Relationship of regional adiposity to serum leptin level in nonobese Japanese type 2 diabetic male patients

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SUMMARY

Background: The aim of the present study was to investigate the relationships between serum leptin levels and regional adipose fat area, BMI, and the measures of variables including serum insulin in nonobese Japanese type 2 diabetic patients.

Methods: A total of 121 nonobese Japanese type 2 diabetic patients (aged 35 to 83 years, body mass index (BMI) (15.4 to 26.8 kg/m²)) were studied. They all were male patients. In conjunction with serum leptin level, BMI, glycosylated hemoglobin (HbA₁c), and fasting concentrations of plasma glucose and serum insulin and lipids (triglycerides, total and HDL cholesterol) were measured.

Results: Univariate regression analysis showed that serum leptin levels were positively correlated to subcutaneous (r = 0.566, P < 0.0001) and visceral (r = 0.481, P < 0.005) fat area in our diabetic patients. Furthermore, serum leptin levels were positively correlated to serum insulin (r = 0.517, P < 0.0001), BMI (r = 0.428, P < 0.0001), serum triglycerides (r = 0.279, P < 0.005), and age (r = 0.225, P < 0.05). There was, however, no relationship between serum leptin levels and measures of other variables including total and HDL cholesterol. Multiple regression analyses showed that serum leptin levels were predicted by subcutaneous fat area (F = 5.92, P < 0.0001) and serum insulin level (F = 5.60, P < 0.0001), which explained 29.0% of the variability of serum leptin concentrations in our nonobese Japanese type 2 diabetic male patients. Visceral fat area, BMI, serum triglycerides, and age, however, were not independently associated with serum leptin levels in our patients.

Conclusions: These results indicate that serum leptin levels are reflective of subcutaneous fat area in nonobese Japanese type 2 diabetic male patients.

Key-words: Regional Adiposity · Leptin · Nonobese · Male · Type 2 Diabetes.

RESUMÉ

Relation entre l’adiposité régionale et le niveau sérique de leptine chez des hommes diabétiques de type 2 Japonais non obèses

Contexte : L’objectif de cette étude était d’investiguer les liens entre les niveaux sériques de leptine et l’adiposité régionale, le BMI, et diverses variables dont l’insulinémie chez des diabétiques de type 2 japonais non obèses.

Méthodes : Un total de 121 diabétiques de type 2 japonais non obèses (âge 35 à 83 ans, index de masse corporelle BMI 15,4 à 26,8 kg/m²) a été étudié. Tous étaient des hommes. Les paramètres mesurés étaient la leptinémie, le BMI, l’hémoglobine glyquée (HbA₁c), et les niveaux à jeun de glycéémie, insulinémie et lipides (triglycérides, cholestérol total et HDL).

Résultats : L’analyse de régression univariée a montré que les niveaux sériques de leptine étaient corrélés positivement à l’aire adipeuse sous-cutanée (r = 0,566, P < 0,0001) et vésiculaire (r = 0,481, P < 0,05) chez nos patients diabétiques. En outre, les leptinémies étaient corrélées positivement à l’insulinémie (r = 0,517, P < 0,0001), au BMI (r = 0,428, P < 0,0001), aux triglycérides circulants (r = 0,279, P < 0,005), et à l’âge (r = 0,225, P < 0,05). Cependant, il n’y avait pas de relation entre la leptinémie et les autres variables dont le cholestérol total et HDL. Les analyses de régression multiple ont montré que les leptinémies étaient prédites par l’aire adipeuse sous-cutanée (F = 5,92, P < 0,0001) et par l’insulinémie (F = 5,60, P < 0,0001), qui expliquent 29,0 % de la variabilité de la leptinémie chez nos patients diabétiques de type 2 japonais non obèses. L’aire adipeuse vésiculaire, le BMI, les triglycérides et l’âge n’étaient cependant pas indépendamment associés avec la leptinémie chez nos patients.

Conclusions : Ces résultats montrent que les taux circulants de leptine reflètent l’aire adipeuse sous-cutanée chez des hommes diabétiques de type 2 japonais non obèses.

Mots-clés : Adiposité régionale · Leptine · Non obèse · Hommes · Diabète de type 2.
Leptin is the neuroregulatory peptide secreted from adipose tissue [1]. Fasting induces a pronounced reduction in leptin whereas overfeeding can increase plasma leptin level. Weight loss and weight gain is associated with a reduction and an increase in plasma leptin concentration, respectively. Such findings suggest that leptin is responsive to changes in energy balance and that leptin is modulated by the degree of body mass index (BMI), adiposity, or of plasma insulin levels. Some investigators have shown a strong association of plasma leptin level with BMI and percent body fat [2, 3]. On the other hand, several lines of literatures suggest that plasma insulin level but not BMI is linked to leptin concentration in man [4-6]. These data raise the hypothesis that body fat distribution is associated with serum leptin level in man.

There are little data regarding the relationship between body fat distribution and leptin level. Ronnemaa et al. [7] showed that visceral but not subcutaneous adipose tissue was correlated with leptin level in Swedish identical twins discordant for obesity. In contrast, Banerji et al. [8] demonstrated that leptin levels are correlated with subcutaneous but not with visceral adipose tissue in Asian Indian healthy males without a known history of diabetes. Snop et al. [9] very recently found that subcutaneous fat deposition correlated with circulating leptin levels, while intra-abdominal fat did not in healthy nondiabetic subjects. The reason for the discrepant results among the groups is currently unknown. It may be due to the racial difference.

To the best of our knowledge, however, the relationship between regional body fat distribution and leptin level was not fully investigated in type 2 diabetic patients yet. In this regard, a major problem is that the degree of being overweight or of hyperglycemia per se affects serum leptin level and regional body fat distribution in man. Moreover, it is well recognized that leptin level is influenced by gender. To overcome this difficulty, we recruited nonobese well recognized that leptin level is influenced by gender. To overcome this difficulty, we recruited nonobese well controlled unique Japanese type 2 diabetic male patients taking into account of HbA1c and fasting glucose level, and investigated the relationships between body fat distribution and serum leptin level. This is the first description that subcutaneous but not visceral abdominal fat area is the strongest predictor of leptin level in nonobese Japanese type 2 diabetic male patients.

**Research design and methods**

One hundred and twenty-one Japanese type 2 diabetic male patients attending at Kansai-Denryoku Hospital were employed for the present study. Type 2 diabetes mellitus was diagnosed based on the criteria of WHO [10]. Anti GAD antibodies were negative in all patients studied. The duration of diabetes was 9.1 ± 0.7 years (mean ± SEM). They all have not received insulin therapy. The patients were treated with diet alone (60 patients) or diet in combination with sulfonylurea (gliclazide) (61 patients). All subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. None of the subjects had significant renal, hepatic, or cardiovascular disease. Thirty-five of 121 patients had hypertension that was treated with angiotensin converting enzyme inhibitors (21/35), calcium channel blockers (13/35), or both (1/35). They did not consume alcohol or perform heavy exercise for at least one week before the study.

The blood was drawn at the morning after a 12-hour fast. Plasma glucose, triglycerides, total and HDL cholesterol were measured. Serum insulin was measured using a two-site immunoradiometric assay (Insulin Ribead II, Dainabot, Japan). Coefficients of variation were 4% for insulin > 25 µU/ml and 7% for insulin < 25 µU/ml, respectively. There was no detectable crossreactivity of proinsulin in the insulin assay. Serum leptin concentration was measured with a radioimmunoassay kit (Linco Research, St Charles, MO) using specific human leptin antibody [11]. The intraassay and interassay coefficients of variation were less than 5% for leptin [3].

All subjects underwent computed tomography (TSX-012A, X-Vigor, Toshiba Co. Ltd. Japan) to measure cross-sectional abdominal subcutaneous and visceral fat areas using commercially available software (Fat Scan, N2 System Corporation, Osaka, Japan) as described previously [12, 13].

**Statistical analysis**

Data were presented as mean ± SEM. Statistical analyses were conducted using the StatView 5 system (Statview, Berkeley, CA). Simple (Spearman’s rank) correlation coefficient and stepwise multiple regression analyses were used to examine the relationships between serum leptin level and subcutaneous or visceral abdominal fat area, BMI, or the measures of variables including serum insulin. P < 0.05 was considered as significant. In multivariate analysis, F value ≥ 4 was considered significant.

**Results**

The subjects studied were all male Japanese type 2 diabetic patients with an age range of 35 to 83 years (59.4 ± 1.0 yr) and a BMI of 15.4 to 26.8 kg/m² (22.8 ± 0.2 kg/m²). They all were nonobese. The fasting plasma glucose was 147 ± 4 mg/dl and glycosylated hemoglobin (HbA1c) was 7.0 ± 0.1%. Serum triglycerides, and total and HDL cholesterol levels were 121 ± 9 mg/dl (range, 30 to 919), 196 ± 4 mg/dl (range, 118 to 446), and 54 ± 2 mg/dl (range, 27 to 218), respectively. Serum insulin level was 6.4 ± 0.3 µU/ml (range, 1.3 to 22) (Table I).

There was a wide variation not only in leptin level but also in subcutaneous and visceral abdominal fat areas. Serum leptin level was 3.3 ± 0.2 mg/dl (range, 1.0 to 11.2 mg/dl). Subcutaneous and visceral abdominal fat areas were 108 ± 4 cm² (range 27.6 to 218.7 cm²) and 96 ± 4 cm² (range 12.2
Regional adiposity and leptin level in nonobese type 2 diabetes

Table I
Clinical characteristics and clinical profile in diabetic male patients.

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<tbody>
<tr>
<td>Number of subjects</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>59.4±1.0</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
<td>9.1±0.7</td>
<td></td>
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<tr>
<td>Diet/SU/insulin</td>
<td>60/61/0</td>
<td></td>
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<tr>
<td>BMI (kg/m²)</td>
<td>22.8±0.2</td>
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<tr>
<td>Fasting glucose (mg/dl)</td>
<td>147±4</td>
<td></td>
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<tr>
<td>HbA₁c (%)</td>
<td>7.0±0.1</td>
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</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>121±9</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>196±4</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>54±2</td>
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<tr>
<td>Fasting insulin (µU/ml)</td>
<td>6.4±0.3</td>
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</tr>
<tr>
<td>Leptin (µg/dl)</td>
<td>3.3±0.2</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat area (cm²)</td>
<td>108±4</td>
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<tr>
<td>Visceral fat area (cm²)</td>
<td>96±4</td>
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to 205.6 cm², respectively. There was a positive correlation between subcutaneous and visceral abdominal fat (r = 0.686, P < 0.001). A positive correlation was observed between BMI and subcutaneous (r = 0.762, P < 0.0001) and visceral (r = 0.529, P < 0.0001) fat area, also.

Table II demonstrates the correlation between serum leptin level and subcutaneous or visceral fat areas, BMI, or the measures of variables including serum insulin in our diabetic patients. Serum leptin levels were positively correlated to subcutaneous (r = 0.566, P < 0.0001) and visceral (r = 0.481, P < 0.0001) fat area in our diabetic patients. Furthermore, serum leptin levels were positively correlated to serum insulin (r = 0.517, P < 0.0001), BMI (r = 0.428, P < 0.0001), serum triglycerides (r = 0.279, P < 0.005), and age (r = 0.225, P < 0.05). There was, however, no relationship between serum leptin levels and measures of other variables including total and HDL cholesterol (Tab II).

Multiple regression analyses showed that serum leptin levels were predicted by subcutaneous fat area (F = 5.92, P < 0.0001) and serum insulin level (F = 5.60, P < 0.0001), which explained 29.0% of the variability of serum leptin concentrations in our nonobese Japanese male type 2 diabetic patients. Visceral fat area (F = 0.22), BMI (F = 1.85), serum triglycerides (F = 0.05), and age (F = 0.18), however, were not independently associated with serum leptin levels in our patients (Tab II).

Discussion

In distinct from white populations, nonobese Japanese type 2 diabetic patients are unique in that they are divided into two variants: one with insulin resistance and the other with normal insulin sensitivity [14-18]. We very recently demonstrated that subcutaneous and visceral fat areas are independently associated with insulin resistance in nonobese Japanese type 2 diabetic patients [13]. This is not in agreement with the data shown by Abate et al. [19] that subcutaneous fat volume but not intraperitoneal nor retroperitoneal one is associated with insulin resistance in non-Hispanic whites with type 2 diabetes. Thus, there seems to be ethnic difference in the relationship between insulin resistance and regional adiposity in type 2 diabetic patients.

Our main question was that which adipose tissue component is associated with leptin secreted from adipose tissue in Japanese type 2 diabetic patients, since discrepant results are reported in nondiabetic subjects [7-9]. To accomplish this, we recruited nonobese well controlled unique Japanese type 2 diabetic male patients since the degree of being overweight, hyperglycemia, or gender per se is considered to be associated with body fat distribution and serum leptin levels. Body mass index in our patients was 22.8±0.2 kg/m² (range, 15.4 to 26.8), indicating that they were nonobese [20]. Their fasting glucose and HbA₁c levels were 147±4 mg/dl and 7.0±0.1%, respectively.

In the present study, we first disclosed that subcutaneous but not visceral abdominal fat area was independently associated with serum leptin level in nonobese Japanese type 2 diabetic male patients. In contrast, serum triglycerides level was associated with visceral fat area (r = 0.345, P < 0.005) but not with subcutaneous one (r = 0.124, P = 0.311) in our nonobese male patients, which also reconfirms our very recent study [15]. It may be argued that hyperinsulinemia per se could interact with leptin secretion [4-6]. However, multiple regression analysis revealed that serum leptin levels were independently associated with subcutaneous fat area and serum insulin level in our diabetic patients. Cnop et al. [9] very recently reported similar results in healthy nondiabetic sub-
jects. Thus, serum leptin level might be reflective of subcutaneous fat area in nonobese Japanese type 2 diabetic male patients.

The bases of this relationship between serum leptin level and subcutaneous fat area have yet to be clarified. It would suggest that the secretion of leptin by the adipose tissue might differ according to the site concerned with a higher secretion in the subcutaneous adipose tissue when compared to the visceral one. In this respect, fat cell size seems to be an important regulator of leptin mRNA expression. Zachwieja et al. [21] demonstrated that both the expression and secretion of leptin were influenced by exercise training in rodents and that exercised animals had an increased percentage of smaller fat cells. Leptin secretion rates have been noted to be 2 to 3 times greater in subcutaneous than in visceral fat tissue in both obese and nonobese humans [22], which was attributed to a 50% increase in fat cell size [22, 23].

In summary, although our present study was performed on a limited number of patients (n = 121), our results suggest that serum leptin level was independently associated with subcutaneous adipose tissue area in nonobese Japanese type 2 diabetic male patients. A study on a larger population should be undertaken to clarify the relationship between serum leptin level and regional abdominal fat area in Japanese type 2 diabetic patients.

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