Estimation of thigh muscle cross-sectional area by single- and multifrequency segmental bioelectrical impedance analysis in the elderly

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Yamada Y, Ikenaga M, Takeda N, Morimura K, Miyoshi N, Kiyonaga A, Kimura M, Higaki Y, Tanaka H. Estimation of thigh muscle cross-sectional area by single- and multifrequency segmental bioelectrical impedance analysis in the elderly. J Appl Physiol 116: 176–182, 2014. First published October 10, 2013; doi:10.1152/japplphysiol.00772.2013.—Bioelectrical impedance analysis (BIA) has been used to estimate skeletal muscle mass, but its application in the elderly is not optimal. The accuracy of BIA may be influenced by the expansion of extracellular water (ECW) relative to muscle mass with aging. Multifrequency BIA (MFBIA) can evaluate the distribution between ECW and intracellular water (ICW), and thus may be superior to single-frequency BIA (SFBIA) to estimate muscle mass in the elderly. A total of 58 elderly participants aged 65–85 years were recruited. Muscle cross-sectional area (CSA) was obtained from computed tomography scans at the mid-thigh. Segmental SFBIA and MFBIA were measured for the upper legs. An index of the ratio of ECW and ICW, and thus measurement of muscle cell mass or muscle mass with aging. The relative expansion of ECW relative to muscle volume with aging (56). The accuracy of MFBIA for estimating thigh muscle CSA higher than SFBIA in the elderly.

Bioelectrical impedance analysis (BIA) is an affordable, noninvasive, easy-to-operate, portable, and fast (within 5–10 min) alternative for assessing segmental or whole-body SMM (18, 21, 35, 48, 49). This BIA may be a practical method for assessing SMM in the elderly. However, the accuracy of BIA in the elderly is questionable because of limited research in this area, and most previous studies that developed and validated BIA for estimating SMM against MRI or CT were performed on healthy young adults. BIA has therefore been notoriously fraught with concerns of unreliability in the elderly population.

BIA can be performed as single-frequency BIA (SFBIA) or multifrequency BIA (MFBIA). Most previous studies that examined the validity of BIA for estimating SMM against CT or MRI have used SFBIA. In these studies, bioelectrical impedance index (L2/Z50), calculated as squared segment length (L) divided by impedance (Z50) or resistance (R50) at 50 kHz, is significantly related with SMM in healthy young adults (18, 21, 35, 43, 48, 49).

Skeletal muscle tissue holds a large amount of water that is partitioned into intracellular water (ICW) and extracellular water (ECW) fractions. The impedance of low-frequency current (<100 kHz; for example, 5 (Z2) or 50 (Z50) kHz) mainly reflects ICW (1, 25). A previous study reported an expansion in ECW relative to muscle volume with aging (56). The accuracy of SFBIA may be influenced by the expansion in ECW relative to muscle mass with aging. The relative expansion of ECW could attenuate the correlation between SMM and L2/Z50 or L2/Z50 in the elderly. By contrast, the impedance of the intracellular component calculated by MFBIA (Z250–5) reflects ICW, which could enhance the correlation between SMM and MFBIA compared with the correlation between SMM and SFBIA. A recent study indicated that skeletal muscle strength is more strongly correlated with the ICW component of MFBIA than L2/Z50 of SFBIA in the elderly (57). Skeletal muscle contains ECW that is not directly related to muscle function, and thus measurement of muscle cell mass or ICW in upper legs would be important. The purpose of the present study was to compare SFBIA and MFBIA for estimating thigh muscle CSA measured by CT in the elderly. The hypothesis was that the relative expansion of ECW in the elderly may mask a decrease in the cellular component of SMM.

METHODS

Subjects. A total of 58 community-dwelling, healthy, elderly Japanese subjects (65–85 years; 31 men, 27 women) participated in this study after providing written, informed consent. Inclusion criteria were 1) ability to walk more than 400 m; 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; and
4) absence of any definite kidney, digestive, or other acute disease. The study protocol was approved by the ethics committee of Fukuoka University. Barefoot standing stature 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, Japan) (35).

**Segmental BIA.** Impedance measurements were obtained with the subjects in a relaxed supine position on a padded bed, arms slightly abducted from the body, forearms pronated, and legs slightly apart. Subjects were instructed to refrain from vigorous exercise for the 24-h period prior to the experiment. Room temperature was adjusted to maintain a thermoneutral environment. Impedance was measured within 5 to 10 min of rest (3). This rest period was necessary to avoid the immediate (1–2 min) effect of transition from a standing to a supine position on the shift in body fluid from the extremities to the thorax, and to avoid the slow phase of this shift that continues for up to 3–12 h (27, 47).

An eight-channel battery-operated impedance instrument (Physion Z, Kyoto, Japan) had been updated to a multifrequency instrument (5, 50, and 250 kHz) from the previously used instrument (Muscle-a, Kyoto, Japan) (19, 35, 49, 54, 55). Before the test, calibration of the system was checked against a series of precision resistors provided by the manufacturer. Disposable tab-type monitoring electrodes (2 cm × 2 cm, Red Dot; 3M) were used. For upper leg measurement, two injecting electrodes were placed on both sides of the body on the dorsal surface of the feet proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints. Sensing electrodes were placed on both sides of the body at the articular cleft between the femoral and tibial condyles. Segment L was measured as the sum of both upper leg lengths measured from the articular cleft between the femoral and tibial condyles and the greater trochanter of the femur. In the present study, to compare BIA with muscle CSA rather than volume, the impedance indexes of L/Z were calculated instead of L/Z (44). For MFBIA, the impedance of the ICW compartment (Z250–5) was calculated as 1/[L/Z250] – (1/Z5)], and the impedance index of L/Z250–5 was calculated. Z5 mainly reflects ECW, and Z250–5 reflects ICW. Thus, the ECW/ICW index was calculated as Z250–5/Z5. The ratio of impedance was highly correlated with ECW/ICW (6).

**Computed tomography.** The CT scanning protocol was performed as previously described (58). With the subject supine, a 10-mm cross-sectional scan (2-mm × 5 slices) of both legs in each subject was obtained at the midpoint between the medial edge of the greater trochanter and the intercondylar fossa. Imaging was obtained with a CT scanner (Aquilion; Toshiba Medical Systems, Japan) using 250 mA, 120 kV, 0.5-s scanning time, 512 × 512 matrix, and 48-cm field of view, thereby attaining a pixel resolution of 0.94 mm. Skeletal muscle attenuation was measured for each subject as the mean attenuation value from all pixels within the range of 0–100 HU, as previously described (14), thereby excluding most of the intramuscular, or marbled, adipose tissue in the analysis. Because of the limited resolution of CT, however, depots of extracellular adipose tissue smaller than the pixel resolution were not completely excluded.

**Statistical analysis.** Results are presented as mean ± SD. All variables were judged to be distributed normally according to the Kolmogorov-Smirnov test (P > 0.1), and thus Pearson’s correlation coefficients were calculated between thigh muscle CSA and impedance indexes. Correlation coefficients were statistically compared using the methods described by Meng et al. (32). Pearson’s correlation analysis was used to examine the relationship between age and ECW/ICW index. Simple regression analyses were used to examine the relationship between thigh muscle CSA and impedance indexes in SFBIA and MFBIA. The residual error of the regression analyses was calculated and the relationship between the residual error and ECW/ICW index or age. Multiple regression analyses were applied to examine the contribution of impedance index, age, weight, and sex for estimating thigh muscle CSA in SFBIA and MFBIA. All analyses were performed using SPSS (PASSW) 18.0 for Windows (SPSS, Chicago, IL). For all analyses, P < 0.05 was used to indicate statistical significance.

**RESULTS**

Table 1 displays participant physical characteristics. Figure 1 shows the relationship between thigh muscle CSA measured using CT and impedance indexes of L/Z5, L/Z50, and L/Z250–5 in the upper legs. The relationship between muscle CSA and SFBIA (L/Z5 and L/Z50) was moderate (r = 0.682 and 0.735, respectively), whereas the relationship between muscle CSA and MFBIA (L/Z250–5) was strong (r = 0.853). Statistical comparison between correlation coefficients was performed using Meng’s z-test, and the correlation coefficients were significantly larger in L/Z250–5 than in SFBIA (L/Z5 and L/Z50; P < 0.01).

Figure 2 shows the relationship between age and ECW/ICW index. Significant and positive correlation was observed between age and ECW/ICW index (P < 0.001), with the index increasing with age. Figure 3 shows the relationship between ECW/ICW index and the residual error of estimating thigh muscle CSAs by BIA. SFBIA significantly tends to overestimate muscle CSA of subjects who had relative expansion of ECW in the thigh segment (P < 0.001). By contrast, this trend was not observed for MFBIA (P = 0.416). The correlation coefficients between age and the residual error of estimating thigh muscle CSAs by BIA were also calculated. SFBIA (5 and 50 kHz) tends to overestimate the muscle CSA of the older subjects (P ≤ 0.05). By contrast, this trend was not observed for MFBIA (P = 0.876).

Table 2 shows the results of multiple regression analysis for estimating thigh muscle CSA by BIA. For SFBIA, age, weight, and sex were included as significant independent variables. L/Z5 was not a significant independent variable for estimating thigh muscle CSA in SFBIA5kHz. L/Z50 was a significant independent variable in SFBIA50kHz, but the standardized coefficient of L/Z50 (β = 0.239) was smaller than those of weight (β = 0.359) and sex (β = −0.381). By contrast, L/Z250–5 was a significant independent variable (P < 0.001) in MFBIA, and the standardized coefficient of L/Z250–5 (β = 0.503) was larger than weight (β = 0.347) and sex (β = −0.223). In addition, age was no longer a significant independent variable in MFBIA (β = −0.024, P = 0.687). The coefficient of determination

**Table 1. Physical characteristics of study participants**

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 31)</th>
<th>Women (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>75.0 ± 5.8</td>
<td>750 ± 4.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163.7 ± 3.6</td>
<td>(155.9–170.0)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.7 ± 9.1</td>
<td>(43.3–80.5)</td>
</tr>
<tr>
<td>Thigh muscle CSA, cm²</td>
<td>246.8 ± 41.6</td>
<td>(160.0–331.5)</td>
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</table>

Thigh muscle cross-sectional area (CSA), mid-thigh muscle CSA as the sum of both legs.
was significantly higher in MFBIA ($R^2 = 0.860$) than in SFBIA. The final equation obtained after excluding age from independent variables is as follows: estimated thigh muscle CSA (cm$^2$) = 608.9 \times \text{L/Z}_{250-5} + 1.566 \times \text{W} - 20.0 \times \text{Sex} + 58.1, where L is segment length (cm); \text{Z}_{250-5} is impedance obtained from MFBIA (Ω); \text{W} is weight (kg); and Sex is male = 1 and female = 2. The relationship between measured thigh muscle CSA by CT and the estimated thigh muscle CSA by MFBIA is shown in Fig. 4.

DISCUSSION

The purpose of this study was to compare SFBIA and MFBIA for estimating thigh muscle CSA in the elderly. Results clearly indicated the significant difference between SFBIA and MFBIA for estimating thigh muscle CSA in the elderly. For SFBIA, the correlation between impedance index and muscle CSA was moderate, and the contribution of impedance index was smaller compared with weight and sex in multiple regression analysis. The estimation was significantly affected by a relative expansion of ECW in the thigh segment in SFBIA. Contrastingly, the correlation between impedance index and muscle CSA was high in MFBIA, and the contribution of impedance index was larger compared with weight and sex in multiple regression analysis. Relative expansion of ECW did not affect the estimation of thigh muscle CSA in MFBIA.

Although a few studies have suggested no age-related differences (41), many others have indicated age-related increases in the ratio of ECW/ICW at the whole-body level using chemical dilution technique, whole-body counting, or neutron activation analysis (11, 46, 52). The results of the present study and previous studies (56, 57) support the hypothesis that the relative expansion of ECW in the elderly masks a decrease in the cellular component of skeletal muscle mass at the segment level as well. The discrepancy might explain in part the disproportionate functional decline observed in elderly patients. Importantly, BIA is a clinically applicable tool.

BIA has become commonly used in both clinical and epidemiological settings. Despite considerable experimentation and theoretical advances, BIA remains something of a black box method for estimating in vivo body composition (4). A large number of studies have been published to examine the
validity of many different commercially supplied BIA devices, with inconsistent conclusions being reported (5, 12, 22, 23, 40, 45). This inconsistency may come about because the output body composition data (e.g., fat mass, fat-free mass, skeletal muscle mass, water distribution, bone mass, and visceral fat) of most commercially supplied BIA devices is based on undisclosed equations. Such equations commonly contain weight, sex, and age, and the contributing ratio of each variable is uncertain. Research into BIA should be based on raw impedance data and disclosure of equations used to obtain these findings. Undisclosed equations do not allow scientists to discuss results theoretically, biologically, and physiologically.

Traditionally, BIA has been assessed with SFBIA using lower frequency (e.g., 50 kHz). Because a lower-frequency current will not completely penetrate cells, SFBIA does not actually measure the entire muscle volume and is theoretically influenced by the relative expansion of ECW. No study, however, has demonstrated the influence of relative expansion of ECW on estimating skeletal muscle CSA or volume using SFBIA. Current results clearly indicated that SFBIA is strongly influenced by relative expansion of ECW and overestimates skeletal muscle CSA in elderly individuals who had a high ECW/ICW index (Fig. 3). The ECW/ICW index is positively correlated with age (Fig. 2), and thus SFBIA needs age as an independent variable in a multiple regression model (Table 2).

Janssen et al. (20, 21) established one of the most commonly used equations for SFBIA estimation of whole-body SMM as follows: skeletal muscle mass (kg) = [(height^2/R50 × 0.401) + (gender × 3.825) + (age × −0.071)] + 5.102, where height is measured in centimeters; R50 is measured in ohms between the right wrist and ankle in a supine position; for gender, men = 1 and women = 0; and age is measured in years. The present study provides a clear explanation of why age is incorporated into the previous SFBIA equation.

MFBIA uses several frequency currents on the principle that the body’s impedance is dependent on the frequency of the alternating current applied. ICW and ECW are separated by cell membranes. Cell membranes act as capacitors that insulate ICW at low frequencies so that predominantly ECW is measured. At higher frequencies, the membranes are permeable to the current so that ICW and ECW are measured (28). Thus the L/Z^250 obtained by MFBIA is theoretically independent of relative expansion of ECW. SFBIA demonstrated a systematic error in the prediction of thigh skeletal muscle, as shown in its relationship to the ECW/ICW index. In contrast, the slope of the relationship of the MFBIA-CT residual against ECW/ICW index was not significantly different from zero, indicating no effect of changes in water volumes on the prediction on skeletal muscle mass. The current results indicate that MFBIA is not influenced by relative expansion of ECW and does not need age as an independent variable in the multiple regression model.

The measured impedance is related not only to fluid volume but also to its intrinsic resistivity. Thus, conductance and impedance are qualities of ions in the aqueous space, not the water itself. The predictive ability of BIA depends on the assumption of constant, or at least randomly variable, ionic concentrations, mainly of sodium and potassium. It is possible that changes in intracellular or extracellular ion concentration might have produced the change in the ECW/ICW index obtained by MFBIA in aging. Total body potassium (TBK) decreases during aging, as do body cell mass (BCM) and ICW. TBK/[fat free mass] or TBK/[total body water] decreases during aging, but the TBK/BCM or TBK/ICW remains constant (53). This is supported by direct observation of rat muscle.
Table 2. Coefficients of multiple regression analysis for estimating thigh muscle cross-sectional area (cm²) by SFBIA and MFBIA

<table>
<thead>
<tr>
<th>Bioelectrical impedance index, L</th>
<th>SFBIA (L/Z5)</th>
<th>MFBIA (L/Z250–5)</th>
<th>SFBIA (L/Z50)</th>
<th>MFBIA (L/Z250–50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstandardized (B)</td>
<td>Standardized (β)</td>
<td>P</td>
<td>Unstandardized (B)</td>
<td>Standardized (β)</td>
</tr>
<tr>
<td>Age, years</td>
<td>−1.56</td>
<td>−0.174</td>
<td>0.018</td>
<td>−1.54</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>1.76</td>
<td>0.395</td>
<td>&lt;0.001</td>
<td>1.601</td>
</tr>
<tr>
<td>Sex, Male: 1; Female: 2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Constant</td>
<td>232.6</td>
<td>−0.410</td>
<td>&lt;0.001</td>
<td>219.3</td>
</tr>
</tbody>
</table>

Unstandardized (B) | Standardized (β) | P | Unstandardized (B) | Standardized (β) | P |
| 117.9 | 0.169 | 0.078 | 135.5 | 0.239 | 0.016 |
| −38.5 | −0.410 | <0.001 | −35.9 | −0.38 | <0.001 |

r² = 0.763

r² = 0.775

r² = 0.860

SFBA, single-frequency bioelectrical impedance analysis; MFBIA, multifrequency bioelectrical impedance analysis.

cells (30), which examined the water and electrolyte content of the heart and skeletal muscle cell of young and old rats. The water content of heart myocytes was significantly lower in old animals than in young animals, but the water content of skeletal muscle cells was not different between the two. Cations (Na⁺ and K⁺) showed an age-dependent increase in heart myocytes but not in skeletal muscle cells. Total monovalent ion concentration was increased in skeletal muscle cells. Increased ion concentration induces overestimation of water volume, but not underestimation. Thus, the present data support the hypothesis of a change in ECW/ICW water distribution in aging rather than a change in ion concentration.

It must be noted that another form of BIA exists, called bioelectrical impedance spectroscopy (BIS). BIS usually uses a current of rectangular wave form, which contains a broad spectrum of frequencies (1–1,000 kHz) and immediately obtains resistance and reactance of each frequency. BIS uses mathematical modeling and mixture equations (e.g., Cole-Cole plot and Hanai formula) to generate relationships between resistance and body fluid compartments (28). Thus, BIS is more strictly based on an electrophysiological theory. However, the reactance of high frequency using this method has more strictly based on an electrophysiological theory. How-ever, the reactance of high frequency using this method has less precision and a larger margin of error. By contrast, MFBIA is based on the measured impedance, which creates less margin of error but is less strictly based on theory. Thus, both BIS and MFBIA have advantages and disadvantages. Recently, we compared BIS and MFBIA, and indicated that the ability of assessing skeletal muscle is almost the same or slightly better in MFBIA compared with BIS (57). Hannan et al. (15) compared the accuracy of MFBIA and BIS to estimate water distribution (ECW and TBW) in surgical patients and concluded that MFBIA is almost the same or slightly better than BIS when used to measure this variable. Thus whatever their minor differences, both MFBIA and BIS can be useful.

Skeletal muscle mass estimated by imaging methods such as MRI, CT, and dual-energy X-ray absorptiometry cannot differentiate intracellular from extracellular water within muscle tissue. Skeletal muscle area measured by CT reflects both intracellular and extracellular water. In fact, many studies have indicated that skeletal muscle mass estimated by these imaging methods is poorly associated with physical function and mortality (29, 39, 50). These results may be affected by the relative expansion of ECW during aging. For future studies, it will be important to validate MFBIA on the basis of multiple techniques including total body 40K counting or bromide dilution method with imaging methods.

In the present study the final multiple regression equation of MFBIA still contains weight and sex as significant independent variables, although their standardized coefficients are smaller than L/Z250–5. A possible reason for this observation may be the morphological differences of the thigh muscle groups and fat between subjects. A limitation of the present study is using single-slice imaging at the mid-thigh can represent but not fully explain whole-thigh muscle volume (8). The CSA of a limb shows nonuniformity along its length. Moreover, the changes induced by weight reduction (24) or resistance training (37, 38) in the CSA of a muscle vary from site to site (35). BIA is based on models of the limb as a set of concentric cylindrical conductors consisting of subcutaneous adipose tissue, muscle, and bone (4). The assumption may be invalid, and thus further independent variables are still needed. Another explanation could be the effect of diversity on skeletal muscle quality. Structural changes in the extracellular matrix include an increase in collagen concentration, a change in the elastic fiber system, and an increase in fat accumulation within skeletal muscle (13, 14, 26, 31, 33, 34). These changes could affect the relationship between BIA and SMM. However, muscle quality was not examined in the present study, revealing another limitation. The effect of morphological difference in thigh muscle groups and muscle quality between subjects on MFBIA requires further exploration in future studies.
The difference between the impedance of low- and high-frequency currents may provide an estimate of intracellular water, but the variance of the difference may be higher than the physiological variances. The application of MFBIA still requires cautious use. In addition, we studied only a healthy elderly population without a young control group, and thus it would not be clear from this study at what age the change might start. Further studies are needed.

Conclusions. Single-frequency BIA is strongly influenced by relative expansion of ECW and overestimates skeletal muscle CSA in the elderly, who have a larger relative expansion of ECW. The relative expansion of ECW is related to age, and provides a physiological explanation of why age is incorporated into previous SFBIA equations to estimate body composition. By contrast, relative expansion of ECW did not affect the estimation of thigh muscle CSA in MFBIA. MFBIA, therefore, provides a more valid estimation of thigh muscle CSA than SFBIA in the elderly.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.Y., M.I., N.T., A.K., M.K., Y.H., and H.T. conceived and designed the experiments; Y.Y. prepared figures; Y.Y. drafted manuscript; Y.Y., M.I., N.T., K.M., N.M., A.K., M.K., Y.H., and H.T. approved final version of manuscript.

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