

# Abdominal visceral fat accumulation is associated with the results of $^{123}\text{I}$ -metaiodobenzylguanidine myocardial scintigraphy in type 2 diabetic patients

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## Abstract

**Purpose** We tested the hypothesis that increased abdominal visceral accumulation (VFA) is associated with insulin resistance and cardiovascular autonomic dysfunction in type 2 diabetic patients not receiving insulin treatment.

**Methods** The fat distribution was evaluated by measuring the VFA by abdominal computed tomography at the umbilical level. The study group consisted of 24 type 2 diabetic patients with high VFA ( $\geq 100 \text{ cm}^2$ , age  $60 \pm 8$  years, high VFA group). The control group consisted of 19 age-matched type 2 diabetic patients with normal VFA ( $< 100 \text{ cm}^2$ , age  $60 \pm 7$  years, normal VFA group). Cardiovascular autonomic function was assessed by baroreflex sensitivity, heart rate variability, plasma norepinephrine concentrations, and cardiac  $^{123}\text{I}$ -metaiodobenzylguanidine (MIBG) scintigraphy.

**Results** Early and delayed  $^{123}\text{I}$ -MIBG myocardial uptake values were lower ( $p < 0.005$  and  $p < 0.0001$ , respectively)

and the percent washout rate of  $^{123}\text{I}$ -MIBG was higher ( $p < 0.0005$ ) in the high VFA group than in the normal VFA group. The fasting plasma insulin concentrations ( $p < 0.005$ ) and the homeostasis model assessment (HOMA) index values ( $p < 0.0005$ ) were higher in the high VFA group than in normal VFA group. Multiple regression analysis revealed that the level of VFA was independently predicted by the HOMA index values and the myocardial uptake of  $^{123}\text{I}$ -MIBG during the delayed phase.

**Conclusion** Our results demonstrate that the level of VFA is associated with depressed cardiovascular autonomic function and insulin resistance in patients with type 2 diabetes mellitus.

**Keywords** Visceral fat accumulation ·  $^{123}\text{I}$ -metaiodobenzylguanidine scintigraphy · Cardiac autonomic function · Insulin resistance · Type 2 diabetes

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## Introduction

A central pattern of body fat distribution, rather than regional or generalized obesity, is now generally considered to play an important role in the metabolic syndrome, which involves insulin resistance, hyperinsulinemia, dyslipidemia, obesity, diabetes mellitus, and hypertension [1–4].

An increased visceral fat accumulation (VFA) is a risk factor for cardiovascular disease [5, 6] and is associated with insulin resistance in healthy subjects [7] and type 2 diabetic patients [8]. Also, impaired autonomic neural activity has been recognized as a crucial risk factor of cardiac dysfunction and is strongly associated with an increased risk for harmful events and overall mortality in

diabetic patients [9, 10]. Recently, we have reported that depressed cardiovascular autonomic function is related to insulin resistance in type 2 diabetic patients [11–13]. However, the relationship among VFA levels, insulin resistance, and cardiac autonomic dysfunction has not been adequately investigated.

Cardiac autonomic function has been assessed by measurements of baroreflex sensitivity (BRS), heart rate variability (HRV), and the concentration of norepinephrine, but the reliability coefficients of these parameters have been shown to be around 50% [14]. A reduction in myocardial uptake of  $^{123}\text{I}$ -metaiodobenzylguanidine (MIBG) reflects a reduction in the concentration of norepinephrine at presynaptic sites or a reduction in the neural density, whereas an enhanced washout rate of  $^{123}\text{I}$ -MIBG reflects enhanced release of norepinephrine from presynaptic sites [15].

Recent technical advances such as  $^{123}\text{I}$ -MIBG scintigraphy allow cardiac autonomic function to be assessed more precisely [16, 17]. For the analysis, furthermore, VFA measured through computed tomography (CT) has been used [18].

We hypothesized that increased VFA is associated with cardiovascular autonomic dysfunction and insulin resistance in type 2 diabetic patients. To test our hypothesis, we compared BRS, HRV, plasma norepinephrine concentrations, and cardiac  $^{123}\text{I}$ -MIBG scintigraphy in addition to the metabolic profiles of Japanese type 2 diabetic patients with normal VFA and those with high VFA, and independent predictors of the level of VFA in these populations were evaluated.

## Materials and methods

We screened 105 consecutive Japanese patients with type 2 diabetes mellitus who were admitted to our department between January 2005 and May 2006. Among these subjects, we enrolled 65 patients who did not have organic heart disease as determined by physical examinations, chest X-rays, 12-lead electrocardiography (ECG), echocardiography, treadmill exercise ECG, and  $^{201}\text{Tl}$  cardiac scintigraphy. All patients underwent clinical examinations to exclude the presence of secondary hypertension. Essential hypertension was defined as diastolic blood pressure  $\geq 90$  mmHg, systolic blood pressure  $\geq 140$  mmHg, or self-reported use of antihypertensive medication [19].

Blood was taken at 7:00 a.m. from the antecubital vein with the patient in the recumbent position after an overnight fast. All patients underwent routine laboratory tests including assays for serum electrolytes, serum total cholesterol, serum triglycerides, serum high-density lipoprotein, fasting plasma glucose, and fasting immunoreactive insulin. Insulin resistance was evaluated by the homeostasis model assess-

ment (HOMA) index: [fasting plasma insulin ( $\mu\text{U/ml}$ )  $\times$  fasting plasma glucose (mmol/l)]/22.5 [20].

All subjects underwent CT at the level of the umbilicus to measure cross-sectional abdominal visceral fat areas; the scans were analyzed with Fat scan version 3 software (N2 Systems, LT, Osaka, Japan). Details of the procedures have been described previously [21]. This method has been validated by other determinations of VFA [22] and widely adopted as a practical method to evaluate regional adiposity.

Twenty-four patients were determined to have high VFA ( $\geq 100$  cm<sup>2</sup>; high VFA group). We also included 19 age-matched patients from the original 65 enrolled patients who had normal VFA ( $< 100$  cm<sup>2</sup>; normal VFA group), the classification of which has previously been validated [23]. The clinical characteristics of patients in the normal and high VFA groups are summarized in Table 1. Sixteen of the 24 patients in the high VFA group and 12 of the 19 patients in the normal VFA group met the criteria for essential hypertension and all of these patients were being treated with calcium channel antagonists, angiotensin-converting enzyme inhibitors, and/or angiotensin II receptor blockers with diuretics. None of the patients was being treated with beta-blockers or insulin. Dyslipidemia was defined as a fasting triglycerides level  $\geq 200$  mg/dl or an HDL-cholesterol concentration  $< 45$  mg/dl for women and  $< 35$  mg/dl for men [19]. Eight of the 24 patients in the high VFA group and six of the 19 patients in the normal VFA group met the criteria for dyslipidemia. Patients with abnormal plasma creatinine concentrations ( $\geq 1.5$  mg/dl) were excluded from the study.

All subjects gave their written informed consent to participation in the study, and the study protocol was approved by the ethics committee of the Oita University Hospital.

## Echocardiography

M-mode and two-dimensional echocardiography and cardiac Doppler recordings were obtained using a phase-array echo-Doppler system. Echocardiograms were obtained using standard parasternal, short axis, and apical views. The left ventricular mass was calculated as:  $1.04 \times ([\text{LVIDd} + \text{IVSTd} + \text{PWTd}]^3 - \text{LVIDd}^3) - 14$  g, where LVIDd is the left ventricular internal diameter at end-diastole, IVSTd is the interventricular septal thickness at end-diastole, and PWTd is the posterior wall thickness at end-diastole. The left ventricular mass was divided by the body surface accumulation to calculate the left ventricular mass index. Pulsed Doppler recordings were made from a standard apical four-chamber view. Mitral inflow velocity was recorded with the sample volume at the mitral annulus level taking the average from at least three cardiac cycles. The peak velocity of early (E) and late ventricular filling

**Table 1** Clinical characteristics of the studied patients

	Normal VFA group	High VFA group	<i>p</i> value
Age (years)	60±8	60±7	ns
Gender (men/women)	9/10	14/10	ns
Visceral fat accumulation (cm <sup>2</sup> )	69.9±17.3	178.2±53.9	<0.0001
Duration of diabetes (years)	7.7±3.3	8.2±4.7	ns
Hypertension (%)	63	67	ns
Dyslipidemia (%)	37	42	ns
Drug use (%)			
Sulfonylurea	47	50	ns
Alpha-glucosidase inhibitors	37	33	ns
Pioglitazone	6	8	ns
Statin	32	38	ns
Calcium channel antagonists	37	42	ns
ACE inhibitors	21	25	ns
Angiotensin receptor blocker	37	46	ns
Diuretic	16	17	ns
Body mass index (kg/m <sup>2</sup> )	25.2±2.4	27.2±3.2	0.0385
Waist circumference (cm)	83.3±7.9	93.0±10.3	0.0015
Hip circumference (cm)	94.9±5.8	99.0±9.2	ns
Waist-to-hip ratio	0.88±0.10	0.94±0.09	0.0389
Systolic blood pressure (mmHg)	129±11	134±13	ns
Diastolic blood pressure (mmHg)	77±9	78±8	ns
Heart rate (bpm)	68±8	69±6	ns
Total cholesterol (mg/dl)	199±30	206±37	ns
Triglyceride (mg/dl)	128±43	156±50	0.0490
HDL-cholesterol (mg/dl)	47±9	41±8	0.0256
Fasting plasma glucose (mg/dl)	135±27	162±50	0.0379
Fasting immunoreactive insulin (μU/ml)	5.9±1.9	8.2±2.3	0.0011
Homeostasis model assessment index	2.0±0.8	3.2±1.1	0.0003
Hemoglobin A1c (%)	7.7±1.3	7.8±0.9	ns
Uric acid (mg/dl)	5.5±1.6	6.6±1.3	0.0116
Creatinine (mg/dl)	0.74±0.23	0.85±0.20	ns

Data are means±SD

VFA visceral adiposity, HDL high-density lipoprotein, ns not significant

(A) was determined, and the ratio (E/A) and deceleration time were recorded.

#### Cardiovascular autonomic function tests

Autonomic function was assessed according to methods described in previous studies [10–12]. During the tests, which were performed between 9:00 and 11:00 a.m., all

subjects were in the supine position in a quiet room with dimmed lights. Autonomic function tests were performed in the morning after an overnight (≥12 h) fast. A blood sample was obtained from a venous catheter for measurement of the plasma norepinephrine concentration. The catheter was inserted in the right cubital vein, and arterial blood pressure was recorded noninvasively through a tonometric sensor (Jentow-7700; Nihon Colin, Komaki, Japan) attached over the left radial artery. The accuracy of continuous blood pressure monitoring has been demonstrated previously [24]. Arterial blood pressure and a standard 12-lead ECG were monitored simultaneously; data were stored in a PCM data recorder (RD-200T; TEAC, Tokyo). Three-lead precordial Holter ECG recordings (model-459; Del Mar Avionics, Irvine, CA) were also obtained throughout the procedure for analysis of HRV.

After an interval of 30 min to permit stabilization of the cardiovascular baroreflex mechanism, the patient was asked to breathe at a rate of 15 breaths/min using a metronome to stabilize the relationship between respiration and cardiovascular function. BRS was assessed by the phenylephrine method. Briefly, phenylephrine (2–3 μg/kg) was injected for 15 s to obtain a 15–40-mmHg rise in systolic blood pressure. BRS was calculated as the slope of the linear regression function relating systolic blood pressure changes to changes in the RR interval. Regression lines with more than 20 data points and a correlation coefficient (*r*) greater than 0.8 were accepted for analysis. The average of the two slopes was taken as the BRS value.

HRV was analyzed using Holter ECG recordings (Marquette Electronics Inc., Milwaukee, WI, USA). The power spectrum of the RR interval was computed by a fast Fourier transformation and expressed as the accumulation under the power spectrum. We calculated the power of two spectral bands: the normal frequency (LF) component at 0.04–0.15 Hz and the high-frequency (HF) component at 0.15–0.40 Hz. Based on their skewed distribution, the measured values of HRV were transformed to natural logarithmic values. The ratio of LF to HF (LF/HF) was also computed.

Planar and single-photon emission computed tomography studies were performed 15 min (early) and 4 h (delayed) after the injection of 111 MBq of <sup>123</sup>I-MIBG using a rotating gamma camera (ZLC 7500; Siemens, Munich, Germany). Data were analyzed with analysis software (SCINTIPAC; Shimadzu, Kyoto, Japan). The anterior planar images from early and delayed <sup>123</sup>I-MIBG studies were analyzed visually. For semiquantitative analysis, regions of interest were identified within the whole heart and a 10×10 mm accumulation over the upper mediastinum on the early and delayed planar images was used to calculate the mean heart-to-mediastinum (H/M) ratio. After correcting for the physical decay of <sup>123</sup>I, the

percent washout rate (WR) of the tracer from the myocardium was determined over a 4-h period.

#### Anthropometric and body composition measurements

The anthropometric and body composition characteristics of the patients were evaluated using the following parameters: height, body weight, body mass index (BMI), waist circumference, hip circumference, and waist-to-hip ratio. BMI was calculated as weight/(height<sup>2</sup>) (kg/m<sup>2</sup>). The waist circumference was measured midway between the lower rib margin and the iliac crest, and the hip circumference was measured at the widest circumference over the trochanter in standing subjects after normal expiration.

#### Statistical analysis

Data are presented as means±SD. Differences between two groups were analyzed with the unpaired Student's *t* test, chi-square test, or Fisher's exact probability test. A *p* value <0.05 was considered statistically significant. Simple (Spearman's rank) correlation coefficients between VFA and various parameters were calculated. Stepwise multiple regression analysis was then used to evaluate the association between the levels of VFA and other factors, such as the BMI, waist circumference, waist-to-hip ratio, E/A ratio, triglyceride levels, HDL-cholesterol levels, uric acid levels, fasting plasma glucose, and plasma insulin concentrations, HOMA index values, urinary albumin excretion, BRS, the percent WR of <sup>123</sup>I-MIBG, and the H/M ratio during the early and delayed phase after <sup>123</sup>I-MIBG administration. In our multivariate analysis, *F* values ≥4 were considered significant.

## Results

As shown in Table 1, the mean ages of the high and normal VFA groups were similar, and there were no significant differences between the groups with respect to gender, duration of diabetes, or administered medications. The BMI values, waist circumferences, and waist-to-hip ratios were larger in the high VFA group than in the normal VFA group (*p*=0.0385, *p*=0.0015, and *p*=0.0389, respectively). Regarding glucose metabolism, fasting plasma glucose and insulin concentrations and HOMA index values were higher in the high VFA group than in the normal VFA group (*p*=0.0379, *p*=0.0011, and *p*=0.0003, respectively). There was, however, no significant difference in the levels of hemoglobin A1c. With regard to lipid metabolism, the concentration of serum triglyceride was higher and the concentration of serum HDL-cholesterol was lower in the high VFA group than in the normal VFA group (*p*=0.0490 and *p*=0.0256, respectively), whereas serum total cholesterol levels were not significantly

different between the groups. The concentration of uric acid was higher in the high VFA group than in the normal VFA group (*p*=0.0116). Renal function tests showed no significant difference in serum concentration between the two groups. The hemodynamic data listed in Table 1 were obtained immediately before BRS assessment. The resting heart rate and the systolic and diastolic blood pressures were not significantly different between the two groups.

Table 2 presents a summary of the echocardiographic findings. The left ventricular dimensions at end-diastole and end-systole, the intraventricular septal and posterior wall thicknesses at end-diastole, the ejection fraction, and the left ventricular mass index values were similar in the two groups. With regard to left ventricular diastolic function, the peak velocity of late ventricular filling (A) was higher and the E/A ratio was lower in the high VFA group compared with the normal VFA group (*p*=0.0136 and *p*=0.0060, respectively). The deceleration time was longer in the high VFA group than in the normal VFA group (*p*=0.0102).

Figure 1 summarizes the results of the cardiovascular autonomic function tests. The BRS was lower in the high VFA group than in the normal VFA group (high VFA group, 8.4±4.2 vs normal VFA group, 12.0±5.1 ms/mmHg, *p*=0.0141; Fig. 1a). The plasma norepinephrine concentration was similar in both groups (high VFA group, 242±83 pg/ml; normal VFA group, 207±65 pg/ml; *p*=ns; Fig. 1b). Furthermore, analysis of HRV in the high and normal VFA groups revealed that the HF power (3.4±1.0 and 3.9±1.2 ln-ms<sup>2</sup>, respectively; *p*=ns) and the LF/HF

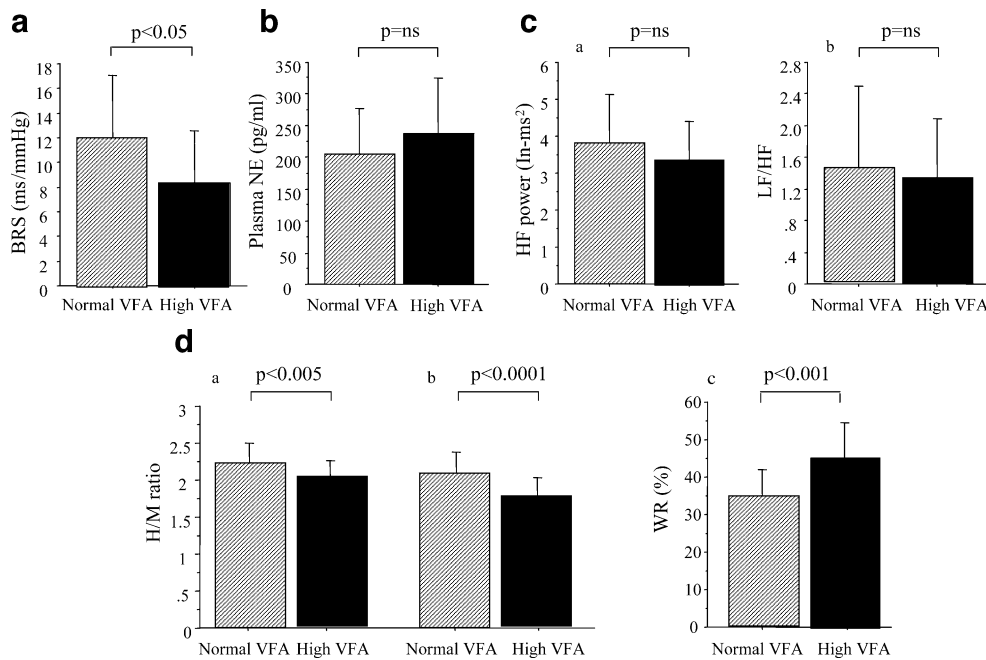
**Table 2** Echocardiographic findings

	Normal VFA group	High VFA group	<i>p</i> value
EF (%)	68±5	67±4	ns
LVIDd (mm)	47±3	48±3	ns
LVIDs (mm)	31±2	32±2	ns
IVSTd (mm)	8.7±0.7	9.0±1.2	ns
PWTd (mm)	9.1±0.8	9.6±1.1	ns
LVMI (g/m <sup>2</sup> )	106±18	112±21	ns
E-peak velocity (cm/s)	64±10	62±12	ns
A-peak velocity (cm/s)	69±12	78±15	0.0136
E/A ratio	0.93±0.19	0.79±0.14	0.0060
Deceleration time (ms)	230±24	251±28	0.0102

Data are means±SD

VA visceral adiposity, EF ejection fraction, LVIDd left ventricular internal dimension at end-diastole, LVIDs left ventricular internal dimension at end-systole, IVSTd interventricular septal thickness at end-diastole, PWTd posterior wall thickness at the end-diastole, LVMI left ventricular mass index, ns not significant





**Fig. 1** Comparison of autonomic function tests of type 2 diabetes mellitus patients with normal visceral fat accumulation (*normal VFA*) or high visceral fat accumulation (*high VFA*). **a** Baroreflex sensitivity (*BRS*). **b** Plasma norepinephrine (*NE*) concentration. **c** Heart rate variability (*HRV*). Power of the high-frequency component (*HF*; 0.15–0.40 Hz; **a**) and the ratio of the low-frequency power (*LF*; 0.04 to 0.15 Hz) to HF power (*LF/HF*, **b**). The distribution of HRV values was

skewed and the values were thus transformed to natural logarithmic values. **d** Cardiac <sup>123</sup>I-MIBG scintigraphic findings. Myocardial uptake of <sup>123</sup>I-MIBG during the early (**a**) and delayed (**b**) phases. Myocardial uptake of <sup>123</sup>I-MIBG is expressed as the mean heart-to-mediastinum (*H/M*) ratio. **c** Percent washout rate (*WR*) of <sup>123</sup>I-MIBG. Data are mean±SD. *ns* not significant

ratios ( $1.2 \pm 0.7$  and  $1.4 \pm 1.1$ , respectively;  $p = ns$ , Fig. 1c) were not significantly different between the two groups. Cardiac <sup>123</sup>I-MIBG scintigraphy disclosed that the H/M ratios at early and delayed phases in the high VFA group were significantly smaller than those in the normal VFA group (early phase:  $2.03 \pm 0.15$  vs  $2.25 \pm 0.27$ , respectively;  $p = 0.0021$ ; delayed phase:  $1.88 \pm 0.18$  vs  $2.20 \pm 0.21$ , respectively,  $p < 0.0001$ ; Fig. 1d). The percent WR of <sup>123</sup>I-MIBG was higher in the high VFA group than in the normal VFA group ( $44.8 \pm 8.5\%$  vs  $35.7 \pm 7.6\%$ ,  $p = 0.0005$ ; Fig. 1d).

Table 3 depicts the correlation between the VFA level and age, the body mass index, and other variables in both the high and the normal VFA group. The VFA levels were positively correlated with body mass index values, waist circumference, waist-to-hip ratio, triglyceride levels, uric acid levels, fasting plasma glucose and insulin concentration, HOMA index values, urinary albumin excretion, and percent WR of <sup>123</sup>I-MIBG, and were negatively correlated with HDL-cholesterol levels, BRS, the E/A ratio, and the H/M ratio during the early and the delayed phase.

Figure 2 shows the positive correlation between the VFA level and the HOMA index values ( $r = 0.569$ ,  $p < 0.0001$ ; Fig. 2a) and the negative correlation between the VFA level and the H/M ratios measured using cardiac <sup>123</sup>I-MIBG scintigraphy during the delayed phase ( $r = -0.509$ ,  $p = 0.0005$ ; Fig. 2b) in type 2 diabetes mellitus.

Multiple regression analysis was performed using the stepwise procedure. The level of VFA was predicted from the HOMA index values and the H/M ratio during the delayed phase (Table 4). In the other model analysis, the H/M ratio during the delayed phase was independently predicted from the level of VFA and the HOMA index values (data not shown).

## Discussion

In this study, type 2 diabetic patients with high VFA levels had decreased uptake and enhanced clearance of <sup>123</sup>I-MIBG in the heart relative to the values measured in type 2 diabetic patients with normal VFA levels. In addition, multiple regression analysis revealed that the level of VFA in the patients could be independently predicted by the HOMA index values and the H/M ratio during the delayed phase.

There are several reports indicating that an elevated VFA concentration is associated with insulin resistance in healthy subjects [7] and patients with type 2 diabetes mellitus [8]. Raji et al. [7] investigated the association between VFA levels and insulin resistance using a hyperinsulinemic–euglycemic clamp in non-obese healthy subjects. They found a significant increase in the VFA levels of healthy subjects with insulin resistance. Gastaldelli et al. [8] demonstrated that VFA has a significant negative impact on

**Table 3** Correlations of VFA with other variables

Parameters	Univariate analysis	
	<i>r</i>	<i>p</i> value
Age	0.101	0.5186
Duration of diabetes mellitus	0.119	0.4463
Body mass index	0.424	0.0046
Waist circumference	0.406	0.0069
Hip circumference	0.051	0.7450
Waist-to-hip ratio	0.398	0.0082
Systolic blood pressure	0.138	0.3972
Diastolic blood pressure	0.139	0.3907
Heart rate	0.206	0.2017
EF	0.080	0.6092
LVIDd	0.188	0.2279
LVIDs	0.184	0.2372
IVSTd	0.229	0.1392
PWTd	0.294	0.0561
LVMI	0.129	0.4080
E/A ratio	-0.327	0.0332
Deceleration time	0.296	0.0541
Total cholesterol	0.247	0.1107
Triglyceride	0.389	0.0099
High-density lipoprotein cholesterol	-0.425	0.0045
Uric acid	0.348	0.0223
Fasting plasma glucose	0.407	0.0068
Fasting immunoreactive insulin	0.458	0.0020
Homeostasis model assessment index	0.569	<0.0001
Hemoglobin A1c	0.084	0.5944
Creatinine	0.267	0.8400
Baroreflex sensitivity	-0.385	0.0141
Plasma norepinephrine	0.264	0.0870
HF power	-0.046	0.7690
LF/HF	-0.102	0.5170
H/M ratio at early phase	-0.357	0.0188
H/M ratio at delayed phase	-0.509	0.0005
Washout rate	0.321	0.0357

HF high frequency, LH low frequency, H/M heart-to-mediastinum ratio. See Table 2 for other abbreviations

**Table 4** Stepwise regression analysis between VFA and other parameters

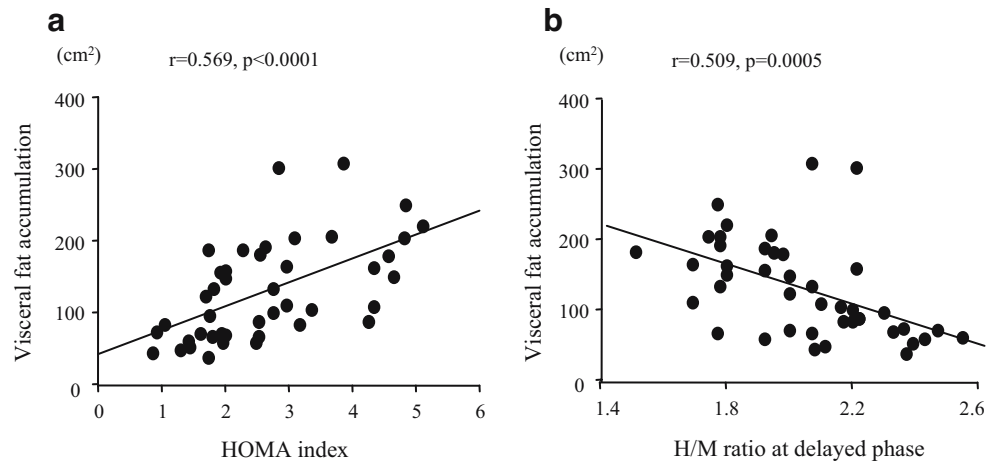
Independent variable	Regression coefficient	Standard error	Standard regression coefficient	F-value
To VFA intercept	253.410			
H/M ratio during the delayed phase	-94.814	36.134	-0.341	6.885
HOMA index	25.703	7.633	0.438	11.339

VFA visceral fat accumulation, H/M heart-to-mediastinum ratio, HOMA homeostasis model assessment

glycemic control through a decrease in peripheral insulin sensitivity in type 2 diabetic patients. In the present study, the level of VFA correlated with the body mass index, triglyceride levels, HDL-cholesterol levels, the fasting plasma insulin concentration, and the HOMA index values.

Although the specific mechanism that links the VFA level and insulin resistance remains to be elucidated, several mechanisms could explain our observations. Arner [25] has suggested that the flux of lipid from the visceral fat depot to liver might account for hepatic insulin resistance. In a canine model, development of insulin resistance occurred concomitantly with visceral adiposity because of a modest fat content in the diet but without increased calories [26]. The other possible mechanism is the contribution of an adipocyte-associated bioactive substance such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), adiponectin, or resistin. TNF- $\alpha$  levels are essentially dependent on VFA [27] and inversely correlated with insulin sensitivity in obese type 2 diabetic patients [28]. Adiponectin decreases as VFA increases [29], and hypo adiponectinemia in patients with increased VFA is closely related to insulin resistance and hyperinsulinemia [30]. Gene and protein expression of resistin increased in abdominal fat and may also contribute

**Fig. 2 a** Correlation between VFA and HOMA index values in type 2 diabetes mellitus patients. **b** Comparisons of VFA and cardiac  $^{123}\text{I}$ -MIBG scintigraphic results disclosed a correlation between the VFA and H/M ratios during the delayed phase in type 2 diabetes mellitus



to insulin resistance [31]. Resistin levels have been reported to be markedly elevated in obese mice and to be decreased by insulin sensitizers, such as thiazolidinediones [32].

The relationship between VFA and cardiovascular autonomic function has been examined in previous studies using HRV analysis or BRS [33, 34]. Lindmark et al. [33] have shown that VFA is associated with insulin resistance and signs of a high ratio of sympathetic versus parasympathetic reactivity by HRV. Beske et al. demonstrated reduced BRS associated with the level of VFA [34]. However, these parameters had reliability coefficients of around 50% [14]. In the present study, HRV and the plasma norepinephrine concentrations were not different between the two groups. But, a significant difference was seen in  $^{123}\text{I}$ -MIBG parameters. These results may suggest association of VFA in diabetic patients with impairment of uptake-1 (norepinephrine transporter) as well as the acceleration of norepinephrine turnover in sympathetic nerve terminal. Impairment in uptake-1 in diabetic rats has been reported [35]. However,  $^{123}\text{I}$ -MIBG uses the same uptake system and storage site as norepinephrine [36]. Our and others' previous studies [11–13, 37] have demonstrated that  $^{123}\text{I}$ -MIBG scintigraphy is a fairly sensitive method for detecting cardiac sympathetic dysfunction in diabetic patients. The present results support the potential of  $^{123}\text{I}$ -MIBG scintigraphy for diagnosis of cardiovascular autonomic dysfunction.

Although the precise mechanisms underlying the interactions between high VFA and impaired autonomic function remain unclear, in our opinion there are several possible mechanisms. First, VFA may affect autonomic function through endothelial dysfunction and impairment of the nitrate oxide system. Visceral fat obesity is associated with endothelial dysfunction by oxidant stress [38]. Nitric oxidant damage and endothelial dysfunction are associated with cardiac autonomic dysfunction and increased HRV [39]. Moreover, we have recently reported that the HOMA index and the myocardial uptake of  $^{123}\text{I}$ -MIBG during the delayed phase were independent predictors of high-sensitivity C reactive protein (HSCRP) [12]. In fact, in a recent report demonstrating the association between increased VFA, insulin resistance, and HSCRP, the authors stressed the central role of endothelial dysfunction [40]. Second, VFA may cause dysregulation of autonomic nervous system through enhanced release of free fatty acids. Insulin resistance is strongly associated with VFA and free fatty acids [33, 41]. An increased release of free fatty acids from visceral adiposity into the portal circulation acts on the liver and secondarily leads to sympathetic activation [42].

Taken together, it is possible that VFA, insulin resistance, and autonomic dysfunction interact and reinforce each other through mechanisms that may be associated with endothelial dysfunction.

Compared with the normal VFA group, patients in the high VFA group showed cardiac diastolic dysfunction. Consistent with the present results, a previous study demonstrated that cardiac diastolic dysfunction is associated with cardiovascular autonomic dysfunction and insulin resistance [19]. Morricone et al. [43] reported that increased VFA is associated with cardiac left ventricular diastolic dysfunction in diabetic patients. Although the precise mechanism is unclear, diastolic dysfunction may interact with cardiac sympathetic nervous function through insulin resistance.

There are several limitations to this study. Firstly, subjects in the present study population had essential hypertension, which was treated with one or more antihypertensive drugs. These characteristics of the patients' backgrounds have been reported to affect insulin resistance [44, 45] and sympathetic nerve function [46, 47]. Nonetheless, the results indicate that VFA in diabetic patients is related to cardiac depression and insulin resistance. Secondly, we have previously reported that HSCRP and hyperhomocysteine are associated with insulin resistance as well as cardiac sympathetic nerve function assessed by  $^{123}\text{I}$ -MIBG [12, 13]. Therefore, further studies are required to evaluate the association among the levels of VFA, HSCRP, hyperhomocysteine, HOMA index values, and  $^{123}\text{I}$ -MIBG parameters. In addition, the prognostic implications of cardiac autonomic function as assessed by  $^{123}\text{I}$ -MIBG scintigraphy remain to be addressed, although there are prognostic studies of heart failure patients using cardiac  $^{123}\text{I}$ -MIBG imaging [48].

In conclusion, our findings suggest that increased VFA in patients with type 2 diabetes are associated with depressed cardiovascular autonomic function and insulin resistance.

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