Clinical Implication of Serial Leptin Measurement in Subjects with Type 2 Diabetes Mellitus

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Abstract. Plasma leptin concentration is closely associated with body fat in humans, with energy restriction inducing a greater decrease in plasma leptin than in body fat. Since adequate energy restriction is mandatory in diet therapy of diabetes mellitus especially in obese subjects, the present study was undertaken to evaluate the clinical implication of serial leptin measurement in the management of diabetic patients. Fifty-four consecutive subjects with type 2 diabetes, who were subjected to adjusted energy restriction during hospitalization, were enrolled in the study. During their hospitalization period (24±4 days), plasma leptin concentrations decreased from 6.9±0.7 to 5.7±0.6 µg/l (P <0.0001) in the overall subjects, and the %change in plasma leptin (−13.9%) was greater than the %changes in body mass index (BMI) and percent body fat (−1.7% and −4.7%, respectively). The %change in plasma leptin was positively correlated with the %changes in BMI and plasma C-peptide (r = 0.526, P < 0.0001 and r = 0.446, P < 0.002, respectively) and negatively with a %change in plasma ketone bodies (r = −0.516, P <0.005). Multiple regression analysis revealed that the %changes in BMI and plasma C-peptide were independent determinants of the %change in plasma leptin. In addition, 38 subjects were followed up after discharge. Three months after discharge, plasma leptin concentrations significantly increased by 25.6%, which was again much greater than the %change in BMI (+0.9%). In 28 subjects who showed increase in plasma leptin levels after discharge, BMI was also increased. In contrast, the remaining 10 subjects without the increase in plasma leptin kept their BMI unchanged. Throughout the observation period, the changes in plasma leptin were prominent in the subjects with BMI greater than 25 kg/m². In conclusion, plasma leptin concentrations showed greater changes than the alterations in anthropometric indexes during the observation period. Serial leptin measurement may be useful to estimate adherence to energy restriction especially in obese subjects with type 2 diabetes.

Key words: Leptin, Diet therapy, Insulin, Type 2 diabetes mellitus

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Leptin, encoded by the obese gene in adipose tissue, is a circulating hormone, which plays a significant physiological role in modulating eating behavior and energy expenditure in rodents [1]. While it is still controversial whether leptin plays such a significant role in humans [2], serious leptin or leptin receptor gene mutations are known to cause morbid obesity in humans [3, 4]. Plasma leptin concentrations have thus been investigated in numerous physiological and pathological conditions in humans [5], and all the studies agree that body fat store and sexuality are the strongest determinants of its circulating concentrations. With regard to humoral factors, insulin and glucocorticoids seem to have the most important physiological roles in modulating obese gene expression and circulating leptin levels [6, 7]. Insulin is crucial in the maintenance of circulating leptin levels...
Some studies suggest that insulin resistance could also raise plasma leptin levels [13], whereas there is no significant difference in plasma leptin concentrations between subjects with type 2 diabetes mellitus and non-diabetic controls [10, 14].

Acute fasting less than 60 hours promptly decreases circulating leptin concentrations. The decrease seems to be due to the decline in insulin levels [8, 15], because the maintenance of glucose and insulin levels abolished the decrease in plasma leptin during fasting [8, 15]. Studies of long-term energy restriction for several days or weeks demonstrate that circulating leptin levels show a prior and greater magnitude of decrease than the values expected by the decline in body fat store [16]. This relative "leptin deficiency" may cause the rebound gain in body weight after intensive dietary restriction in obese people [16]. Adequate energy restriction is mandatory to achieve good glycemic control and improve insulin resistance-associated abnormalities such as dyslipidemia and hypertension in subjects with type 2 diabetes [17]. In consideration of the dissociated decrease in body fat and circulating leptin levels by energy restriction [16], serial leptin measurement may provide unique information on energy intake, although little prospective data are available in subjects with type 2 diabetes. The present study was undertaken to evaluate the clinical implication of serial leptin measurement in subjects with type 2 diabetes, who were subjected to strictly adjusted energy intake during their hospitalization and followed up after discharge.

Subjects and Methods

Subjects

Fifty-four patients with type 2 diabetes mellitus, who were admitted to Jichi Medical School Hospital for hyperglycemia, were enrolled in the present study during May and August, 1999. They consisted of 24 males and 30 females with ages ranging from 25 to 76 years (53 ± 2 years old, mean ± SEM). They were diagnosed as having type 2 diabetes, based on their typical clinical courses, negative anti-GAD anti-bodies and preserved urinary C-peptide excretion (22.5 ± 2.3 nmol/day). Estimated duration of the disease was 10 ± 1 years. Diabetic retinopathy was present in 26 patients; 12 had simple, and 14 proliferative retinopathy. Persistent proteinuria (exceeding 300 mg/day) was found in 6 patients, microalbuminuria (30-300 mg/day) in 18, and symptomatic diabetic neuropathy in 34. Serum creatinine concentrations were below 136 μmol/l in all subjects. Subjects with liver cirrhosis and endocrine disorders were all excluded. Nine subjects were treated with diet therapy alone, 31 with sulfonylurea and 14 with insulin on admission. During the hospitalization, sulfonylurea was introduced in 2 out of the 9 subjects treated with diet therapy alone, and insulin (20 ± 2 units/day) was substituted in 12 out of the 31 subjects on sulfonylurea therapy. Insulin dose was increased by 2-14 units/day in 10 out of the 14 subjects treated with insulin on admission. Troglitazone and metformin were not prescribed throughout the study period because of possible confounding effects of these insulin-sensitizing agents on circulating leptin concentrations [18, 19].

Energy intake was maintained at 25 kcal per ideal body weight (kg) in patients with BMI greater than 25 kg/m² (n=22), and at 27 kcal per ideal body weight in the remaining 32 patients. Ideal body weight (kg) was calculated by the formula; (height in m)² × 22 [20]. The ratio of carbohydrate, protein and fat was fixed at 53-58, 17-20 and 22-26% of the daily energy intake, respectively. Adherence to diet therapy was strictly encouraged throughout the hospitalization period. In addition, they were put on a walking regimen of 10,000 paces/day as much as possible. Exercise was prohibited in 6 subjects with persistent proteinuria and 3 subjects with active proliferative retinopathy.

On the day after admission and a few days before discharge (day 24 ± 4), antecubital venous blood was taken at 8:00 AM after an overnight fast to measure plasma glucose, HbA1c, C-peptide, total cholesterol, HDL-cholesterol, triglyceride, total ketone bodies and leptin. At the same time, body weight and percent body fat were measured in each subject with a bioelectrical impedance analyzer (TBF-305, Tanita, Tokyo, Japan).

After discharge, 38 subjects (16 males and 22 females) out of the 54 patients were followed up at Jichi Medical School Hospital, and the remaining 16
subjects were referred to other hospitals. In the 38 subjects, plasma leptin concentrations were measured at 3 months after the discharge. BMI and HbA1c were also determined at 3 and 6 months after discharge. There were no differences in ages, sex, BMI and the therapeutic modes at discharge between the 38 subjects followed up and the overall 54 patients (data not shown).

Seventy-four non-diabetic control subjects (39 males and 35 females, aged 44±2 years), selected from healthy subjects who had been examined for a regular health checkup, served as controls to examine the relationship between BMI and plasma leptin.

The protocol was approved by the local ethics committee at Jichi Medical School, and informed consent was obtained from all subjects.

**Measurements**

Plasma glucose concentrations were measured by glucose-oxidase method, and HbA1c by HPLC method. Plasma levels of total cholesterol, HDL-cholesterol, triglyceride and total ketone bodies were analyzed by enzymatic methods. Plasma C-peptide and leptin concentrations were measured by radio-immunoassay kits (Daiichi-Radioisotope, Tokyo, Japan, and Linco Research Inc., St. Charles, MO, respectively). The intra- and inter-assay coefficients of variation were less than 5% for C-peptide and leptin.

**Table 1.** Clinical and biochemical characteristics of the 54 patients with type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>on admission</th>
<th>at discharge</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2±0.6</td>
<td>23.8±0.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>percent body fat (%)</td>
<td>27.6±1.5</td>
<td>26.1±1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fasting glucose (mmol/l)</td>
<td>9.8±0.4</td>
<td>6.3±0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.2±0.2</td>
<td>6.8±0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>fasting C-peptide (mmol/l)*</td>
<td>0.66±0.04</td>
<td>0.55±0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>total cholesterol (mmol/l)</td>
<td>5.6±0.2</td>
<td>4.7±0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2±0.1</td>
<td>1.2±0.0</td>
<td>NS</td>
</tr>
<tr>
<td>triglyceride (mmol/l)</td>
<td>1.7±0.1</td>
<td>1.3±0.1</td>
<td>&lt;0.0004</td>
</tr>
<tr>
<td>total ketone bodies (umol/l)*</td>
<td>156±18</td>
<td>242±39</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>leptin (µg/l)</td>
<td>6.9±0.7</td>
<td>5.7±0.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Fasting C-peptide and total ketone bodies were determined in 47 and 33 out of the 54 patients, respectively. NS, not significant.

**Statistical analysis**

All data were shown as mean±SEM. Simple linear regression analysis was performed to calculate correlation. Student's paired t-test, or one-way analysis of variance (ANOVA) followed by Fisher's t-test were appropriately used to compare differences. Multiple regression analysis was used to evaluate determinants of the %change in plasma leptin concentrations. The statistical package StatView (Abacus Concepts, Berkeley, CA) for Macintosh version 4.5 was employed for these analyses. A p-value less than 5% was considered significant.

**Results**

Clinical and biochemical characteristics of the 54 subjects with type 2 diabetes mellitus enrolled in the present study are shown in Table 1. On admission, plasma leptin concentrations were 6.9±0.7 µg/l, ranging from 1.3 to 26.7 µg/l, and showed a strong correlation with BMI (r=0.662, P<0.0001) and with percent body fat (r=0.644, P<0.0001), respectively. Plasma leptin concentrations in female subjects were higher than those in male subjects with the same BMI or percent body fat (data not shown). Regression lines between plasma leptin and BMI were equivalent between the diabetic and control subjects in both sexes (data not shown). During hospitalization, BMI, percent body fat, fasting glucose, HbA1c, fast-
ing C-peptide, plasma total cholesterol and triglyceride concentrations were significantly decreased, whereas plasma levels of total ketone bodies were increased (Table 1). Plasma leptin concentrations at discharge (5.7±0.6 μg/l) were significantly lower

\begin{table}
\centering
\begin{tabular}{|l|c|c|}
\hline
Parameter & Correlation coefficient (r) & p \\
\hline
BMI & 0.526 & <0.0001 \\
percent body fat & 0.302 & 0.054 \\
fasting glucose & 0.214 & 0.120 \\
HbA1c & 0.105 & 0.523 \\
fasting C-peptide & 0.446 & <0.002 \\
total cholesterol & 0.436 & <0.001 \\
HDL cholesterol & 0.116 & 0.413 \\
triglyceride & 0.487 & <0.001 \\
total ketone bodies & -0.516 & <0.005 \\
\hline
\end{tabular}
\caption{Correlation between %change in plasma leptin and %changes in other variables during hospitalization.}
\end{table}

Fig. 1. Relationship between %change in plasma leptin concentrations and %changes in BMI (A), fasting plasma C-peptide (B) and plasma total ketone bodies (C) during hospitalization.

than those on admission (Table 1). BMI, percent body fat and plasma leptin were decreased by 1.7%, 4.7% and 13.9%, respectively. The decrease in plasma leptin was prominent in subjects with BMI greater than 25 kg/m² (from 9.5±1.4 to 6.6±1.0
Table 2 summarizes the correlations between the %change in plasma leptin concentrations and %changes in the other variables during hospitalization. The %change in plasma leptin was positively correlated with %changes in BMI, fasting C-peptide, plasma total cholesterol, and triglyceride concentrations, whereas it was negatively related to a %change in plasma ketone bodies (Fig. 1). Multiple regression analysis revealed that the %changes in BMI and fasting C-peptide were independently related to the %changes in plasma leptin concentrations (Table 3).

After discharge, the BMI of the 38 subjects who were followed up was 24.5 ± 0.7 kg/m² at discharge, remained unchanged at 3 months (24.6 ± 0.7 kg/m²) and then gradually increased to 24.9 ± 0.7 kg/m² at 6 months after discharge (P < 0.05 as compared to values at discharge) (Fig. 2A). Plasma leptin concentrations at discharge were 6.0 ± 0.8 µg/l in these subjects, and then increased to 7.2 ± 0.8 µg/l at 3 months (P < 0.05, as compared to values at discharge) (Fig. 2A). The %change in plasma leptin (+25.6%) was again greater than that in BMI (+0.9% at 3 months and +1.9% at 6 months, respectively) (Fig. 2B). There were positive correlations between the %change in plasma leptin concentrations during the 3 months, and that in BMI during the 3 and 6 months after discharge (Fig. 3). The increase in plasma leptin was prominent in the subjects with BMI greater than 25 kg/m² (from 6.7 ± 1.1 to 8.8 ± 1.4 µg/l, by 42.6%, n = 16).

As compared to the levels at discharge, plasma leptin levels 3 months later were increased in 28 subjects (from 5.1 ± 0.7 to 7.6 ± 1.0 µg/l, by 53.6%) and decreased in 10 subjects (from 8.8 ± 2.1 to 6.4 ± 1.4 µg/l, by 24.7%). In the former, BMI was 24.6 ± 1.0 kg/m² at discharge, remained unchanged at 3 months (25.0 ± 0.9 kg/m²), and then increased to

Table 3. Multiple regression analysis of the relationship between %change in plasma leptin and %changes in other variables during hospitalization.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>4.190</td>
<td>1.298</td>
<td>0.002</td>
</tr>
<tr>
<td>fasting C-peptide</td>
<td>0.312</td>
<td>0.115</td>
<td>0.009</td>
</tr>
<tr>
<td>fasting plasma glucose</td>
<td>0.078</td>
<td>0.135</td>
<td>0.566</td>
</tr>
</tbody>
</table>

N=47, r²=0.36. SE, standard error of parameter estimate.
25.4±0.9 kg/m² at 6 months after discharge (P < 0.005). In contrast, in the latter subjects BMI remained unchanged (24.2±0.8 at discharge, 23.6±0.7 at 3 months, and 23.7±0.6 kg/m² at 6 months, respectively).

Among the 38 subjects followed up, 17 subjects were finally treated with insulin. Throughout the observation period, there were no differences in time course change in plasma leptin levels between the 17 insulin-treated subjects and the remaining 21 subjects (data not shown). Plasma leptin levels at discharge and 3 months after discharge were slightly higher in insulin-treated subjects than the remaining subjects despite the same BMI (data not shown).

**Discussion**

During hospitalization, the decrease in plasma leptin showed a significant correlation with the decrease in BMI in both simple and multiple regression analyses, but its magnitude was greater than the values expected by the decrease in BMI and body fat. These results were consistent with the previous observations in short-term (less than 60 hours) fasting studies [8, 15] and in long-term (several days or weeks) energy restriction studies [16]. This would indicate that factor(s) other than body fat are involved in the decrease in circulating leptin by energy restriction.

First, the decrease in plasma leptin was associated with the decrease in fasting C-peptide concentrations in both simple and multiple regression analyses. Basal insulin secretion is strongly associated with circulating leptin concentrations [21], and insulin plays a crucial role in the maintenance of circulating leptin levels [8, 9]. Recent data also reveal that the decrease in plasma leptin by energy restriction is correlated with the decrease in plasma insulin concentrations in non-diabetic subjects [22]. Therefore, the decreased insulin secretion during hospitalization might be involved in the decrease in plasma leptin.

Secondly, we found a significant correlation between the increase in plasma ketone bodies and the decrease in plasma leptin. The increase in plasma ketone bodies implies accelerated lipolysis due to glucose deprivation as an energy source. Kolaczynski et al. reported that ketone bodies per se do not affect circulating leptin concentrations [23]. These data suggest that some unknown signals, which sense glucose deprivation, might decrease obese gene expression or the release of leptin into circulation, along with accelerating lipolysis and ketogenesis [24]. Free fatty acid itself might be a candidate for such signals [25], although the exact mechanisms have yet to be clarified.

Thirdly, markedly improved glycemic control may be a causative factor in the decrease in plasma leptin. It has been reported that glucosamine is one of the key factors that upregulate the obese gene expression [26], and that diabetes mellitus itself is associated with increased glucosamine accumulation. How-
ever, we found no difference in the relationship between plasma leptin and BMI according to the presence or absence of diabetes, nor was the decrease in fasting glucose associated with the decrease in plasma leptin. Therefore, it seems unlikely that improved glycemic control decreased circulating leptin concentrations.

In the follow-up study, we found a slight but significant increase in BMI and a moderate increase in plasma leptin. These results indicate that increased energy intake after discharge might increase both plasma leptin concentrations and BMI. In fact, the 28 subjects, in whom plasma leptin levels were increased, did show a gain in their body weight, whereas the remaining 10 subjects without the increase in plasma leptin kept their body weight unchanged. Therefore, we assumed that the change in plasma leptin levels reflects adherence to diet therapy in those subjects.

Adequate energy restriction is mandatory for the improvement of glycemic control in subjects with type 2 diabetes [17]. In the present study, we showed that effective energy restriction was associated with the greater decrease in plasma leptin concentration than the decrease in BMI and body fat. The rebound increase in plasma leptin after discharge was closely related to the increase in BMI, but was again greater in magnitude than BMI. These changes were prominent especially in subjects with BMI greater than 25 kg/m². We propose that serial leptin measurement may be useful to estimate adherence to energy restriction in obese subjects with type 2 diabetes.

References

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