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

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Submitted 5 July 2013; accepted in final form 6 October 2013

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). Thus BIA may be a practical method for assessing SMM in the elderly. However, the application 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 (Z5) or 50 (Z50) kHz) mainly reflects ECW (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-s) 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
Variables were judged to be distributed normally when the pixel resolution was smaller than the pixel resolution of the instrument used. Impedance measurements were obtained in the morning, and limb lengths were measured to the nearest 0.5 cm using a flexible tape.

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 SFBA 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 SFBA and MFBIA. All analyses were performed using SPSS (PASW) 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 SFBA (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 SFBA (L2/Z5 and L2/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. SFBA 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. SFBA (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 SFBA, age, weight, and sex were included as significant independent variables. L/Z5 was not a significant independent variable for estimating thigh muscle CSA in SFBA at 5 kHz. L/Z50 was a significant independent variable in SFBA at 50 kHz, 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>
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</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>75.0 ± 5.8</td>
<td>75.0 ± 4.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163.7 ± 3.6</td>
<td>149.5 ± 6.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.7 ± 9.1</td>
<td>52.9 ± 9.3</td>
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<tr>
<td>Thigh muscle CSA, cm²</td>
<td>246.8 ± 41.6</td>
<td>181.0 ± 22.7</td>
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**Note:** 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 × $L/Z_{250-5}$ + 1.566 × W − 20.0 × Sex + 58.1, where L is segment length (cm); $Z_{250-5}$ is impedance obtained from MFBIA (Ω); 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

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Fig. 1. Relationship between thigh muscle cross-sectional area (CSA) measured by CT and impedance indexes of $L/Z_5$ (A), $L/Z_{50}$ (B), and $L/Z_{250-5}$ (C) in upper legs. The relationship between muscle CSA and SFBIA ($L/Z_5$ and $L/Z_{50}$) was moderate, whereas the relationship between muscle CSA and MFBIA ($L/Z_{250-5}$) was strong.

Fig. 2. Relationship between age and ECW/ICW index. A significant and positive correlation was observed ($r = 0.434, P < 0.001$).
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) = \( \frac{\text{height}^2/R_{50}^{0.401}}{\text{gender} \times 3.825} \times \frac{\text{age} \times -0.071}{0.071} + 5.102 \), where height is measured in centimeters; \( R_{50} \) 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 \( L/Z_{250-5} \) 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

![Fig. 3. Relationship between ECW/ICW index and residual error of estimating thigh muscle CSA by BIA. SFBIA significantly tends to overestimate the muscle CSA of subjects who had relative expansion of ECW in the thigh segment (A and B). By contrast, the trend was not observed for MFBIA (C).](image-url)
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 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 $L/Z_{250.5}$. 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 the morphological differences of the thigh muscle groups and 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.

### 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>Unstandardized (B)</th>
<th>Standardized (β)</th>
<th>P</th>
<th>Unstandardized (B)</th>
<th>Standardized (β)</th>
<th>P</th>
<th>Unstandardized (B)</th>
<th>Standardized (β)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>SFBIA (L/Z₁₀)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Unstandardized (B)</td>
<td>117.9</td>
<td>0.169</td>
<td>0.078</td>
<td>135.5</td>
<td>0.239</td>
<td>0.016</td>
<td>593.8</td>
<td>0.503</td>
<td>&lt;0.001</td>
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<tr>
<td>Standardized (β)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>−1.56</td>
<td>−0.174</td>
<td>0.018</td>
<td>−1.54</td>
<td>−0.17</td>
<td>0.017</td>
<td>−0.22</td>
<td>−0.02</td>
<td>0.687</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>1.76</td>
<td>0.395</td>
<td>&lt;0.001</td>
<td>1.601</td>
<td>0.359</td>
<td>&lt;0.001</td>
<td>1.546</td>
<td>0.347</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, Male: 1; Female: 2</td>
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<tr>
<td>Constant</td>
<td>232.6</td>
<td>0.410</td>
<td>&lt;0.001</td>
<td>−35.9</td>
<td>−0.38</td>
<td>&lt;0.001</td>
<td>−21.0</td>
<td>−0.22</td>
<td>0.002</td>
</tr>
</tbody>
</table>

$R^2 = 0.763 \quad r^2 = 0.775 \quad r^2 = 0.860$

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

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**Fig. 4. Relationship between thigh muscle CSA measured by CT and estimated thigh muscle CSA by MFBIA.**

**J Appl Physiol • doi:10.1152/japplphysiol.00772.2013 • www.jappl.org**
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**

Support for this study was provided by Technology Scientific Research Budget Basic Research Grant A19200069 (Strategic Research Infrastructure) from the Japanese Government’s Ministry of Education, Culture, Sports, Science and Technology to the Fukuoka University Institute for Physical Activity. Support was also provided by Research Fellowship Grant 23-333 to Y. Yamada and Research Grant 24240091 to M. Kimura, both from the Japan Society for the Promotion of Science for Young Scientists, and by a grant from the Takeda Science Foundation.

**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


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