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Estimation of visceral fat area using criteria for metabolic syndrome: A cross-sectional study

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ABSTRACT

Background and aims: The aim of this study was to calculate the visceral fat area (VFA) based on the criteria for metabolic syndrome (MetS).
Methods: A multiple regression analysis was performed to determine the estimated VFA using data from Japanese participants (2315 men and 1684 women). Receiver operating characteristic curve (ROC) analyses were performed to determine the optimal estimated VFA cutoff for the diagnosis of central obesity. The cutoff was also applied to a second cohort to validate the model.
Results: The estimated VFA was calculated using the MetS criteria, age, and body mass index (adjusted coefficient of determination = 0.682 for men and 0.726 for women). The area under the ROC curve for waist circumference, VFA, and estimated VFA were 0.669, 0.741, and 0.749, respectively, for men and 0.711, 0.787, and 0.803, respectively, for women. The optimal cutoffs for estimated VFA were 128.1 cm^2 for men and 82.2 cm^2 for women. Multivariate logistic regression for heart disease revealed that estimated VFA, rather than waist circumference, was associated with a high risk of heart disease.
Conclusion: The estimated VFA is a better index of central obesity than waist circumference and VFA for the diagnosis of MetS.

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1. Introduction

The harmonized criteria for metabolic syndrome (MetS) define central obesity using race- and gender-specific waist circumference (WC) cutoffs [1]. For Japanese populations, the WC cutoffs are ≥85 cm and ≥90 cm for men and women, respectively. These values were defined based on cross-sectional studies that have shown that these cutoffs were equivalent to 100 cm^2 of visceral fat area (VFA) [2]. However, these cutoffs have been criticized, because only Japan sets higher cutoffs for women than for men. However, setting lower cutoffs for WC and VFA in women than in men for defining central obesity is needed to identify subjects with MetS in Japanese and other Asian populations [3]. To measure VFA for the assessment of MetS, computed tomography (CT) is required, which has several limitations such as cost, convenience of use, and radiation exposure. On the other hand, artificial intelligence (AI) will likely revolutionize body composition measurements, supporting CT-based measurements, and facilitating larger population-based studies [4]. Furthermore, the AI approach is expected to facilitate many body composition measurements beyond VFA, including subcutaneous adipose tissue, liver, and muscle measurements [5].

In the present study, we tried to calculate the VFA based on the criteria of MetS such as WC, blood pressure (BP), triglyceride (TG) level, high-density lipoprotein cholesterol (HDL-C) level, and fasting plasma glucose (FPG) level. We also investigated the optimal cutoffs for the diagnosis of central obesity. We applied these cutoff points to a cohort without VFA measurements to validate the model.

2. Material and methods

2.1. Study population

We recruited adult male Japanese participants who visited our center between January 2017 and December 2019 for medical checkup (n = 23,987) and adult female Japanese participants who visited our center between January 2014 and December 2019 for medical checkup (n = 37,989). Of the enrolled participants, the

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MetS criteria were measured in 23,987 men and 33,238 women. The cohort used to calculate VFA based on the MetS criteria consisted of 2315 men and 1684 women who underwent VFA measurements via CT. The cohort used to validate the model consisted of 21,672 men and 31,554 women who did not undergo VFA measurements. This study was approved by the Ethics Committee of Aichi Prefectural University (31-1-58) and Social Medical Corporation Daiyukai (2019−020). Informed consent was obtained from all individual participants included in the study.

2.2. Measurements

The height and weight were measured in a standing position, and body mass index (BMI) was then calculated from these measures. The WC and VFA were computed and measured using commercial software on a CT scanner (SCENARIA, FUJIFILM Healthcare Systems Corporation, Tokyo, Japan). All measurements were taken at the umbilical level in the supine position. The BP was measured with a standard mercury sphygmomanometer on the right or left arm after the participants had rested in a sitting position for at least 10 min. After an overnight fast, venous blood samples were collected while the participants were in a seated position for measurement of TG, HDL-C, and plasma glucose.

The nonadipose components of MetS were defined using the harmonized criteria [1] as the presence of two or more of the following components: 1) HDL-C < 40 mg/dL in men or <50 in women; 2) TG $\geq$ 150 mg/dL; 3) systolic BP $\geq$ 130 mmHg and/or diastolic BP $\geq$ 85 mmHg; and 4) FPG $\geq$100 mg/dL. The participants who were on medication for diabetes, elevated TG, reduced HDL-C, or hypertension were included as having those risk factors.

2.3. Statistical analyses

A multiple regression analysis was performed in a stepwise manner for each gender with all criteria for MetS, age, and BMI as explanatory variables and VFA as the objective variable. Based on this analysis, we developed estimation formulas for VFA. Two adjustment models were used: Model 1 included age, WC, HDL-C, TG, systolic BP, diastolic BP, and FPG as explanatory variables; Model 2 included the variables in Model 1 plus BMI as explanatory variables. The receiver operating characteristic curve (ROC) analyses were performed to determine the appropriate cutoffs of WC, VFA and estimated VFA (eVFA) in identifying subjects with two or more nonadipose components of MetS. The optimal cutoffs were obtained from the Youden index [maximum (sensitivity + specificity − 1)]. We conducted sensitivity analyses using WC as an adipose component of MetS. The ROC analyses were performed to determine the appropriate cutoffs of WC, VFA and eVFA in identifying subjects with three or more components of MetS.

Continuous variables are presented as mean ± standard deviation. The clinical parameters were compared using two sample *t* tests or Chi-square tests, as appropriate. The statistical significance level was defined as $P < 0.05$ or the absolute adjusted residual value of >1.96. A logistic regression model was used to calculate the adjusted odds ratio (OR) with a 95% confidence interval (CI) for risks of heart disease. The multivariate logistic regression model using the forward selection method (likelihood ratio) was performed with adjustments for the following potential confounding factors: WC, TG, HDL-C, BP, FPG, and eVFA. All statistical analyses were performed with SPSS 26.0 software (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Baseline data of participants with VFA measurement

Table 1 shows the clinical characteristics of the participants with VFA measurements. We found 1123 (48.5%) and 473 (38.7%) participants who had with two or more nonadipose components of MetS in 2315 men and 1684 women, respectively. The participants in both genders with two or more nonadipose components of MetS had higher WC, BMI, and VFA than those without MetS.

Table 1
Clinical characteristics of participants with VFA measurement.

	Men			Women		
		Two or more nonadipose components other than WC			Two or more nonadipose components other than WC	
	Total	Absent	Present	Total	Absent	Present
n	2315	1192	1123	1684	1211	473
Age (year)	57 ± 11	53 ± 12	60 ± 10	57 ± 11	55 ± 11	64 ± 8
BMI (kg/m^2)	24.3 ± 3.2	23.4 ± 2.9	25.2 ± 3.3	22.0 ± 3.4	21.4 ± 3.1	23.6 ± 3.7
WC (cm)	87.0 ± 9.2	84.3 ± 8.8	89.8 ± 8.9	81.1 ± 10.2	79.0 ± 9.5	86.5 ± 10.0
SBP (mmHg)	122 ± 14	118 ± 13	127 ± 14	115 ± 17	111 ± 15	125 ± 17
DBP (mmHg)	76 ± 11	73 ± 10	78 ± 11	67 ± 11	66 ± 10	72 ± 11
TC (mg/dL)	207 ± 35	207 ± 32	207 ± 37	216 ± 34	218 ± 35	209 ± 32
HDL-C (mg/dL)	58 ± 15	61 ± 14	55 ± 15	73 ± 18	76 ± 18	64 ± 16
TG (mg/dL)	133 ± 95	101 ± 48	167 ± 118	95 ± 56	81 ± 40	128 ± 72
LDL-C (mg/dL)	127 ± 31	129 ± 28	126 ± 33	130 ± 32	131 ± 32	126 ± 30
FPG (mg/dL)	101 ± 18	95 ± 13	108 ± 20	92 ± 14	89 ± 10	100 ± 20
S−Cr (mg/dL)	0.90 ± 0.17	0.89 ± 0.13	0.91 ± 0.20	0.65 ± 0.11	0.65 ± 0.10	0.66 ± 0.12
UA (mg/dL)	6.3 ± 1.2	6.1 ± 1.2	6.4 ± 1.3	4.7 ± 1.0	4.5 ± 1.0	5.1 ± 1.2
VFA (cm^2)	120.5 ± 54.8	98.9 ± 49.4	143.3 ± 50.9	67.9 ± 43.1	55.8 ± 36.1	99.0 ± 44.0
Treatment						
Hypertension	609 (26.3)	95 (8.0)	514 (45.8)	244 (14.5)	61 (5.0)	183 (38.7)
Diabetes	189 (8.2)	33 (2.8)	156 (13.9)	70 (4.2)	11 (0.9)	59 (12.5)
Hyperlipidemia	478 (20.6)	0 (0.0)	478 (42.6)	324 (19.2)	0 (0.0)	324 (68.5)
Complications						
Stroke	79 (3.4)	24 (2.0)	55 (4.9)	16 (1.0)	6 (0.5)	10 (2.1)
Heart disease	130 (5.6)	31 (2.6)	99 (8.8)	49 (2.9)	22 (1.8)	27 (5.7)

Data are presented as means ± standard deviation or n (percentage).
VFA, visceral fat area; WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; S−Cr, serum creatinine; UA, uric acid.

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Table 2
Results of ROC analyses in participants with VFA measurement.

ROC curve	Cutoff value	Sensitivity	Specificity	Area	95%CI
Men					
WC (cm)	87.4	0.599	0.658	0.669	0.648–0.691
VFA (cm^2)	119.0	0.679	0.689	0.741	0.721–0.761
eVFA (cm^2)	128.1	0.608	0.747	0.749	0.729–0.768
Women					
WC (cm)	79.2	0.778	0.528	0.711	0.684–0.737
VFA (cm^2)	60.9	0.812	0.631	0.787	0.764–0.811
eVFA (cm^2)	82.2	0.668	0.791	0.803	0.780–0.825

ROC, receiver operating characteristics; VFA, visceral fat area; CI, confidence intervals; WC, waist circumference; eVFA, estimated VFA.

3.2. Calculation of eVFA and difference between VFA and eVFA

The eVFA (cm^2) for men was calculated using the following formula: $-340.611 + 5.040 \times$ WC (cm) $+ 1.164 \times$ age (year) $+ 0.051 \times$ TG (mg/dL) $- 2.889 \times$ BMI (kg/m^2) $+ 0.234 \times$ FPG (mg/dL) $- 0.263 \times$ HDL-C (mg/dL) $+ 0.097 \times$ systolic BP (SBP) (mmHg). Meanwhile, the following formula was used for women: $-209.257 + 2.246 \times$ WC (cm) $+ 0.123 \times$ TG (mg/dL) $+ 0.636 \times$ age (year) $- 0.250 \times$ HDL-C (mg/dL) $+ 0.168 \times$ SBP (mmHg) $+ 1.451 \times$ BMI (kg/m^2) $+ 0.150 \times$ FPG (mg/dL). The adjusted coefficient of determination (R^2) in this multiple regression analysis was 0.682 ($P < 0.001$) and 0.726 ($P < 0.001$) for men and women, respectively. If the value of the eVFA was negative, the eVFA was set to 0. Results were similar in a sensitivity analysis excluding BMI from explanatory variables (Model 1, Supplementary Table 1).

The mean eVFA was 120.6 ± 45.1 cm^2 and 67.9 ± 36.6 cm^2 for men and women, respectively. The correlation coefficient between the VFA and eVFA was 0.827 ($P < 0.001$) and 0.854 ($P < 0.001$) for men and women, respectively. The median difference between the VFA and eVFA in men was −0.045 cm^2,with the 10th, 25th, 75th, and 90th percentiles being −38.8, −19.0, 20.4, and 38.1 cm^2, respectively, whereas that in women was 1.5 cm^2 with the 10th, 25th, 75th, and 90th percentiles being −29.4, −13.4, 14.5, and 26.1 cm^2, respectively. The median difference between absolute values of VFA and eVFA in men was 19.9 cm^2,with the 10th, 25th, 75th, and 90th percentiles being 3.7, 9.3, 33.6, and 49.8 cm^2, respectively, whereas that in women was 14.0 cm^2 with the 10th, 25th, 75th, and 90th percentiles being 2.9, 6.6, 24.4, and 36.7 cm^2, respectively.

3.3. ROC analyses

Table 2 presents the area under the ROC curves to identify subjects with two or more nonadipose components of MetS. The eVFA showed the greatest areas in both men and women. The optimal cutoffs for WC, VFA, and eVFA in men were 87.4 cm, 119.0 cm^2, and 128.1 cm^2, respectively. Meanwhile, those of women were 79.2 cm, 60.9 cm^2, and 82.2 cm^2, respectively. Results were similar in a sensitivity analysis using WC as an adipose component of MetS (Supplementary Table 2).

3.4. Baseline data and group comparisons based on the presence of heart disease in participants without VFA measurements

Table 3 shows the clinical characteristics of the participants without VFA measurements. Heart disease occurred in 495 (2.3%) and 271 (0.9%) men and women, respectively. In both genders, the participants with heart disease had significantly higher WC, SBP, TG, FPG, and eVFA and lower HDL-C compared with these parameters in participants without heart disease.

3.5. Multivariate logistic regression for heart disease

Table 4 shows the adjusted OR for heart disease for eVFA and MetS components. Categorical cutoffs for WC and eVFA were ≥90 cm and >128 cm^2 in men and ≥80 cm and >82 cm^2 in women, respectively. Categorical cutoffs for MetS components were based on the harmonized MetS criteria [1]. The categorical cutoffs for eVFA were based on the ROC analyses in the present study. Higher eVFA or MetS components were associated with a high risk of heart disease. The adjusted ORs in men were 2.62 for BP (95%CI: 2.12–3.23, $P < 0.001$), 6.41 for HDL-C (95%CI: 5.27–7.80, $P < 0.001$), 1.61 in FPG (95%CI: 1.33–1.96, $P < 0.001$), and 1.38 for eVFA (95%CI: 1.04–1.81, $P = 0.024$). The adjusted ORs in women were 3.40 for BP (95%CI: 2.59–4.45, $P < 0.001$), 1.47 for TG (95%CI: 1.01–2.15, $P = 0.047$), 2.10 for HDL-C (95%CI: 1.45–3.05, $P < 0.001$), and 1.49 for eVFA (95%CI: 1.13–1.98, $P < 0.001$). In contrast, WC (>90 cm) was associated with a low risk of heart disease in men, with an adjusted OR of 0.60 (95%CI: 0.45–0.79, $P < 0.001$).

4. Discussion

To the best of our knowledge, 11 studies estimating the VFA from anthropometric variables have been previously published (Supplementary Table 3) [6–16]. It is true that measuring additional parameters other than the criteria for MetS, such as waist-to-hip ratio [8–10,12,14,15] and sagittal abdominal diameter [8–10], improve the adjusted R^2, but measuring these parameters is troublesome. This study revealed that the VFA can be estimated from the criteria for MetS, and that the VFA showed a strong positive correlation to the eVFA. The correlation between the VFA and eVFA was stronger and the error between the VFA and eVFA was smaller in women than those in men. Previous studies have shown that visceral adiposity is closely associated with an increased risk of cardiovascular morbidity and mortality [17]. The WC is indeed a criterion of MetS that indicates central obesity; however, it does not differentiate between subcutaneous fat and visceral fat. On the other hand, the VFA can accurately distinguish between subcutaneous fat and visceral fat. Visceral adipose tissue (VAT), but not VFA, has also been used to describe the CT area measurement [5,18]. VFA measurement is recommended [2,19] and generally used in Japan [3,15,20–24]; thus, VFA was used to describe the CT area measurement in the present study. The lumber level rather than the umbilical level may be preferred when measuring VFA and WC, given its variable position in the supine and upright configurations. However, the umbilical level VFA and WC measurements are recommended [2,19] and generally used in Japan [3,15,20–39]; therefore, VFA and WC were measured at the umbilical level in the supine position in the present study.

The Japanese WC cutoffs, which are ≥85 cm in men and ≥90 cm in women, are different from those of other Asian populations, which are ≥90 cm in men and ≥80 cm in women [1]. To the best of our knowledge, 21 studies of Japanese men [3,20–39] (Supplementary Table 4) and 20 studies of Japanese women [3,20–26,28–39] (Supplementary Table 5) have previously estimated the WC cutoffs using ROC analyses. The median WC cutoffs in previous studies were 85.3 cm and 80.0 cm in Japanese men and women, respectively. The former was close to the WC cutoff in the Japanese population and the latter was consistent with the WC cutoff in Asians. In the present study, the WC cutoff for men (87.4 cm) was near the middle between the Japanese and Asian cutoffs, whereas that for women (79.2 cm) was close to that of Asians. On the other hand, the median VFA cutoffs in previous studies (19, 22, 23, 35–37) were 107.2 cm^2 (range 92.0–132.6 cm^2) and 74.9 cm^2 (range 60.2–98.3 cm^2) in Japanese men and women, respectively. Although the Japanese WC cutoff points were based

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Table 3
Clinical characteristics of participants without VFA measurement.

	Men				Women			
		Heart disease				Heart disease		
	Total	Absent	Present	*P* value	Total	Absent	Present	*P* value
n	21,672	21,177 (97.7)	495 (2.3)	–	31,554	31,283 (99.1)	271 (0.9)	–
Age (year)	49 ± 11	49 ± 11	59 ± 10	<0.001	47 ± 11	47 ± 11	55 ± 13	<0.001
BMI (kg/m^2)	23.7 ± 3.6	23.7 ± 3.6	24.7 ± 4.2	<0.001	22.0 ± 3.8	21.9 ± 3.7	23.8 ± 5.1	<0.001
WC (cm)	83.8 ± 9.7	83.7 ± 9.7	87.0 ± 10.3	<0.001	76.8 ± 9.5	76.7 ± 9.5	82.6 ± 12.3	<0.001
SBP (mmHg)	121 ± 15	121 ± 15	125 ± 16	<0.001	112 ± 16	112 ± 16	120 ± 18	<0.001
DBP (mmHg)	75 ± 12	75 ± 12	75 ± 10	0.353	67 ± 11	67 ± 11	69 ± 11	0.002
TC (mg/dL)	204 ± 34	204 ± 34	183 ± 37	<0.001	204 ± 35	204 ± 35	198 ± 31	0.001
HDL-C (mg/dL)	59 ± 15	59 ± 15	55 ± 15	<0.001	72 ± 16	72 ± 16	68 ± 18	<0.001
TG (mg/dL)	119 ± 93	119 ± 93	122 ± 103	0.222	79 ± 52	79 ± 52	97 ± 43	<0.001
LDL-C (mg/dL)	124 ± 30	125 ± 30	106 ± 30	<0.001	118 ± 30	118 ± 30	114 ± 27	0.004
FPG (mg/dL)	97 ± 19	97 ± 19	108 ± 26	<0.001	89 ± 14	89 ± 14	94 ± 15	<0.001
S−Cr (mg/dL)	0.88 ± 0.30	0.88 ± 0.27	0.97 ± 0.78	<0.001	0.64 ± 0.17	0.64 ± 0.17	0.68 ± 0.16	<0.001
UA (mg/dL)	6.1 ± 1.2	6.1 ± 1.2	5.9 ± 1.2	<0.001	4.4 ± 1.0	4.4 ± 1.0	4.9 ± 1.3	<0.001
eVFA (cm^2)	95.2 ± 48.4	95.2 ± 48.2	124.4 ± 48.4	<0.001	49.2 ± 34.6	49.0 ± 34.5	75.3 ± 42.5	<0.001
Treatment								
Hypertension	3498 (16.1)	3196 (15.1)	302 (61.0)	<0.001	2781 (8.8)	2658 (8.5)	123 (45.4)	<0.001
Diabetes	1144 (5.3)	1014 (4.8)	130 (26.3)	<0.001	632 (2.0)	611 (2.0)	21 (7.7)	<0.001
Hyperlipidemia	2162 (10.0)	1887 (8.9)	275 (55.6)	<0.001	2215 (7.0)	2120 (6.8)	95 (35.1)	<0.001
Complications								
Stroke	327 (1.5)	295 (1.4)	32 (6.5)	<0.001	260 (0.8)	250 (0.8)	10 (3.7)	<0.001

Data are presented as means ± standard deviation or n (percentage).
VFA, visceral fat area; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; S−Cr, serum creatinine; UA, uric acid; eVFA, estimated visceral fat area.

Table 4
Multiple logistic regression analysis for heart disease in participants without VFA measurement.

	Reference	Odds ratio	95%CI	*P* value
Men				
WC	≥90 cm	0.60	0.45−0.79	<0.001
BP	≥130/85 mmHg or medication for hypertension	2.62	2.12−3.23	<0.001
HDL-C	<40 mg/dL or medication for reduced HDL-C	6.41	5.27−7.80	<0.001
FPG	≥100 mg/dL or medication for diabetes	1.61	1.33−1.96	<0.001
eVFA	>128 cm^2	1.38	1.04−1.81	0.024
Women				
BP	≥130/85 mmHg or medication for hypertension	3.40	2.59−4.45	<0.001
TG	≥150 mg/dL or medication for elevated TG	1.47	1.01−2.15	0.047
HDL-C	<50 mg/dL or medication for reduced HDL-C	2.10	1.45−3.05	<0.001
eVFA	>82 cm^2	1.49	1.13−1.98	<0.001

Categorical cutoffs for WC and eVFA were ≥90 cm and >128 cm^2 in men and ≥80 cm and >82 cm^2 in women, respectively. Categorical cutoffs for MetS components were based on the harmonized MetS criteria [1]. The categorical cutoffs for eVFA were based on the ROC analyses in the present study.
VFA, visceral fat area; CI, confidence intervals; WC, waist circumference; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; eVFA, estimated VFA; TG, triglyceride; MetS, metabolic syndrome; ROC, receiver operating characteristics.

on 100 cm^2 of VFA in both men and women [2], previous studies have demonstrated that the VFA cutoffs for men were higher than those for women. In the present study, the cutoffs of the VFA and eVFA for men were also higher than those for women. These findings suggest that the WC cutoff points in the Japanese population should be reevaluated.

We applied the eVFA cutoffs from a cohort with VFA measurement to a cohort without VFA measurement to validate the model. Multivariate logistic regression analyses for heart disease revealed that eVFA, but not WC, was associated with a high risk of heart disease in both genders. WC was associated with a low risk of heart disease in men, which may be because the average WC in both groups with and without heart disease were below the reference MetS level (<90 cm), and the distribution of WC in these groups overlapped.

5. Limitations

There are several limitations in this study. First, because the WC was measured using commercial software on a CT scanner in this study, it is possible that there was a difference between the WC in this study and the WC measured at the level of the umbilicus with a tape while standing, which might cause an erroneous eVFA. Second, the values of BP, TG, HDL-C, and FPG in participants taking medications of hypertension, hyperlipidemia, or hyperglycemia, respectively, were different from those in participants who would not have taken these medications. Since it is possible that the values of BP, TG, and FPG were underestimated and that of HDL-C was overestimated in participants taking these medications, their eVFA could be underestimated compared with that of participants without medications. Third, because the CT scans included liver and skeletal muscle, it was a lost opportunity not to consider ectopic fat deposition in these organs (i.e., hepatic and myosteatosis) [18].

6. Conclusion

The VFA can be calculated based on anthropometric variables related to obesity. Furthermore, the eVFA could be an excellent

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index as a criterion for central obesity compared with the WC and VFA in the diagnosis of MetS. The currently recommended WC thresholds for abdominal obesity differ in different populations and ethnic groups [1]. That is because heterogeneity of composition of abdominal tissues and their location-specific and changing relations with metabolic factors and cardiovascular risk factors in different ethnic groups do not allow a simple definition of abdominal obesity that could be applied uniformly [40]. The present study was conducted in the Japanese population, but if the eVFA is estimated in other ethnic groups, the ethnic differences for central obesity as a criterion of MetS may be organized and integrated into standard practice.

Declaration of competing interest

No, there are no competing interests for any author.

Contributiorship statement

The authors' contributions were as follows: M.K. designed the research; M.K. and S.O. conducted the research; M.K. and S.M. analyzed the data and M.K. wrote the paper. The manuscript was drafted and prepared, reviewed and revised by all authors. All authors made substantial contributions to the paper and approved the final version of the manuscript.

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Appendix A. Supplementary data

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