Abstract  The present study aimed to compare the accuracy of estimating the percentage of total body fat (%TBF) among three bioelectrical impedance analysis (BIA) devices: a single-frequency BIA with four tactile electrodes (SF-BIA4), a single-frequency BIA with eight tactile electrodes (SF-BIA8) and a multi-frequency BIA with eight tactile electrodes (MF-BIA8). Dual-energy x-ray absorptiometry (DXA) and hydrostatic weighing (HW) were used as references for the measured values. Forty-five healthy college student volunteers (21 males: 172.9±5.5 cm and 65.8±9.1 kg and 24 females: 160.7±6.6 cm, 52.6±6.2 kg) were the subjects. Correlation coefficients between the BIA measurements and the references were calculated. The standard error of estimation (SEE) was calculated by regression analysis when estimating the reference measures (DXA and HW) from the predictor (SF-BIA4, SF-BIA8 and MF-BIA8). The differences in %TBF between the reference and the predictor, calculated by the reference minus the predictor, were plotted against the %TBF measured by the references. The MF-BIA 8 here showed the highest correspondence to the reference and the least estimation error compared with the other BIA methods. It is considered that there is a limit to directly estimate FFM from a regression equation using impedance, weight, height and age as independent variables, and that %TBF can be more accurately estimated by measuring segmental impedances using eight electrodes and multi-frequency electric currents and then estimating total body water from these impedances.

Introduction  Bioelectrical impedance analysis (BIA) is a practical method for assessing human body composition. Its validity and utility for various sample groups including a general adult population, the young and the obese have been reported (Gray et al., 1989; Oppliger et al., 1992; Segal et al., 1988; Baumgartner, 1996; Tanaka et al., 1999). Recently, inexpensive automatic BIA devices have become available commercially. These BIA devices automatically display body composition measures after impedance measurement. Because of their inexpensiveness and simple operation they are useful for establishing habits of daily body fat measurement for ordinary people, and for increasing their awareness of body fat mass.

Several BIA devices have been developed, and are characterized by differences in electrodes (the number, type and placement), electric current frequency, and body position at measurement. Single-frequency BIA devices with four tactile electrodes (SF-BIA4) have generally been used; but recently, single- and multi-frequency BIA devices with eight tactile electrodes have been developed for assessing segmental body composition, in addition to total body composition. Many single-frequency BIA devices with four tactile electrodes use hydrostatic weighing (HW) measure as reference to predict percent total body fat (%TBF). On the other hand, BIA devices with eight tactile electrodes have been developed using dual-energy x-ray absorptiometry (DXA) measures as reference. Furthermore, the single frequency BIA with eight tactile electrodes (SF-BIA8) used in this study (BC-118, TANITA Corp. Ltd.) estimates %TBF using a hand-to-leg impedance, while the multi frequency BIA with eight tactile electrodes (MF-BIA8) used in this study (InBody 3.0, Biospace) estimates %TBF using segmental impedance values at the right arm, left arm, right leg, left leg and trunk for four electrical frequencies. As mentioned above, although prediction methods differ among BIA devices, their estimation

Keywords: Single-frequency BIA, multi-frequency BIA, percent body fat, Bland-Altman
accuracies have not been sufficiently compared from the viewpoints of differences in electrical current frequencies, number of electrodes and prediction principles. This study applied three automatic BIA devices, a SF-BIA4, a SF-BIA8 and an MF-BIA8, and compared the accuracies of their %TBF estimates using DXA and HW as references.

Methods

Subjects
Forty-five healthy college students (21 males and 24 females) volunteered for this study. Their heights and weights were: 172.9±5.5 cm and 65.8±9.1 kg for males, 160.7±6.6 cm and 52.6±6.17 kg for females. Mean height and weight of the subjects did not differ significantly from the Japanese standard for the same age (Tokyo Metropolitan University, 2000).

Measurement tools and procedures

The study was approved by the Human subject ethical committee of Kanazawa University. All participants signed a letter of informed consent before the study. After explanations of the measurement procedures, subjects were measured using DXA, HW and the three BIA devices (SF-BIA4, SF-BIA8 and MF-BIA8). To examine the test-retest reliability, all subjects underwent two trials for DXA and the three BIA devices. One set of DXA measurements and the BIA trials were conducted in a single day, followed by the second series of DXA measurements, which were conducted on another day but in the same time zone as the first trial, for each subject. HW measurements were conducted in five trials on another day. All measurements were conducted between 10:00 AM and 15:00 PM. Room temperature was kept from 24°C to 25°C. Before measurements, the subjects were not allowed to eat or exercise for two hours, and were asked to excrete. And during the measurements, subjects wore only swimming suits or a light cotton shirt. Although the three automatic BIA devices used in this study were designed for a user to self-measure without technical skills and specific knowledge, a nominated tester conducted the BI measurements for all subjects for the purposes of the study.

Single-frequency BIA with four tactile electrodes (SF-BIA4) measurement

A single-frequency bioelectrical impedance analyzer using four-point tactile electrodes (TBF-101, TANITA Corp., Tokyo, Japan) was used. This device uses an alternating current (AC) of 500 μA with a single frequency of 50 kHz. A total of eight tactile electrodes are built into the right hand grip, left hand grip, right foot plate and left foot plate. %TBF is estimated as follows: FFM in the whole of the body is estimated from a multiple-regression equation using measured values of impedance between right hand and right leg and weight, and pre-entered values of height and age as independent variables. This prediction equation is developed for Japanese using DXA measurement as reference. Then, the %TBF is calculated using the following equations: fat mass (FM, kg) = weight (kg) – FFM (kg); %TBF = FM / weight × 100%.

For taking measurements, technical skills and specific knowledge are unnecessary. The subjects stand on the foot plates with bare feet while holding the hand grips. Their arms are stretched along their trunk. Two trials were carried out. After the first trial, the subjects stepped off the foot plates, and then stood on them again. Each trial was completed within 1 min.

Multi-frequency BIA with eight tactile electrodes (MF-BIA8) measurement

A multi-frequency BIA with eight-point tactile electrodes (InBody 3.0, Biospace Co., Seoul, Korea) was used. This analyzer uses an AC of 250 μA with multi frequency of 5, 50, 250 and 500 kHz. As with the SF-BIA8, in each grip and plate, two electrodes are built, respectively. This analyzer measures segmental impedances at the right arm, left arm, right leg, left leg and trunk for all frequencies, and can automatically display measurements of FFM, FM, %TBF, total body water, internal cell fluid, external cell fluid and the segmental fluid distribution.

An AC of 250 μA (I) is applied between the right hand and the right foot. The recorded voltage drop between the right hand and the left hand is divided by I to obtain the impedance of the right arm. The same operation is conducted for the voltage drop between the left hand and the left foot to obtain the impedance of the trunk, and the voltage drop between the right and left foot to obtain the impedance of the right leg. Then, the AC is applied between the left arm and the left leg and the voltage drop between the right hand and the left hand
is measured to obtain the impedance of the left arm. The voltage drop between the right and the left foot is measured to obtain the impedance of the left leg. The impedance value of the total body is calculated by summing the segmental impedance values. These values of impedance are calculated for all frequencies.

For estimation of %TBF, five segmental impedances (right hand, left hand, right leg, left leg and trunk) are measured for all frequencies, and intracellular water and total body water are estimated using these impedance measurements (the prediction equation and its reference have not been published). Muscle mass is estimated from total water based on the assumption that hydration of FFM is 73.2%. Then, bone mass is estimated using the predicted muscle mass, and this prediction equation uses DXA measurements as references (the detailed prediction equation has not been published). FFM is calculated by summing predicted muscle mass and bone mass, and the fat mass is calculated by subtracting the FFM from the weight. These prediction equations of total body water and FFM were developed for Asians, including Japanese.

For taking measurements, technical skills and specific knowledge are unnecessary. Two trials were carried out in a similar manner to that used in the SF-BIA8 measurement procedure. Each trial was completed within 2 min.

**Dual-energy x-ray absorptiometry (DXA) measurement**

Total body scanning was performed using a dual-energy x-ray absorptiometry analyzer (model DPX-L, Lunar Radiation Corp., Madison, WI). DXA measurements were performed by a trained radiology technician based on a standard technique while the subject was lying in a supine position on a table according to the manufacturer’s guidelines. The scanner was calibrated daily against the standard calibration block supplied by the manufacturer to control the possible baseline drift. DXA measured the R value, which is theoretically related to the soft tissue composition concerning bone mineral content, lean and fat tissues. Pixels of soft tissue were used to calculate the ratio of mass attention coefficients at 40 to 50 keV (low energy) and 80 to 100 keV (high energy), using software version 1.3Z. Subjects wore only a standard light cotton shirt to minimize clothing absorption. The duration of total body scanning was about 20 min, and the total x-ray irradiation absorbed by the organism was 5 mrems or lower, equivalent to 10% of a standard chest film.

**Hydrostatic weighing (HW)**

The subjects sat on a chair attached to a weighing scale (AD-6204, A&D). After exhaling, the subjects were submerged and their hydrostatic weights were measured. The residual volume (RV) was measured in the water without head submersion with a nitrogen washout technique (system 9, Minato Medical Corp.) based on the open-circuit method. The hydrostatic weight was measured 5 times. The mean value of the middle three measurements was used as a representative value. The prediction of body density (Db) and %TBF was calculated using the following prediction equation (Goldman and Buskirk, 1961).

\[
 Db = W / \left\{ (W - HW)/0.9937 - RV - VGI \right\} \\
 %BF = (4.57/Db - 4.142) \times 100
\]

W: body weight, HW: hydrostatic weighing, RV: residual volume, VGI: gas in the viscera assumed to be 100 ml, %BF: percent body fat

The water temperature was maintained between 35°C and 37°C and the gas volume in the viscera was assumed to be 100 ml. The subjects wore only swimming suits during the measurements.

**Statistical analyses**

The test-retest reliability of the %TBF of each method was examined by calculating the intra-class correlation coefficient (ICC). Significant differences of mean values of DXA, HW and three BIA devices were examined by ANOVA. If the significant differences were found, multiple comparisons were conducted by the Tukey HSD method. The estimation accuracies of the BIA devices were compared in references to the DXA and HW measurements. The correlation coefficients between the BIA measurements and the references were calculated. The standard error of estimation (SEE) was calculated by regression analysis when estimating the reference (DXA or HW) from the predictor (SF-BIA4, SF-BIA8 or MF-BIA8). The systematic error for predicting %TBF by the BIA devices was examined by Bland-Altman plots (Bland and Altman 1986). The differences in the %TBF between the reference and the predictor, calculated by the reference minus the predictor, were plotted against the %TBF measured by DXA or HW. In addition, the limits of agreement, defined as a range of the mean ±2 SD of the difference between the two different methods, were calculated to examine agreement between the reference and the predictor.

The significance level in this study was set at p<0.05.

**Results**

**The test-retest reliability of the %TBF measurement in each method**

Table 1 shows the ICC of each technique. ICC values were 0.994 or over (SF-BIA4: 0.995, SF-BIA8: 0.994, MF-BIA8: 0.995, DXA: 0.996, HW: 0.996), and the test-retest reliability of each technique was considered to be high.

**The comparisons of the %TBF among the five methods**

Table 1 shows the mean and standard deviation of the %TBF for each of the methods. The %TBF measured by DXA (%TBF\textsubscript{DXA}) was 16.04±6.16%, and the lowest and highest value were obtained in the %TBF\textsubscript{HW} (15.25±5.27%) and in the %TBF\textsubscript{SF-BIA4} (19.49±4.47%). When referenced to the %TBF\textsubscript{DXA}, all BIA methods tended to overestimate the %TBF. In examining the significant mean difference, the %TBF\textsubscript{SF-BIA4} and %TBF\textsubscript{SF-BIA8} were significantly greater than the %TBF\textsubscript{DXA} and %TBF\textsubscript{HW}. There were no significant differences between...
%TBF\textsubscript{MF-BIA8} and %TBF\textsubscript{DXA} or %TBF\textsubscript{HW}. These trends were similar in male and female groups.

Figure 1 shows the correlation coefficients between the %TBF values of the five methods. In total, the correlations with DXA were found to be 0.90 or over. The SEE values when estimating the %TBF\textsubscript{DXA} by the %TBF of the other BI methods were all found to be 3.0 or less, and the lowest and highest values were found in SF-BIA\textsubscript{8} (2.14%) and SF-BIA\textsubscript{8} (2.64%), respectively. In the female group, the correlations with DXA were found to be 0.80 or over, while those in male group were from 0.66 (SF-BIA\textsubscript{8}) to 0.71 (MF-BIA\textsubscript{8}). The SEE values were 3.0 or less in both groups, and tended to be less in the male than in the female group. Compared with the SF-BIA\textsubscript{4}, the MF-BIA\textsubscript{8} and SF-BIA\textsubscript{8} showed greater correlations and smaller SEE values. The MF-BIA\textsubscript{8} showed a greater correlation and a smaller SEE than the SF-BIA\textsubscript{8}.

Correlations with HW were from 0.78 (SF-BIA\textsubscript{4}) to 0.81 (MF-BIA\textsubscript{8}), and SEE values were from 3.16% (MF-BIA\textsubscript{8}) to 3.31% (SF-BIA\textsubscript{4}). Although in the SF-BIA\textsubscript{4} measurements the correlations and SEE values were almost equal between male and female groups, the correlation with HW for the MF-BIA\textsubscript{8} and SF-BIA\textsubscript{8} measurements tended to be smaller in males than females. In females, the SF-BIA\textsubscript{4} showed a smaller correlation with HW and a greater SEE as compared with other BIA devices. In males, the correlations with HW for the MF-BIA\textsubscript{8} and SF-BIA\textsubscript{8} measurements were smaller but the SEE values were greater than for the SF-BIA\textsubscript{4}.

Figure 2 shows the mean and standard deviations of the differences between the references (DXA or HW) and the three BIA methods, and the limits of agreements. The value of the limits of agreement was 10% or under the SF-BIA\textsubscript{8} and MF-BIA\textsubscript{8}, but was 10% or over the SF-BIA\textsubscript{4}. In total, the SF-BIA\textsubscript{4} and SF-BIA\textsubscript{8} tended to overestimate in %TBF compared with DXA, but not so the MF-BIA\textsubscript{8}, which showed the least error of the BIA devices. However, the MF-BIA\textsubscript{8} tended to underestimate in males (mean different was 1.38%) and overestimate in females (mean different was 1.04%); so that the trends in error with DXA differed between males and females. Compared with HW, the error in the MF-BIA\textsubscript{8} was the least, being the nearest to 0.0. The SF-BIA\textsubscript{4} and SF-BIA\textsubscript{8} tended to overestimate, with limits of agreement slightly greater in the SF-BIA\textsubscript{8} than the SF-BIA\textsubscript{4}.

Discussion

The prediction equation used in the SF-BIA\textsubscript{4} was developed using HW measurements as references, while those in the SF-BIA\textsubscript{8} and MF-BIA\textsubscript{8} used DXA measurements as their references (though the MF-BIA\textsubscript{8} uses DXA just in part). This study comprehensively compared the accuracy of estimating %TBF among these three BIA devices using DXA and HW as our references.

BIA device estimates of the %TBF from total body water are calculated from the bioelectrical impedance. In single-frequency BIA, a low frequency electric current of 50 kHz is

<table>
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<th>Table 1</th>
<th>Descriptive measurements of %TBF and test-retest reliability of each method</th>
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<tr>
<td>Total</td>
<td>SF-BIA\textsubscript{4}</td>
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<tr>
<td>Mean</td>
<td>19.49</td>
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<tr>
<td>SD</td>
<td>4.47</td>
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<tr>
<td>Male</td>
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<tr>
<td>SD</td>
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<tr>
<td>SD</td>
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<tr>
<td>ICC</td>
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<table>
<thead>
<tr>
<th>Test-retest reliability</th>
<th>SF-BIA\textsubscript{4}</th>
<th>SF-BIA\textsubscript{8}</th>
<th>MF-BIA\textsubscript{8}</th>
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<tbody>
<tr>
<td>ICC</td>
<td>0.994</td>
<td>0.995</td>
<td>0.996</td>
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SF-BIA\textsubscript{4}: Single-frequency BIA with 4-point tactile electrodes, SF-BIA\textsubscript{8}: Single-frequency BIA with 8-point tactile electrodes, DXA: Dual-energy x-ray absorptiometry, HW: Hydrostatic weighing, ICC: Intra class correlation, **: p<0.01.
generally used (Baumgartner, 1996). However, single-frequency BIA tends to overestimate the fat free mass (FFM) of the obese, and to underestimate that of athletes (Gray et al., 1989; Oppliger et al., 1992; Segal et al., 1988). One of the reasons for these problems is considered to be in the assumption that body water in the FFM or the ratio of the intracellular water (ICW) to extracellular water (ECW) is constant. Indeed, a previous study has reported that ECW increases with advancing obesity, and that ECW decreases and ICW increases relative to increasing FFM (Baumgartner, 1996). Therefore, it is possible in single-frequency BIA that measurement errors increase with variations in the physical characteristics of the sample population (Baumgartner, 1996; Deurenberg et al., 1989). In addition, single frequency BIA devices used in this study directly estimate FFM from regression equations using impedance, weight, height and age as independent variables. Therefore, the accuracies of estimating %TBF in these BIA devices depends on the accuracies of the regression equations; and this is considered as a part of the reasons for errors in estimating %TBF.

Comparing the estimation accuracy of the SF-BIA4 with that of the SF-BIA8; the correlations and SEE values were almost equal but errors from references tended to be less in the SF-BIA8 than with the SF-BIA4. Because both errors from DXA and HW were less with the SF-BIA8 than with the SF-BIA4, and errors from references with the SF-BIA4 were
greater in HW than in DXA, the estimation accuracy of the SF-BIA8 is considered superior to that of the SF-BIA4. The difference in the measurement principle between these devices is that the SF-BIA4 measures the leg-to-leg impedance while the SF-BIA8 measures hand-to-leg impedance. The validity of the leg-to-leg BIA device has been reported (Cable et al., 2001), although the individual differences in measurements became large with variations in age in the sample (Jartti et al., 2000). Therefore, it was not clarified whether hand-to-leg BIA can more accurately estimate %TBF of young Japanese than leg-to-leg BIA from just the results in this study. Since the SF-BIA8 used in this study is a successor BIA device to the SF-BIA4, the results obtained in this study may be caused by the improvements in the prediction equation used in the SF-BIA8 as compared with the SF-BIA4 rather than by any differences in the pathways of the impedance measurements.

A multi-frequency BI method avoids the problems encountered in a single-frequency BIA device by using low and high frequency electric currents. A low frequency electric current, such as 1 kHz or 5 kHz, sends currents only within the ICW, while a high frequency electric current, such as 100 kHz or 200 kHz, sends currents in both the ECW and ICW (Baumgartner, 1996; Cha, 1995; Kanai et al., 1987). Multi-frequency BIA estimates total body water (TBW), ECW and ICW using these characteristics of electric currents. In single-frequency BIA, the length of the conductor is needed to estimate the body composition, and it is substituted by the height or a value predicted from sex and age. This then is one of the reasons for measurement errors. However, since multi-frequency BIA can directly estimate body water composition from only the ratio of the low to high frequency impedances, this method can accurately estimate the body compositions of the obese and the athlete (Battistini et al., 1995; Chumlea et al., 1994; Deurenberg et al., 1996; Van Loan et al., 1992). Especially, the SF-BIA8 directly estimates FFM from a multiple regression equation using impedance, weight, height and age as independent variables. The results in this study, concerning of correlations with references, SEE and error, showed that the estimation accuracy of an MF-BIA8 was superior to other BIA devices. However, although the error from DXA was small, characteristics of errors from DXA differed between males and females. An MF-BIA8 tended to overestimate %TBF in males and underestimate that in females. The %TBF measured by DXA of many males in this study were 15% or less, and that of many females were 15% or more. Therefore, it is not clear that this result is influenced by the factor of sex or the differences in degree of obesity (systematic error). This will need to be determined using a sample population with a variety of physical characteristics. It is considered that the MF-BIA8 used in this study can accurately measure segmental impedances by using eight electrodes, and that this results in more accurate estimations of %TBF. Although the SF-BIA8 in this study also uses eight electrodes and can measure segmental impedances, only the impedance measurement between the right leg and the right hand is used to estimate %TBF. The estimation accuracy of %TBF in the SF-BIA8 could be improved by adding other segmental impedances measurements to the prediction equation as independent variables.

In conclusion, the MF-BIA8 used in this study showed the highest correspondence to the references used, and also showed the least estimation error compared to the other BIA methods tested. Although the estimation error of the SF-BIA8 used here was almost equal to that of the MF-BIA8, it tended to overestimate the %TBF measured by DXA, as compared with the MF-BIA8. Although the characteristics of errors from DXA differed between males and females, the estimation accuracy of a MF-BIA8 is considered to be superior to other BIA methods. It is considered that there is a limit to directly estimate FFM from a regression equation using impedance, weight, height and age as independent variables, and that %TBF can be more accurately estimated by measuring segmental impedances using eight electrodes and multi-frequency electric currents and by then estimating total body water from these impedances.

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