Relationship between Visceral Fat Accumulation and Anti-Lipolytic Action of Insulin in Patients with Type 2 Diabetes Mellitus

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Abstract. Insulin resistance is closely related to developing type 2 diabetes mellitus. Visceral fat accumulation is associated with insulin resistance, which affects the free fatty acid (FFA) metabolism. We investigated the interactions among visceral fat accumulation, FFA metabolism and insulin resistance in 20 patients with type 2 diabetes mellitus, including 11 obese and 9 non-obese subjects. Body fat distribution was estimated by measuring the areas of both subcutaneous and visceral fat mass on abdominal computed tomography at the umbilical level. Glucose infusion rate (GIR) and plasma FFA responses to insulin were determined as an index of insulin resistance and anti-lipolytic action, respectively, in a euglycemic hyperinsulinemic clamp study. There was an inverse correlation between GIR and insulin-induced decrease in plasma FFA in all diabetic patients ($r = -0.653$, $P < 0.01$). Visceral fat mass area was well correlated with GIR ($r = -0.583$, $P < 0.01$) and insulin-induced decrease in plasma FFA ($r = 0.724$, $P < 0.001$), whereas subcutaneous fat mass area was not correlated either with GIR or plasma FFA decrease. These findings suggest that visceral fat accumulation results in increasing the resistance against the anti-lipolytic action of insulin, and that FFA metabolism is closely related with glucose utilization in patients with type 2 diabetes mellitus.

Key words: Visceral fat, Insulin resistance, Free fatty acid, Type 2 diabetes mellitus, Euglycemic glucose clamp


IT has been reported that the accumulation of fat mass in the intra-abdominal lesion (visceral fat mass) is associated with insulin resistance in developing type 2 diabetes mellitus [1–4]. This may be partly attributed to the impaired metabolism of free fatty acids (FFA) originating from the adipose tissue [5, 6]. Santomauro et al. [7] recently reported that the lowering of plasma FFA levels with a potent long-acting nicotinic acid analog could reduce insulin resistance and hyperinsulinemia, resulting in improved glucose tolerance in diabetic patients.

It is known that the effect of insulin on systemic lipolysis is impaired in type 2 diabetes mellitus. Groop et al. [8] reported that the suppressive effect of insulin on plasma FFA turnover was decreased in lean patients with type 2 diabetes mellitus compared with age- and weight-matched controls. However, there has been limited information regarding the implications of obesity in the effect of insulin on FFA metabolism. Chen et al. [9] observed that plasma FFA levels were suppressed in obese subjects to a similar extent as non-obese subjects under high concentrations of insulin.

It was previously demonstrated in vitro that visceral adipose tissue was resistant to the anti-lipolytic action of insulin compared with subcutaneous adipose tissue [10, 11]. In addition, it was shown that the visceral adipose tissue lipolysis was more resistant to insulin suppression than in the leg in normal subjects in vivo, suggesting a regional heterogeneity of insulin-regulated FFA release [12]. It remains to be elucidated, however, whether the visceral fat mass is
implicated in insulin-regulated systemic FFA release in diabetic patients. In comparing obese diabetic patients with equally obese non-diabetic subjects, Basu et al. [13] found that splanchnic palmitate release was similar in the two groups under high concentration of insulin although the diabetic subjects had much greater amount of visceral fat. On the other hand, it has been shown that upper body non-splanchnic [13] and abdominal deep subcutaneous fat content [14] play important roles in the rate of systemic lipolysis.

The aim of the present study was to investigate the relationship between visceral fat mass and insulin-induced decrease in plasma FFA levels under euglycemic hyperinsulinemic clamp in patients with type 2 diabetes mellitus. Here we show clear in vivo evidence that the anti-lipolytic action of insulin is reduced by accumulation of visceral fat mass.

Subjects and Methods

Subjects

Twenty patients with type 2 diabetes mellitus (10 males, 10 females) with mean (± SD) age of 55±13 yr were examined. Degree of obesity was estimated by body mass index (BMI). Type 2 diabetes mellitus was diagnosed according to the 1998 WHO guidelines [15].

In all subjects, body weight had been stable for more than three months before the study and any medication that affects insulin sensitivity and FFA metabolism was not allowed during the study. The study was approved by the ethics committee of our institution, and informed written consent was obtained from all subjects.

Methods

Intra-abdominal visceral and subcutaneous fat mass areas were estimated by abdominal computed tomography (CT) at the umbilical levels as described previously [16]. Plasma glucose, plasma FFA and blood HbA1c levels were determined by glucose oxidase method, enzymatic method (Determiner NEFAT755, Kyowa Medix Co., Tokyo, Japan) and high performance liquid chromatography (HPLC), respectively. Serum insulin levels were determined by enzyme linked immunosorbent assay (ELISA).

Euglycemic hyperinsulinemic clamp study was performed as described previously [17], using an artificial pancreas (STG-22, Nikkiso Co., Tokyo, Japan). After overnight fasting, indwelling catheters were inserted into bilateral antebrachial veins at 9 a.m. Regular human insulin (800 mU, Eli Lilly Co., Indianapolis, IN, U.S.A.) was infused for 10 min as a priming dose, followed by constant infusion of insulin at a rate of 40 mU/m²/min for 120 min. Blood glucose levels were monitored and euglycemia (5.5 mmol/l) was maintained with a variable-rate infusion of 10% glucose solution. The infusion rate of glucose solution was corrected every min and recorded. GIR was defined as the mean glucose infusion rate to maintain euglycemia during the last 30 min. Blood samples were obtained immediately before insulin infusion and every 30 min thereafter to determine plasma FFA levels. Anti-lipolytic action of insulin was estimated by % decrease in plasma FFA levels during 120 min from the basal value.

Statistical analysis

All the data were expressed as mean ± SD values. Regression analysis on % decrease in plasma FFA levels and GIR was performed by the least square method for continuous variables (age; BMI; duration of diabetes; fasting plasma glucose; fasting serum insulin; HbA1c; total cholesterol; triglyceride; fasting plasma FFA; systolic and diastolic blood pressure; and visceral and subcutaneous fat area). The interactions between % decrease in plasma FFA levels and various clinical characteristics of the subjects were examined by multiple stepwise regression analysis. The F value was set at 4.0 at each step. A probability of P<0.05 was considered significant.

Results

The clinical characteristics of the subjects are summarized in Table 1. Eleven patients were obese (BMI≥25) and nine were non-obese (BMI<25). Basal plasma FFA levels were higher in obese patients with type 2 diabetes mellitus than in non-obese patients. Both visceral fat mass area and subcutaneous fat mass area were greater in obese patients with type 2 diabetes mellitus than in non-obese patients.
Plasma FFA levels were considerably decreased and stabilized at the last two points (90 and 120 min) during the euglycemic hyperinsulinemic clamp. There was a significant correlation between insulin-induced decrease in plasma FFA levels (percent decrease in plasma FFA) and fasting serum insulin ($r=0.533$, $P<0.05$), triglyceride ($r=0.452$, $P<0.05$), and visceral fat mass area ($r=0.724$, $P<0.001$) (Fig. 1A), whereas it was not correlated with age, BMI, duration of diabetes, fasting plasma glucose, HbA1c, total cholesterol, or subcutaneous fat mass area ($r=0.171$, $P=0.471$) (Fig. 1B). When the data of men and women were analyzed separately, the percent decrease in plasma FFA was significantly correlated with visceral fat both in men ($r=0.785$, $P<0.01$) and women ($r=0.684$, $P<0.05$).

There was an inverse correlation between GIR and triglyceride ($r=-0.450$, $P<0.05$), the percent de-

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**Table 1. Clinical Characteristics of the Subjects Examined**

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Non-obese</th>
<th>Obese</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>M/F</td>
<td>10/10</td>
<td>5/4</td>
<td>5/6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55 ± 13</td>
<td>57 ± 8</td>
<td>54 ± 17</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 5.8</td>
<td>21.3 ± 3.3</td>
<td>29.7 ± 4.5**</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>4.3 ± 2.8</td>
<td>4.3 ± 2.9</td>
<td>4.4 ± 2.9</td>
</tr>
<tr>
<td>Treatment (D/OHD)</td>
<td>6/14</td>
<td>2/7</td>
<td>4/7</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>6.3 ± 1.1</td>
<td>6.2 ± 0.8</td>
<td>6.3 ± 1.3</td>
</tr>
<tr>
<td>Fasting serum insulin (pmol/l)</td>
<td>56 ± 43</td>
<td>41 ± 40</td>
<td>69 ± 43</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.9 ± 0.9</td>
<td>7.0 ± 0.9</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.84 ± 0.86</td>
<td>4.84 ± 0.96</td>
<td>4.84 ± 0.83</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.74 ± 1.34</td>
<td>1.07 ± 0.15</td>
<td>2.28 ± 0.50</td>
</tr>
<tr>
<td>Fasting plasma FFA (mEq/l)</td>
<td>0.77 ± 0.22</td>
<td>0.62 ± 0.05</td>
<td>0.89 ± 0.06*</td>
</tr>
<tr>
<td>Abdominal fat area: visceral (cm²)</td>
<td>66 ± 47</td>
<td>33 ± 27</td>
<td>94 ± 43*</td>
</tr>
<tr>
<td>subcutaneous (cm²)</td>
<td>112 ± 70</td>
<td>61 ± 42</td>
<td>154 ± 59*</td>
</tr>
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D, diet therapy; OHD, oral hypoglycemic drugs. *$P<0.01$, **$P<0.01$ vs. non-obese

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**Fig. 1.** Relationship between % decrease in plasma FFA after insulin infusion and visceral (A) and subcutaneous fat mass area (B) in 20 patients with type 2 diabetes mellitus. Open and closed circles indicate obese patients and non-obese patients, respectively.
crease in plasma FFA \( (r = -0.652, P < 0.01) \) (Fig. 2), and visceral fat mass area \( (r = -0.583, P < 0.01) \) (Fig. 3A). GIR was not correlated with age, BMI, duration of diabetes, fasting plasma glucose, fasting serum insulin, HbA1c, total cholesterol and subcutaneous fat mass area \( (r = -0.309, P = 0.186) \) (Fig. 3B). When the data of men and women were analyzed separately, GIR was significantly correlated with visceral fat both in men \( (r = -0.668, P < 0.05) \) and women \( (r = -0.680, P < 0.05) \).

To characterize the clinical variables for determining the percent decrease in plasma FFA, stepwise regression analysis was performed employing possible risk factors (BMI, fasting plasma glucose, fasting serum insulin, total cholesterol, and triglyceride). Both GIR (\( \beta \)-value = 0.54; F value = 10.1) and fasting serum insulin (\( \beta \)-value = -0.37; F value = 4.8) were identified as significant independent variables for determining the percent decrease in plasma FFA \( (r^2 = 0.56; P < 0.001) \).

![Fig. 2. Correlation between % decrease in plasma FFA levels and glucose infusion rate (GIR) in euglycemic hyperinsulinemic clamp in 20 patients with type 2 diabetes mellitus. Open and closed circles indicate obese patients and non-obese patients, respectively.](image)

![Fig. 3. Relationship between GIR and visceral (A) and subcutaneous fat mass area (B) in 20 patients with type 2 diabetes mellitus. Open and closed circles indicate obese patients and non-obese patients, respectively.](image)
Discussion

Roust and Jensen [18] demonstrated in non-diabetic premenopausal women that postprandial suppression of endogenous FFA release was reduced in upper body-obesity estimated by waist to hip circumference ratio. In the present study, we estimated the visceral and the subcutaneous fat mass by abdominal CT scan and measured plasma FFA levels during euglycemic hyperinsulinemic study to evaluate anti-lipolytic action of insulin in vivo. The percent decrease in plasma FFA was well correlated with visceral fat mass area, but not with subcutaneous fat mass area in either men and women in these diabetic patients. These findings provide in vivo evidence that the visceral adipose tissue was more resistant to the anti-lipolytic action of insulin than the subcutaneous adipose tissue.

It was reported that subcutaneous adipose tissue was more sensitive to anti-lipolytic action of insulin than visceral adipose tissue in vitro [10, 11]. Bolinder et al. [10] reported that the affinity of insulin for its receptor was higher in the subcutaneous fat cells than in the omental fat cells, although there was no difference in the number of receptors. Richelsen et al. [11] reported that insulin showed much reduced anti-lipolytic property in omental adipose tissue than in subcutaneous adipose tissue. These in vitro findings support our present observations.

Recently, Basu et al. [13] reported that visceral fat area was correlated with systemic palmitate flux during hyperinsulinemic hyperglycemic clamp when data from both diabetic and non-diabetic subjects were included in the analysis. However, when the diabetic and non-diabetic subjects were analyzed separately, they failed to demonstrate any correlation between the parameters. The apparent discrepancy could be attributed to the characteristics of the diabetic patients examined. In the study of Basu et al. [13] the diabetic patients were obese (mean BMI: 30.1 \pm 1 kg/m²) and small in number (n = 14). In the present study, we demonstrated a relationship between visceral fat and systemic FFA metabolism by insulin in twenty diabetic patients including both obese and non-obese (BMI range: 16.0–41.4 kg/m²) subjects.

Insulin is a potent anti-lipolytic hormone and decreases plasma FFA concentrations. The reduced anti-lipolytic action of insulin could lead to accelerated lipolysis, resulting in elevated plasma FFA levels. Obesity is associated with elevated fasting and postprandial plasma FFA levels in the presence of hyperinsulinemia [19, 20]. There is a line of evidence suggesting that an increase in plasma FFA levels plays a role in the development of fasting hyperglycemia in patients with type 2 diabetes mellitus [9, 20, 21]. The magnitudes of the defect in insulin suppression of plasma FFA levels and on glucose uptake in the tissue are roughly comparable [9]. Kelley et al. [22] have shown that the maintenance of elevated plasma FFA by lipid infusion during euglycemic hyperinsulinemic clamp decreased muscle glucose uptake and glucose storage in healthy subjects. Dresner et al. [23] reported that raising plasma FFA levels from 0.5 mmol/l to 1.8 mmol/l acutely induced insulin resistance. Our present findings that GIR and fasting plasma insulin levels, both of which were insulin resistance indexes, were identified as significant independent variables for determining the percent decrease in plasma FFA during euglycemic hyperinsulinemic clamp and could provide further evidence for a close association between insulin-induced decrease in plasma FFA and insulin sensitivity in the skeletal muscle.

In conclusion, it was suggested that accumulation of visceral fat mass was associated with reduced anti-lipolytic action of insulin in vivo. The impairment of FFA metabolism was closely related to glucose uptake in patients with type 2 diabetes mellitus.

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References


