Plasma dipeptidyl peptidase 4 activity correlates with body mass index and the plasma adiponectin concentration in healthy young people

Yasushi Kirino1), 2), Masako Sei1), Kazuyoshi Kawazoe2), Kazuo Minakuchi2) and Youichi Sato1), 3)

1) Department of Human Genetics and Public Health, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, Japan
2) Department of Clinical Pharmacy, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, Japan
3) Department of Pharmaceutical Information Science, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8505, Japan

Abstract. We previously found that plasma dipeptidyl peptidase 4 (DPP4) activity was associated with the development of obesity, type 2 diabetes, and type 1 diabetes using animal models. In this study, we investigated whether DPP4 activity is correlated with the clinical parameters of obesity and/or diabetes in healthy young subjects. Body mass index (BMI), plasma DPP4 activity, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, fasting blood glucose, adiponectin concentration, and body fat were measured in 165 subjects (110 males and 55 females, age 23.2 ± 2.4 years). In correlation analyses, DPP4 activity displayed strong positive correlations with BMI ($p = 5.5 \times 10^{-5}$) and total cholesterol ($p = 0.0014$), and a negative correlation with the plasma adiponectin concentration ($p = 0.013$), but not fasting blood glucose. Our findings suggest that plasma DPP4 activity is correlated with the clinical parameters of obesity rather than diabetes in young people.

Key words: DPP4 activity, Healthy young subject, Body mass index, Adiponectin

DPP4 (CD26, EC3.4.14.5) is a complex enzyme that is present on the surface of most cell types including kidney, liver, pancreas, and fat cells as well as in soluble form in the circulation [1, 2]. DPP4 is a serine protease that cleaves the penultimate L-proline or L-alanine found at the N-termini of several polypeptides, such as GLP-1, GIP, neuropeptides, and many chemokines. GLP-1 and GIP are members of the incretin hormone family, which stimulate pancreatic insulin secretion. GLP-1 and GIP were found to be involved in insulin biosynthesis, β-cell proliferation, and the inhibition of food intake in preclinical studies [3, 4]. The peripheral and central GLP-1 sensitive pathways appear to be organized to co-operatively control food intake and body weight [5]. However, these peptides are rapidly degraded and inactivated by DPP4. Therefore, it is suggested that DPP4 activity might be associated with the onset and/or severity of obesity and diabetes.

Previously, we found that plasma DPP4 activity was significantly greater in impaired glucose tolerance rats fed a high-fat or high-sucrose diet than in normal rats [6]. Additionally, using Otsuka Long-Evans Tokushima fatty rats, which display a spontaneously hyperphagic and obese phenotype in combination with hyperglycemia and hyperinsulinemia, we showed that plasma DPP4 activity changes in accordance with the progression of hyperinsulinemic obesity and pancreatic islet atrophy [7]. Furthermore, we reported that the plasma DPP4 level and DPP4 mRNA expression in some tissues increased progressively with the development of streptozotocin-induced type 1 diabetes [8].

Although several clinical studies have investigated whether DPP4 activity is correlated with the onset or severity of diabetes and/or obesity, they have produced mixed results; i.e., plasma DPP4 activity levels were found to increase [9, 10] and decrease [11-13] in patients with type-2 diabetes. Moreover, it has been
reported that the degree of plasma DPP4 activity was significantly higher in obese subjects than in lean subjects [14, 15]; however, the DPP4 hyperactivity in obese individuals did not seem to be affected by their degree of overweightness [14]. Moreover, it has been reported that the degree of plasma DPP4 activity was affected by widely used anti-diabetic agents, such as metformin [16], and ageing [12]. Taking these findings together, it might be difficult to define the relationship between DPP4 activity and the levels of blood glucose and BMI because of the diverse clinical backgrounds of subjects; therefore, data from healthy young subjects would help us to identify whether DPP4 activity is physiologically involved in diabetes and/or obesity.

In this study, we investigated the correlations between plasma DPP4 activity and various risk factors for obesity and diabetes in healthy young subjects.

Materials and Methods

Subjects and measurement of their clinical parameters

One hundred and sixty-five young Japanese students (110 males and 55 females) from the University of Tokushima (Tokushima, Japan) participated in this study. Prior to their inclusion, they were confirmed to be generally healthy and free from medical problems. Informed consent was obtained from all participants, and the study was approved by the medical ethics committee of the University of Tokushima, Tokushima, Japan. BMI, total cholesterol, HDL cholesterol, triglycerides, and fasting blood glucose were automatically measured, as described previously [17], and body fat was measured using the TBF-310 Body Fat Analyzer (Tanita Corp., Tokyo, Japan). After overnight fasting, 10 ml of venous blood were taken from each participant under sterile conditions. Plasma samples were obtained by centrifugation and stored at -80 °C until analysis. Total cholesterol, HDL cholesterol, triglycerides, and blood glucose were automatically measured using an automatic analyzer (Model No.7150; Hitachi High-Technologies Corp., Tokyo, Japan). The plasma adiponectin concentration was measured using a human adiponectin enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical, Tokyo, Japan).

DPP4 enzyme assay

DPP4 activity was determined as the rate of cleavage of 7-amino-4-methylcoumarin (AMC) from the synthetic substrate H-glycyl-prolyl-AMC (Gly-Pro-AMC; Sigma, St. Louis, MO, U.S.A.), as described previously [6-8]. Briefly, 5 μL of plasma were mixed with 35 μL of assay buffer (25 mmol/L HEPES, 140 mmol/L NaCl, 80 mmol/L MgCl2, and 1 % BSA; pH 7.8). After 5-min preincubation at room temperature, the reaction was initiated by the addition of 40 μL of assay buffer containing 0.1 mmol/L of the Gly-Pro-AMC substrate. After 20 min incubation, fluorescence was determined using a spectrofluorometer (Tecan Infinite™ M200, Tecan Japan, Yokohama; excitation: 380 nm/emission: 460 nm). A standard curve for free AMC was generated using 0-50 μmol/L solutions of AMC (Sigma). Plasma DPP4 activity is expressed as the amount of cleaved AMC per minute per mL (nmol/min/mL).

Statistical analysis

Values are expressed as the mean ± SD. Pearson’s correlation and partial correlation analysis were used to examine the correlations between DPP4 activity and BMI, body fat, total cholesterol, triglycerides, adiponectin, etc. All statistical analyses were performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL), and statistical significance was indicated by p values of < 0.05.

Results

The mean age of the recruited subjects was 23.2 ± 2.4 years. Their mean BMI, total cholesterol, HDL cholesterol, triglycerides, body fat, and fasting blood glucose values were 21.2 ± 2.8 kg/m2, 172.5 ± 27.8 mg/dL, 59.8 ± 11.6 mg/dL, 70.9 ± 31.4 mg/dL, 19.3 ± 5.6 %, and 88.5 ± 5.9 mg/dL, respectively (Table 1). They were confirmed to be healthy and free from medical problems. The mean plasma adiponectin concentration was 17.2 ± 9.2 μg/mL and showed strong negative correlations with BMI (r = - 0.14, p = 0.0017) and total cholesterol (r = 0.27, p = 0.0006) and a positive correlation with HDL cholesterol (r = 0.25, p = 0.0014) and a positive correlation with HDL cholesterol (r = 0.28, p = 0.00029) (Table 2).

In these subjects, plasma DPP4 activity displayed strong positive correlations with BMI (r = - 0.21, p = 0.0067), and triglycerides (r = - 0.19, p = 0.014) and a positive correlation with HDL cholesterol (r = 0.28, p = 0.00029) (Table 3).

The correlations between DPP4 activity and BMI (r = 0.24, p = 0.0017) and total cholesterol (r = 0.27, p = 0.0006) remained significant after adjusting for age and sex (Table 3), but not adiponectin (r = -0.14, p = 0.08). On the other hand, plasma DPP4 activity was not corre-
Table 1 The subjects' characteristics and biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>n (Male/Female)</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>Total cholesterol (mg/dL)</th>
<th>HDL cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>Body fat (%)</th>
<th>Fasting blood glucose (mg/dL)</th>
<th>Adiponectin (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>165 (110/55)</td>
<td>23.2 ± 2.4</td>
<td>21.2 ± 2.8</td>
<td>172.5 ± 27.8</td>
<td>59.8 ± 11.6</td>
<td>70.9 ± 31.4</td>
<td>19.3 ± 5.6</td>
<td>88.5 ± 5.9</td>
<td>17.2 ± 9.2</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.

Table 2 Correlations between adiponectin concentration and the subjects' characteristics

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.05</td>
<td>0.49</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.44</td>
<td>3.8 × 10⁻⁹</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.07</td>
<td>0.93</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.28</td>
<td>0.00029</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.19</td>
<td>0.014</td>
</tr>
<tr>
<td>Body fat</td>
<td>-0.14</td>
<td>0.073</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>-0.21</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

**Fig. 1** Correlations between DPP4 activity and (a) BMI, (b) plasma adiponectin, and (c) total cholesterol
lated with the fasting blood glucose level, HDL cholesterol, triglycerides, or body fat.

**Discussion**

Adiponectin is a 30-kDa hormone that is produced exclusively by adipocytes. It is well known that the plasma adiponectin concentration is associated with BMI, the risk of type 2 diabetes, lipid profile, and cardiovascular disease (CVD). However, previous studies have shown that the association between the adiponectin concentration and BMI differed according to age, gender, and ethnicity. Hirose et al. reported that the serum adiponectin level was negatively correlated with BMI and glucose and positively correlated with HDL cholesterol in healthy Japanese individuals ranging in age from 30-65 [18]. In addition, Medina-Bravo et al. reported that in a prepubertal population, the obese children displayed lower adiponectin concentrations than the non-obese subjects, but this was not the case in the pubertal group [19]. The present results showed that the plasma adiponectin concentration was negatively correlated with BMI, fasting blood glucose, and triglycerides and a positive correlation with HDL cholesterol in healthy young subjects with a mean age of 23.2 ± 2.4 years. It is suggested that the secretion and/or synthesis of adiponectin is affected by each individual’s BMI, lipid profile, and blood glucose levels, even in healthy Japanese in their twenties.

As described in the Introduction, it is disputed whether DPP4 activity is correlated with the onset or severity of obesity. Recently, Lamers et al. reported that DPP4 is an adipokine that might be linked to obesity, and the serum DPP4 concentration is correlated with various clinical parameters such as age; BMI; the sizes of subcutaneous and visceral adipocytes; and the levels of insulin, adiponectin, and leptin in lean (n = 20) and morbidly obese (n =20) men [15]. Our data are consistent with those of previous studies and support the evidence obtained from obese and healthy young subjects. Although we did not detect a significant correlation between DPP4 activity and body fat, high DPP4 activity at a young age might induce the subsequent accumulation of body fat, but further studies are required to confirm this.

In this study, plasma DPP4 activity was not correlated with the fasting blood glucose level in healthy young subjects. Clinical studies have been reported that DPP4 activity showed significant correlations with plasma glucose and HbA1c levels in diabetic patients [9, 10]. Moreover, an in vitro study determined that exposure to high levels of glucose enhanced the biosynthesis of DPP4 in human glomerular endothelial cells [20]. Pala et al. reported that oral glucose loading did not induce DPP4 activation in normal, impaired glucose tolerance, or type 2 diabetes subjects [21]. In addition, Ryskjaer et al. reported that plasma DPP4 activity is not altered following meal ingestion or acute changes in the plasma glucose level [10]. Taking these findings together, we assume that DPP4 biosynthesis is correlated with long-term exposure to a high glucose concentration.

In conclusion, plasma DPP4 activity was found to be associated with BMI, the plasma adiponectin concentration, and total cholesterol, but not fasting blood glucose in healthy young subjects. Long-term obesity and lipid accumulation cause insulin resistance and hyperglycemia through abnormal adipocytokine secretion from adipocytes [22]. The present study suggests that plasma DPP4 activity is physiologically involved in the early stages of adiposity prior to hyperglycemia. Longitudinal follow-up studies are needed to ascertain whether plasma DPP4 activity is an early marker of the progression from obesity to metabolic syndrome and/or type 2 diabetes.

**Acknowledgements**

We are grateful to the late Prof. Yutaka Nakahori of the University of Tokushima for his helpful advice. There is no relationship which may lead to a conflict of interest.

**References**


DPP4 activity correlates with BMI


