td>
</tr>
<tr>
<td>AT areas (cm²)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Visceral</td>
<td>42.8 ± 36.6</td>
<td>124.4 ± 43.5*</td>
<td>145.1 ± 61.2*</td>
<td>151.9 ± 37.6*</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>67.2 ± 46.0</td>
<td>217.0 ± 41.2*</td>
<td>227.0 ± 43.2*</td>
<td>191.5 ± 45.7*</td>
</tr>
<tr>
<td>VWR (cm²/kg)</td>
<td>0.76 ± 0.24</td>
<td>1.35 ± 0.41*</td>
<td>1.66 ± 0.55*</td>
<td>1.86 ± 0.33*</td>
</tr>
</tbody>
</table>

* P < 0.05, significant differences among four groups.

Discussion

In our study, GHBP level was most highly correlated with the relative amount of visceral fat (VWR) among anthropometric parameters such as IBW and WHR and total body fat amount irrespective of the degree of glucose tolerance. Recently, Roelen et al. (18) demonstrated that in patients with GH deficiency, visceral fat may be involved in the regulation of GHBP levels. Rasmussen et al. (19) demonstrated that GHBP elevated in obesity was restored to normal after weight loss and changes in GHBP were mainly associated with a reduction of abdominal fat mass. These findings suggest that visceral fat may have GH receptors which mediate direct metabolic actions such as stimulation of lipolysis in AT (20), although the liver has a very high density of GH receptors and most circulating GHBP can be assumed to arise from the liver (21, 22).

Table 2 The response of glucose, insulin and FFA during an OGTT, HbA1c, GH secretion stimulated by L-dopa and IGF-I level of the subjects. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Lean NGT</th>
<th>Obese NGT</th>
<th>Obese IGT</th>
<th>Obese DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-AUC (pmol/l×h)</td>
<td>12.0 ± 2.1</td>
<td>14.7 ± 3.4*</td>
<td>19.5 ± 4.5*</td>
<td>25.4 ± 3.3*</td>
</tr>
<tr>
<td>Insulin-AUC (pmol/l×h)</td>
<td>283.6 ± 137.3</td>
<td>1056.8 ± 441.5*</td>
<td>835.9 ± 245.4*</td>
<td>477.8 ± 288.0*</td>
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<tr>
<td>FFA-AUC (mmol/l×h)</td>
<td>580.8 ± 227.7</td>
<td>834.4 ± 200.5*</td>
<td>949.2 ± 241.9*</td>
<td>1075.2 ± 369.3*</td>
</tr>
<tr>
<td>GH-AUC (ng/ml×h)</td>
<td>17.5 ± 13.8</td>
<td>11.3 ± 5.9*</td>
<td>7.3 ± 4.2*</td>
<td>5.1 ± 2.6*</td>
</tr>
<tr>
<td>IGF-I (ng/dl)</td>
<td>213.0 ± 85.3</td>
<td>200.1 ± 48.2</td>
<td>234.8 ± 111.9</td>
<td>241.2 ± 102.8</td>
</tr>
</tbody>
</table>

* P < 0.05, significant differences among four groups.
Considering that most GHBP mainly results from proteolysis of the extracellular part of the receptors in humans (23), the regulation mechanism for proteolysis of the GH receptors in hepatic and extrahepatic tissues is important in determining the changes of GHBP observed in visceral fat obesity. The protease involved in this cleavage remains to be identified. There is speculation that some metabolites associated with visceral fat obesity such as FFA and insulin may influence GHBP release. We found that GHBP levels were still correlated with insulin-AUC and FFA-AUC after adjusting for the relative amount of visceral fat, which suggests that insulin and FFA draining to the portal circulation can influence the release of hepatic GHBP.

In our study, the obese DM group had a significantly lower insulin-AUC during OGTT than the obese NGT group. The obese DM group had not only hyperinsulinemia associated with visceral obesity but also impaired insulin secretion. In previous reports (13, 23), GHBP could be regulated by the degree of insulinopenia in diabetics. Based on their results, we could speculate that GHBP levels of the obese DM group should be lower than the obese NGT group. Interestingly we found that GHBP levels were higher in the obese DM group. Although these results indicated that the impact of VWR exceeded the impact of decreased insulin secretion, we could not exclude that insulin might influence GHBP levels because the insulin-AUC was significantly correlated with the variation of GHBP by multiple regression analysis in all subjects.

Some reports showed that there was a negative correlation between GHBP levels and GH secretion, which suggested that a decrease in GH secretion could increase GH sensitivity and the up-regulation of the GH receptor compensating for impairment of GH secretion (24, 25). In our study, IGF-I levels did not decrease in obese subjects, despite GH hyposcretion, and they were still correlated with insulin-AUC and FF A-AUC in obese subjects. Portal circulation can influence GHBP release. We found that GHBP levels did not influence GHBP levels, whereas insulin and FFA could also play a role in regulation of GHBP; and (iii) although GH was not the main regulator of GHBP, the unchanged IGF-I level despite GH hyposcretion suggests that increased GHBP levels reflect GH hypersensitivity in order to compensate for decreased GH secretion in obesity.

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References


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