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Development of a New Method for Estimating Visceral Fat Area with Multi-Frequency Bioelectrical Impedance

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Excessive visceral fat area (VFA) is a major risk factor in such conditions as cardiovascular disease. In assessing VFA, computed tomography (CT) is adopted as the gold standard; however, this method is cost intensive and involves radiation exposure. In contrast, the bioelectrical impedance (BI) method for estimating body composition is simple and noninvasive and thus its potential application in VFA assessment is being studied. To overcome the difference in obtained impedance due to measurement conditions, we developed a more precise estimation method by selecting the optimum body posture, electrode arrangement, and frequency. The subjects were 73 healthy volunteers, 37 men and 36 women, who underwent CT scans to assess VFA and who were measured for anthropometry parameters, subcutaneous fat layer thickness, abdominal tissue area, and impedance. Impedance was measured by the tetrapolar impedance method using multi-frequency BI. Multiple regression analysis was conducted to estimate VFA. The results revealed a strong correlation between VFA observed by CT and VFA estimated by impedance ($r = 0.920$). The regression equation accurately classified VFA ≧ 100 cm² in 13 out of 14 men and 1 of 1 woman. Moreover, it classified VFA ≧ 100 cm² or < 100 cm² in 3 out of 4 men and 1 of 1 woman misclassified by waist circumference (W) which was adopted as a simple index to evaluate VFA. Therefore, using this simple and convenient method for estimating VFA, we obtained an accurate assessment of VFA using the BI method. ——— bioelectrical impedance; visceral fat; computed tomography; body composition; waist circumference.
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Further clarification of the relationship between obesity and various diseases has recently been provided (Kopelman 2000; Rubenstein 2005), including a report that excessive visceral fat area (VFA) is a major risk factor in such conditions as cardiovascular disease (Matsuzawa et al. 1995; Scholze et al. 2007). In 2005, the definition and diagnostic standard for metabolic syndrome (MS) in Japan was presented through the collaborative efforts of eight societies. In the criterion, VFA ≧ 100 cm² is a crucial item for diagnosing MS, and computed tomography (CT) was

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adopted as the gold standard for VFA assessment (Tokunaga et al. 1983; Yoshizumi et al. 1999). When umbilicus-level VFA exceeds 100 cm^2, the risk factor increases markedly. However, this method has its drawbacks, including high cost and exposure to radiation. VFA of 100 cm^2 corresponds to waist circumference (W) of 85 and 90 cm in Japanese men and women, respectively. Therefore, not only CT, but also W exceeding 85 cm in men and 90 cm in women, was adopted as a simple index for classifying VFA $\geqq$ 100 cm^2 (The Examination Committee of Criteria for "Obesity Disease" in Japan 2002).

The bioelectrical impedance (BI) method is used to estimate body composition by analyzing the impedance obtained when a weak current flows through the body. The BI method is noninvasive, simple, and inexpensive. Various studies have demonstrated the reliability of impedance measurements and the validity of BI equations for estimating intracellular and extracellular fluid distribution and lean body mass have been shown to influence BI estimates of body composition (Lukaski et al. 1986; Van Loan et al. 1992). Methods for estimating VFA using BI have been studied in recent years (Hainer et al. 1995; Scharfetter et al. 2001; Miwa et al. 2005). As the obtained impedance differs according to measurement conditions such as body posture (Pinilla et al. 1992; Roos et al. 1992; Scharfetter et al. 1997), electrode arrangement, (Baker 1989; Caterine et al. 1997) and frequency (Zhu et al. 2005), these properties must be considered when estimating VFA by the BI method. Here, we developed a more precise method for estimating VFA by selecting the optimum body posture, electrode arrangement, and arbitrary frequency for the measurement conditions.

Subjects and Methods

Subjects

The study subjects were 73 healthy volunteers including 37 men (mean age ± s.d.: 34.6 ± 12.4 years, range: 20-64 years) and 36 women (mean age ± s.d.: 33.4 ± 10.0 years, range: 20-57 years) recruited from the student body and the teaching and clerical staff at our university. All subjects were fully informed of the procedures, risk and discomfort involved in the CT abdominal scan, and the study protocol was approved by the Bioethics Committee of Utsunomiya University.

Procedure

Physical examination included height, body mass, W, hip circumference (HIP), sagittal abdominal diameter, and frontal abdominal diameter. Body mass index (BMI) was calculated as body mass (kg) divided by the square of height (m). Waist-hip ratio (WHR) was calculated as W (cm) divided by HIP. Waist-height ratio (WHtR) was calculated as W (cm) divided by height. CT scans taken at the umbilical level used Signa HiSpeed DX/i (GE Yokogawa Medical Systems, Tokyo). VFA and subcutaneous fat area (SFA) were measured using image analysis software Fat Scan Version 3.0 (N2 System Corporation, Hyogo). Areas of muscle (M.) rectus abdominis, M. obliquus abdominis, M. psoas major, M. quadratus lumborum, M. paraspinal, and columna vertebralis were calculated by planimeter, Super PLANIX β (Tamaya Technics Inc., Tokyo). Abdominal subcutaneous fat layer thickness (SFL) at front (T1), side (T2), diagonally back (T3), and back (T4) were calculated from CT images.

Impedance was measured by the tetrapolar impedance method using multi-frequency BI (MFBIA-07, Tanita Corporation, Tokyo). Repeatability was confirmed using the same subject. Sensing electrodes were symmetrically centered on the body axis. The distance between sensing electrodes was 10 cm (Fig. 1). Current electrodes were positioned to the right and left of the sensing electrodes, and at two different distances: 7 cm (electrode arrangement A), and 10 cm (electrode arrangement B). Electrocardiography electrodes were used (Red Dot™, 3M Health Care, St. Paul, MN, USA). Impedance was taken at 5, 25, 50, 100, 250, and 500 kHz with subjects in the supine, sitting, and standing posture, respectively. Exercise, eating, and drinking prior to impedance measurement was prohibited for all subjects.

Statistical analysis

The correlation between impedance and each measurement value was examined by Pearson's correlation coefficient. Stepwise multiple regression analysis was used to estimate VFA. Independent variables were sex, age, each anthropometry parameter, and impedance. All subjects were used in this analysis. Cross-validation using 20 other volunteers was employed to evaluate the

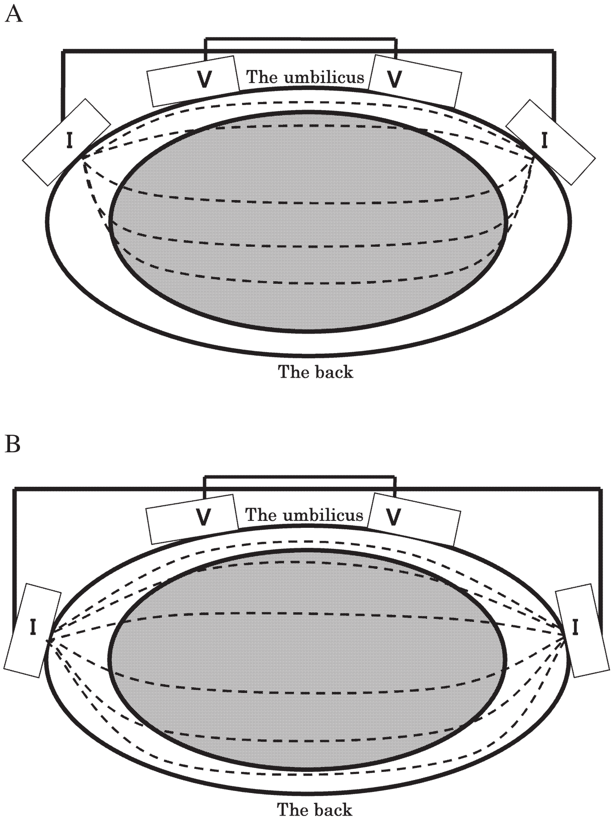

Fig. 1. Electrode arrangement A and electrode arrangement B.
Above figure shows electrode arrangement A. Sensing electrodes (V) were symmetrically centered on the body axis. The distance between V was 10 cm. Current electrodes (I) were positioned to the right and left of V. The distance between V and I was 7 cm.
Under figure shows electrode arrangement B. V were symmetrically centered on the body axis. The distance between V was 10 cm. I were positioned to the right and left of V. The distance between V and I was 10 cm.

precision of multiple regression equations. The Bland-Altman method was used to compare VFA observed by CT and VFA estimated by impedance (Bland and Altman 1986). The upper and lower limits of agreement, defining the range within which 95% of the differences between methods are expected to lie, were calculated as bias ± 1.96 standard deviation (S.D.). The bias and the upper and lower limits of agreement are reported as the 95% confidence interval. A p value of less than 0.05 was regarded as being statistically significant.

Results

The data is expressed as mean ± S.D. (Table 1). A total of 14 men (19.2%) showed VFA ≧ 100 cm^2; 16 men (21.9%) showed W ≧ 85 cm; 13 men (17.8%) showed VFA ≧ 100 cm^2 and W ≧ 85 cm. One woman (1.4%) showed VFA ≧ 100 cm^2; one woman (1.4%) showed W ≧ 90 cm; none of the women showed VFA ≧ 100 cm^2 and W ≧ 90 cm.

The strongest and weakest correlation coefficients between impedance obtained for 36 measurement conditions and each measurement value were SFA (r = 0.718–0.821), WHtR (r = 0.652–0.813), sagittal abdominal diameter (r = 0.664–0.740), T1 (r = 0.718–0.810), W (r = 0.637–0.749), and VFA (r = 0.675–0.744). Stronger correlation was observed for VFA at low frequency (5 kHz: r = 0.695–0.739) compared to high frequency (500 kHz: r = 0.675–0.736). The highest correlation for posture was standing, followed by sitting, and supine (r = 0.715–0.744, r = 0.718–0.735, r = 0.675–0.714, respectively). Other values did not show a strong correlation with impedance (r = –0.03–0.706). Especially, impedance correlated with muscular area and columna vertebralis area (r = 0.021–0.347, r = 0.179–0.268, respectively).

The results of multiple regression analysis are shown in Table 2. In this analysis, VFA was taken as a dependent variable and sagittal abdominal diameter, WHR, sex, age, body mass, and impedance (50, 100, 250, or 500 kHz) were taken as independent variables. The regression equations using impedance had a stronger correlation (r = 0.913–0.920) compared to those using only anthropometry parameters (r = 0.906). In the regression equations, electrode arrangement B had a stronger correlation than electrode arrangement A (r = 0.914–0.920, r = 0.913–0.918, respectively). Supine posture had the strongest correlation of all postures (supine: r = 0.917–0.920; sitting: r = 0.913–0.914; standing: r = 0.918, respectively). Therefore, we obtained the strongest correlation (regression equation: VFA = 3.74 × Sagittal abdominal diameter + 132.77 × WHR − 17.61 × Sex + 2.26 × Impedance (100 kHz) + 0.93 × Age + 0.85 × Body mass − 214.54, r = 0.920,

TABLE 1. Anthropometric indices, area of abdominal tissue and subcutance fat thickness (mean ± S.D.).

Anthropometric indices		Area of abdominal tissue		Subcutance fat thickness	
Age (years)	34.03 ± 11.23	SFA (cm^2)	116.49 ± 62.25	The front of abdomen	1.50 ± 0.70
	34.59 ± 12.43		117.26 ± 69.17	(cm)	1.48 ± 0.79
	33.44 ± 10.00		115.71 ± 55.21		1.52 ± 0.61
Height (cm)	165.59 ± 8.24	VFA (cm^2)	59.33 ± 48.97	The side of abdomen	1.12 ± 0.74
	171.32 ± 6.02		86.76 ± 53.99	(cm)	0.90 ± 0.49
	159.69 ± 5.65		31.14 ± 17.94		1.34 ± 0.89
Weight (kg)	62.08 ± 13.65	M. rectus abdominis	11.22 ± 3.54	The diagonally back	4.29 ± 1.67
	71.39 ± 12.46	(cm^2)	12.99 ± 3.59	of abdomen (cm)	4.24 ± 1.75
	52.53 ± 6.18		9.39 ± 2.41		4.34 ± 1.62
W (cm)	79.32 ± 9.83	M. obliquus abdominis	39.58 ± 13.63	The back of abdomen	0.87 ± 1.17
	83.82 ± 10.38	(cm^2)	50.02 ± 9.79	(cm)	0.62 ± 0.49
	74.71 ± 6.70		28.84 ± 7.05		1.12 ± 1.57
HIP (cm)	91.46 ± 7.00	M. psoas major (cm^2)	23.04 ± 8.69		
	94.23 ± 7.24		29.89 ± 6.36		
	88.61 ± 5.50		16.00 ± 3.63		
Sagittal abdominal diameter	18.62 ± 2.85	M. quadratus lumborum	11.70 ± 4.47		
(cm)	20.12 ± 2.94	(cm^2)	14.06 ± 4.70		
	17.07 ± 1.71		9.27 ± 2.53		
Frontal abdominal diameter	28.60 ± 2.85	M. paraspinal (cm^2)	39.74 ± 8.82		
(cm)	29.84 ± 2.88		45.32 ± 7.67		
	27.32 ± 2.20		33.99 ± 5.71		
BMI (kg / m^2)	22.44 ± 3.39	Columna vertebralis	25.06 ± 5.09		
	24.25 ± 3.50	(cm^2)	27.48 ± 5.48		
	20.58 ± 2.01		22.58 ± 3.16		
WHtR	0.48 ± 0.05				
	0.49 ± 0.06				
	0.47 ± 0.04				
WHR	0.87 ± 0.06				
	0.89 ± 0.06				
	0.84 ± 0.05				

above: both sexes (n = 73); middle: men (n = 37); under: women (n = 36);
W, waist circumference; HIP, hip circumference; BMI, body mass index; WHtR, waist-height ratio; WHR, waist-hip ratio; SFA, subcutaneous fat area; VFA, visceral fat area; M., muscle.

TABLE 2. Correlation between VFA observed by CT and VFA estimated by impedance of different posture and electrode arrangement.

Posture		Result of multiple regression analysis	Correlation
Supine posture	A	VFA = 3.70 × Sagittal abdominal diameter + 124.34 × WHR - 18.40 × Sex + 1.02 × 250 kHz Impedance + 0.92 × Age + 0.83 × Body mass - 194.58	r = 0.917
	B	VFA = 3.74 × Sagittal abdominal diameter + 132.77 × WHR - 17.61 × Sex + 2.26 × 100 kHz Impedance + 0.93 × Age + 0.85 × Body mass - 214.54	r = 0.920
Sitting posture	A	VFA = 4.55 × Sagittal abdominal diameter + 134.88 × WHR - 18.41 × Sex + 0.71 × 500 kHz Impedance + 0.82 × Age + 0.71 × Body mass - 202.30	r = 0.913
	B	VFA = 4.28 × Sagittal abdominal diameter + 145.19 × WHR - 18.49 × Sex + 1.62 × 250 kHz Impedance + 0.79 × Age + 0.71 × Body mass - 208.65	r = 0.914
Standing posture	A	VFA = 3.59 × Sagittal abdominal diameter + 132.03 × WHR - 18.03 × Sex + 0.96 × 50 kHz Impedance + 0.84 × Age + 0.79 × Body mass - 196.35	r = 0.918
	B	VFA = 3.76 × Sagittal abdominal diameter + 142.18 × WHR - 18.47 × Sex + 2.14 × 250 kHz Impedance + 0.84 × Age + 0.75 × Body mass - 205.58	r = 0.918
Anthropometric		VFA = 6.47 × Sagittal abdominal diameter + 186.81 × WHR - 10.77 × Sex + 0.94 × Age + 0.83 × Body mass - 290.31	r = 0.906

(n = 73)
Anthropometric: it is not used impedance in independent variables when calculated multiple regression analysis;
A and B: the distance of between sensing electrodes and current electrodes; A, 7 cm; B, 10 cm;
VFA, visceral fat area; WHR, waist-hip ratio; Sex, man is 1 and woman is 2.

$p < 0.01$, Fig. 2) when the measurement conditions were electrode arrangement B and supine posture. Also, we obtained a strong correlation by cross-validation (r = 0.899, $p < 0.01$). The Bland-Altman method for comparison between VFA observed by CT and VFA estimated by impedance showed a mean bias and 1.96 S.D. of 0.00 ± 37.58 cm^2 (Fig. 3). There was no difference between the mean VFA observed by CT and the mean VFA estimated by impedance.

DISCUSSION

CT is presently the most popular and trusted method for measuring VFA. However, the method is costly and involves radiation exposure. In contrast, the BI method is simple and noninvasive (Scharfetter et al. 2001; Miwa et al. 2005); however, the relationship between impedance and VFA is dependent on the measurement conditions (Baker 1989; Pinilla et al. 1992; Roos et al. 1992; Caterine et al. 1997; Scharfetter et al. 1997; Zhu et al. 2005). We tried to develop a more precise estimation method for VFA by selecting two types of electrode position, three types of body posture, and arbitrary frequency using multiple regression analysis.

Scharfetter et al. (2001) showed that the impedance is correlated with SFL of the abdomen when electrodes are placed on the abdomen. We also obtained a strong correlation between T1 and impedance (r = 0.718–0.810). This could indicate that VFA estimated by impedance is calculated as a higher value than VFA observed by CT when T1 is thicker. Therefore, it is necessary to select the electrode arrangement and frequency whereby the effects of SFL can be reduced or eliminated. Baker (1989) showed that impedance reflects information from just below the surface when using the electrode arrangement where the distance between sensing and current electrodes is narrow. Therefore, impedance better reflects VFA information from the deep part of tissue when the distance between sensing and current electrodes is wide. In this study, electrode arrangement B had a stronger correlation than electrode arrangement A by multiple regression analysis (r = 0.914–0.920, r = 0.913–0.918, respectively). In addition,

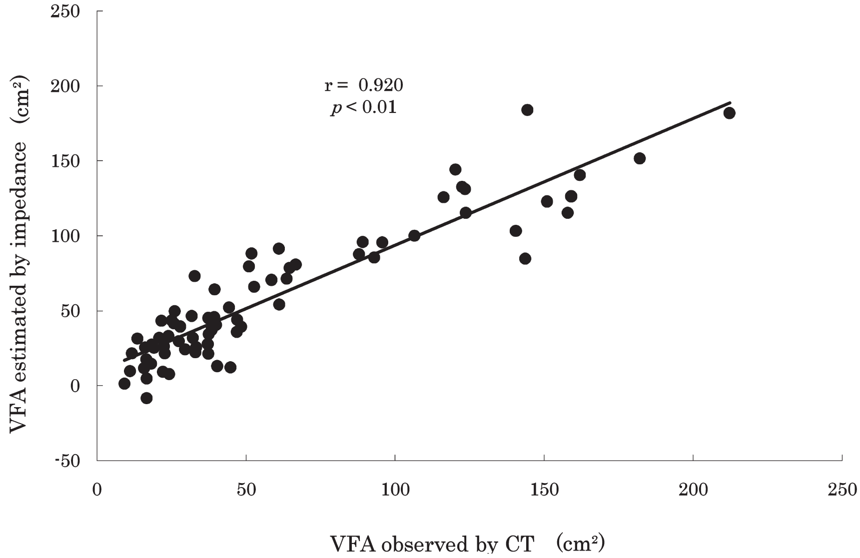

Fig. 2. The relationship between VFA observed by CT and VFA estimated by impedance.
Transverse axis is VFA observed by CT. Vertical axis is VFA estimated by impedance which measurement condition is supine posture, 100 kHz, and electrode arrangement B. It is shown the relationship between VFA observed by CT and VFA estimated by impedance. Its correlation coefficient is r = 0.920.

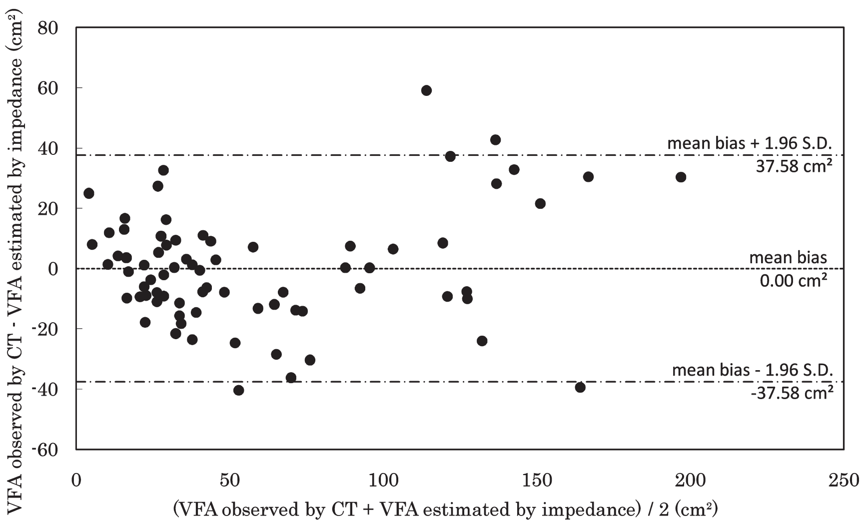

Fig. 3. Bland–Altman plot for comparison between VFA observed by CT and VFA estimated by impedance when measurement condition was supine posture, 100 kHz, and electrode arrangement B.
Transverse axis is (VFA observed by CT + VFA estimated by impedance) / 2. Vertical axis is VFA observed by CT – VFA estimated by impedance. The zero line on the vertical axis represents the mean difference between mean VFA observed by CT and mean VFA estimated by impedance. There is no difference between both mean values. Lines between ± 20 and ± 40 on vertical axis represent the limits of agreement (bias ± 1.96 S.D.). The error was admitted ± 37.58 cm^2, between both values.

it showed a weak correlation with muscular area and columna vertebralis area (r = 0.021–0.347, r = 0.179–0.268, respectively). Thus, it was possible to reduce the effects of SFL, muscular area, and columna vertebralis in the shallow part of tissues by adopting electrode arrangement B, and we consider it possible to selectively obtain information on VFA by using impedance.

The BI method may possibly be affected by measurement posture (Pinilla et al. 1992; Roos et al. 1992), which would explain why intracellular and extracellular fluid distribution shifts by measurement posture (Scharfetter et al. 1997). Therefore, the correlation between impedance and VFA observed by CT differed. Moreover, VFA distribution differs according to measurement posture. When the measurement posture is sitting or standing, the visceral organs droop compared to the supine posture. Consequently, the supine posture shows the same VFA distribution at the umbilicus level as in the CT. Therefore, the supine posture is the optimum posture for estimating VFA by the BI method.

Impedance depends upon many tissue characteristics and their electrical properties (Zhu et al. 2005). Low frequency current cannot flow through adipose tissue due to the high resistance. On the other hand, high frequency current makes it possible to obtain information not only on adipose tissue but also on other tissue because it can flow through all tissue. Therefore, 100 kHz is the proper frequency for considering these electrical properties even though various frequencies (50, 100, 250 or 500 kHz) were used for each measurement condition by multiple regression analysis. Therefore, we consider that the supine posture, electrode arrangement B, and 100 kHz frequency are the most precise measurement conditions for estimating VFA by BI. In fact, we obtained the strongest correlation (r = 0.920, $p < 0.01$) between VFA observed by CT and VFA estimated by impedance using these measurement conditions.

Although adipose tissue distribution differs between men and women (The Examination Committee of Criteria for 'Obesity Disease' in Japan 2002), we did not base the multiple regression analysis on gender. Instead, we divided the VFA estimated by impedance into men and women. There was no difference between mean VFA observed by CT (men: 86.76 cm^2, women: 31.14 cm^2) and mean VFA estimated by impedance (men: 86.76 cm^2, women: 31.14 cm^2). As the error between these values is less in both genders, the regression equation was highly precise regardless of gender.

The regression equation used in this study must solve the problems described above. However, we were able to confirm that VFA estimated by impedance had a strong correlation with VFA observed by CT. There was no difference between the mean VFA observed by CT and the mean VFA estimated by impedance, even though the upper and lower limits of agreement were ± 37.58 cm^2 by the Bland-Altman method when the measurement conditions were supine posture, frequency at 100 kHz, and electrode arrangement B. The regression equation showed a strong correlation by cross-validation (r = 0.899, $p < 0.01$). Consequently, we accurately classified VFA ≧ 100 cm^2 for 13 out of 14 men and 1 of 1 woman. In addition, there was no misclassification of VFA ≧ 100 cm^2 for subjects who were not VFA < 100 cm^2 by using this regression equation. Moreover, we precisely classified VFA ≧ 100 cm^2 or < 100 cm^2 for 3 out of 4 men and 1 of 1 woman misclassified using the index of W ≧ 85 or 90 cm, respectively.

In conclusion, we propose that this is a simple and convenient method for accurately estimating VFA by using the BI method when the measurement conditions are supine posture, 100 kHz and electrode arrangement B, which uses a wider distance between sensing and current electrodes. However, several participants in this study were extremely thin or suffered from obesity (BMI = 16.1–34.1). In the future, we hope to investigate subjects with different body types and develop a regression equation applicable to a wide range of physiques.

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