Visceral fat obesity increases serum DPP-4 levels in men with type 2 diabetes mellitus

Short title: DPP-4 and visceral fat

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Abbreviations

DPP-4: dipeptidyl peptidase-4, T2DM: type 2 diabetes mellitus, MetS: metabolic syndrome: GLP-1, glucagon-like peptide 1: GIP, gastric inhibitory polypeptide

Abstract

Objective: The relationship between serum DPP-4 level and visceral fat mass is still unclear in type 2 diabetes mellitus (T2DM). This study thus aimed to examine the association of visceral fat accumulation and metabolic syndrome with serum DPP-4 levels in T2DM.

Methods: Visceral and subcutaneous fat areas were evaluated by performing computed tomography scan in 135 men with T2DM, who had never taken DPP-4 inhibitors or GLP-1 receptor agonists. We investigated the association between serum DPP-4 levels and visceral fat area as well as the presence of metabolic syndrome.

Results: Multiple regression analysis adjusted for age, duration of T2DM, body mass index, serum creatinine, and HbA1c showed that serum DPP-4 levels were positively associated with visceral fat area (β=0.25, p=0.04), but not subcutaneous fat area (β=-0.18, p=0.13). In logistic regression analyses adjusted for the confounding factors described above, serum DPP-4 levels were positively associated with visceral fat obesity and metabolic syndrome [odds ratio (OR)=1.63, 95% confidence interval (CI)=1.00-2.66 per standard deviation (SD) increase, p=0.04; OR=1.77, 95%CI=1.09-2.88 per SD increase, p=0.02, respectively].

Conclusions: The present study showed that serum DPP-4 level was positively and specifically associated with accumulation of visceral fat and the presence of metabolic syndrome in men with T2DM.

Introduction

Dipeptidyl peptidase-4 (DPP-4) is a ubiquitously expressed transmembrane glycoprotein and rapidly cleaves N-terminal dipeptides from a variety of substrates including incretin hormones such as glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), resulting in inactivation of the incretin hormones [1-3]. Incretin has been shown to increase insulin secretion and suppress glucagon secretion as well as food intake, and the action of these incretin hormones are reported to be impaired in patients with type 2 diabetes mellitus (T2DM) [4, 5]. Because the inhibition of DPP-4 leads to an increase in circulating endogenous intact GLP-1 as well as GIP and improves glycemic control in patients with T2DM, DPP-4 has gained considerable interest as a therapeutic target. DPP-4 inhibitors that prolong the insulinotropic effects of incretin are now worldwidely available for treatment of type 2 diabetes.

Several studies on adipocyte function have revealed that not only is adipose tissue an energy-storing organ but also it secretes a variety of biologically active molecules, which are named adipokines [6]. Adipose tissue has crosstalk with various organs such as the brain, liver, pancreas, immune system, muscle, and others through the adipokines [7]. The disruption of the adipokines by adipose tissue dysfunction is now considered to be a main cause of obesity-associated diseases such as metabolic syndrome (MetS) and T2DM. Recently, Lamers et al. have shown that DPP-4 is expressed in adipocytes during their differentiation and may impair insulin sensitivity directly in fat, skeletal, and smooth muscle cells [8]. In addition, it has been shown that the expression and secretion of DPP-4 in visceral fat are greater than in subcutaneous fat [8, 9]. These findings suggest that DPP-4 is one of adipokines and that elevated serum DPP-4 level is associated with visceral fat

accumulation and obesity-associated diseases. Indeed, several studies reported that serum DPP-4 levels were significantly elevated in obese subjects [10] and patients with T2DM [11]. However, it remains unclear whether or not serum DPP-4 levels are associated specifically with visceral fat mass and MetS in patients with T2DM.

In the present study, we aimed to investigate the association of serum DPP-4 level with visceral fat accumulation defined by computed tomography (CT) scan as well as the presence of MetS in men with T2DM.

Subjects and methods

Subjects

This cross-sectional study was approved by the ethical review board of Shimane University Faculty of Medicine and complied with the Helsinki Declaration. We consecutively enrolled the patients who had no hepatic or renal dysfunction and had never taken DPP-4 inhibitors, GLP-1 receptor agonists or thiazolidinediones, if informed consent was obtained after a detailed explanation of the study purpose and methods. All patients visited Shimane University Hospital for evaluation or treatment of T2DM, which has been established by attending physicians, from 2009 to 2013. The subjects in this study were a total of 135 Japanese male patients with T2DM.

Anthropometric and biochemical measurements

Body height (cm) was measured with a Martin metal anthropometer to the nearest 0.1 cm according to the standard technique, and body weight (kg) was measured using a medical electronic scale and recorded with 0.05 kg precision with the subject wearing light clothes. Body mass index (BMI; kg/m^2) was calculated by the following formula; weight/height in $meter^2$. Blood pressure (mmHg) was measured after a 5-minute rest in the supine position using mercury sphygmomanometer.

After fasting overnight, blood samples were collected. HbA1c was determined by high performance liquid chromatography. The value for HbA1c is estimated as an NGSP (National Glycohemoglobin Standaridization Program) equivalent value calculated by the formula HbA1c (%) = HbA1c

(JDS) (Japan Diabetes Society) (%) + 0.4 % [12]. Total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), fasting plasma glucose (FPG), alanine transaminase (ALT), and creatinine were evaluated using standard enzymatic methods. Serum C-peptide was measured by enzyme linked immunosorbent assay (ELISA).

Measurement of serum DPP-4 concentration

Serum DPP-4 concentration was determined using commercially available diagnostic kits (R&D Systems, Minneapolis, MN) as previously reported [13,14]. Serum samples were diluted 100-fold and added to the wells of microtitre plates coated with a monoclonal antibody against DPP-4. After incubation of the sample for 2 h at room temperature, a horseradish-peroxidase–conjugated polyclonal antibody against DPP-4 was added, and the plates were incubated for another 2 h. The assay was developed with peroxidase substrate for 30 min at room temperature in the dark. Absorbance was measured at 450 nm, and a reference wavelength of 540 nm was used. Standard curves for DPP-4 were prepared by using serial dilutions of exogenous recombinant human DPP-4. The coefficient of variation of DPP-4 measurements was <10%.

Radiography

As previously described [15], visceral and subcutaneous fat areas at the level of the umbilicus were measured using commercially available CT (Toshiba medical systems, Tokyo, Japan), which determined

adipose tissue area electronically by setting the attenuation values for the region of interest within a range of -150 and -50 Hounsfield units. Visceral fat area and subcutaneous fat area were determined separately with the use of a trace function, which manually defined the boundary between the visceral and subcutaneous fat with a cursor.

Diagnosis of the metabolic syndrome

We diagnosed patients as MetS based on the guideline in Japan [16], when they have visceral obesity with at least one of the following two conditions: hypertension and dyslipidemia. Although waist circumference is generally used for evaluating visceral obesity in epidemiological studies, we precisely defined visceral obesity as visceral fat area at the umbilicus equal to or more than 100 cm^2, which was calculated using CT [17]. Hypertension was defined as systolic blood pressure (SBP) equal to or more than 130 mmHg, diastolic blood pressure (DBP) equal to or more than 85 mmHg, or previous treatment for hypertension [16]. Dyslipidemia was defined as TG concentrations equal to or more than 150 mg/dL, HDL-C less than 40 mg/dL, or current treatment for dyslipidemia [16].

Statistical analysis

Baseline data of subjects were expressed as mean ± standard deviation (SD). It was checked that all variables except for ALT were normally distributed before performing statistical analysis. Logarithmic (log) transformation of ALT was carried out before examining multiple regression analysis. Statistical significance

between two groups was determined using Student's *t*-test. Simple, multiple, and logistic regression analyses were performed using the statistical computer program StatView (Abacus Concepts, Berkeley, CA). A $P < 0.05$ was considered to be significant.

Results

Baseline characteristics of the subjects

Baseline characteristics of the subjects are shown in Table 1. The averages of age and diabetes duration were 57.4 and 9.8 years, respectively. The cumulative numbers of patient who had been taking insulin, sulfonylurea, metformin, α-glucosidase inhibitors, and glinide were 68, 35, 23, 8 and 6, respectively (several patients were treated with multiple therapies). Of 135 patients, 57 had no anti-diabetic medication, 51 took monotherapy, 23 took two drugs, and 4 took three drugs. Of 135 subjects, 78 had visceral obesity (57.8%), and 74 had metabolic syndrome (54.8%).

Association between serum DPP-4 levels and metabolic parameters

First, to investigate the association between serum levels of DPP-4 and various metabolic parameters, we performed multiple regression analysis adjusted for age, duration of diabetes, body mass index (BMI), log(ALT), and serum creatinine (Table 2). Surprisingly, serum DPP-4 levels were not associated with any parameters of diabetes, blood pressure, or dyslipidemia. Serum DPP-4 levels were significantly and positively associated with visceral fat area (β=0.25, p=0.043), but not subcutaneous fat area (β=-0.18, p=0.133). Moreover, the association between serum DPP-4 levels and visceral fat area was still significant even after additional adjustment for HbA1c (β=0.25, p=0.046).

Association of serum DPP-4 levels with visceral obesity and metabolic syndrome

Next, to investigate the association of serum DPP-4 levels with the presence of visceral obesity and MetS, we performed unpaired Student *t*-tests. Serum DPP-4 was slightly, but not significantly, higher in patients with visceral obesity than without it (p=0.096) (Table 3). Fasting C-peptide, serum creatinine, TC, TG, and LDL-C were significantly higher in patients with visceral obesity than those without it, while HDL-C was significantly lower in patients with visceral obesity than those without it. Serum DPP-4 was significantly higher in patients with MetS than without it (p=0.046) (Table 4). Fasting C-peptide, serum creatinine, TC, TG, and LDL-C were significantly higher in patients with MetS than those without it, while HDL-C was significantly lower in patients with MetS than those without it.

In logistic regression analyses without any adjustment, serum DPP-4 levels tended to be positively associated with visceral obesity and MetS (Table 5). Moreover, after adjusting for age, duration of diabetes, BMI, ALT, serum creatinine, and HbA1c, serum DPP-4 levels were significantly and positively associated with visceral obesity and MetS [odds ratio (OR)=1.63, 95% confidence interval (CI)=1.00-2.66 per SD increase, p=0.049; OR=1.77, 95%CI=1.09-2.88 per SD increase, p=0.022, respectively]. However, when visceral fat area was added as an independent variable, the association between serum DPP-4 levels and MetS turned into insignificant (OR=2.02, 95%CI=0.86-4.73 per SD increase, p=0.105).

Discussion

In the present study, we showed a significant and positive association between serum DPP-4 levels and visceral fat area even after adjusting for BMI in 135 men with T2DM. Moreover, logistic regression analysis adjusted for various parameters showed a significant association between serum DPP-4 levels and the presence of visceral obesity. Previously, Lamers et al. showed that serum DPP-4 levels were significantly increased in 20 male obese patients than in 20 lean controls, and that serum DPP-4 levels were significantly and positively correlated with BMI when a correlation analysis was performed in all subjects [8]. Although multiple regression analysis adjusted for confounding factors was not performed in the study, they performed adipose tissue biopsies from the same patients and showed that DPP-4 expression in visceral fat was significantly higher than that in subcutaneous in obese patients, but not lean controls. Aso et al. also reported a clinical study with 52 patients with T2DM [10]. Serum DPP-4 levels were positively correlated with body weight and BMI in simple correlation analyses, but the association of serum DPP-4 levels with body weight and BMI became insignificant in a stepwise regression analysis. Strengths of the present study are that the number of subjects was relatively larger than those of previous studies, and that multiple regression analysis adjusted for suggested confounding parameters was performed. In addition, we evaluated fat area separately in visceral and subcutaneous by using CT scan. Therefore, these findings suggest that serum DPP-4 is specifically associated with accumulation of visceral fat in T2DM, and that the assessment of visceral fat mass rather than BMI may be useful to assess the concentration of serum DPP-4.

Previous studies have shown that obesity attenuates the efficacy of DPP-4 inhibitors on blood

glucose control [10, 18, 19]. It is suggested that obesity increases DPP-4 levels, and that obesity-related increased DPP-4 may weaken the effects of DPP-4 inhibitors. Kim et al. reported a meta-analysis using 55 clinical trials and showed that baseline BMI was significantly correlated with the HbA1c-lowering efficacy of DPP-4 inhibitors [19]. Furthermore, Aso et al. previously demonstrated that baseline serum DPP-4 levels were positively associated with changes in HbA1c levels after 24-week treatment with sitagliptin [10]. In the present study, visceral fat area was positively associated with serum DPP-4 levels independently of BMI, suggesting that accumulation of visceral fat mass may be an important factor for the efficacy of DPP-4 inhibitors.

MetS is known to be associated with the risk of developing cardiovascular disease and T2DM based on insulin resistance and disruption of the adipokines [20]. Previous studies have shown that serum DPP-4 concentration and its expression in visceral fat are related to insulin resistance, and that serum DPP-4 levels were increased in patients with MetS [8]. In addition, increased plasma DPP-4 activities were associated with chronic inflammation and predicted new-onset hyperglycemia [21], and inhibition of DPP-4 by sitagliptin reduced low-grade inflammation markers and cell adhesion molecules [22]. These findings suggest that serum DPP-4 levels may be involved in the metabolic diseases other than diabetes. In the present study, we found a significant association between serum DPP-4 levels and the presence of MetS although the association became insignificant when it was additionally adjusted for visceral fat area. However, serum DPP-4 levels were not associated with blood pressure or serum lipids levels. Many participants in this study received medications for hypertension, dyslipidemia, and diabetes; thus, further large scale epidemiological studies are

needed to examine the association between serum DPP-4 and MetS.

There are several limitations in this study. First, healthy subjects were not examined in this study. Second, we analysed only subjects who visited Shimane University Hospital, a tertiary centre, for treatment of diabetes mellitus. Therefore, the participants enrolled in this study might have relatively severe states of the disorder and might not be representative of all Japanese patients with the disorder. Also, the subjects in this study were only Japanese. Capacity of insulin secretion and degree of obesity in Asian are known to be different from Western people [23]. Therefore, it needs to be clarified whether or not our findings are universal. Third, more than 50% of the subjects were treated. Therefore, we cannot exclude the possibility that the diabetes treatment affected serum DPP-4 levels. Fourth, we could not evaluate DPP-4 activity. However, several studies demonstrated that the serum level of DPP-4 is strongly correlated with circulating DPP-4 activity in humans [24, 25], suggesting that serum DPP-4 concentration may reflect DPP-4 activity. Fifth, the conclusions of this study are weakened by its cross-sectional design. In a cross-sectional study, causal relationships cannot generally be inferred. Finally, we unfortunately did not measure the hormones such as GLP-1 and GIP cleaved by DPP-4 in this study.

In conclusion, serum DPP-4 levels were independently and positively associated with visceral fat area in men with T2DM, and associated with the presence of visceral obesity and MetS.

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Authors' Contributions

ST, IK, and MN conceived the study, participated in its design, coordination and acquisition of data, and performed the statistical analysis. IK wrote the manuscript. TS participated in its design interpreted the findings and revised the manuscript critically. All authors read and approved the final manuscript.

Conflicts of Interest and Disclosure

Sayuri Tanaka, Ippei Kanazawa, Masakazu Notsu, and Toshitsugu Sugimoto declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Table 1 Baseline characteristics of men with type 2 diabetes

Number of subjects		135	
Age (years)	57.4	±	15.2
Duration of diabetes (years)	9.8	±	9.2
Body height (cm)	165.6	±	7.9
Body weight (kg)	69.5	±	15.6
BMI (kg/m^2)	25.2	±	4.6
SBP (mmHg)	131.0	±	18.6
DBP (mmHg)	80.8	±	10.2
FPG (mg/dL)	161.8	±	47.4
HbA1c (%)	9.3	±	2.2
Fasting C-peptide (ng/mL)	2.0	±	1.1
ALT (U/L)	31	±	23
Creatinine (mg/dL)	0.89	±	0.34
TC (mg/dL)	194.5	±	57.1
TG (mg/dL)	157.0	±	85.3
HDL-C (mg/dL)	47.3	±	12.6
LDL-C (mg/dL)	119.7	±	41.4
DPP-4 (ng/mL)	809.1	±	183.6
Visceral fat area (cm^2)	119.5	±	55.6
Subcutaneous fat area (cm^2)	159.4	±	101.0
Insulin		68	
Sulfonylurea		35	
Metformin		23	
Alpha-glucosidase inhibitor		8	
Glinide		6	
Number of anti-diabetic medication			
0		57	
1		51	
2		23	
3		4	
Visceral obesity		78	
Metabolic syndrome		74	

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; ALT, alanine transaminase; TC, total cholesterol; TG, triglyceride, HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; DPP-4, dipeptidyl peptidase-4

Table 2 Association between serum DPP-4 levels and various parameters in men with type 2 diabetes

	β	p value
FPG	-0.07	0.447
HbA1c	-0.01	0.904
Fasting C-peptide	0.01	0.926
SBP	0.01	0.895
DBP	0.02	0.794
TC	0.07	0.458
TG	0.11	0.245
HDL-C	0.15	0.134
LDL-C	0.04	0.643
Visceral fat area	0.25	0.043
Subcutaneous fat area	-0.18	0.133

Multiple regression analysis was performed between serum DPP-4 levels and metabolic parameters adjusted for age, duration of diabetes, body mass index, log(ALT), and serum creatinine.

DPP-4, dipeptidyl peptidase-4; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride, HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol

Table 3 Comparison of various parameters between patients with and without visceral fat obesity in men with type 2 diabetes

Visceral obesity	No			Yes			p value
Number of subjects	57			78			
Age (years)	56.8	±	14.0	57.8	±	16.0	0.695
Duration of diabetes (years)	10.8	±	9.8	9.2	±	8.8	0.321
BMI (kg/m^2)	22.8	±	3.1	27.0	±	4.7	<0.001
SBP (mmHg)	129.3	±	19.4	132.2	±	18.0	0.379
DBP (mmHg)	80.0	±	9.2	81.4	±	11.0	0.452
FPG (mg/dL)	161.3	±	45.5	162.2	±	48.9	0.915
HbA1c (%)	9.3	±	2.1	9.3	±	2.3	1.000
Fasting C-peptide (ng/mL)	1.7	±	0.9	2.2	±	1.1	0.006
ALT (U/L)	27	±	22	34	±	23	0.119
Creatinine (mg/dL)	0.82	±	0.28	0.94	±	0.37	0.041
TC (mg/dL)	176.9	±	38.0	207.0	±	64.8	0.003
TG (mg/dL)	118.4	±	45.9	185.0	±	96.0	<0.001
HDL-C (mg/dL)	50.5	±	13.5	45.1	±	11.5	0.014
LDL-C (mg/dL)	106.8	±	34.1	128.8	±	43.8	0.002
DPP-4 (ng/mL)	778.3	±	178.9	831.7	±	184.8	0.096
Visceral fat area (cm^2)	70.9	±	21.8	155.1	±	44.7	<0.001
Subcutaneous fat area (cm^2)	115.7	±	74.9	191.3	±	106.0	<0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; ALT, alanine transaminase; TC, total cholesterol; TG, triglyceride, HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; DPP-4, dipeptidyl peptidase-4

Table 4 Comparison of various parameters between patients with and without metabolic syndrome in men with type 2 diabetes

Metabolic syndrome	No			Yes			p value
Number of subjects	61			74			
Age (years)	57.3	±	14.1	57.4	±	16.1	0.973
Duration of diabetes (years)	10.8	±	9.7	9.0	±	8.8	0.275
BMI (kg/m^2)	23.0	±	3.1	27.1	±	4.9	<0.001
SBP (mmHg)	128.1	±	19.5	133.3	±	17.6	0.106
DBP (mmHg)	79.6	±	9.1	81.9	±	11.0	0.197
FPG (mg/dL)	160.0	±	44.5	163.3	±	49.8	0.688
HbA1c (%)	9.2	±	2.1	9.4	±	2.3	0.621
Fasting C-peptide (ng/mL)	1.7	±	0.9	2.2	±	1.2	0.003
ALT (U/L)	27	±	21	35	±	24	0.064
Creatinine (mg/dL)	0.81	±	0.27	0.96	±	0.37	0.014
TC (mg/dL)	178.2	±	37.3	207.7	±	66.4	0.003
TG (mg/dL)	116.7	±	44.9	190.1	±	96.0	<0.001
HDL-C (mg/dL)	51.1	±	13.8	44.3	±	10.7	0.002
LDL-C (mg/dL)	107.7	±	33.2	129.3	±	44.9	0.003
DPP-4 (ng/mL)	774.5	±	177.7	837.7	±	184.6	0.046
Visceral fat area (cm^2)	75.3	±	27.8	156.0	±	45.3	<0.001
Subcutaneous fat area (cm^2)	119.2	±	73.8	192.5	±	108.6	<.0001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; ALT, alanine transaminase; TC, total cholesterol; TG, triglyceride, HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; DPP-4, dipeptidyl peptidase-4

Table 5 Association of serum DPP-4 levels with visceral fat obesity and metabolic syndrome in men with type 2 diabetes

	Visceral obesity			Metabolic syndrome		
	Odds ratio	95% CI	p value	Odds ratio	95% CI	p value
Serum DPP-4	1.41	0.98 - 1.97	0.099	1.45	0.88 - 1.75	0.050
Serum DPP-4*	1.63	1.00 - 2.66	0.049	1.77	1.09 - 2.88	0.022

*Adjusted for age, duration of diabetes, body mass index, ALT, serum creatinine, and HbA1c
CI, confidential intervals
Unit of change; SD per increase