J Atheroscler Thromb, 2016; 23: 1067-1079.
doi: 10.5551/jat.33399

Original Article

Visceral Adiposity is Preferentially Associated with Vascular Stiffness Rather than Thickness in Men with Type 2 Diabetes

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***Aim*: Visceral fat accumulation is known to underlie the clustering of cardiovascular risk factors. However, it is not completely understood how visceral fat accumulation influences the development of cardiovascular disease. In this study, we investigated the clinical impact of visceral adiposity on vascular stiffness and thickness in patients with type 2 diabetes (T2D).**

***Methods*: One hundred and sixty-one patients with T2D, including 92 men and 69 women, were included in this cross-sectional study. Visceral fat area (VFA) and subcutaneous fat area (SFA) were measured by dual bioelectrical impedance analysis. Stiffness parameter β and intima-media thickness (IMT) of the common carotid artery were measured by ultrasonography.**

***Results*: The mean age and duration of diabetes in the study population were 61 years and 13.9 years, respectively. In men, VFA and waist circumference (WC) were positively correlated with stiffness parameter β, whereas body mass index (BMI), WC, and SFA were negatively correlated with IMT. In contrast, in women, none of the obesity-related indices were significantly correlated with stiffness parameter β or IMT. In multiple regression analyses, VFA as well as WC, BMI, and SFA were independently associated with stiffness parameter β after adjustment for age and other potential confounders in men but not in women. None of the obesity-related indices were independently associated with IMT for either sex.**

***Conclusion*: In men with T2D, visceral adiposity is associated with carotid arterial stiffness but not thickness.**

Key words: Obesity, Type 2 diabetes, Visceral fat accumulation, Stiffness parameter β, IMT

Introduction

Visceral obesity, which is a key component of the metabolic syndrome, underlies the clustering of cardiovascular risk factors including hypertension, dyslipidemia, and glucose intolerance[1)] and is related to the risk of cardiovascular morbidity and mortality[2)]. Visceral fat accumulation was also shown to be independently associated with the incidence of coronary artery diseases in several population-based studies[3, 4)]. Moreover, several studies showed that the presence of metabolic syndrome was associated with the risk of cardiovascular morbidity and mortality even in subjects with type 2 diabetes (T2D)[5, 6)], indicating an important role for visceral adiposity in the development and progression of atherosclerotic cardiovascular disease (CVD) in such patients.

Alterations in vascular morphology and function can be assessed by ultrasonography as intima-media thickness (IMT) ("atherosclerosis") and vascular wall stiffness ("arteriosclerosis"), respectively[7)], both of which are shown to predict adverse CVD events[8-10)]. Importantly, carotid arterial stiffness predicts CVD

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Received: October 3, 2015
Accepted for publication: December 29, 2015

mortality independent of simultaneously measured carotid IMT[11]. Our research group previously reported that both IMT and stiffness parameter β of the carotid artery were higher in T2D subjects than in non-diabetic subjects[12, 13]. Previous community-based studies showed that presence of the metabolic syndrome was an independent determinant of both carotid IMT and stiffness[14]. Visceral adiposity estimated by computed tomography (CT), magnetic resonance imaging (MRI), or waist-to-hip ratio was also shown to be related to IMT[15] and distensibility/stiffness[16-18] of the carotid artery in healthy or non-diabetic subjects. However, regarding the link between visceral adiposity and the risk of CVD, only a few studies have focused on the differential impacts of visceral adiposity on vascular thickness and stiffness in subjects with T2D[19-21].

Recently, the dual bioelectrical impedance analysis (dual BIA) instrument was developed for measuring visceral fat area (VFA), and its clinical utility in detecting severity of the metabolic syndrome and effectiveness of weight reduction therapy in obese patients has been confirmed in pilot studies[22, 23]. Apart from the study of Bouchi *et al.*[24], which used aortic pulse wave velocity (PWV), no study has so far investigated the association between VFA estimated by dual BIA and surrogate markers of CVD.

Aim

In this study, we evaluated visceral adiposity using a dual BIA instrument and investigated the clinical impact of visceral adiposity on arterial wall thickness and stiffness in patients with T2D in a cross-sectional manner.

Methods

Subjects

We consecutively enrolled 161 subjects with T2D (92 men and 69 women) who were admitted to the Diabetes Center of the Osaka City University Hospital for the purpose of glycemic control, education, and/or evaluation of diabetic complications between February 2013 and September 2014. T2D was diagnosed based on the criteria of the American Diabetes Association[25]. Subjects with type 1 diabetes and other types of diabetes were excluded from this study. We also excluded subjects with waist circumference (WC) >130 cm because their VFA cannot be measured by a dual BIA instrument. In our analyses, a smoker was defined as a current smoker. All subjects provided written informed consent, and the ethical review board of our institution approved this study protocol (No. 164).

Physical and Laboratory Measurements

Blood pressure was determined by the conventional cuff method with an automatic sphygmomanometer after subjects had rested for at least 15 min. WC was measured to the nearest centimeter at the level of the umbilicus in a standing position at the end of gentle expiration. Blood was drawn after an overnight fast, and biochemical parameters were analyzed by a standard laboratory method as previously described[26].

Measurement of VFA by Dual BIA

VFA, along with subcutaneous fat area (SFA), was measured by a dual BIA instrument (HDS-2000, Omron Healthcare Co. Ltd., Kyoto, Japan) in the morning after an overnight fast. Dual BIA calculates the cross-sectional area of intra-abdominal fat at the level of the umbilicus based on two different impedance values, as described elsewhere in detail[22, 23]. A close correlation between VFA determined by dual BIA and that obtained by a CT scan was previously demonstrated[22, 23].

Measurement of carotid IMT and stiffness parameter β by ultrasonography

IMT and stiffness parameter β of the common carotid artery were measured by an ultrasonic phase-locked echo-tracking system, which was equipped with a high-resolution real-time 13-MHz linear scanner (Prosound F75; Hitachi Aloka Medical, Ltd., Tokyo, Japan) as previously reported[13, 27]. In brief, IMT of bilateral carotid arteries was measured at the sites of the most advanced atherosclerotic lesions in longitudinal and transverse projections. The greatest IMT, including plaque, was utilized as a marker of atherosclerotic changes in the carotid arteries.

Stiffness parameter β, an index of arterial wall stiffness, was calculated as $\ln(Ps/Pd) \times Dd/(Ds - Dd)$, where Ps and Pd are systolic and diastolic blood pressure and Ds and Dd are systolic and diastolic inner diameters of the carotid artery, respectively[12, 13]. The greater stiffness parameter β between the left and right carotid arteries was utilized as a marker of arteriosclerotic changes. Those measurements were performed in the morning after an overnight fast and with a resting period of 15 min in a quiet room air-conditioned at 25℃ throughout the year. The subjects were also refrained from smoking, alcohol, and beverages containing caffeine a few hours before measurement according to the recommendations of the international task force[28].

Statistics

Data were expressed as number (%), mean ± standard deviation (SD), or median (interquartile range) as appropriate. For comparisons between men and women, the χ^2-test, Student's *t*-test, or Wilcoxon rank-sum test was performed as appropriate. Simple and multiple linear regression analyses were performed to evaluate the relationship between stiffness parameter β or IMT and various clinical parameters, including obesity-related indices [body mass index (BMI), WC, VFA, and SFA]. Skewed parameters, including plasma triglycerides levels, were logarithmically transformed before regression analyses. In multiple regression analyses, stiffness parameter β or IMT was the dependent variable, and obesity-related indices (BMI, WC, VFA, or SFA), age, systolic blood pressure, glycated hemoglobin A1c (HbA1c) level, serum creatinine level, high-density lipoprotein (HDL) cholesterol level, low-density lipoprotein (LDL) cholesterol level, smoking status, presence of treatment with statins, and presence of treatment with angiotensin-II receptor blockers or angiotensin-converting enzyme inhibitors (ARB/ACEI) were the independent variables. A p value of <0.05 was considered to indicate statistical significance. Statistical analyses were performed using the JMP® 10 software (SAS Institute Inc., Cary, NC).

Results

Clinical Characteristics of the Subjects

The clinical characteristics of the total study population as well as men and women separately are shown in **Table 1**. Twenty (12%) subjects with T2D were treated with dietary therapy alone; 119 (74%) with oral hypoglycemic agents, including 90 with dipeptidyl peptidase-4 inhibitors, 62 with sulfonylureas, 60 with biguanides, 22 with thiazolidinediones, 19 with α-glucosidase inhibitors, 5 with insulin secretagogues (glinides); 22 (14%) with insulin alone; 26 (16%) with a combination of insulin and oral hypoglycemic agents; and 3 (2%) with glucagon-like peptide-1 analogs. The mean age and known duration of diabetes in the study population were 61 years and 13.9 years, respectively. There were significantly more male smokers than female smokers. Plasma HDL cholesterol levels were lower in men than in women. Age, duration of diabetes, BMI, WC, blood pressure, presence of treatment with statins or ARB/ACEI, renal function, glycemic control, and lipid profiles other than HDL cholesterol were not significantly different between men and women.

Abdominal Adiposity and Carotid Arterial Stiffness and Thickness in the T2D Subjects

The mean VFAs estimated by dual BIA in men and women were 99.3 ± 53.2 cm^2 and 88.7 ± 45.3 cm^2, respectively, without a significant difference between the sexes (**Table 1**). Similarly, no significant difference in SFA was found between the sexes. In good agreement with a pilot study in obese patients[23], VFA estimated by dual BIA was closely correlated with BMI ($r=0.807$, $p<0.001$) and WC ($r=0.881$, $p<0.001$) in subjects with T2D (**Supplemental Table 1**). Moreover, VFA by dual BIA showed significant correlation with metabolic parameters, such as plasma immunoreactive insulin levels ($r=0.415$, $p<0.001$) and triglycerides levels ($r=0.331$, $p<0.001$) in all subjects and with HDL cholesterol levels ($r=-0.483$, $p<0.001$) and diastolic blood pressure ($r=0.280$, $p=0.022$) in women (**Supplemental Table 1**).

The mean value of carotid stiffness parameter β in the total population was 11.6 (range, 2.5–34.9), and there was no significant difference between men and women. The mean carotid IMT for the total population was 1.05 mm (range, 0.37–2.41), without a significant difference between the sexes (**Table 1**).

Association of Visceral Adiposity with Carotid Stiffness and Thickness

We first examined the association of carotid stiffness or thickness with obesity-related indices, including BMI, WC, VFA, and SFA, and other risk factors for CVD by simple linear regression analyses separately in men and women. In men, stiffness parameter β was positively correlated with WC and VFA but not with BMI or SFA (**Fig. 1** and **Table 2**). IMT was negatively correlated with BMI, WC, and SFA but not with VFA (**Supplemental Fig. 1** and **Table 2**). In contrast, in women, none of the obesity-related indices were significantly correlated with stiffness parameter β (**Supplemental Fig. 2**) or IMT (**Supplemental Fig. 3**) (**Table 2**).

Among the other known risk factors for CVD, including age, blood pressure, fasting glucose level, HbA1c level, serum creatinine level, triglycerides level, HDL cholesterol level, and LDL cholesterol level, age was significantly correlated with stiffness parameter β and IMT in both men and women. In addition, an inverse correlation between HDL cholesterol level and stiffness parameter β was found in women (**Table 2**). None of the other cardiometabolic risk factors were significantly associated with stiffness parameter β or IMT in each sex, presumably because a large number of the subjects were receiving medications for hypertension, dyslipidemia, and diabetes.

Table 1. Clinical characteristics, abdominal adiposity, and carotid stiffness and thickness in all subjects with type 2 diabetes as well as in men and women separately

	All	Men	Women	*p*
N	161	92	69	
Age (years)	61 ± 14	60 ± 13	63 ± 14	0.204
Duration of diabetes (years)	13.9 ± 11.1	14.6 ± 11.4	13.0 ± 10.6	0.394
BMI (kg/m^2)	27.1 ± 5.8	26.7 ± 5.3	27.7 ± 6.5	0.273
WC (cm)	92 ± 14	92 ± 13	91 ± 14	0.631
Systolic blood pressure (mmHg)	132 ± 19	130 ± 16	136 ± 22	0.055
Diastolic blood pressure (mmHg)	76 ± 11	77 ± 10	74 ± 11	0.074
Smoker *n* (%)	29 (18.0)	24 (34.8)	5 (9.3)	0.001
Statin *n* (%)	66 (41.0)	33 (35.9)	33 (47.8)	0.127
ARB/ACEI *n* (%)	65 (40.4)	38 (41.3)	27 (39.1)	0.871
Fasting glucose (mg/dL)	131 ± 39	131 ± 40	130 ± 38	0.711
HbA1c (%)	8.6 ± 1.9	8.6 ± 1.9	8.6 ± 1.9	0.894
Immunoreactive insulin (μU/mL)	7.4 (4.6–11.55)	7.1 (4.4–11.1)	7.5 (4.6–11.9)	0.407
Serum creatinine (mg/dL)	1.02 ± 0.85	1.13 ± 0.73	0.88 ± 0.97	0.062
Triglycerides (mg/dL)	126 (92–163)	130 (100–171)	116 (85–159)	0.179
HDL-cholesterol (mg/dL)	43 ± 12	40 ± 10	46 ± 14	0.004
LDL-cholesterol (mg/dL)	115 ± 39	114 ± 42	115 ± 36	0.896
VFA (cm^2)	94.7 ± 50.1	99.3 ± 53.2	88.7 ± 45.3	0.186
SFA (cm^2)	216.7 ± 109.2	208.2 ± 100.1	228.0 ± 120.1	0.255
Stiffness parameter β	11.6 ± 5.3	11.0 ± 4.7	12.3 ± 5.9	0.159
IMT (mm)	1.05 ± 0.34	1.03 ± 0.37	1.08 ± 0.30	0.377

Data are expressed as mean ± SD, median (interquartile range), or *n* (%). *P*-values were determined by Student's *t*-test, Wilcoxon rank-sum test, or χ^2-test, as appropriate. Abbreviations: BMI, body mass index; WC, waist circumference; smoker, prevalence of current smokers; statin, prevalence of subjects treated with statins; ARB/ACEI, prevalence of subjects treated with angiotensin-II receptor antagonists or ACE inhibitors; HbA1c, glycated hemoglobin A1c level; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VFA, visceral fat area; SFA, subcutaneous fat area; IMT, intima-media thickness.

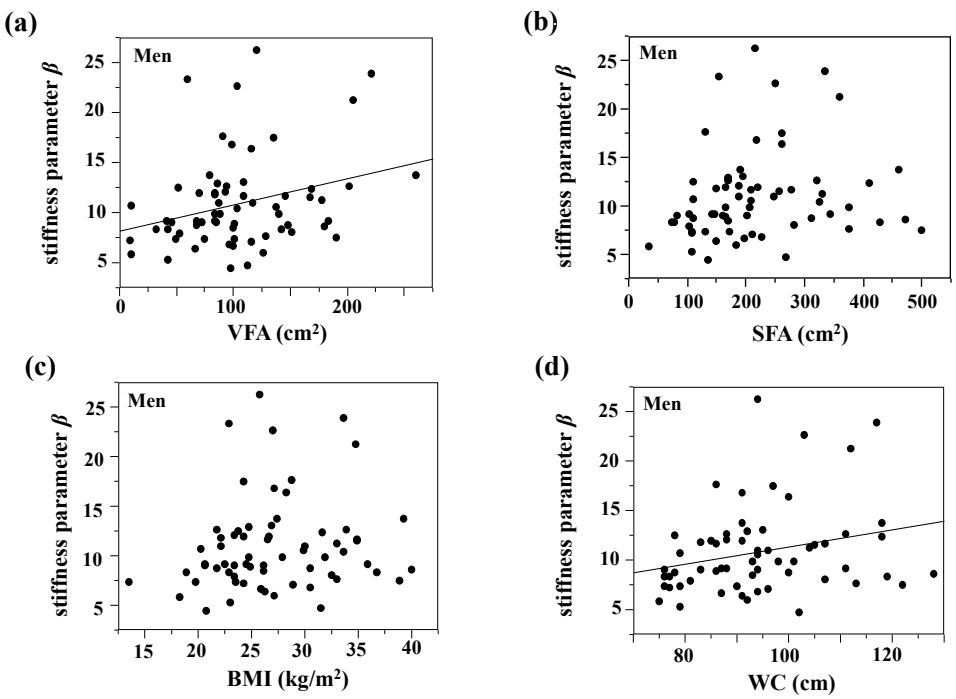

Fig. 1. Association of visceral fat area (VFA) (a), subcutaneous fat area (SFA) (b), body mass index (BMI) (c), and waist circumference (WC) (d) with stiffness parameter β of the common carotid artery in men with type 2 diabetes (T2D)

In men with T2D, VFA (r=0.290, p=0.019) and WC (r=0.257, p=0.044), but not SFA (r=0.186, p=0.139) and BMI (r=0.145, p=0.248), showed significant positive correlations with stiffness parameter β.

Table 2. Correlations between carotid stiffness and thickness and clinical variables in subjects with type 2 diabetes

	Stiffness parameter β		IMT	
	Men	Women	Men	Women
Age	0.335**	0.323*	0.425**	0.358**
BMI	0.145	0.072	−0.267*	−0.098
WC	0.257*	0.153	−0.265*	−0.073
VFA	0.290*	0.183	−0.173	−0.098
SFA	0.186	0.048	−0.259*	−0.043
Systolic blood pressure	0.094	−0.072	0.013	0.165
Diastolic blood pressure	0.021	−0.140	−0.115	−0.031
Fasting glucose	−0.092	0.035	−0.011	−0.161
HbA1c	−0.084	0.165	−0.091	−0.145
Serum creatinine	0.074	0.096	−0.043	0.114
Log [triglycerides]	0.090	0.158	−0.028	−0.012
HDL-cholesterol	0.070	−0.424**	−0.149	−0.078
LDL-cholesterol	−0.155	−0.196	0.024	0.046

Values are correlation coefficients determined by simple regression analyses. *$p<0.05$; **$p<0.01$. BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin A1c level; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VFA, visceral fat area; SFA, subcutaneous fat area; IMT, intima-media thickness.

Table 3. Associations of obesity-related indices with carotid stiffness and thickness in subjects with type 2 diabetes

	Stiffness parameter β		IMT	
	Men	Women	Men	Women
BMI (kg/m^2)	0.443**	0.080	−0.141	0.013
WC (cm)	0.527**	0.048	−0.106	0.027
VFA (cm^2)	0.417**	0.159	−0.105	0.024
SFA (cm^2)	0.488**	0.103	−0.139	0.080

Values are standard regression coefficients determined by multiple regression analyses. In men or women, body mass index (BMI), waist circumferences (WC), visceral fat area (VFA), or subcutaneous fat area (SFA) was set as an independent variable after adjustment for age, systolic blood pressure, HbA1c level, serum creatinine level, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, smoking status, and presence of treatment with statins and ARB/ACEI. **$p<0.01$.

Multivariate Analyses of the Determinants for Carotid Stiffness and Thickness

Age was correlated not only positively with carotid stiffness parameter β and IMT but also negatively with obesity-related indices (BMI, $r=-0.387$, $p<0.001$; WC, $r=-0.305$, $p=0.001$; VFA, $r=-0.249$, $p=0.001$; SFA, $r=-0.381$, $p<0.001$), suggesting that the correlations of obesity-related indices with stiffness parameter β and IMT were confounded at least by age. Therefore, multiple regression analyses were performed to identify independent associations of visceral adiposity with carotid stiffness and thickness after adjusting for age and other potential confounders, including systolic blood pressure, HbA1c level, serum creatinine level, HDL cholesterol level, LDL cholesterol level, smoking status, and presence of treatment with statins and ARB/ACEI (**Table 3**). Among all the variables, obesity-related indices, such as VFA ($\beta=0.417$, $p=0.001$), SFA ($\beta=0.488$, $p=0.001$), WC ($\beta=0.527$, $p<0.001$), and BMI ($\beta=0.443$, $p=0.002$), along with age, were independently and positively associated with stiffness parameter β in men (**Table 3**), whereas none of these obesity-related indices were found to be a determinant of IMT (**Table 3**). In women, none of the obesity-related indices were independently associated with carotid stiffness parameter

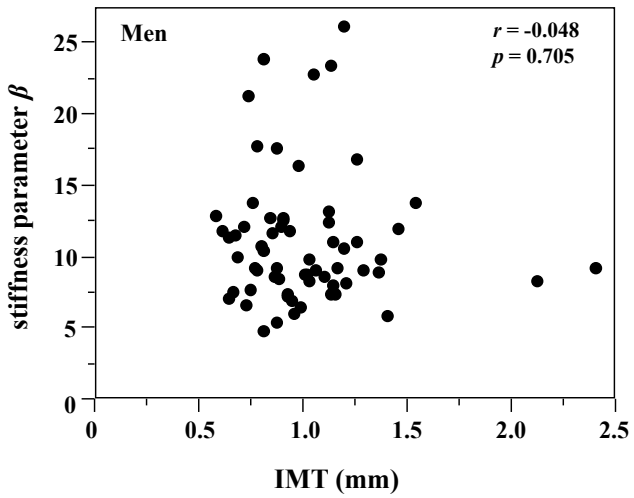

Fig. 2. Association between stiffness parameter β and intima-media thickness (IMT) of the common carotid artery in men with type 2 diabetes

In men with type 2 diabetes, stiffness parameter β was not significantly correlated with IMT ($r = -0.048$, $p = 0.705$).

β or IMT (**Table 3**).

Association between Carotid Stiffness and Thickness in Men

Finally, to understand the reason why visceral adiposity is associated with carotid arterial stiffness differently from thickness in men with T2D, we examined the association between carotid stiffness parameter β and IMT. We found no significant correlation between stiffness parameter β and IMT in men (**Fig. 2**). Then, we divided the men into four groups according to the median values of carotid stiffness parameter β (9.8) and IMT (0.93 mm) and compared clinical parameters between the men with high IMT but low stiffness parameter β ("IMT-advanced") and those with high stiffness parameter β but low IMT ("stiffness-advanced"). As expected from the results of multivariate analyses, subjects in the stiffness-advanced group were much more obese with greater BMI, WC, VFA, and SFA than those in the IMT-advanced group (**Supplemental Table 2**). In contrast, the other parameters, including age, duration of diabetes, blood pressure, medications, glycemic control, and lipid profile, were similar between the groups (**Supplemental Table 2**).

Discussion

This study explored the relationship between visceral adiposity and carotid arterial stiffness and thickness in patients with T2D and demonstrated that VFA estimated by dual BIA was closely associated with carotid stiffness parameter β, but not with IMT, in men. Importantly, the association between VFA and carotid stiffness was independent of known CVD risk factors. Moreover, in women, visceral adiposity was associated with neither carotid stiffness parameter β nor IMT. These findings indicate an important role of visceral fat accumulation in the pathogenesis of arteriosclerosis or functional changes in arteries in men with T2D.

We did not find a significant association between visceral adiposity and IMT in our male population. This finding is in disagreement with previous studies showing a positive association of visceral adiposity with IMT[15, 20, 29-31]. There are at least two possible explanations for the conflicting results. First, the methods of estimating visceral fat mass were different. A number of studies using ultrasonography have associated visceral fat thickness with IMT[20, 29-31], whereas a few studies using CT, the gold standard for quantifying visceral fat mass, have provided inconsistent results on the relationship between VFA and IMT[15, 21]. Although both visceral fat thickness measured by ultrasonography[32] and VFA measured by dual BIA[22, 23] are reportedly well correlated with VFA determined by CT, estimates of local fat thickness and the total cross-sectional area of visceral fat may be differently related to clinical parameters. Second, there were large differences between the study populations. The subjects of the previous studies were healthy[15, 31], mostly non-diabetic[29, 30], or much younger patients with T2D than those included in our study[20]. In contrast, our study included subjects with T2D with a relatively higher age and a long duration of the disease. In this regard, Konishi *et al.*[21] did not find a significant relationship between VFA and IMT in patients with T2D whose mean age, duration of diabetes, and IMT were similar to those of our subjects.

Despite no significant association between VFA and IMT, this study clearly demonstrated a positive correlation between VFA and carotid stiffness parameter β in men with T2D. The association between visceral adiposity and carotid arterial stiffness measured by ultrasonography has been demonstrated in healthy subjects[16] and the general population[17]. Presence of the metabolic syndrome[14] or high waist-to-hip ratio[18] was also associated with increased carotid arterial stiffness in non-diabetic women[18] and community-dwelling subjects[14]. There are few studies investigating the impact of visceral fat mass on carotid arterial stiffness in patients with T2D[19, 21]. Diamant *et al.*[19] showed that carotid stiffness expressed as the distensibility coefficient was inversely associated with VFA measured by MRI in 16 patients with uncomplicated T2D

but not in age-matched healthy controls. Konishi *et al.*[21] also demonstrated that VFA measured by CT was independently associated with carotid stiffness parameter β in 151 patients with T2D but not in age-matched non-diabetic controls. These studies indicate that a strong association between abdominal obesity or visceral adiposity and carotid arterial stiffness can be observed in a wide range of subjects from the general population to patients with T2D and that this association is stronger in subjects with T2D than in those without it.

Differential impact of cardiovascular risk factors on arterial stiffness and IMT were also observed in Japanese patients with T2D[21, 33, 34]. Consistent with our results, several studies reported that the association with obesity-related measures was different between arterial stiffness and IMT[21, 35]. In Japanese T2D patients, VFA was significantly associated with carotid stiffness parameter β but not with carotid IMT[21]. Moreover, BMI was a strong determinant of aortic PWV, but not carotid IMT, in obese T2D patients in the U.S[35]. On the other hand, dyslipidemia[21, 33] and hyperglycemia[34, 35], but not obesity-related indices, were preferentially associated with carotid IMT in subjects with T2D. In addition, we found that only obesity-related indices were significantly different between the men with higher stiffness but lower IMT and the men with lower stiffness but higher IMT. These observations indicate a preferential association of obesity with arterial stiffness over arterial thickness in T2D. Because the correlation between IMT and stiffness index is relatively low[14], we speculate the dominant role of obesity in arterial stiffening regardless of arterial wall thickness.

Insulin resistance may be one of the mechanisms linking visceral adiposity to arterial stiffness. Our research group has extensively investigated the association between insulin resistance and carotid arterial stiffness and thickness in patients with T2D[12, 36-39]. We[12] and others[16, 40] showed that insulin sensitivity evaluated by the euglycemic hyperinsulinemic clamp technique was inversely associated with arterial stiffness of the carotid[12, 40] and femoral[12, 16] arteries in healthy women[16] and subjects with T2D[12, 40]. Moreover, we found that improved carotid stiffness parameter β was associated with increased clamp-measured insulin sensitivity after short-term exercise[39] and with the increase in adiponectin levels after treatment with an insulin sensitizer pioglitazone or metformin[38] in patients with T2D. On the other hand, only a few studies[36, 41] including ours[36] showed significant and inverse relationship between carotid IMT and insulin sensitivity as determined by the euglycemic hyperinsulinemic clamp in patients with T2D. In T2D subjects of our previous studies, correlation with clamp-measured insulin sensitivity index was higher in carotid stiffness parameter β ($r=-0.393$, $p=0.002$)[12] than in IMT ($r=-0.225$, $p=0.014$)[36]. Because visceral adiposity evidently underlies insulin resistance[1], it is conceivable that accumulated visceral fat predominantly affects carotid arterial stiffness over wall thickness, at least partly through impaired insulin sensitivity in T2D men. Nevertheless, several studies indicate possible implications of adipocytokines or inflammatory factors, such as adiponectin[21, 38], leptin[42], TNF-α[21], C-reactive protein[19], and interleukin-6[19], in carotid arterial stiffness in subjects with T2D. Further studies are needed to completely understand how visceral adiposity regulates arterial stiffness and differently impacts carotid stiffness and thickness in diabetic individuals.

In contrast to men, women exhibited no significant association between visceral adiposity and carotid arterial stiffness in this study. Sex hormone is one of the possible explanations. Arterial stiffness is known to be affected by age-related loss of estrogen action in women[43, 44]. Moreover, the detrimental impact of diabetes on aortic and carotid arterial stiffness was reported to be greater in women than in men[45]. However, vascular endothelial function and arterial stiffness could be fluctuated with a menstrual cycle in premenopausal women[46]. Because we performed vascular measurements without considering menstrual cycle, it is possible that we could not detect the association between visceral adiposity and stiffness parameter β in women in this study.

We noticed that SFA and VFA were differently correlated with IMT in men, but not in women, in univariate regression, although this correlation disappeared after multivariate adjustment. Similarly, univariate models showed different association of SFA and VFA with stiffness parameter β in men, but not in women, although both were independent explanatory factors for stiffness parameter β in multivariate models. We do not know the importance of these univariate findings nor the precise mechanisms. However, these findings may be related to the differential roles of SFA and VFA on insulin resistance, abnormal cytokine profile[1] and arterial wall thickness[29-31] and stiffness[16, 47], as shown in the previous studies, and also indicate a possible difference in the adiposity–artery relationship between the sexes.

This study provides a clinical evidence supporting the critical role of visceral adiposity in carotid arterial stiffness in patients with T2D. Compared with the precedent studies in T2D subjects[19, 21], novel findings of this study are that visceral adiposity is strongly associated with stiffness as a functional property, rather

than with wall thickness as a morphological property, of the carotid artery and that the association is found only in men. Because both carotid IMT and arterial stiffness independently predict CVD events[8-10], this study indicates that visceral adiposity regulates arterial stiffness, regardless of arterial thickness, to potentially modulate the risk of CVDs in patients with T2D. This study further proposes that VFA, which can be repeatedly assessed by the dual BIA instrument, is a potential marker for monitoring the effect of weight-reducing therapy on vascular health in patients with obesity and diabetes.

This study has several limitations. First, because this was a cross-sectional study, a causal relationship between visceral fat accumulation and carotid arterial stiffness could not be confirmed. Second, our subjects were receiving anti-hypertensive drugs and statins, which could have affected arterial stiffness and the related atherosclerotic risk factors. To minimize the effect of such treatments, we adjusted for the presence of these therapies in the multivariate analyses. Third, because the subjects with T2D were hospitalized largely with inadequate glycemic control, the current results cannot be generalized. Fourth, non-diabetic controls were not used to compare the findings, and we could not confirm that carotid stiffness parameter β was increased in subjects with T2D. In our previous study, we used the same procedure to confirm that carotid stiffness parameter β was higher in T2D patients than in non-diabetic subjects[37]. Finally, visceral adiposity was evaluated not by CT, the gold-standard method, but by dual BIA. However, previous studies reported a good correlation between VFA values obtained by CT and dual BIA[22, 23].

Conclusion

This study demonstrates that visceral adiposity is independently associated with stiffness parameter β of the carotid artery in men with T2D. Our data may indicate that the accumulated visceral fat preferentially affects arterial stiffness, known as an independent predictor of CVD. Further studies are required to identify the factors linking visceral fat accumulation and arterial stiffening in diabetes. In addition, interventional studies are warranted to clarify whether reduction of visceral adiposity could reverse arterial stiffness, a functional change of the arterial wall, in patients with diabetes at a high risk of CVDs.

Acknowledgments

The authors wish to acknowledge the excellent technical assistance of Ms. Mika Sakaki from the research laboratory of the Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine. This study was supported by a Grant-in-Aid for Scientific Research (No. 20591068) from the Japan Society for the Promotion of Science (to M.E. and K. Mori).

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this study.

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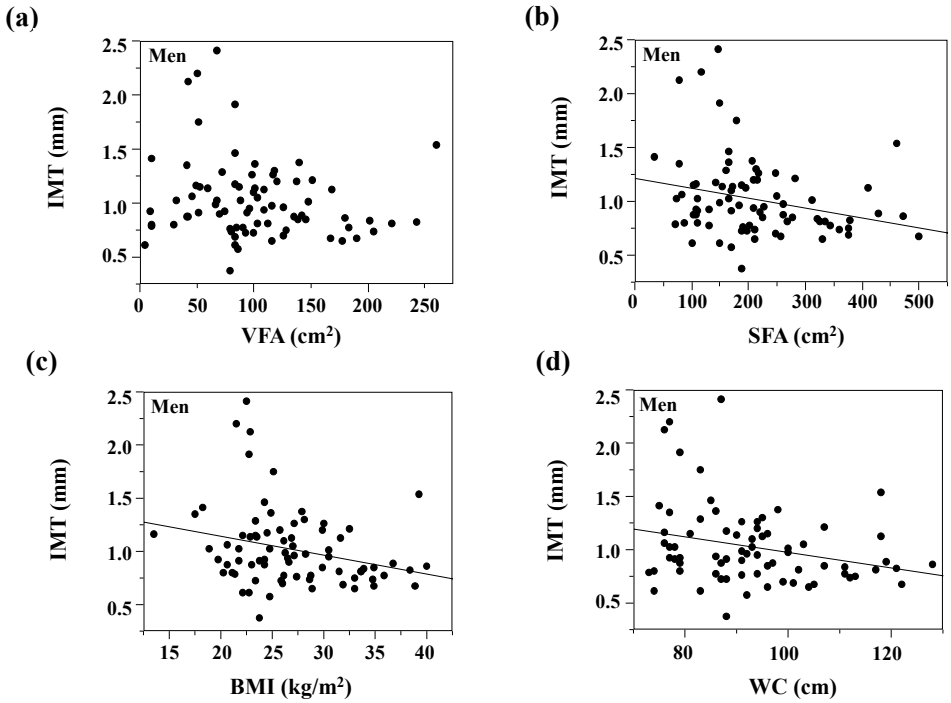
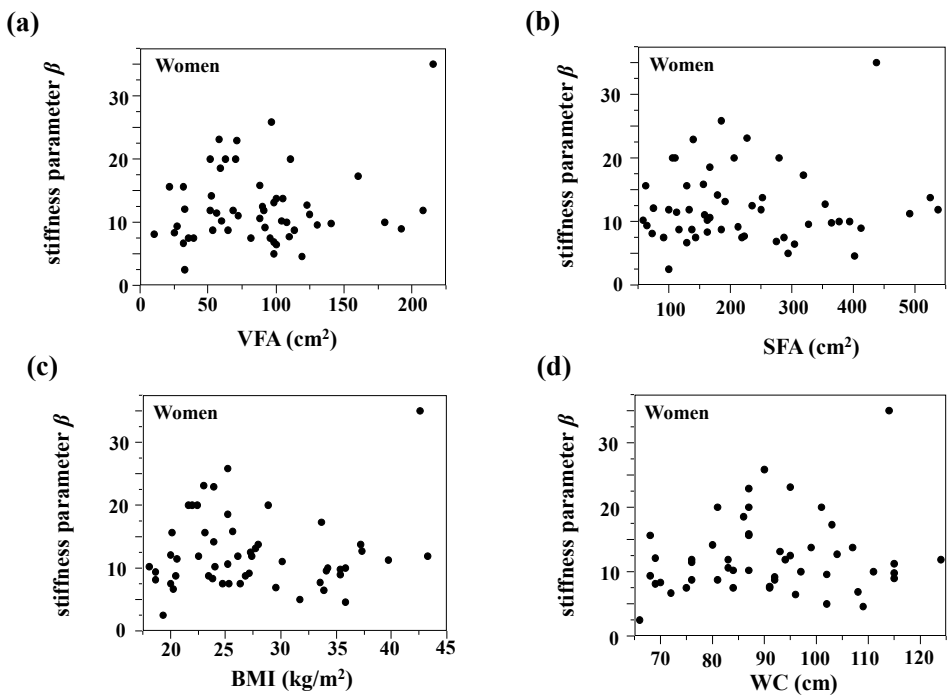


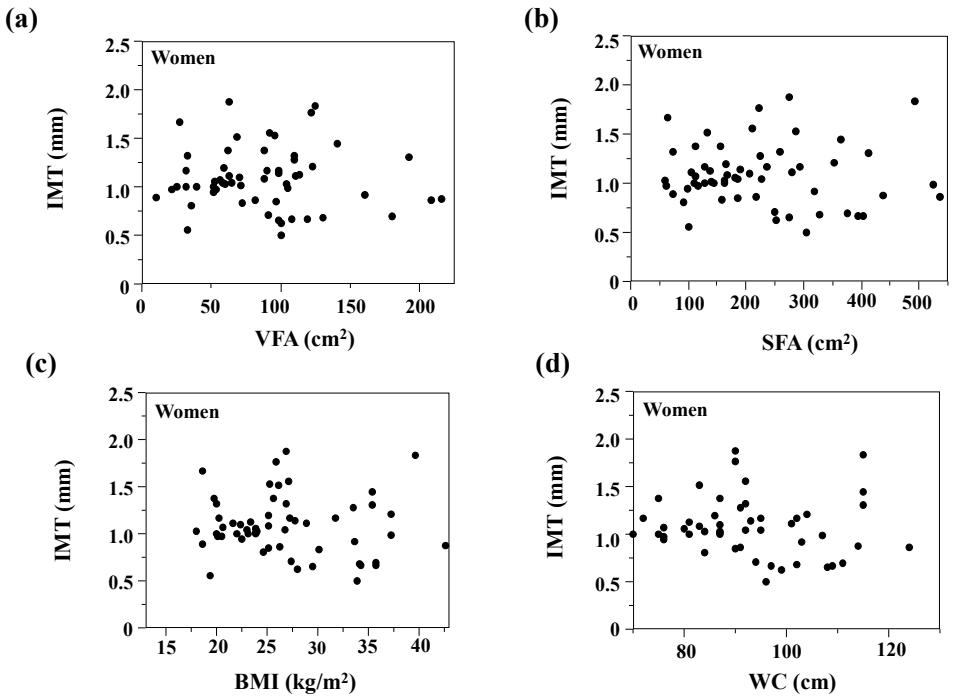
Supplemental Fig. 1. Association of visceral fat area (VFA) (a), subcutaneous fat area (SFA) (b), body mass index (BMI) (c), and waist circumference (WC) (d) with intima-media thickness (IMT) of the common carotid artery in men with type 2 diabetes

In men with type 2 diabetes, SFA ($r = -0.259$, $p = 0.023$), BMI ($r = -0.267$, $p = 0.019$) and WC ($r = -0.265$, $p = 0.023$), but not VFA ($r = -0.173$, $p = 0.134$), showed significant correlations with IMT.



Supplemental Fig. 2. Association of visceral fat area (VFA) (a), subcutaneous fat area (SFA) (b), body mass index (BMI) (c), and waist circumference (WC) (d) with stiffness parameter β of the common carotid artery in women with type 2 diabetes

In women with type 2 diabetes, none of the obesity-related indices were significantly correlated with stiffness parameter β (VFA, $r = 0.183$, $p = 0.191$; SFA, $r = 0.048$, $p = 0.735$; BMI, $r = 0.072$, $p = 0.610$; WC, $r = 0.153$, $p = 0.288$).



Supplemental Fig. 3. Association of visceral fat area (VFA) (a), subcutaneous fat area (SFA) (b), body mass index (BMI) (c), and waist circumference (WC) (d) with intima-media thickness (IMT) of the common carotid artery in women with type 2 diabetes

In women with type 2 diabetes, none of the obesity-related indices were significantly correlated with carotid IMT (VFA, $r = -0.046$, $p = 0.737$; SFA, $r = -0.043$, $p = 0.753$; BMI, $r = -0.098$, $p = 0.468$; WC, $r = -0.073$, $p = 0.598$).

Supplemental Table 1. Correlations of visceral fat area with obesity-related indices and metabolic parameters in subjects with type 2 diabetes

	ALL		Men		Women	
	r	*p*	*r*	*p*	*r*	*p*
Age	−0.249	0.001	−0.255	0.014	−0.225	0.063
BMI	0.807	<0.001	0.825	<0.001	0.852	<0.001
WC	0.881	<0.001	0.893	<0.001	0.879	<0.001
Systolic blood pressure	0.086	0.285	0.034	0.755	0.193	0.117
Diastolic blood pressure	0.166	0.039	0.065	0.545	0.280	0.022
Fasting glucose	0.077	0.336	0.044	0.679	0.128	0.302
HbA1c	−0.029	0.715	−0.121	0.251	0.117	0.344
Log [immunoreactive insulin]	0.415	<0.001	0.461	<0.001	0.385	0.002
Log [triglycerides]	0.331	<0.001	0.281	0.007	0.401	0.001
HDL-cholesterol	−0.284	<0.001	−0.101	0.338	−0.483	<0.001
LDL-cholesterol	−0.021	0.793	−0.059	0.577	0.050	0.683

r, correlation coefficients determined by simple regression analyses. BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin A1c level; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Supplemental Table 2. Comparison of obesity-related indices and clinical parameters between IMT-advanced and Stiffness-advanced subjects in men with type 2 diabetes

	Stiffness β -High IMT-Low	IMT-High Stiffness β -Low	*p*
N	18	18	
Age (years)	57.8 ± 10.8	63.4 ± 11.4	0.139
Duration of diabetes (years)	14.2 ± 2.5	10.0 ± 2.5	0.249
BMI (kg/m^2)	28.5 ± 5.3	23.9 ± 4.6	0.009
WC (cm)	96 ± 12	86 ± 9	0.010
VFA (cm^2)	116.4 ± 58.2	78.4 ± 38.6	0.027
SFA (cm^2)	237.9 ± 89.1	149.8 ± 71.2	0.002
Systolic blood pressure (mmHg)	133 ± 20	131 ± 17	0.779
Diastolic blood pressure (mmHg)	82 ± 12	78 ± 9	0.213
Smoker *n* (%)	4 (22.2)	7 (38.9)	0.275
Statin use *n* (%)	4 (22.2)	6 (33.3)	0.456
Insulin use *n* (%)	8 (44.4)	5 (27.8)	0.296
ARB/ACEI use *n* (%)	6 (33.3)	9 (50.0)	0.309
Fasting glucose (mg/dL)	138 ± 10	125 ± 10	0.360
HbA1c (%)	9.1 ± 2.8	8.5 ± 1.7	0.420
Immunoreactive insulin (μU/mL)	8.2 (3.5 – 12.2)	7.2 (4.6 – 11.1)	0.882
Serum creatinine (mg/dL)	1.37 ± 1.03	1.12 ± 0.60	0.388
Triglycerides (mg/dL)	150 (104 - 210)	117 (85 - 153)	0.164
HDL-cholesterol (mg/dL)	40.6 ± 9.3	37.9 ± 7.0	0.330
LDL-cholesterol (mg/dL)	125 ± 43	114 ± 47	0.426

Data are expressed as mean ± SD, median (interquartile range), or *n* (%). *P*-values were determined by Student's *t*-test, Wilcoxon rank-sum test, or χ^2-test, as appropriate. Abbreviations are the same as in Table 1.