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# Proximal electrode placement improves the estimation of body composition in obese and lean elderly during segmental bioelectrical impedance analysis

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Abstract Bioelectrical impedance analysis (BIA) is an affordable, non-invasive, easy-to-operate, and fast alternative method to assess body composition. However, BIA tends to overestimate the percent body fat (%BF) in lean elderly and underestimate %BF in obese elderly people. This study examined whether proximal electrode placement eliminates this problem. Forty-two elderly men and women (64-96 years) who had a wide range of BMI  $[22.4 \pm 3.3 \text{ kg/m}^2 \text{ (mean} \pm \text{SD}), \text{ range } 16.8-33.9 \text{ kg/m}^2]$ and %BF (11.3-44.8%) participated in this study. Using <sup>2</sup>H and <sup>18</sup>O dilutions as the criterion for measuring total body water (TBW), we compared various BIA electrode placements; wrist-to-ankle, arm-to-arm, leg-to-leg, elbow-toknee, five- and nine-segment models, and the combination of distal (wrists or ankles) and proximal (elbows or knees) electrodes. TBW was most strongly correlated with the square height divided by the impedance between the knees and elbows  $(H^2/Z_{\text{proximal}}; r = 0.965, P < 0.001)$ . In the wrist-to-ankle, arm-to-arm, leg-to-leg, and five-segment

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Y. Hashii · S. Ando · Y. Okayama · T. Morimoto · M. Kimura Kyoto Prefectural University of Medicine, Kyoto, Japan models, we observed systematic errors associated with %BF (P < 0.05). After including the impedance ratio of the proximal to distal segments (P/D) as an independent variable, none of the BIA methods examined showed any systematic bias against %BF. In addition, all methods were able to estimate TBW more accurately (e.g., in the wrist-to-ankle model, from  $R^2 = 0.90$ , SEE = 1.69 kg to  $R^2 = 0.94$ , SEE = 1.30 kg). The results suggest that BIA using distal electrodes alone tends to overestimate TBW in obese and underestimate TBW in lean subjects, while proximal electrodes improve the accuracy of body composition measurements.

**Keywords** Muscle mass distribution · Doubly labeled water · Elderly · Obesity · Physical activity level · Systematic bias

# Introduction

In middle-aged and aged populations, the amount and distribution of fat, muscle, and water compartments is associated with functional status, disability, mortality, and established risk factors for a variety of chronic diseases (Wannamethee et al. 2005; Calling et al. 2006; Nakamura et al. 2006; Ruhl et al. 2007). Therefore, the accurate assessment of body composition is particularly important in these individuals. Bioelectrical impedance analysis (BIA) has gained recognition as an affordable, non-invasive, easy-to-operate, and fast alternative method for the assessment of body composition (Janssen et al. 2000; Kyle et al. 2001). However, it has limitations with respect to precision and accuracy. In particular, BIA tends to overestimate fat-free mass (FFM) and total body water (TBW) in obese individuals and underestimate these measures in

lean individuals (Baumgartner et al. 1998; Frisard et al. 2005; Mitsui et al. 2006; Neovius et al. 2006; Pateyjohns et al. 2006).

The human body is composed of several segments between the wrist and ankle with different cross-sectional areas (CSAs) (Lukaski 1993; Organ et al. 1994; Stahn et al. 2007). In a series circuit of multiple impedance elements (Z), the composite impedance is the sum of the individual Z measurements. The total Z from wrist to ankle is influenced mostly by the forearm and lower leg, because they have smaller diameters (Lukaski 1993; Organ et al. 1994) and relatively smaller proportions of muscle tissue, which has a greater electrolyte content and conductivity of electricity than do the other tissues (bone + fat tissues) (Schwan and K1az 1956; Reddy and Saha 1984) compared to the proximal segments (Dempster and Gaughran 1967). Therefore, although the equation used in wrist-to-ankle (whole-body) methods can be applied to individuals who have body morphologies similar to that used in the development of the equation, traditional BIA may be less accurate for people who have atypical morphologies, such as obese or frail elderly individuals.

To solve this problem and to improve the reliability of BIA, researchers have proposed dividing the human body into different segments or regions (Lukaski 1993). One of the most often used segmental methods is the five-segment model (both arms, both legs, and the trunk; (Organ et al. 1994). Each of these segments, except the trunk, which has a heterogeneous composition (Ishiguro et al. 2006), can be considered cylindrical in shape and to have a homogenous composition. However, this approach may not resolve these issues because the limbs involve segments with different CSAs (thigh vs. lower leg and upper arm vs. forearm). In addition, this method does not account for potential differences in the composition of these segments (Lukaski 1993).

A large amount of TBW is distributed throughout the proximal segments; however, the total Z is most influenced by the distal segments in traditional BIA. Thus, individual differences in the Z ratio of the proximal to distal segments (P/D) may be related to systematic errors in the estimation of BIA (Ishiguro et al. 2005). We hypothesized that obese individuals have a smaller muscle volume (MV) ratio from the thigh to the lower leg because, compared to lean people, obese individuals use greater ankle joint torque, but not knee and hip torque, during walking (DeVita and Hortobagyi 2003). In addition, obese individuals tend to engage in less daily physical activity (Levine et al. 2005). Because of this physical inactivity, thigh muscle mass decreases at a greater rate compared to lower leg muscle mass (Ishida et al. 1997; Hughes et al. 2004). If this hypothesis is correct, using P/D ratios to determine body composition will provide more reliable estimates. The aims of the present study were to determine why BIA methods tend to overestimate TBW in obese individuals, and to solve this problem using a combination of distal and proximal electrodes in BIA measurements.

The choice of the criterion method is also important in the assessment of BIA validity. Body water has the highest conductance of any material in the human body, and the hydrated portion of the body is proportional to the conductance. We selected total body water (TBW) as the criterion method, which was analyzed using stable isotope dilutions of  $^{2}$ H and  $^{18}$ O. This is one of the most accurate and valid tools for assessing TBW, with a theoretical precision well below 1% (Schoeller et al. 1980; Racette et al. 1994).

## Subjects and methods

## Subjects

A total of 42 elderly Japanese individuals (64–96 years; 20 men and 22 women; %BF 11.3-44.8%) participated in this study, after first providing written, informed consent. Thirty-two healthy and relatively active elderly individuals (14 men and 18 women) were recruited from among the participants of an ongoing health study conducted at Kyoto Prefectural University of Medicine; an additional ten elderly subjects (six men and four women) were recruited from the public Long-Term Care (LTC) insurance system (Tsutsui and Muramatsu 2007) at two geriatric health facilities. The eligibility criteria were as follows: (1) ability to walk more than 10 m with a cane, (2) ability to provide informed consent and no sign of dementia, (3) no history of any replacement arthroplasty or current use of an artificial pacemaker, (4) absence of a diagnosis of pathological edema or lymphedema, (5) absence of any definite kidney, digestive, or other current acute disease. The study protocol was approved by the ethics committee of Kyoto Prefectural University of Medicine. Barefoot standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. The body mass of each subject was measured to the nearest 0.1 kg, with the subjects dressed in light clothing without shoes. Anthropometric measurements were obtained in the morning and limb lengths were measured to the nearest 0.5 cm using a flexible tape (Flat rule, KDS Co., Ltd., Japan) with the subjects in a standing position (Miyatani et al. 2001).

#### TBW measurement

TBW was measured using the  ${}^{2}$ H and  ${}^{18}$ O dilutions method, as previously described (Yamada et al. 2009). Briefly, after collection of a baseline urine sample,





Fig. 1 Schematic representation of electrode position for each bioelectrical impedance analysis (BIA) (additional details shown in the text)

participants drank a premixed dose containing approximately 0.12 g/kg predicted TBW of <sup>2</sup>H<sub>2</sub>O (99.8 atom%, Isotec<sup>TM</sup>, Ohio, USA) and 2.5 g/kg predicted TBW of H<sup>18</sup><sub>2</sub>O (10.0 atom%, Taiyo Nippon Sanso, Tokyo, Japan). Urine samples were collected the next morning (day 1) and up until the morning of day 15. The <sup>18</sup>O and <sup>2</sup>H dilution spaces ( $N_{O}$  and  $N_{d}$ ) were determined by dividing the dose of the administered tracer (as moles of <sup>2</sup>H- or <sup>18</sup>O-water) by the intercept (<sup>2</sup>H and <sup>18</sup>O enrichments at time zero) (Coward 1990). These calculations were performed due to the influence of delayed isotopic equilibration on the accuracy of the DLW method in elderly subjects (Blanc et al. 2002). The  $N_d/N_O$  of the present study was  $1.038 \pm 0.012$  (mean  $\pm$  SD), which is consistent with previous studies (Racette et al. 1994). TBW was calculated as  $N_{\rm d}$  divided by 1.041 for the dilution space measured by <sup>2</sup>H, and  $N_{\rm O}$  divided by 1.007 for the dilution space measured by <sup>18</sup>O (Racette et al. 1994). The correlation coefficient was 0.998 between TBW measured by <sup>18</sup>O and TBW measured by <sup>2</sup>H. Percent body fat (%BF) was calculated as follows: (body weight  $-0.732 \times \text{TBW}$ )/body weight  $\times$ 100 (Racette et al. 1994).

#### **BIA** measurements

Segmental bioelectrical impedance analysis (SBIA) measurements were conducted according to previous studies (Miyatani et al. 2001; Ishiguro et al. 2005; Tanaka et al. 2007). All measurements were taken with the participants in a relaxed supine position on a padded wooden table, arms slightly abducted from the body, forearms pronated, and legs slightly apart. An eight-channel battery-operated impedance instrument (Muscle- $\alpha$ ; Art Haven 9, Kyoto, Japan) was used because it was capable of simultaneously measuring and displaying values obtained from four electrode pairs (Miyatani et al. 2000; Miyatani et al. 2001; Ishiguro et al. 2005; Tanaka et al. 2007; Yamada et al. 2007). This system applies a constant current of 500 µA at 50 kHz through the body and measures impedance (Z), not resistance. Prior to the test, the system was calibrated against 10, 100, and 1,000  $\Omega$  and was checked against a series of precision resistors provided by the manufacturer: errors were less than 1% for all measurements. The subjects were instructed to refrain from vigorous exercise and alcohol intake during the 24-h period and from eating a meal or drinking in excess of 0.51 of water during the 4-h period preceding the experiments (Baumgartner 1996; Evans et al. 1998). The room temperature was adjusted to maintain a thermoneutral environment. The impedance was measured within 5-10 min after the subject had lain down (Baumgartner 1996). This was done in order to avoid the immediate (1-2 min) effect of the transition from a standing to a supine position on the shifts in body fluids from the extremities to the thorax and to avoid the slow phase of the shifts that continues for up to 3-12 h (Gudivaka et al. 1994; Kushner et al. 1996; Slinde et al. 2003).

Pre-gelled electrocardiogram tab-type monitoring electrodes  $(2 \text{ cm} \times 2 \text{ cm}, \text{Red Dot}, 3 \text{ M})$  were used. Current injection electrodes were placed on both sides of the body on the dorsal surface of the hands and feet proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively. Voltage measurement electrodes were placed on both sides of the body on the mid-dorsum of the wrist and centered on a line joining the bony prominences of the radius and ulna. Additionally, the midanterior ankle centered on a line joining the malleolus lateralis and malleolus medialis, the lateral epicondyle of the humerus, the articular cleft between the femur- and tibiacondyles, the greater trochanter of the femurs, and the head of the radius and the acromion process of the shoulders (Fig. 1; (Tanaka et al. 2007). In addition to measurements for the traditional whole-body model, measurements for the arm-to-arm, legto-leg, and the five-segment models were also obtained.

Table 1Physicalcharacteristics, bioelectricalimpedance indices, andbioelectrical impedance indexratios in the participants

	Men $(N = 20)$		Women $(N = 2$	2)
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Physical characteristics				
Age (years)	$76.2\pm7.4$	65-87	$76.7 \pm 7.9$	64–96
Weight (kg)	$57.5\pm9.6$	42.0-76.5	$49.8\pm7.8$	37.1–72.3
Height (cm)	$160.4\pm7.0$	145.8-175.4	$148.5\pm5.5$	141.0-162.3
BMI (kg/m <sup>2</sup> )	$22.2\pm2.7$	16.8-27.5	$22.6\pm3.8$	17.0-33.9
TBW (kg)	$30.4 \pm 4.1$	23.7-37.1	$23.2\pm2.7$	19.1–30.3
%BF	$26.1\pm 6.0$	11.3-33.6	$35.4\pm7.0$	14.7-44.8
Bioelectrical impedance indices				
$H^2/Z_{\rm wholebody} \ (\rm cm^2/\Omega)$	$55.0\pm9.0$	39.3-73.3	$41.8\pm5.0$	33.0-55.8
$H^2/Z_{\rm arm}~({\rm cm}^2/\Omega)$	$50.3\pm7.5$	35.8-61.3	$37.2 \pm 4.1$	29.8-46.4
$H^2/Z_{\text{leg}} \text{ (cm}^2/\Omega)$	$68.5 \pm 15.6$	46.5-116.6	$53.7\pm9.3$	41.0-81.4
$L_{\rm arm}^2/Z_{\rm arm} + L_{\rm leg}^2/Z_{\rm leg} \ ({\rm cm}^2/\Omega)$	$39.8\pm7.5$	28.4-58.7	$29.9\pm4.7$	22.9-43.4
$ \begin{array}{l} L_{\mathrm{UA}}^2/Z_{\mathrm{UA}} + L_{\mathrm{FA}}^2/Z_{\mathrm{FA}} + L_{\mathrm{TH}}^2/Z_{\mathrm{TH}} \\ + L_{\mathrm{LL}}^2/Z_{\mathrm{LL}} \ (\mathrm{cm}^2/\Omega) \end{array} $	44.1 ± 7.6	30.9-62.1	$32.2 \pm 4.4$	26.2–44.3
$H^2/Z_{\rm proximal} \ ({\rm cm}^2/\Omega)$	$123.1\pm18.6$	89.8-152.8	$88.8 \pm 10.0$	72.5-108.4
Bioelectrical impedance index ratios				
P/D ratio in the arm	$2.12\pm0.21$	1.64-2.43	$1.96\pm0.18$	1.58-2.33
P/D ratio in the leg	$2.08\pm0.29$	1.34-2.56	$1.89\pm0.29$	1.42-2.49
P/D ratio in the whole-body	$2.25\pm0.12$	1.94-2.40	$2.13\pm0.13$	1.91-2.35

*BMI* body mass index, *TBW* total body water measured by doubly labeled water, *%BF* percent body fat, *H* height, *Z* impedance of the indicated segment, *P/D* ratio the impedance index ratio of the proximal to distal segments

Measurements for the proximal and nine-segment models were then performed (Scheltinga et al. 1992; van Marken Lichtenbelt et al. 1994). We applied a computerized program in order to finish an entire measurement of one person's six models within 5 min.

The anthropomorphic distinction between the trunk and upper or lower limb segments is sometimes difficult. Therefore, we compared the impedance with and without shoulder and hip electrodes in the 32 elderly subjects. When  $Z_1$  was defined as the impedance with shoulder and hip electrodes and  $Z_2$  was defined as the impedance without shoulder and hip electrodes using electrodes in the contralateral limbs; (Organ et al. 1994; Cornish et al. 2000),  $Z_2$  was very strongly correlated (r = 0.977 to 0.981, P < 0.001) with Z<sub>1</sub>. Z<sub>1</sub> can be calculated using the following equations: for the upper arm,  $Z_1 = 0.827 \times Z_2$  $(R^2 = 0.962$ , standard error of the mean [SEE] = 3.71  $\Omega$ ); and for the thigh,  $Z_1 = 0.876 \times Z_2$  ( $R^2 = 0.961$ , SEE = 1.98  $\Omega$ ). No significant difference was observed between the right and left limbs. These results indicate that  $Z_1$  and  $Z_2$  are proportionate to each other and either value can be used. Therefore,  $Z_2$  was used in all further analyses in the present study.

The repeatability of the impedance measurements for each segment was assessed on two separate days in a pilot study with 14 men. The intra-class correlation coefficients (ICC<sub>[3,1]</sub>) for the test–retest was 0.943–0.978 for each measurement. No significant difference was observed in any of the Z measurements between the two tests. The bioelectrical impedance (BI) index of the examined segment was calculated using the following equation: BI index = segment length<sup>2</sup>/Z. We assumed that the segment lengths are reflected by the distance between the two detector electrodes in the segmental models and by height in the whole-body, leg-to-leg, arm-to-arm, and proximal models.

MV was estimated using previously validated equa-72.71; forearms,  $MV = 110.41(L^2/Z) + 54.238$ ; thighs,  $MV = 131.19(L^2/Z) - 152.86$ ; and lower legs, MV = $126.35(L^2/Z) + 31.35$ . The estimated standard errors against the MVs measured by MRI were as follows: forearm, 38.4 cm<sup>3</sup>; upper arm, 40.9 cm<sup>3</sup>; lower leg, 107.2 cm<sup>3</sup>; and thigh, 362.3 cm<sup>3</sup> (Miyatani et al. 2001). The MV ratios of the thigh to lower leg and the upper arm to forearm were calculated to examine the relationship between muscle distribution and obesity. We also calculated the following ratios: the BI index of the thigh to lower leg as the P/D ratio in the leg; the ratio of the BI index of the upper arm to forearm as the P/D ratio in the arm; and the ratio of the BI index of the proximal model to the whole-body model as the P/D in the whole body.

#### Statistical analysis

All analyses were performed using SPSS 12.0 for Windows (SPSS; Inc., Chicago, IL). Results were presented as



Fig. 2 Relationships between percent body fat (%BF) and estimated MV ratios of the proximal to distal segments (P/D) in the leg (a) and in the arm (b)

the mean  $\pm$  standard deviation (SD) and the ranges. For all of the analyses, an alpha of 0.05 was used to denote statistical significance. Correlation coefficients were calculated using Pearson's product moment. Correlation coefficients were statistically compared using the methods described by Meng et al. (1992). A single regression analysis and multiple stepwise regression analyses were applied to examine differences in estimation between the

Table 2 Simple regression model for TBW estimation

six models with different electrode placement. The relationships between %BF and the residuals of measured TBW to estimated TBW were calculated. Bootstrapping was performed to validate the developed formula, in which 1,000 sets of data were randomly drawn from the original data set. This procedure represents a reliable model for validating prediction formulas (Efron 1983). For each set of bootstrap samples, an  $R^2$  value between the measured and predicted values of TBW was calculated. The mean  $R^2$  and the 95% confidence interval (CI) of the mean  $R^2$  are given.

# Results

Characteristics of the study population

Physical characteristics of the study subjects are shown in Table 1. Eight subjects were obese  $[BMI > 25 \text{ kg/m}^2$ , according to criteria for Asian populations (Kanazawa et al. 2002)], and six subjects were underweight (BMI < 18.5 kg/m<sup>2</sup>). Table 1 also presents descriptive statistics of the bioelectrical impedance indices and the ratios used in this study.

The relationships between %BF and the estimated MV ratio of the thigh to lower leg and the ratio of the upper arm to forearm are shown in Fig. 2. The P/D ratios were significantly correlated with %BF and obese individuals tended to have smaller P/D ratios in both the arms and legs.

## Single correlations between TBW and BI indices

TBW measured via isotope dilutions was better correlated with all of the BI indices (r = 0.840-0.965, P < 0.001) than weight (r = 0.825, P < 0.001) or height (r = 0.838, P < 0.001). The correlation coefficient for TBW and  $H^2/Z_{\text{proximal}}$  showed the strongest relationship (r = 0.965, P < 0.001). The equations for predicting TBW using simple regression analyses are shown in Table 2. SEE was

Electrode placements	Simple regression models	SEE			Bootstrap analysis		
		$R^2$	kg	%	Mean $\pm$ SD	95% CI	
Whole-body (wrist-to-ankle)	$0.493 (H^2/Z_{\text{wholebody}}) + 3.258$	0.90	1.69	6.3	$0.8992 \pm 0.0008$	(0.8975, 0.9009)	
Arm-to-arm	$0.535 (H^2/Z_{arm}) + 3.718$	0.87	1.84	6.9	$0.8698 \pm 0.0013$	(0.8672, 0.8723)	
Leg-to-leg	$0.293 (H^2/Z_{leg}) + 9.196$	0.70	2.80	10.4	$0.7244 \pm 0.0021$	(0.7203, 0.7284)	
Segmental model 1	$0.589 (BI_{(ARM + LEG)}) + 6.584$	0.84	2.06	7.7	$0.8412 \pm 0.0012$	(0.8388, 0.8436)	
Segmental model 2	$0.562 (BI_{(UA + FA + TH + LL)}) + 5.688$	0.89	1.73	6.5	$0.8891 \pm 0.0009$	(0.8874, 0.8908)	
Proximal (elbow-to-knee)	$0.217 (H^2/Z_{\text{proximal}}) + 4.182$	0.93	1.36	5.1	$0.9281 \pm 0.0007$	(0.9269, 0.9294)	

*H* height, *Z* impedance of the indicated segment, *BI* summation of the bioelectrical impedance indices of the indicated segments (see Table 1),  $R^2$  coefficients of determination, *SEE* standard error of the estimate



**Fig. 3** Relationships between percent body fat (%BF) and the residuals of measured and estimated TBW using a single regression model for each BIA method (Table 2)

smallest in the proximal model (1.36 kg) and largest in the leg-to-leg model (2.80 kg). Validation of the prediction equations using bootstrap sampling methods produced mean  $R^2$  values, which are shown in Table 2, indicating the

most acceptable validation of the developed formula of the proximal model.

In addition, %BF was significantly correlated with the residuals of measured TBW to estimated TBW in the whole-body model, arm-to-arm model, leg-to-leg model, and segmental model 1 (P < 0.05). We found that the estimated TBW tended to be higher in obese individuals and lower in lean individuals (Fig. 3). In contrast, the residuals of the proximal model and segmental model 2 were not significantly correlated with %BF.

## Multiple regression models

To improve the regression models, BI indices and BI index ratios were further examined in stepwise regression analyses. The whole-body P/D ratio was highly correlated with the P/D ratio in the leg and arm (r = 0.861 and 0.863,respectively, P < 0.001). Therefore, we used the wholebody P/D ratio as a representative variable in the wholebody model, segmental models 1 and 2, and the proximal model. In addition, we used the P/D ratio in the arm as a representative variable in the arm-to-arm model, and the P/D ratio in the leg as a representative variable in the legto-leg model. In all models, TBW estimation improved significantly when using the P/D ratios (Table 3), and the residuals were no longer significantly correlated with %BF in any model (Fig. 4). Validation of prediction equations using bootstrap sampling methods produced mean  $R^2$  values, which are shown in Table 3. These results indicated the most acceptable validation of the developed formula of the whole-body or proximal model using the P/D ratio as an independent variable.

# Discussion

In this study, BIA accuracy was significantly improved using proximal electrode placement (Table 2) or a combination of distal and proximal methods (Table 3). Differences in P/D ratios are one of the main reasons why BIA tends to overestimate TBW in obese individuals. After including the P/D ratio as an independent variable, none of the BIA methods examined showed any systematic bias against %BF. Additionally, all methods were able to more accurately estimate TBW.

In the literature, a large number of equations have been established for estimating body composition using BIA (Kyle et al. 2004). In multiple regression analyses, many of these equations use body weight as a predictor, independently of the impedance index ( $H^2/Z$ ). Moreover, some equations use age, sex, or the circumference of a given area as additional predictors (Kyle et al. 2004); however, these variables were included simply because they improved the

#### Table 3 Multiple equations for TBW

Table 5 Multiple equations for TBW							
Electrode placements	Simple regression models	SEE			Bootstrap analysis		
		$R^2$	kg	%	Mean $\pm$ SD	95% CI	
Whole-body (wrist-to-ankle)	$0.487 (H^2/Z_{wholebody})^{\dagger\dagger\dagger} + 7.745 (P/D_{wholebody})^{\dagger\dagger\dagger} - 13.375^{\dagger\dagger\dagger}$	0.94	1.32	4.9	0.9355 ± 0.0199	(0.9343, 0.9367)	

Whole-body (wrist-to-ankle)	0.487 $(H^2/Z_{\text{wholebody}})^{\dagger\dagger\dagger}$ + 7.745 $(P/D_{\text{wholebody}})^{\dagger\dagger\dagger}$ - 13.375 <sup>†††</sup>	0.94	1.32	4.9	$0.9355 \pm 0.0199$	(0.9343, 0.9367)
Arm-to-arm	$0.523 (H^2/)Z_{arm})^{\dagger\dagger\dagger} + 3.000 (P/D_{arm})^{\dagger} - 1.880$	0.89	1.75	6.5	$0.8855 \pm 0.0328$	(0.8834, 0.8875)
Leg-to-leg	$0.312 (H^2/Z_{\text{leg}})^{\dagger\dagger\dagger} + 4.696 (P/D_{\text{leg}})^{\dagger\dagger\dagger} - 1.272$	0.78	2.45	9.1	$0.7940 \pm 0.0488$	(0.7910,  0.7970)
Segmental model 1	$0.595 (BI_{(ARM+LEG)})^{\dagger\dagger\dagger} + 10.897 (P/D_{wholebody})^{\dagger\dagger\dagger} - 17.642^{\dagger\dagger\dagger}$	0.93	1.42	5.3	$0.9254 \pm 0.0205$	(0.9241, 0.9267)
Segmental model 2	0.551 $(BI_{(UA+FA+TH+LL)})^{\dagger\dagger\dagger}$ + 5.716 $(P/D_{wholebody})^{\dagger\dagger}$ - 6.400	0.91	1.56	5.8	$0.9109 \pm 0.0220$	(0.9095, 0.9122)
Proximal (elbow-to-knee)	$0.224 (H^2/Z_{\text{proximal}})^{\dagger \dagger \dagger} - 3.363 (P/D_{\text{wholebody}})^{\dagger} + 10.775^{\dagger \dagger}$	0.94	1.30	4.9	$0.9372 \pm 0.0193$	(0.9360, 0.9384)

*H* height, *Z* impedance of the indicated segment, *RPD* the impedance index ratio of the proximal to distal segments, *BI* summation of the bioelectrical impedance indices of the indicated segments (see Table 1);  $R^2$  coefficients of determination, *SEE* standard error of the estimate <sup>†, ††, †††</sup> Significant variables for estimating TBW (<sup>†</sup>P < 0.05, <sup>††</sup>P < 0.01, <sup>†††</sup>P < 0.001)

precision of the prediction equation, and not because there was a theoretical model (Deurenberg et al. 1990; Baumgartner et al. 1991; Haapala et al. 2002). Therefore, we used only impedance indices in this study, which are based on Ohm's law. All of the impedance indices (wrist to ankle, armto-arm, leg-to-leg, sum of segments, and elbow-to-knee) were highly correlated with TBW, and all of the simple regression models, excluding the leg-to-leg model, showed relatively good agreement with TBW (Table 2; Fig. 3). The leg-to-leg model, which was one of the most convenient measurements, did not accurately estimate body composition using only the impedance index. Although the proximal model was better correlated with TBW than the whole-body model, none of the segmental models was more accurate than the whole-body model. The proximal model eliminated the effects of small diameter segments in the body (i.e., the forearm and lower leg) and instead emphasized the trunk segment. These results were not surprising considering that most of the patient's TBW is in the trunk, whereas the forearms and lower legs have high impedance and little water.

Several studies have reported TBW and FFM overestimation in obese individuals when employing the wholebody BIA method (Frisard et al. 2005; Neovius et al. 2006; Pateyjohns et al. 2006). The results of the present study are consistent with previous results and also indicate that legto-leg, arm-to-arm, and traditional five-segment models tend to overestimate TBW in obese individuals (Fig. 3). Differences between TBW obtained using the dilution and impedance methods increased with obesity in these models. These results are not surprising, given that these individuals have atypical morphologies.

Estimated MV ratios of the thigh to lower leg and the upper arm to forearm tended to be lower in obese individuals and higher in lean individuals (Fig. 2). Obese individuals tend to spend less time performing middle- to high-intensity activities than lean people and these activities (e.g., walking quickly, running, and exercising) recruit larger muscles in the proximal sites of limbs (Cooper et al. 2000; Hansen and Allen 2002; Calling et al. 2006; Yang et al. 2006). Furthermore, obese individuals have altered gait biomechanics that involve an 88% higher ankle joint torque than lean people moving at the same speed; however, then have equal knee and hip joint torque and power while at the same speed as lean people, despite their greater weight (DeVita and Hortobagyi 2003). Those factors account for the lower MV ratios of the thigh to lower leg in obese individuals.

In comparison, coefficients for the proximal and ninesegment models, both of which used proximal electrode placement, were not significantly correlated with TBW. Residuals obtained from the nine-segment model depend less upon body fat, the one model that considers morphology; however, the nine-segment model is not a better predictor than the whole-body model. One possible reason for this is that the impedance of each segment is much smaller than the whole-body impedance. Relative measurement errors are also increased in the segmental model. Further research is required to clarify these issues.

The P/D ratio improved TBW estimation in all of the BIA methods. Proximal segments have low impedance and high water volumes, whereas the forearm and lower leg have high impedance and small water volumes. Therefore, whole-body BIA overestimates TBW in individuals who have a lower P/D ratio and underestimates TBW in individuals who have a lower P/D ratio and underestimates TBW in individuals who have a higher P/D ratio. Thus, the P/D ratio is an important variable that fills in the gap between the whole-body impedance index and TBW. The P/D ratio coefficients in the whole-body multiple regression models should be positive.



**Fig. 4** Relationship between percent body fat (%BF) and the residuals of measured and estimated TBW using the multiple regression models for each BIA method (Table 3)

The present study demonstrates that impedance measured using proximal electrodes (elbow and knee) improved measurement accuracy compared to traditional BIA methods. The proximal placement of electrodes has been tested previously in healthy and young adults. Results from these past studies suggested that the proximal placement of electrodes provided slightly more precise estimates of whole-body composition in young subjects (Scheltinga et al. 1992; Lukaski 1993; van Marken Lichtenbelt et al. 1994; Franckowiak et al. 2003; Ishiguro et al. 2005). To our knowledge, this is the first such study using elderly subjects, and the first study to examine the physiological parameters that limit the precise measurements with distal electrode placement.

The isotope dilution method for measuring TBW appears to be one of most reliable methods. Specifically, if <sup>18</sup>O and isotope ratio mass spectrometry (IRMS) are used, measurement errors are much smaller (Schoeller et al. 1980). In elderly subjects, however, Blanc et al. (2002) observed that 10% of the study population had delayed <sup>18</sup>O and deuterium equilibration in the urine. This delay induced a drastic TBW overestimation of 18%. Therefore, the authors suggested that, when plasma sampling is not possible, the intercept method using the first urine sample 24-h post dose should be used to calculate TBW. The  $N_{\rm d}/N_{\rm O}$  in the present study was 1.038  $\pm$  0.012, which is consistent with previous studies (Racette et al. 1994). In addition, the correlation coefficient was 0.998 between TBW measured by  ${}^{18}$ O and the TBW measured by  ${}^{2}$ H. Thus, the measured TBW is reliable within the measurement error of isotope analysis (1-2%; (Racette et al. 1994).

Selection of an electric current frequency is one of the essential problems of BIA. Elderly subjects have variable water distributions (ratio of extracellular to intracellular water) because of aging, disease, or both (Ritz 2001). It is possible to obtain water distribution data using bioelectrical impedance spectroscopy (BIS) or multi-frequency impedance analysis (MFBIA). However, in this study, such methods would have complicated the data analysis, for which reason we used a single frequency of 50 kHz. It would be valuable to examine the influence of current frequency on BIA in future studies.

Evaluation of the trunk region is one limitation of BIA (Ishiguro et al. 2006). In this study, when we added the BIA index of the trunk to the segmental models, the correlation coefficients against TBW were decreased (in the traditional five-segment model, r decreased from 0.926 to 0.894; in the new nine-segment model, r decreased from 0.953 to 0.906; data not shown). These results suggested that modeling the trunk as one cylinder is too simplistic for estimating body composition. The trunk has a heterogeneous composition (e.g., the thorax vs. the abdomen) and Ishiguro et al. (2006) demonstrated that the muscle volume of the trunk can be estimated as a specialized segmental BIA by dividing the trunk into upper, middle, and lower segments. However, this would complicate our data

analysis and this is outside the scope of this study. Further research into this is needed.

In conclusion, the present study demonstrated that BIA using distal (wrists and/or ankles) electrodes alone tends to overestimate TBW in obese subjects and underestimate TBW in lean subjects. We showed that measurements taken using proximal electrode placement (elbow and knee) improved the precision of traditional BIA models estimations, and it was possible to estimate TBW with higher accuracy. We also found that the impedance ratio of the proximal to distal (P/D) segments was significantly correlated with obesity.

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