Table 2 Clinical and Biochemical Data Relating to In-Stent Restenosis in T2DM Patients

Characteristic	Restenosis (+) (n=19)	Restenosis (−) (n=24)	Univariate p value	Multivariate p value
Age (years)	66 (11)	69 (11)	0.442	NS
Sex (M/F)	17/2	12/13	0.012*	NS
OMI/SAP	8/11	12/13	0.607	NS
Body mass index (kg/m²)	23.7 (3.1)	26.0 (4.8)	0.085	NS
Hypertension	41%	58%	0.282	NS
Smoking	35%	17%	0.179	NS
Insulin treatment	35%	33%	0.903	NS
Medication of statin	21%	54%	0.032*	NS
Fasting plasma glucose (mg/dl)	121 (19)	131 (35)	0.351	NS
Glycohemoglobin (%)	7.2 (2.0)	6.9 (1.3)	0.576	NS
LDL-cholesterol (mg/dl)	119 (21)	104 (19)	0.180	NS
Triglyceride (mg/dl)	140 (65)	116 (41)	0.165	NS
HDL-cholesterol (mg/dl)	40.5 (8.7)	49.4 (12.5)	0.024*	NS
Remnant-like particle (mg/dl)	4.2 (1.7)	4.4 (1.8)	0.790	NS
Lipoprotein(a)	25.6 (17.1)	25.4 (21.7)	0.978	NS
Uric acid (mg/dl)	6.4 (1.7)	5.8 (1.2)	0.199	NS
hs-CRP	0.104 (0.096)	0.158 (0.167)	0.251	NS
MDA-LDL (U/L)	117.2 (32.2)	88.2 (24.1)	0.008**	0.008**
Stent diameter (mm)	3.0 (0.2)	2.9 (0.3)	0.207	NS
Stent length (mm)	16.0 (4.2)	15.8 (3.6)	0.446	NS
MSA (mm²)	6.35 (1.07)	5.98 (1.25)	0.252	NS

*Data are means (SD) or percentages. Statistical analysis was conducted using a stepwise multiple regression analysis. Values of p<0.05 indicate significant differences. *p<0.01; **p<0.05.*
Abbreviations see in Table 1.

healthy volunteers. In the preliminary study, serum MDA-LDL level in the healthy volunteers was 58.8±17.9 U/L (n=86) (unpublished data).

Statistical Analysis

A simultaneous and a stepwise multiple regression analysis were used to evaluate the independent association of various risk factors with ISR. A comparison of the variables among 4 groups (Figs 1,3) was performed using 1-way analysis of variance with a post-hoc test (Fisher's PLSD). Linear regression analysis and Pearson correlation coefficients was used for comparisons between the glycohemoglobin and MDA-LDL concentrations (Fig 2). Student's unpaired t-test was used for comparison of MDA-LDL level before and after PCI (Fig 4). Statistical significance was defined as $p<0.05$.

Results

Clinical and laboratory characteristics of the enrolled patients are shown in Table 1. Of 131 patients, 38 developed ISR within 6 months after stenting and 93 patients did not. A stepwise multiple regression analysis was used to evaluate the independent association of various risk factors with ISR. Sex difference (male gender), T2DM, and elevation of glycohemoglobin (HbA_{1c}) were positive univariate factors, and medication of HMG-CoA reductase inhibitor (statin) was a negative univariate factor for ISR. However, T2DM was the only independent risk factor for ISR (Table 1).

The analysis shown in Table 1 clearly indicates that T2DM is a significant risk factor for ISR. In fact, the percentage ISR rate was 44.2% in diabetic patients (19/43 patients) compared with 21.6% in non-diabetic patients (19/88 patients). Thus, we investigated the diabetes-related risk factors that promote ISR. A stepwise multiple regression analysis was also used to evaluate the independent association of these variables with ISR in diabetic patients (Table 2). Male gender and elevated serum MDA-LDL concentration were positive univariate factors, and medication with HMG-CoA reductase inhibitor (statin) and the HDL-cholesterol concentration were negative univariate factors for ISR. However, only elevation of the serum MDA-LDL concentration was an independent risk factor for ISR (Table 2).

Fig 1 compares the average concentration of serum MDA-LDL in diabetic and non-diabetic patients with or without ISR. A significant elevation of serum MDA-LDL concentration was observed only in the diabetic patients who developed ISR. We examined whether the serum MDA-LDL level relates to the control of diabetes and it was positively correlated to HbA_{1c} in diabetic patients (Fig 2). Interestingly, this trend was not observed in non-diabetic patients. In contrast, the HbA_{1c} level was not statistically different between the ISR and non-ISR groups of diabetic patients (Fig 3). These results suggest the possibility that an elevated plasma glucose level does not directly promote ISR, but rather the metabolic abnormality caused by a high plasma glucose level increases serum MDA-LDL, which results in the increased rate of ISR. To exclude the possibility that an elevated serum MDA-LDL is simply derived from the restenosis site (ie, neointima in the stent), we investigated whether the serum MDA-LDL level is altered after treatment of ISR, but as shown in Fig 4, it was not significantly decreased after treatment (ie, mechanical crushing of the neointima with a PCI balloon). This result strongly suggests that an elevated level of serum MDA-LDL in diabetic patients is not merely a consequence of restenosis.

Discussion

T2DM is a serious risk factor for poor outcome after percutaneous transluminal coronary angioplasty.[21] Although coronary stents improve the acute results and decrease the rate of restenosis compared with balloon angioplasty, diabetes remains a key independent predictor of ISR.[5,21,22] After stenting of native coronary arteries, diabetic patients

have significantly more restenosis of the lumen than non-diabetic patients, as determined by quantitative coronary angiographic analysis, regardless of the treatment modality for diabetes[6] Serial intravascular ultrasound analysis has shown that accelerated intimal hyperplasia in the stented lesion is the main cause of the increased restenosis in diabetic patients[23] In contrast, most risk factors for CAD are not associated with an increased risk of restenosis[2,3] In the present study, we found no significant correlation of the various risk factors for CAD to ISR, except for diabetes. Thus, our result is quite consistent with previous reports in which diabetes is a potent risk factor for restenosis after coronary stenting[3,5,24]

The mechanisms involved in the development of ISR in diabetic patients have been extensively investigated in animal models. The metabolic alterations caused by diabetic hyperglycemia or hyperinsulinemia are considered to be involved in many of the pathophysiologic processes leading to restenosis. Long-term hyperglycemia leads to the formation of advanced glycation endproducts (AGEs); the accumulation of AGEs and their receptors in the vessel wall has been implicated in the neointimal formation after vascular injury in diabetic rats[25] Hyperinsulinemia rather than hyperglycemia may be of crucial importance in promoting the exaggerated neointimal hyperplasia after balloon injury. Hyperinsulinemia has been shown to induce cell proliferation through the activation of the Ras/MARK pathway in diabetic animals[26] Park et al evaluated 2 different models of DM: streptozotocin (STZ)-treated Sprague-Dawley rats (type 1 DM) and obese Zucker rats (type 2 DM). Neointimal hyperplasia was assessed by computerized morphometry after carotid balloon injury. Whereas there was no difference in the neointimal area in the STZ-treated rats compared with the controls, the neointimal area was markedly increased in the obese Zucker rats, which suggested the possibility that insulin resistance is associated with a propensity for neointimal proliferation[8] Although the data from animal studies have suggested several possible pathways that might underlie the exacerbation of ISR in diabetes, the precise mechanisms of ISR in clinical observations remain poorly defined.

Local and mechanical factors, such as implantation of multiple stents, longer stents, and small stent diameter, have been reported as independent predictors of ISR after coronary intervention[1-3,27] Kastrati et al reported that stent placement procedures yielding a final MLD of less than 3 mm increased the likelihood of restenosis by 50% and doubled the likelihood of target lesion revascularization! The lesion length was also reported to be a significant correlate of lumen loss at 6-month angiographic follow-up[28] However, factors related to the implanted stents did not seem to be the prime cause of ISR observed in the diabetic patients in the present study.

The present study showed for the first time that the markedly increased rate of post-stenting restenosis observed in diabetic patients is associated with an increased serum MDA-LDL concentration. This trend was not observed in the non-diabetic patients, suggesting the adverse effects of elevated serum MDA-LDL to ISR are peculiar to diabetic patients. MDA-modified LDL, one of the major oxidized LDL, has been known to play key roles in the progression of atherosclerosis[29,30] The serum level of MDA-LDL is increased in CAD patients, and is positively correlated with the thickness of the intima media in the carotid arteries[31] Circulating MDA-LDL is distributed in serum fractions containing small, dense LDL[18] and the elevated circulating MDA-LDL is considered to be a potent risk factor for atherosclerosis[32] On the other hand, the level of circulating MDA-LDL is reported to be elevated in patients with diabetes[33] and in STZ-induced diabetic rats[34] The mechanisms underlying the increase in MDA-LDL in diabetes have not been fully clarified, although enhancement of lipoperoxidation and decreased glutathione levels are reportedly involved in the elevated level of MDA-LDL[35] Interestingly, treatment with pravastatin, an HMG-CoA reductase inhibitor, can normalize elevated MDA-LDL levels without affecting the LDL-cholesterol level, suggesting that its effects might be attributable to decreased LDL oxidation[34] The precise pathway between elevated serum MDA-LDL and increased ISR was not defined in this study. Oxidized LDL has been reported to exert direct cytotoxicity on endothelial cell, to promote synthesis and secretion of adhesion molecules, and to increase monocyte chemotaxis and adhesion[11,12] Oxidized LDL increases vascular smooth muscle cell proliferation[13,14] and the mitogenic effect of oxidized LDL on vascular smooth muscle cells is mediated by the activation of the Ras/Raf/MEK/MAPK pathway[36] The inflammatory and proliferative effects of oxidized LDL may be accountable for the enhancement of neointimal cell proliferation that leads to ISR in diabetic patients.

The source of the elevated serum MDA-LDL in the diabetic patients who developed restenosis was uncertain in this study; however, the observed increase in MDA-LDL did not seem to be derived from local restenosis site (ie, neointima in the stent). First, pathology of the restenosis site consists of proliferated neointima and lacks a lipid core of oxidized LDL that might be the source of circulating MDA-LDL. Second, increased serum MDA-LDL levels were not observed in non-diabetic patients, including those who developed ISR (Fig 1). Finally, serum MDA-LDL levels were not altered after treatment of restenosis with balloon angioplasty to mechanically abrogate the neointima at the restenosis site (Fig 4). Interestingly, serum MDA-LDL was positively correlated with HbA1c level in diabetic patients (Fig 2), although HbA1c did not relate to ISR itself (Fig 3). These results support the notion that the elevation of serum MDA-LDL is attributable to the metabolic abnormalities of T2DM and acts as a promoter of ISR in diabetic patients.

The present study clearly showed that an elevated level of serum MDA-LDL in diabetic patients relates to ISR. However, because measurement of MDA-LDL was performed during the follow-up period in this study, it is uncertain whether the MDA-LDL level at the time of PCI predicts future ISR. In this respect, diabetic patients tend to have no apparent angina symptoms, even when significant restenosis has occurred. Thus, measurement of serum MDA-LDL during follow-up period, seems to be useful as an indicator of the potential development of ISR in these less symptomatic diabetic patients.

In conclusion, an elevated serum MDA-LDL concentration appears to be a major adverse factor for ISR in diabetic patients. Further studies are necessary to investigate the upstream and downstream pathways leading to ISR associated with elevated MDA-LDL, and to determine the origin of the increased serum MDA-LDL in diabetic patients.

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Hypoadiponectinemia in type 2 diabetes mellitus in men is associated with sympathetic overactivity as evaluated by cardiac ^{123}I-metaiodobenzylguanidine scintigraphy

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Abstract

Hypoadiponectinemia is associated with insulin resistance. However, there is very limited information about the relationship between plasma adiponectin and cardiac autonomic nervous function. We tested the hypothesis that hypoadiponectinemia is associated with cardiac sympathetic overactivity in patients with type 2 diabetes mellitus. Thirty-three male type 2 diabetic patients not on insulin treatment were classified into a hypoadiponectinemia group (plasma adiponectin concentration, <4.0 μg/mL; age, 58.6 ± 8.6 years [mean ± SD]; n = 14) and an age-matched normoadiponectinemia group (serum adiponectin concentration, ≥4.0 μg/mL; age, 58.2 ± 8.1 years; n = 19). In each patient, baroreflex sensitivity, heart rate variability, plasma norepinephrine concentration, and cardiac ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphic findings were assessed. Compared with the normoadiponectinemia group, the hypoadiponectinemia group had a higher body mass index ($P < .01$), higher plasma concentrations of glucose and insulin ($P < .05$ and $P < .01$, respectively), higher homeostasis model assessment of insulin resistance (HOMA-IR) values ($P < .005$), higher plasma triglyceride levels ($P < .05$), and lower plasma high-density lipoprotein cholesterol levels ($P < .05$). In the hypoadiponectinemia group, the autonomic function measurements included a lower baroreflex sensitivity ($P < .05$) and a lower delayed myocardial uptake of ^{123}I-MIBG ($P < .01$) with a higher washout rate ($P < .05$). Multiple regression analysis revealed that the plasma adiponectin level was independently associated with HOMA-IR ($F = 9.916$) and the percent washout rate of ^{123}I-MIBG ($F = 5.985$). Our results suggest that in middle-aged men with type 2 diabetes mellitus, hypoadiponectinemia is associated with cardiac sympathetic overactivity as determined by ^{123}I-MIBG scintigraphy.
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1. Introduction

Adiponectin is a hormone that is produced by adipocytes [1]. In patients with type 2 diabetes mellitus, low plasma adiponectin levels are associated with insulin resistance [2,3]. Low plasma adiponectin levels have also been shown to be an independent predictor of type 2 diabetes mellitus [4]. Most importantly, plasma adiponectin and insulin resistance are strongly associated with the development of coronary artery disease [5,6]. Cardiac autonomic nerve dysfunction is strongly related to cardiovascular mortality in type 2 diabetic patients [7]. Although insulin resistance depresses cardiac autonomic nervous function in these patients [8-10], there is limited information on the relationship between plasma adiponectin and cardiac autonomic nervous function [11]. In subjects with insulin resistance, sympathetic overactivity may play a central role in pathogenesis [12,13]. In this regard, cardiac ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy is a sensitive diagnostic tool that allows the direct assessment of sympathetic nervous function [14]. Plasma adiponectin levels have been found to be lower in men than in women, probably because of the effects of androgen [15]. In the present study, we investigated the association between plasma adiponectin levels and cardiac autonomic function in relation to insulin resistance in middle-aged male patients with type 2 diabetes mellitus.

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2. Methods and design

2.1. Subjects

Sixty-five consecutive Japanese male patients with type 2 diabetes mellitus who were admitted to our department in 2003 were screened. Type 2 diabetes mellitus was defined as a fasting plasma glucose concentration of 126 mg/dL or greater or a 2-hour plasma glucose concentration of 200 mg/dL or greater after a 75-g oral glucose load, or the self-reported use of antidiabetic medication [16]. Of the 65 patients, the 33 patients who did not have organic heart disease were enrolled. Patients with macroalbuminuria (>300 mg/d) or abnormal plasma creatinine concentrations (≥ 1.2 mg/dL) were excluded. Patients treated with insulin were also excluded. Plasma adiponectin concentrations were measured in venous blood obtained between 6:00 and 7:00 AM after an overnight fast by using a commercially available enzyme-linked immunosorbent assay kit (Otsuka Pharmaceuticals, Tokyo, Japan) [6]. Hypoadiponectinemia was defined as a plasma adiponectin concentration of less than 4.0 μg/mL [6]. Based on these results, 33 patients were classified as belonging to either the hypoadiponectinemia group (age, 58.6 ± 8.6 years [mean ± SD]; n = 14) or the normoadiponectinemia group (age, 58.2 ± 8.1 years; n = 19). The clinical characteristics of the studied patients are summarized in Table 1. After secondary hypertension was excluded, essential hypertension was defined as a diastolic blood pressure of 90 mm Hg or higher, a systolic blood pressure of 140 mm Hg or higher, or self-reported use of antihypertensive medication [17]. Dyslipidemia was defined as fasting triglyceride level of 200 mg/dL or higher or high-density lipoprotein (HDL) cholesterol level less than 35 mg/dL [17]. Insulin resistance was evaluated by using the homeostasis model assessment of insulin resistance (HOMA-IR) according to the following formula: HOMA-IR = {(fasting plasma insulin [μU/mL] × fasting plasma glucose [mmol/L])/22.5} [18]. Prior written informed consent was obtained from all patients, and the study protocol was approved by the institutional review board of Oita University.

2.2. Echocardiography

M-mode 2-dimensional echocardiography and cardiac Doppler recordings were obtained by using a phase-array echo-Doppler system. The left ventricular mass was calculated according to Devereux et al [19]: left ventricular mass = {1.04([LVIDd + IVSTd + PWTd]^3 − LVIDd^3) − 14 g}, where LVIDd is the left ventricular internal dimension at end diastole; IVSTd the interventricular septal thickness at end diastole, and PWTd the posterior wall thickness at end diastole. To calculate the left ventricular mass index (LVMI), the left ventricular mass was divided by the body surface area. Based on pulsed Doppler recordings, the peak velocity of early (E) and late ventricular filling (A) was determined, and the ratio (E/A) and deceleration time were measured to assess cardiac diastolic function.

2.3. Plasma norepinephrine concentration, heart rate variability, and baroreflex sensitivity

All subjects were studied while in the supine position in a quiet room between 9:00 and 11:00 AM [6-8]. A catheter was inserted into the right cubital vein, and the arterial blood pressure was recorded noninvasively by tonometry (Jentow-7700; Nihon Colin, Komaki, Japan) [20]. Arterial blood pressure and a 12-lead electrocardiogram (ECG) were monitored simultaneously; data were stored in a PCM data recorder (RD-200T; TEAC, Tokyo, Japan). Holter ECG recordings (model 459, Del Mar Avionics, Irvine, CA) were also obtained. After an interval of 30 minutes to allow the patient to stabilize, the patient was asked to breathe at a rate of 15 breaths per minute measured with a metronome. Subsequently, blood samples were obtained from the venous catheter to measure plasma norepinephrine concentration. The baroreflex sensitivity (BRS) was assessed with the phenylephrine method [8-10]. Phenylephrine (2-3 μg/kg) was injected over 15 seconds to increase the systolic blood pressure by 15 to 40 mm Hg. The BRS was calculated as the slope of the linear regression line relating the systolic blood pressure changes to the RR interval changes. Regression lines with more than 20 data points and a correlation coefficient (r) greater than 0.8 were accepted for analysis. The mean of the 2 slope values was taken as the BRS value.

Table 1
Clinical characteristics of the studied patients

	Hypo-AD (n = 14)	Normo-AD (n = 19)	P
Age (y)	58.6 ± 8.6	58.2 ± 8.1	NS
Duration of diabetes (y)	7.9 ± 5.4	7.2 ± 5.8	NS
Essential hypertension (%)	65	74	NS
Dyslipidemia (%)	48	53	NS
Drug use (%)			
Sulfonylurea	48	53	NS
α Glucosidase inhibitors	42	41	NS
Statin	39	41	NS
Calcium-channel antagonists	47	41	NS
Angiotensin-converting enzyme inhibitors	29	24	NS
Angiotensin receptor blockers	52	59	NS
Body mass index (kg/m^2)	27.0 ± 1.4	24.3 ± 3.4	< .01
Systolic blood pressure (mm Hg)	132 ± 18	130 ± 14	NS
Diastolic blood pressure (mm Hg)	77 ± 11	75 ± 9	NS
Heart rate (beats/min)	68 ± 6	67 ± 9	NS
Fasting plasma glucose (mg dL)	156 ± 28	140 ± 25	< .05
Fasting immunoreactive insulin (μU mL)	8.3 ± 2.6	6.1 ± 1.7	< .01
HOMA-IR	3.2 ± 1.0	2.1 ± 0.7	< .005
Hemoglobin A_{1c} (%)	7.7 ± 1.2	7.5 ± 1.0	NS
Total cholesterol (mg/dL)	211 ± 23	196 ± 27	NS
Triglyceride (mg/dL)	165 ± 55	132 ± 32	< .05
HDL cholesterol (mg dL)	37 ± 8	45 ± 13	< .05
Uric acid (mg dL)	6.4 ± 1.6	5.9 ± 1.3	NS
Creatinine (mg/dL)	0.8 ± 0.2	0.7 ± 0.2	NS
Creatinine clearance (mL min)	83 ± 36	104 ± 32	NS

Data are mean ± SD unless otherwise indicated. Hypo-AD indicates hypoadiponectinemia group; Normo-AD, normoadiponectinemia group.

Heart rate variability (HRV) was analyzed by using a 300-second interval on the Holter ECG recordings obtained immediately before phenylephrine injection. The power spectrum of the RR interval was computed by a fast Fourier transform and expressed as the area under the power spectrum [8-10]. We calculated the power of 2 spectral bands, the low-frequency component (LF) at 0.04 to 0.15 Hz and the high-frequency component (HF) at 0.15 to 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. Whereas HF represents cardiac vagal activity, LF is a mixture of vagal and sympathetic activities [21]. The LF/HF was used to estimate cardiac sympathetic activity [21].

2.4. Cardiac ^{123}I-MIBG scintigraphy

Metaiodobenzylguanidine is a guanethidine analogue that is accumulated in the norepinephrine storage granules in postganglionary sympathetic neurons. Radioactive labeling of MIBG allows visualization of the sympathetic neuronal tissue in richly innervated organs such as the heart [14]. Planar and single-photon emission computed tomography studies were performed at 15 minutes and 4 hours after the injection of 111 MBq of ^{123}I-MIBG with a rotating gamma camera (ZLC 7500, Siemens, Munich, Germany). The data were analyzed using analysis software (SCINTIPAC, Shimadzu, Kyoto, Japan). The anterior planar images from the early and delayed ^{123}I-MIBG studies were analyzed visually. To do the semiquantitative analysis, regions of interest were drawn over the whole heart, and a 10 × 10-mm area over the upper mediastinum on the early and delayed planar images was used to calculate the mean heart-mediastinum (H/M) ratio. After correcting for the physical decay of iodine 128, the percent washout rate (WR) of the tracer from the myocardium was determined over a 4-hour period.

2.5. Statistical analysis

Data are presented as mean ± SD. Differences between the 2 groups were analyzed by the unpaired Student t test, χ^2 test, or Fisher exact probability test as appropriate. A value of $P < .05$ was considered statistically significant. Simple (Spearman rank) correlation coefficients between the plasma adiponectin concentration and the various variables were calculated, and a stepwise multiple regression analysis was then used to evaluate the independent association of these variables with plasma adiponectin concentration. On the multivariate analysis, F values of 4 or greater were considered statistically significant.

3. Results

As shown in Table 1, the 2 groups had a similar mean age and there were no significant differences with respect to duration of diabetes, number of patients with essential hypertension or dyslipidemia, and medications administered. The hypoadiponectinemia group had significantly higher body mass index ($P < .01$), fasting plasma glucose concentrations ($P < .05$), and insulin concentrations ($P < .01$), resulting in a higher HOMA-IR ($P < .005$). There was no significant difference in hemoglobin A_{1c} between the 2 groups. The plasma triglyceride was higher ($P < .05$) and the HDL cholesterol was lower ($P < .05$) in the hypoadiponectinemia group. The plasma creatinine and creatinine clearance were not significantly different. The hemodynamic data listed in Table 1 were obtained immediately before BRS assessment. The resting heart rate, as well as the systolic and diastolic blood pressures, was not significantly different. With respect to the echocardiographic findings, there were no significant differences in LVIDd and LVIDs at end systole (48 ± 4 vs 50 ± 4 mm and 31 ± 4 vs 33 ± 3 mm, respectively), IVSTD (9.0 ± 1.3 vs 9.5 ± 1.5 mm), PWTd (9.5 ± 1.2 vs 10.1 ± 1.2 mm), ejection

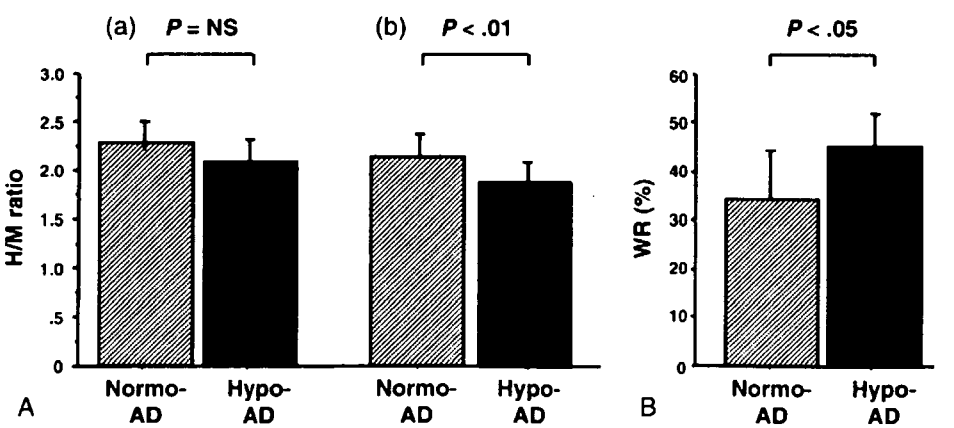

Fig. 1. Comparison of cardiac ^{123}I-MIBG scintigraphic findings in the type 2 diabetic patients with normoadiponectinemia (Normo-AD) and hypoadiponectinemia (Hypo-AD). A, Myocardial uptake of ^{123}I-MIBG in the early (a) and delayed (b) phases. Myocardial uptake of ^{123}I-MIBG is expressed as the mean H/M ratio. B, Percent WR of ^{123}I-MIBG. Data are mean ± SD.

fraction (70% ± 5% vs 69% ± 4%), and LVMI (113 ± 29 vs 121 ± 19 g/m^2). However, the E/A ratio was greater (0.95 ± 0.27 vs 0.77 ± 0.18, $P < .05$) and the deceleration time was longer (253 ± 27 vs 230 ± 31 msec, $P < .05$) in the hypoadiponectinemia group.

With respect to the results of the cardiovascular autonomic function tests, the BRS was lower in the hypoadiponectinemia group (6.0 ± 3.3 vs 9.4 ± 4.8 ms/mm Hg, $P < .05$), whereas plasma norepinephrine concentrations were similar (239 ± 99 vs 217 ± 112 pg/mL, P = not significant [NS]). The HRV analysis showed that the HF power and the LF/HF ratio were not significantly different (4.1 ± 1.7 vs 3.8 ± 1.4 ln ms^2, P = NS; 1.6 ± 0.9 vs 1.3 ± 0.9, P = NS, respectively). On cardiac ^{123}I-MIBG scintigraphy, although the H/M ratio in the early phase was not significantly different (2.12 ± 0.22 vs 2.27 ± 0.25, P = NS; Fig 1A[a]), in the delayed phase, the H/M ratio was lower in the hypoadiponectinemia group (1.94 ± 0.35) than in the normoadiponectinemia group (2.33 ± 0.31, $P < .01$; Fig. 1A[b]). The percent WR of ^{123}I-MIBG was higher in the hypoadiponectinemia group (42.5% ± 9.0% vs 34.5% ± 11.8%, $P < .05$; Fig. 1B). Table 2 shows the correlations between plasma adiponectin concentration and clinical variables for all of the patients in both groups. Plasma adiponectin concentration correlated negatively with body mass index, fasting plasma insulin, HOMA-IR, triglyceride, and percent WR of ^{123}I-MIBG, and positively with plasma HDL cholesterol, BRS, and H/M ratios in the early and delayed phases. Stepwise multiple regression analysis was done using these 9 variables. Plasma adiponectin concentration was found to be independently associated with HOMA-IR (F = 9.196) and the percent WR of ^{123}I-MIBG (F = 5.985) (Table 3).

Table 2
Correlations of plasma adiponectin with other variables

Parameters	Univariate	
	r	P
Age	−0.212	NS
Duration of diabetes mellitus	−0.190	NS
Body mass index	−0.372	.0332
Systolic blood pressure	−0.242	NS
Diastolic blood pressure	−0.120	NS
Heart rate	−0.335	NS
Total cholesterol	−0.089	NS
Triglyceride	−0.369	.0346
HDL cholesterol	−0.422	.0114
Uric acid	−0.228	NS
Fasting plasma glucose	−0.334	NS
Fasting immunoreactive insulin	−0.427	.0132
HOMA-IR index	−0.496	.0033
Hemoglobin A_{1c}	−0.206	NS
Creatinine	−0.202	NS
Creatinine clearance	0.333	NS
EF	0.155	NS
LVIDd	−0.317	NS
LVIDs	−0.236	NS
IVSTd	−0.153	NS
PWTd	−0.316	NS
LVMI	−0.297	NS
E/A ratio	0.151	NS
Deceleration time	−0.189	NS
Baroreflex sensitivity	0.407	NS
Plasma norepinephrine	−0.111	NS
HF power	0.091	NS
LF/HF	−0.252	NS
H/M ratio at early phase	0.401	.0206
H/M ratio at delayed phase	0.482	.0046
Percent WR of ^{123}I-MIBG	−0.423	.0142

Table 3
Stepwise regression analyses between plasma adiponectin and various parameters

Independent variable	Regression coefficient	SE	Standard regression coefficient	F
To adiponectin intercept	19.312			
HOMA-IR	−2.523	0.832	−0.444	9.196
Percent WR of ^{123}I-MIBG	−0.174	0.071	−0.358	5.985

F values equal to or greater than 4 were considered statistically significant.

4. Discussion

In the present study, middle-aged male type 2 diabetic patients with hypoadiponectinemia had a higher body mass index, higher fasting plasma concentrations of glucose and insulin, higher HOMA-IR, higher plasma triglyceride levels, and a lower plasma HDL cholesterol level than patients who had normoadiponectinemia. The body mass index, fasting plasma insulin concentration, HOMA-IR, plasma triglyceride concentration, and plasma HDL cholesterol concentration had a significant correlation with the plasma adiponectin concentration, which suggests a strong association between hypoadiponectinemia and insulin resistance [2,3]. Because there is a substantial association between these variables, it is possible that hypoadiponectinemia is associated with the overall abnormalities seen in glucose and lipid metabolism. Thus, although the present study was designed to assess the impact of hypoadiponectinemia, the obtained results might have been predominantly influenced by insulin resistance rather than hypoadiponectinemia.

In the present study, ^{123}I-MIBG scintigraphy showed that the H/M ratio in the delayed phase was decreased and the percent WR of ^{123}I-MIBG was increased in the hypoadiponectinemia group, and the percent WR of ^{123}I-MIBG was independently associated with plasma adiponectin concentration, which suggests that there is substantial sympathetic overactivity in patients with low plasma adiponectin concentration. These findings are novel. There is a growing body of evidence that sympathetic overactivity may play a central role in the pathogenesis of insulin resistance [22,23]. Recent experimental studies have also suggested that sympathetic overactivity has a role in the regulation of adiponectin expression [24,25]. Fasshauer et al [24] reported that adiponectin messenger RNA expression was inhibited by β-adrenergic stimulation via protein kinase A in 3T3-L1

adipocytes. More recently, Delporte et al [25] demonstrated that β-adrenergic stimulation down-regulated adiponectin messenger RNA in cultured mouse explants from the visceral and subcutaneous regions. Based on these experimental observations, it can be postulated that in patients with insulin resistance substantial sympathetic overactivity might reduce the adiponectin gene expression. However, it is still unclear whether low plasma adiponectin concentration, as observed in the clinical setting, is the cause or the result of sympathetic overactivity. With respect to vagal function, the BRS value was lower in patients with hypoadiponectinemia than in patients with normoadiponectinemia and was positively correlated with plasma adiponectin. Because there is a strong interaction between sympathetic and vagal activity [11], it is uncertain whether the low BRS value observed in the hypoadiponectinemia patients reflects relatively depressed vagal activity in response to sympathetic overactivity.

Until now, very limited information was available on the relationship between plasma adiponectin and cardiac autonomic nervous function. Wakabayashi and Aso [11] studied the relationship between plasma adiponectin concentration and the power spectral analysis of HRV in 105 patients with type 2 diabetes mellitus (51 women and 54 men); they reported that plasma adiponectin concentration showed an independent negative association with the 24-hour LF/HF ratio. Based on this observation, they concluded that a sympathovagal balance favoring relative sympathetic activation was associated with hypoadiponectinemia in patients with type 2 diabetes mellitus [11]. Although our HRV analysis did not show an association with plasma adiponectin concentration, our ^{123}I-MIBG scintigraphic findings appear to support their conclusion.

It is noteworthy that diastolic function, as determined by E/A ratio and deceleration time, is depressed in patients with hypoadiponectinemia. The exact mechanisms that explain the association between hypoadiponectinemia and diastolic dysfunction have not been elucidated. In a recent study demonstrating the association between insulin resistance and diastolic function in patients with type 2 diabetes mellitus and subjects with impaired glucose tolerance [26], the authors speculated that the insulin resistance may be involved in the onset of cardiac fibrosis, as shown in an experimental rat model of the prestage of type 2 diabetes mellitus [27].

Some methodological issues have to be addressed. First, 64% of hypoadiponectinemia patients and 74% of normoadiponectinemia patients had been diagnosed as having essential hypertension. In addition, 48% of hypoadiponectinemia patients and 53% of normoadiponectinemia patients had been diagnosed as having dyslipidemia. All these patients were being treated with one or more antihypertensive drugs and/or a statin, as shown in Table 1. These medications might have affected our results. Second, the present study included a relatively small number of patients because of our strict inclusion criteria. Third, there is currently no "gold standard" for the assessment of human sympathetic nervous activity to use as a comparison with other techniques. In fact, the reason why the patients with hypoadiponectinemia did not show an altered HRV or altered plasma norepinephrine levels, as expected, cannot be explained rationally. Regarding the HRV analysis, we analyzed ECG recording data using a 300-millisecond interval at rest obtained immediately before phenylephrine injection. The analysis using 24-hour data could have detected the sympathetic overactivity such as increased LF/HF. With respect to the levels of plasma norepinephrine, Grassi and Esler [28] mentioned that plasma norepinephrine measurements provide global indices of sympathetic nervous function but provide no information on regional sympathetic nervous system function. The authors, therefore, suggested that the sensitivity of the plasma norepinephrine approach in detecting increased sympathetic activity is not optimal [28]. Based on our observations, it is likely that ^{123}I-MIBG scintigraphy may be fairly sensitive in detecting cardiac sympathetic overactivity, at least in the population of patients that we studied. Finally, we divided the patients into 2 groups based on the criteria used for Japanese patients with coronary artery disease [6]. It remains to be determined whether this cutoff index (<4.0 μg/mL) is valid for use in type 2 diabetic patients.

In conclusion, the present study suggests that, in middle-aged male patients with type 2 diabetes mellitus, hypoadiponectinemia is associated with sympathetic overactivity as evaluated by cardiac ^{123}I-MIBG scintigraphy.

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Soluble tumor necrosis factor receptor 2 is independently associated with pulse wave velocity in nonobese Japanese patients with type 2 diabetes mellitus

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Abstract

The aim of the present study was to investigate the factors contributing to pulse wave velocity (PWV) in patients with type 2 diabetes mellitus. We focused on tumor necrosis factor (TNF) including soluble TNF receptors (sTNF-R1, sTNF-R2) in this study because TNF seems to be associated with the progression of atherosclerosis and because the relationships between PWV and TNF were not yet examined in type 2 diabetic patients. Univariate regression analyses showed that PWV was positively correlated with age ($r = 0.492$, $P < .001$), diabetes duration ($r = 0.251$, $P = .021$), systolic ($r = .595$, $P < .001$) and diastolic ($r = 0.248$, $P = .022$) blood pressure, antihypertensive medication ($r = 0.268$, $P = .013$), and the concentrations of sTNF-R1 ($r = 0.354$, $P = .001$) and sTNF-R2 ($r = 0.415$, $P < .001$). Although there was a positive correlation between TNF-a and sTNF-R1 ($r = 0.382$, $P < .001$) or sTNF-R2 ($r = 0.394$, $P < .001$), TNF-a was not associated with PWV. Other variables including gender were not associated with PWV. Multiple regression analyses showed that PWV was independently predicted by the level of age ($F = 15.1$), systolic blood pressure ($F = 31.6$), and sTNF-R2 ($F = 5.2$), which explained 49.2% of the variability of PWV. From these results, it can be concluded that serum soluble TNF receptor is an important independent factor associated with aortic PWV in type 2 diabetic patients.
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1. Introduction

Type 2 diabetes mellitus is associated with high mortality and morbidity due to atherosclerosis including coronary heart disease (CHD). As regards the risk factors responsible for the evolution of atherosclerosis in diabetic patients, Bierman [1] previously estimated that typical risk factors including blood pressure, cholesterol, and smoking can account for no more than 25% to 30% of excess cardiovascular risk factors in diabetic patients. Thus, other factors seem to play a major role in the progression of atherosclerosis in diabetes.

A number of studies have identified abnormalities of arterial stiffness in subjects with diabetes [2-4]. It has recently been reported that aortic stiffness measured by pulse wave velocity (PWV) is highly predictive of cardiovascular mortality in subjects with type 2 diabetes mellitus [5]. PWV also predicts cardiovascular mortality in nondiabetic subjects [6]. Whereas age and blood pressure are shown to be associated with PWV, age and blood pressure alone do not completely account for the abnormalities of aortic stiffness in subjects with type 2 diabetes mellitus.

Tumor necrosis factor a (TNF-a), the proinflammatory cytokine, seems to be associated with the progression of

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atherosclerosis in type 2 diabetes mellitus. There is, however, a paucity of the literature regarding the relationship between TNF-a and atherosclerosis in type 2 diabetic patients. We recently demonstrated that TNF-a system activity, especially soluble TNF-R1 (sTNF-R1), is strongly and independently associated with albuminuria in type 2 diabetic patients [7]. Klein et al [8] demonstrated that TNF-a influences the metabolism of glycosaminoglycans, which are components of the vascular endothelium and the glomerular basement membrane and are involved in the etiology of microalbuminuria. Shai et al [9] demonstrated that sTNF-R2 is strongly associated with risk of CHD in patients with type 2 diabetes mellitus. It is therefore hypothesized that there is a causal relationship between TNF-a and vascular complications in diabetic patients. To the best of our knowledge, however, the relationships between serum soluble TNF receptor and PWV have not yet been evaluated in type 2 diabetes mellitus.

In this context, a major problem is that atherosclerotic disease such as CHD, renal failure, stroke, and peripheral arterial occlusive disease of the lower extremities might affect PWV. Moreover, it is well recognized that being overweight or hyperglycemic per se might affect serum concentrations of TNF-a and soluble TNF receptor in humans [10,11]. To disclose the mechanisms responsible for the early stage of atherosclerosis, we recruited nonobese, well-controlled, unique Japanese type 2 diabetic patients who had no evidence of vascular complications including CHD, cerebral infarction, renal failure, and peripheral arterial occlusive disease, taking into account body mass index (BMI) and fasting glucose. This is the first description of serum level of soluble TNF receptor being independently associated with PWV in nonobese, well-controlled, unique Japanese type 2 diabetic patients.

2. Subjects and methods

Eighty-six Japanese type 2 diabetic patients who visited Kansai-Denryoku Hospital were enrolled for the present study. They had no abnormal electrocardiogram findings suggestive of ischemic heart disease. They also had normal serum creatinine level (b1.0 mg/dL), ankle brachial index greater than 1.0, and no signs of cerebral stroke. Thus, they were considered to have no major cardiovascular disease at the time of the study. Type 2 diabetes mellitus was diagnosed based on the criteria of the World Health Organization [12]. The subjects had no evidence of current acute illness or infectious process. The duration of diabetes was 11.1 F 0.8 years (mean F SEM). Seventy-five of 86 patients were taking sulfonylureas (gliclazide) and the rest were controlled with diet alone. None had received insulin therapy or any medications known to enhance insulin sensitivity such as biguanide or pioglitazone. Before the study, 2 dietitians confirmed, by checking daily food records, that the subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. Blood pressure was measured twice with Colin BP-103ill (Tokyo, Japan) while the patients were in the sitting position after at least 5 minutes rest and the mean value was used for the analysis. Hypertension was defined as systolic blood pressure of 140 nbsp;mm Hg or higher and/or diastolic blood pressure of 90 mm Hg or higher or current use of antihypertensive medication. On the day of the examination, they were told not to take all medications including sulfonylurea, antihypertensive medications, and lipid-lowering agents. Thirty-four (40%) patients were treated with antihypertensive medications. Twenty-nine (34%) of 86 patients were receiving lipid-lowering agents (bezafibrate, 17; HMG-CoA reductase inhibitor, 12). Cigarette smoking was dichotomized into bneverQ and beverQ (including past and current) by use of a questionnaire. They were told not to smoke at least 1 day before the study. They did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with the glucose oxidase method. Triglyceride, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels were also measured. The low-density lipoprotein (LDL) cholesterol level was calculated with the Friedewald formula [13]. Serum insulin was measured by a two-site immunoradiometric assay (Insulin Riabead II, Dainabot, Osaka City, Japan). Coefficients of variation were 4% and 7% for insulin greater than and insulin less than 25 IU/mL, respectively.

The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the formula: fasting serum insulin (IU/mL) • fasting plasma glucose (mmol/L)/22.5 [14]. HOMA-IR was validated in diabetic subjects with diet therapy alone and in those treated with sulfonylureas [15,16]. HOMA-IR greater than 2.5 was defined as insulin resistance [17,18]. Serum TNF-a concentrations were measured by an enzyme immunoassay kit (Quantikine HS Human TNF-a immunoassay kit, R&D Systems, Minneapolis, MN), and serum concentrations of sTNF-R1 and sTNF-R2 were measured by enzyme-linked immunosorbent assay (BIOTRAK, Amersham Life Sciences, Uppsala, Sweden) as described previously [7,19]. The limits of sensitivity for TNF-a, sTNF-R1, and sTNF-R2 were 0.5, 25, and 50 pg/mL, respectively. The intra-assay coefficients of variation for TNF-a, sTNF-R1, and sTNF-R2 were 5.9%, 4.7%, and 3.2%, respectively. The inter-assay coefficients of variation for TNF-a, sTNF-R1, and sTNF-R2 were 10.8%, 5.8%, and 3.6%, respectively.

2.1. Measurement of PWV

Pulse wave velocity was measured with a volume plethysmographic apparatus (form PWV/ABI version-112, Colin, Komaki, Japan). Briefly, after an overnight fast, the subjects were examined in the early morning while in the supine position. Electrocardiogram electrodes were placed on both wrists. A microphone for detecting heart sounds was placed on the left edge of the sternum. The cuffs were wrapped on both the brachia and ankles. The characteristic

Table 1
Clinical profiles of patients studied

	All patients	Male patients	Female patients
n	86	61	25
Age (y)	62.8 F 1.0	61.3 F 1.1	66.3 F 1.54
Duration of diabetes (y)	11.1 F 0.8	11.1 F 1.0	11.2 F 1.3
Smoking (%)	26	33	844
BMI (kg/m^2)	22.8 F 0.3	23.1 F 0.2	22.2 F 0.64
Systolic blood pressure (mm Hg)	136 F 2	134 F 2	140 F 4
Diastolic blood pressure (mm Hg)	82 F 1	83 F 1	81 F 2
Sulfonylurea/diet	76/10	55/6	21/4
HMG-CoA reductase inhibitor (%)	15	11	244
Bezafibrate (%)	21	21	20
Antihypertensive agent (%)	42	36	56
Fasting glucose (mg/dL)	142 F 3	144 F 3	133 F 5
HbA_{1c} (%)	7.0 F 0.1	7.0 F 0.1	7.1 F 0.2
Fasting insulin (I U/mL)	6.6 F 0.4	6.7 F 0.4	6.3 F 0.7
HOMA-IR	2.32 F 0.15	2.40 F 0.17	2.13 F 0.24
Triglyceride (mg/dL)	122 F 6	128 F 8	105 F 94
HDL cholesterol (mg/dL)	59 F 2	55 F 2	66 F 344
Total cholesterol (mg/dL)	204 F 4	201 F 4	210 F 9
LDL cholesterol (mg/dL)	126 F 4	126 F 4	128 F 8
Serum creatinine (mg/dL)	0.76 F 0.02	0.82 F 0.02	0.63 F 0.0444
Serum urea nitrogen (mg/dL)	15.3 F 0.4	15.2 F 0.4	15.6 F 0.9
TNF-a (pg/mL)	3.3 F 0.2	3.60 F 0.29	2.65 F 0.194
sTNF-R1 (pg/mL)	1184 F 44	1184 F 41	1185 F 100
sTNF-R2 (pg/mL)	2053 F 57	2070 F 59	2013 F 115
PWV (cm/s)	1661 F 34	1663 F 37	1655 F 59

4 P b .05 vs male patients.
44 P b .01 vs male patients.

points of wave forms were determined automatically and the results were printed out. All procedures took about 5 minutes. The interobserver and intraobserver variation coefficients were 8.4% and 10.0%, respectively. Measurements on different days revealed that slight changes in blood pressure did not correlate with changes in PWV. The mean PWV value measured on either side of each patient was used for the analysis.

2.2. Statistical analysis

Data were presented as mean F SEM. Statistical analyses were conducted with the StatView 5 system (Statview, Berkeley, CA). Simple (Spearman rank) correlation coefficients between PWV and measures of variables were calculated, and a stepwise multiple regression analysis was then used to evaluate the independent association of these variables with PWV. The means of the 2 groups (male vs female patients) were compared with Student t test. P b .05 was considered as significant. In multivariate analysis, an F value of 4 or greater was considered significant.

3. Results

Clinical characteristics of all subjects are summarized in Table 1. They were all Japanese type 2 diabetic patients (61 men and 25 women) with an age range of 43 to 84 years and a BMI of 17.1 to 26.7 kg/m^2. They all were nonobese [20]. The ranges of fasting glucose and glycosylated hemoglobin (HbA_{1c}) were from 92 to 194 mg/dL and from 4.9% to 10.1%, respectively. There was a wide variation in insulin resistance calculated from HOMA-IR (range, 0.51-7.17). Thirty-one (36%) of the 86 subjects had HOMA-IR greater than 2.5, indicating that they were insulin resistant [17,18].

Clinical features of male and female patients are shown in Table 1. Although a significant difference was observed in age, smoking status, BMI, triglycerides, HDL cholesterol, creatinine, and TNF-a between the 2 groups, there was no significant difference in some variables including sTNF-R1, sTNF-R2, and PWV between the 2 groups.

Values of PWV ranged from 1139 to 2728 cm/s (mean, 1661 cm/s; SD, 292 cm/s) (Table 1). Only 17 (20%) of 86 patients had PWV less than 1400 cm/s (range, 1139-1385 cm/s). This finding was far different from the recent report by Kim et al [21] in which 90% of the PWV values were between 525 and 1399 cm/s in 2488 healthy individuals. We therefore considered all patients as a group and investigated the relationships between PWV and some variables including TNF-a with univariate and multiple regression analyses.

Table 2
Correlation of brachial-ankle PWV with measures for variables in all diabetic patients

	Univariate		Multivariate
	r	P	F
Age	0.492	b.001	15.1
Diabetes duration	0.251	.021	2.9
Systolic blood pressure	0.595	b.001	31.6
Diastolic blood pressure	0.248	.022	0.4
TNF-a	0.167	.123	–
sTNF-R1	0.354	.001	0.1
sTNF-R2	0.415	b.001	5.2
Gender	• 0.032	.765	–
Smoking	• 0.047	.663	–
BMI	• 0.113	.296	–
Fasting glucose	0.075	.492	–
HbA_{1c}	0.054	.620	–
Insulin	• 0.007	.950	–
HOMA-IR	0.019	.861	–
Triglycerides	• 0.095	.382	–
Total cholesterol	• 0.035	.747	–
HDL cholesterol	0.036	.743	–
LDL cholesterol	• 0.043	.692	–
Serum creatinine	0.079	.465	–
Therapy for diabetes	• 0.023	.831	–
Therapy for hypertension	0.268	.013	2.1
Therapy for triglyceride	0.055	.610	–
Therapy for cholesterol	0.016	.881	–

Table 2 illustrates the correlation between PWV and the measures of variables including age, sex, and TNF in all diabetic patients. PWV was positively correlated with age (r = 0.492, P b .001), diabetes duration (r = 0.251, P = .021), systolic blood pressure (r = 0.595, P b .001), diastolic blood pressure (r = 0.248, P = .022), sTNF-R1 (r = 0.354, P = .001), and sTNF-R2 (r = 0.415, P b .001). The difference in PWV was also observed between the patients taking antihypertensive medications and those who were not (r = 0.268, P = .013). However, other variables including TNF-a, sex, smoking status, BMI, and therapy for diabetes or hyperlipidemia were not associated with PWV.

Multiple regression analyses were carried out by using the stepwise procedure in all diabetic patients (Table 2). The analysis included PWV as a dependent variable and candidate risk factors (age, diabetes duration, systolic blood pressure, diastolic blood pressure, sTNF-R1, sTNF-R2, therapy for hypertension) as independent variables (Table 2). PWV was independently predicted by age (F = 15.1), systolic blood pressure (F = 31.6), and serum concentration of sTNF-R2 (F = 5.2), which explained 49.2% of the variability of PWV in our diabetic patients. Other variables including diabetes duration, diastolic blood pressure, therapy for hypertension, and sTNF-R1 were not independently associated with PWV in our nonobese Japanese type 2 diabetic patients. Finally, smoking status and BMI were incorporated as candidate risk factors. PWV was independently predicted by age (F = 15.1), systolic blood pressure (F = 31.6), and serum concentration of sTNF-R2 (F = 5.2), which explained 49.2% of the variability of PWV in the patients. Smoking status (F = 3.1) and BMI (F = 3.2) were not independently associated with PWV in our patients.

4. Discussion

The main novel finding in the present study is that sTNF-R2 is strongly and independently associated with brachial-ankle PWV in nonobese Japanese type 2 diabetic patients.

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by insulin resistance and/or defective insulin secretion [22]. As distinct from white populations, Japanese type 2 diabetic patients are unique in that they are not always obese, but some individuals are both insulin sensitive and insulin resistant [23-25]. The present study reconfirmed that 36% of the nonobese Japanese type 2 diabetic patients were insulin resistant.

Atherosclerosis is the leading cause of mortality and morbidity in subjects with type 2 diabetes mellitus. Although the mechanisms by which atherosclerosis occurs are not fully clarified, it has been shown that most clinical events result from mild to moderate arterial lesions that abruptly progress to severe obstructions [26]. Thus, detecting the early stage of atherosclerosis is considered to be the first line to clarify the mechanisms responsible for the evolution of atherosclerosis in type 2 diabetic patients.

Pulse wave velocity, a measure of aortic distensibility, is a noninvasive method to detect the early stage of atherosclerosis in humans. There are some reports suggesting that gender per se might affect the value of PWV [27-29]. We could not, however, find any significant relationship between PWV and gender in our patients.

Pulse wave velocity is shown to predict mortality in patients with hypertension and older healthy individuals, independently of known confounding factors [30]. Several studies have demonstrated that PWV correlates with diabetic complications. Tanokuchi et al [31] reported that PWV is related to serum creatinine level in patients with type 2 diabetes mellitus. Okada et al [32] showed the relationship between PWV and autonomic neuropathy in type 2 diabetic patients. Aso et al [33] demonstrated that PWV was associated with retinopathy and albuminuria in type 2 diabetic patients. PWV is also confirmed to be highly predictive of cardiovascular mortality in subjects with type 2 diabetes mellitus [5]. Thus, it may be hypothesized that micro- and macrovascular complications of type 2 diabetes mellitus share the common pathophysiologic mechanisms. This hypothesis is supported by the report from Neil et al [34] that microalbuminuria, a component of microvascular disease, has strongly and independently been associated with the development of cardiovascular disease and mortality in type 2 diabetic patients. Retinopathy, another microvascular complication, has been shown to be associated with increased cardiovascular and all-cause mortality risk in type 2 diabetic patients [35].

It is well recognized that low-grade inflammation per se seems to have a major role in the pathogenesis of

Table 3
Correlation of brachial-ankle PWV with measures for variables in 52 diabetic patients who have not received antihypertensive medications

	Univariate		Multivariate
	r	P	F
Age	0.291	.038	2.8
Diabetes duration	0.086	.537	–
Systolic blood pressure	0.533	b.001	15.2
Diastolic blood pressure	0.297	.034	1.6
TNF-a	0.198	.157	–
sTNF-R1	0.465	b.001	0.5
sTNF-R2	0.482	b.001	11.2
Gender	0.021	.879	–
Smoking	0.084	.549	–
BMI	• 0.045	.747	–
Fasting glucose	• 0.026	.853	–
HbA_{1c}	0.004	.974	–
Insulin	0.035	.804	–
HOMA-IR	• 0.003	.982	–
Triglycerides	• 0.018	.896	–
Total cholesterol	• 0.144	.303	–
HDL cholesterol	• 0.074	.599	–
LDL cholesterol	• 0.153	.275	–
Serum creatinine	0.180	.198	–
Therapy for diabetes	• 0.031	.827	–
Therapy for triglyceride	0.074	.598	–
Therapy for cholesterol	• 0.021	.884	–

atherosclerosis and diabetes [36]. Ridker et al [37] showed that increased levels of inflammatory markers such as the high-sensitivity C-reactive protein (CRP) and interleukin 6 (IL-6) can predict increased risk of cardiovascular disease in humans. Serum IL-6 is shown to be predictive of the development of type 2 diabetes mellitus in women [38]. Stehouwer et al [39] confirmed that increased urinary albumin excretion, endothelial dysfunction, and chronic inflammation are interrelated processes that are associated with risk of death in type 2 diabetic patients. In the present study, however, we could not find any significant relationships between PWV and serum concentrations of CRP or IL-6 in our diabetic patients (data not shown). It may be argued that antihypertensive medications affect PWV by altering blood pressure in our patients. We therefore investigated 52 patients who have not received antihypertensive medications and found that PWV was independently predicted by the level of systolic blood pressure ($F = 15.2$) and sTNF-R2 ($F = 11.2$), which explained 35.6% of the variability of PWV (Table 3). This finding also supports our idea that TNF system activity per se plays an important role in PWV in Japanese type 2 diabetic patients.

Tumor necrosis factor a, the proinflammatory cytokine, seems to be associated with the progression of atherosclerosis in type 2 diabetes mellitus. There is, however, a paucity of the literature regarding the relationship between TNF-a and atherosclerosis in type 2 diabetic patients. As an index of TNF-a system activities, we measured serum TNF-a, serum sTNF-R1, and serum sTNF-R2 and found that serum sTNF-R2 is strongly and independently associated with PWV in nonobese Japanese type 2 diabetic patients. However, we could not find any independent relationship between PWV and serum TNF-a. It should be noted that TNF receptor levels remain elevated for a longer time than TNF-a itself and TNF receptors might reflect the degree of TNF-a activation more accurately than the measurement of TNF-a itself. Soluble TNF receptor is thus suggested to be a more valuable factor for monitoring the degree of TNF-a system activity in humans.

The mechanisms by which TNF-a system activities are associated with PWV in nonobese Japanese type 2 diabetic patients are not known at present. There is some evidence that TNF-a is associated with the evolution of atherosclerosis. TNF-a has been shown to contribute to the synthesis of inflammatory markers such as CRP and fibrinogen in liver [40], to mediate chemotaxis of monocytes and fibroblasts [41], and to enhance the expression of vascular cell adhesion molecules such as intercellular adhesion molecule 1 [42]. Irrespective of this, our present study showed that sTNF-R2 but not sTNF-R1 was independently associated with PWV. The validity of the present study is supported by the recent longitudinal investigation by Shai et al [9], who showed that sTNF-R2 is an independent predictor of CHD events in patients with type 2 diabetes mellitus.

The reason why TNF-R2 but not TNF-R1 was associated with PWV in our patients remains to be clarified. These 2 receptors seem to differ in signaling and functional properties [43]. Most biological responses such as cytotoxicity and nuclear factor j B activation are mediated by TNF-R1 but not by TNF-R2 [44]. There are some data available regarding the potential role of TNF-R2 in studies in humans. TNF-a has shown to up-regulate TNF-R2 expression in humans [45]. Obese subjects are shown to overexpress TNF-a and TNF-R2 in adipose tissue and have higher levels of TNF-R2 compared with lean subjects [46,47]. In contrast, we previously demonstrated that plasma TNF-R2 but not TNF-R1 was significantly higher in patients with bulimia nervosa [18]. Thus, it might be suggested that the adipose tissue is not the immediate source of TNF-a in our nonobese Japanese patients with type 2 diabetes mellitus. Recent studies have demonstrated that the binding of advanced glycation end products to specific cell-surface receptor molecules expressed on kidney cells can induce local cytokine and initiate local inflammatory reaction [48]. Angiotensin II, a substance that is associated with the development of renal injury in diabetic patients is shown to up-regulate expression of TNF-a [49]. The source of TNF-a in our patients, however, has yet to be determined. It should be noted that macrophages from diabetic patients release more TNF-a than do control macrophages [50]. Furthermore, high glucose can activate monocytes and induce the expression of TNF-a via oxidant stress and nuclear factor j B transcription factor [51].

In summary, although our present study was performed on a limited number of patients without major clinical signs of macrovascular complications, serum sTNF-R2 is likely to be involved in the brachial-ankle PWV in nonobese Japanese type 2 diabetic patients.

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Metabolic syndrome, insulin resistance, and atherosclerosis in Japanese type 2 diabetic patients

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Abstract

The aim of the present study was to investigate the relationships between metabolic syndrome and atherosclerosis in 57 Japanese type 2 diabetic patients. Metabolic syndrome was diagnosed based on the criteria raised by the Japan Internal Medicine Society. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment. Ultrasonographically measured carotid atherosclerosis, brachial-ankle pulse wave velocity (ba-PWV), and ankle brachial index (ABI) were used to assess the degree of atherosclerosis. Of 57 patients, 25 were diagnosed as having metabolic syndrome. The patients with metabolic syndrome had significantly higher levels of waist circumference, insulin, insulin resistance index of homeostasis model assessment, systolic and diastolic blood pressures, and serum triglycerides, and lower concentrations of adiponectin. However, there was no significant difference in age, sex, glycosylated hemoglobin (hemoglobin A_{1c}), fasting glucose, leptin, and tumor necrosis factor system activities including tumor necrosis factor α between the 2 groups. Furthermore, no significant difference was observed in the degree of carotid atherosclerosis (intimal-medial thickness in plaque-free segments: 0.72 ± 0.03 vs 0.72 ± 0.02 mm, $P = .435$; carotid stenosis in plaque segments: 6.6% ± 3.0% vs 6.6% ± 1.7%, $P = .497$), ba-PWV (1676 ± 56 vs 1654 ± 44, $P = .380$), and ABI (1.16 ± 0.01 vs 1.15 ± 0.01, $P = .245$) between the 2 groups. From these results, it can be suggested that metabolic syndrome, an insulin-resistant state, is not associated with carotid atherosclerosis, ba-PWV, or ABI in Japanese type 2 diabetic patients.
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1. Introduction

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by insulin resistance and/or defective insulin secretion [1]. There seems to be ethnic difference in insulin resistance in type 2 diabetes mellitus. Using a minimal model approach shown by Bergman [2] and Welch et al [3], we previously demonstrated that 40% of type 2 diabetic patients are insulin resistant in Japanese populations [4-6]. In contrast, Haffner et al [7] used this approach and found that 92% of type 2 diabetic patients are insulin resistant in white populations. Moreover, mean body mass index (BMI) in representative epidemiological studies of Japanese type 2 diabetic patients were 23 to 25 kg/m^2, lower than that found in the studies of the whites [8]. Whereas it is well recognized that BMI is one of the most important factors contributing to insulin resistance in diabetic patients, this unique feature of Japanese type 2 diabetic patients allows us to explore other factors related to insulin resistance.

Taking into account these fascinating features, we previously demonstrated that serum triglycerides is independently associated with insulin resistance in Japanese type 2 diabetic patients [9,10]. Thereafter, we found that

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adiponectin and leptin are also independent factors associated with insulin resistance [11,12]. Moreover, we showed that both triglyceride and adiponectin are associated with visceral fat areas, whereas leptin is associated with subcutaneous fat areas in these patients [11-13]. Thus, abdominal fat areas are likely to be associated with insulin resistance in Japanese type 2 diabetic patients. Not only triglyceride but also leptin and adiponectin are recognized to be associated with atherosclerosis in diabetic patients [14-16].

The metabolic syndrome is reported to be one of the conditions associated with insulin resistance and/or atherosclerosis in humans [17]. The major criteria for the metabolic syndrome, however, are emphasized on the waist circumference. Waist circumference provides a crude but effective measure of visceral fat [18]. Along with increased waist circumference, the minor criteria for the metabolic syndrome such as raised triglyceride/low high-density lipoprotein (HDL) cholesterol, high blood pressure, or high concentration of glucose are suggested to be associated with atherosclerosis in Japanese type 2 diabetic patients. Thus, it may be questioned whether the use of metabolic syndrome to assess atherosclerosis is superior to other risk factors such as hyperglycemia especially in Japanese type 2 diabetic patients. It has been established that hyperglycemia per se is associated with the development of atherosclerosis in diabetic patients. To clarify this, we recruited Japanese type 2 diabetic patients who had no major evidence of atherosclerosis to compare the degree of atherosclerosis between the diabetic patients with and without metabolic syndrome syndrome, taking into account BMI and hemoglobin A_{1c} (HbA_{1c}).

2. Subjects and methods

Fifty-seven Japanese type 2 diabetic patients with BMI of less than 27 kg/m^2 who were well controlled in terms of glycosylated hemoglobin (HbA_{1c}) (7.1% ± 0.1%, mean ± SEM) were enrolled. Type 2 diabetes mellitus was diagnosed based on the World Health Organization criteria [19]. They had no evidence of current acute illness including clinically significant infectious disease. The duration of diabetes was 10.9 ± 1.0 years (range, 1-35 years). Of 57 diabetic patients, 52 were taking sulfonylureas and the rest were treated with diet alone. They had not been treated with insulin or any medications known to alter insulin sensitivity. 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 (CVD). Patients did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Metabolic syndrome was diagnosed by the criteria raised by the Japan Internal Medicine Society. Although the use of waist circumference to assess abdominal adiposity is superior to BMI, the cutoff value for waist circumference is likely to be population-specific as there are clear differences across ethnic populations in the relationship between overall adiposity, abdominal adiposity, and visceral fat accumulation. The major criterion in Japanese population is waist circumference of greater than 85 cm in men and greater than 90 cm in women. The minor criteria is as follows: serum triglyceride of ≧150 mg/dL or HDL cholesterol of <40 mg/dL, blood pressure of ≧130/85 mm Hg, and fasting glucose concentration of ≧110 mg/dL. The patients who had both 1 major criteria and 2 or 3 minor criteria were diagnosed as having metabolic syndrome.

Blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with a glucose oxidase method. The triglycerides, total cholesterol, and HDL cholesterol were also measured. Serum insulin was measured using a 2-site immunoradiometric assay (Insulin Riabead II, Dainabot, Japan). Coefficients of variation were 4% for insulin of greater than 25 μU/mL and 7% for insulin of less than 25 μU/mL, respectively. Serum adiponectin and leptin were measured with a radioimmunoassay kit (Linco Research, St Charles, MO). The intra- and interassay coefficients of variation (CVs) were less than 5% for adiponectin and leptin, respectively. Serum tumor necrosis factor α (TNF-α) concentrations were measured with an enzyme immunoassay kit (Quantikine HS Human TNF-α Immunoassay Kit, R&D Systems, Minneapolis, MN), and serum concentrations of soluble TNF receptor 1 (sTNF-R1) and soluble TNF receptor 2 (sTNF-R2) were measured with an enzyme-linked immunosorbent assay (BIOTRAK, Amersham Life Sciences, Uppsala, Sweden), as described previously [20]. The limits of sensitivity for TNF-α, sTNF-R1, and sTNF-R2 were 0.5, 25, and 50 pg/mL, respectively. The intra-assay CVs for TNF-α, sTNF-R1, and sTNF-R2 were 5.9%, 4.7%, and 3.2%, respectively. The interassay CVs for TNF-α, sTNF-R1, and sTNF-R2 were 10.8%, 5.8%, and 3.6%, respectively. Samples for insulin, adiponectin, leptin, and TNF were prepared, frozen, and stored at −70°C until the assay.

The estimate of insulin resistance index of homeostasis model assessment (HOMA-IR) was calculated with the formula: fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5 [21]. The insulin resistance index of homeostasis model assessment was validated in diabetic patients treated with diet therapy alone and in those treated with sulfonylureas [22,23]. Therefore, we estimated HOMA-IR in diet-treated and sulfonylurea-treated diabetic patients.

Along with ultrasonographically measured carotid atherosclerosis, brachial-ankle pulse wave velocity (ba-PWV) and ankle brachial index (ABI) were used to assess the degree of atherosclerosis.

A carotid sonography was performed with high-resolution B-mode scanning equipment (Logic 500 GE Yokogawa, Milwaukee, WI) with a 7.5-MHz sector scanner probe [24]. The common carotid arteries of both sides were examined with longitudinal and transverse scans because we could not analyze the internal and external carotid arteries fully in all

patients. The CV for interobserver variability was found to be 8.5% and the CV for intraobserver variability was 6.0%. The intimal-medial thickness (IMT) of the common carotid artery was measured in plaque-free segments as the distance from the leading edge of the first echogenic line to that of the second echogenic line. The mean of IMT in plaque-free segments of bilateral common carotid arteries was used for the analysis. The degree of stenosis was also measured in the plaque segments of bilateral common carotid arteries. It was calculated as a percentage ratio between the area of the plaque and that of the lumen using the formula: (lumen area − residual lumen area)/lumen area × 100. Both the areas were automatically measured by the system on a frozen transverse scanning plane at the site of maximal narrowing. When 2 or more plaques were present in the vessel, only that causing the greatest degree of stenosis was considered for analysis.

Brachial-ankle PWV and ABI were measured using a volume-plethysmographic apparatus (from PWV/ABI version-112, Colin, Komaki, Japan). Briefly, after an overnight fast, the subjects were examined in the supine position, with electrocardiogram electrodes placed on both wrists, a microphone for detecting heart sounds placed on the left edge of the sternum, and cuffs wrapped on both the brachia and ankles. The characteristic points of waveforms were determined automatically, and the results were printed out. All procedures took about 5 minutes. The interobserver and intraobserver variation coefficients were 8.4% and 10.0%, respectively. Measurements on different days revealed that slight changes in blood pressure did not correlate with changes in ba-PWV. The mean ba-PWV and ABI values measured on either side of each patient were used for the analysis.

2.1. Data analysis

Data were presented as means ± SEM. Statistical analysis was conducted using the StatView 5 system (Statview, Berkeley, CA). The means of 2 groups were compared using Student *t* test. $P < .05$ was considered as significant.

3. Results

The subjects studied were all Japanese type 2 diabetic patients (40 men and 18 women) with an age range of 43 to 79 years (62.7 ± 1.1 years) and a BMI of 17.1 to 26.7 kg/m^2 (23.0 ± 0.3 kg/m^2). The fasting plasma glucose was 143 ± 3 mg/dL and HbA_{1c} was 7.1% ± 0.1%. Fasting insulin level was 6.8 ± 0.4 μU/mL. Serum triglycerides and total and HDL cholesterol levels were 119 ± 7, 208 ± 5, and 61 ± 2 mg/dL, respectively. Serum adiponectin and leptin concentrations were 13.6 ± 1.2 μg/mL and 5.8 ± 0.5 ng/mL, respectively. The concentrations of TNF-α, sTNF-R1, and sTNF-R2 were 3.1 ± 0.2, 1132 ± 36, and 2009 ± 54 pg/mL, respectively. On the other hand, there was a wide variation in insulin resistance calculated from HOMA-IR in our diabetic patients (range, 0.71-6.10; 2.40 ± 0.16). Of 57 patients, 24 (41%) patients had HOMA-IR of greater than 2.5, indicating that they are insulin resistant [9,10]. Intimal-medial thickness in plaque-free segments of carotid artery, carotid stenosis in plaque segments, ba-PWV, and ABI were 0.72 ± 0.02 mm (range, 0.40-1.10 mm), 6.6% ± 1.6% (range, 0%-54.5%), 1664 ± 35 cm/s (range, 1139-2294 cm/s), and 1.15 ± 0.01 (range, 1.02-1.26), respectively.

Table 1 shows the clinical profile between the patients with and without metabolic syndrome. Of the 57 patients, 25 were diagnosed as having metabolic syndrome. These patients had significantly higher levels of waist circumference, HOMA-IR, systolic and diastolic blood pressures, and serum triglycerides, but significantly lower concentrations of adiponectin as compared with those without metabolic syndrome. No significant difference was observed in age, sex, fasting glucose, leptin, and HbA_{1c} between the two. The concentrations of TNF-α, sTNF-R1, and sTNF-R2 were not significantly different between the 2 groups. There was no significant difference in the degree of carotid atherosclerosis (IMT in plaque-free segments: 0.72 ± 0.03 vs 0.72 ± 0.02 mm, $P = .435$; carotid stenosis in plaque segments: 6.6% ± 3.0% vs 6.6% ± 1.7%, $P = .497$), ba-PWV (1676 ± 56 vs 1654 ± 44, $P = .380$), and ABI (1.16 ± 0.01 vs 1.15 ± 0.01, $P = .245$) between the 2 groups.

Table 1
Clinical characteristics of the diabetic patients included in the study

	Metabolic syndrome (+)	Metabolic syndrome (−)	*P*
No. of subjects	25	33	–
Waist (cm)	89.6 ± 0.8	77.3 ± 1.2	<.001
Age (y)	62.1 ± 1.8	63.2 ± 1.3	.316
Male/female	20/5	20/12	.071
HOMA-IR	2.71 ± 0.23	2.17 ± 0.20	<.05
Diabetes duration (y)	10.3 ± 1.4	11.1 ± 1.4	.351
Smoking (%)	20	21	.486
SU/diet	22/3	29/3	.343
BMI (kg/m^2)	24.0 ± 0.4	22.2 ± 0.4	<.001
Systolic blood pressure (mm Hg)	143 ± 3	133 ± 3	<.05
Diastolic blood pressure (mm Hg)	88 ± 2	81 ± 2	<.005
Fasting glucose (mg/dL)	141 ± 4	145 ± 4	.237
Fasting insulin (μU/mL)	7.7 ± 0.6	6.1 ± 0.6	<.05
HbA_{1c} (%)	7.0 ± 0.2	7.2 ± 0.2	.227
Triglycerides (mg/dL)	134 ± 12	108 ± 9	<.05
Total cholesterol (mg/dL)	208 ± 7	207 ± 7	.473
HDL cholesterol (mg/dL)	57 ± 3	63 ± 3	.062
LDL cholesterol (mg/dL)	131 ± 6	127 ± 6	.347
adiponectin (μg/mL)	10.7 ± 1.1	15.5 ± 1.9	<.05
Leptin (ng/mL)	6.2 ± 0.8	5.4 ± 0.7	.242
TNF-α (pg/mL)	3.4 ± 0.3	2.9 ± 0.2	.065
sTNF-R1 (pg/mL)	1118 ± 46	1143 ± 52	.366
sTNF-R2 (pg/mL)	1971 ± 68	2036 ± 78	.276
IMT (mm)	0.72 ± 0.03	0.72 ± 0.02	.435
Stenosis (%)	6.6 ± 3.0	6.6 ± 1.7	.497
ba-PWV (cm/s)	1676 ± 56	1654 ± 44	.380
ABI	1.16 ± 0.01	1.15 ± 0.01	.245

SU indicates sulfonylurea; LDL indicates low-density lipoprotein.

4. Discussion

Type 2 diabetes mellitus is a syndrome characterized by insulin resistance and/or defective insulin secretion [1]. There seems to be ethnic difference in insulin resistance in type 2 diabetes mellitus. Haffner et al surveyed the prevalence of white type 2 diabetic patients and found that 92% of type 2 diabetic patients were insulin resistant [7]. Chaiken et al [25] reported that 60% of type 2 diabetic patients with BMI of less than 30 kg/m^2 were insulin resistant in African American populations. We recently demonstrated that 40% of type 2 diabetic patients are insulin resistant in Japanese type 2 diabetic patients [9,10]. Thus, Japanese type 2 diabetic patients are considered to have a unique feature, specifying that they are divided into 2 categories: one with insulin resistance and the other with normal insulin sensitivity [4-6,9,10]. This idea was reconfirmed in the present study.

Another unique feature of Japanese type 2 diabetic patients is that they are not always massively obese. We previously showed that the mean BMI in representative epidemiological studies of Japanese type 2 diabetic patients are 23 to 25 kg/m^2, lower than in the studies of other ethnic populations such as whites [8]. Thus, Japanese type 2 diabetic patients are hypothesized to have another fascinating feature in terms of insulin resistance and atherosclerosis as compared with other ethnic populations.

In the present study, we first found that metabolic syndrome is associated with insulin resistance but not always associated with atherosclerosis in Japanese type 2 diabetic patients. This is a surprising finding because it is a commonly held belief that metabolic syndrome is an important cluster of metabolic abnormalities linked with insulin resistance and CVD [17].

One possible explanation is that the waist circumference, the major criteria for the metabolic syndrome, might not be an accurate measure of intra-abdominal fat areas in Japanese type 2 diabetic patients who are not massively obese. Fujimoto et al [26] previously demonstrated that visceral adiposity, blood pressure, and plasma glucose, but not abdominal circumference, are independent risk factors for incident coronary heart disease in Japanese-American diabetic patients. The BMI of their patients (25.8 kg/m^2) was similar to that of our patients.

The second possible explanation is because of the clinical characteristics or to the degree of atherosclerosis in our patients. The patients studied had no significant CVD and were not accompanied by any major significant abnormalities in the ultrasonographically measured carotid atherosclerosis, PWV, and ABI. The range of IMT, carotid stenosis, PWV, and ABI were 0.4 to 1.1 mm, 0% to 54.5%, 1139 to 2294 cm/s, and 1.02 to 1.26, respectively. Therefore, the association between metabolic syndrome and the degree of atherosclerosis would probably be higher in a population-based study in which the patients with CVD were included in this study.

The third possible explanation is that inflammation including TNF-α and/or hyperglycemia rather than insulin resistance may have unfavorable effects on the atherosclerotic change in Japanese type 2 diabetic patients. It is reported that high glucose can activate monocytes and induce the expression of TNF-α via oxidant stress and nuclear factor-κB transcription factor [27]. Shai et al [28] demonstrated that sTNF-R2 is strongly associated with the risk of coronary heart disease in patients with type 2 diabetes mellitus. Rauchhaus et al [29] demonstrated that elevated sTNF-R1 has shown to be predictive of cardiovascular mortality in patients with chronic heart failure. We recently found that sTNF-R1 was associated with albuminuria in Japanese type 2 diabetic patients [30]. In the present study, we could not find any significant differences in TNF-α system activities (TNF-α, sTNF-R1, sTNF-R2) between the 2 groups. It should be noted that TNF-α system activities are not associated with insulin resistance in Japanese type 2 diabetic patients with BMI of less than 27.0 kg/m^2 [20]. Alternatively, the long-standing diabetic state per se is such a powerful factor on atherosclerosis so that the effect of other risk factors including metabolic syndrome is masked. This idea is supported by the results from the recent 11-year follow-up investigation shown by Bruno et al [31] that diabetic patients with metabolic syndrome had similar all-cause and CVD mortality as compared with those without metabolic syndrome.

Irrespective of this, our present study showed that metabolic syndrome, an insulin-resistant state, is not associated with carotid atherosclerosis, ba-PWV, or ABI in Japanese type 2 diabetic patients. In this respect, Kahn et al [32] very recently warns that clinicians should evaluate and treat all CVD risk factors without regard to whether a patient meets the criteria for diagnosis of the metabolic syndrome.

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