Bioimpedance analysis: a useful technique for assessing appendicular lean soft tissue mass and distribution

Serenella Salinari,1 Alessandro Bertuzzi,2 Geltrude Mingrone,3 Esmeralda Capristo,3 Antonio Scarfone,3 Aldo V. Greco,3 and Steven B. Heymsfield4

1Dipartimento di Informatica e Sistemistica, Università di Roma “La Sapienza,” 00184 Roma; 2Istituto di Analisi dei Sistemi ed Informatica del CNR, 00185 Roma; 3Istituto di Medicina Interna e Geriatria, Università Cattolica del Sacro Cuore, 00168 Roma, Italy; and 4Obesity Research Center, St. Luke’s Roosevelt Hospital, Columbia University College of Physicians and Surgeons, New York, New York 10025

Submitted 28 June 2002; accepted in final form 3 December 2002

The aim of the present study was to evaluate the feasibility and reliability of estimates of appendicular lean soft tissue mass obtained by bioimpedance analysis (BIA). BIA estimates were compared with the estimates obtained by dual-energy X-ray absorptiometry (DXA). Ten normal weight and 10 obese women had BIA and DXA evaluations. Lower limb SM mass was then derived from DXA appendicular lean soft tissue estimates. Lower limb SM mass and SM distribution were also estimated from BIA modeling that fits measured resistance values along the leg. SM mass (mean ± SD) was 5.8 ± 1.0 kg by BIA vs. 5.8 ± 1.1 kg by DXA in normal weight subjects and 7.2 ± 1.4 kg by BIA vs. 7.2 ± 1.2 kg by DXA in obese subjects. Mean ± SD of the absolute value of the relative error was 7.0 ± 3.4 and 5.9 ± 3.4% in the two groups, respectively. Similar results were obtained by using five resistance values for the analysis. In conclusion, the proposed BIA model provides an adequate means of evaluating appendicular SM mass.

Body composition; dual-energy X-ray absorptiometry; bioimpedance analysis; nutritional assessment

Measurement of body composition is important in a variety of clinical situations, including weight management. The primary goal of weight-loss programs is to maximize the loss of fat mass while preserving fat-free mass. Valid data on body composition changes are essential to the prescription and evaluation of the efficacy of clinical weight-loss interventions.

Skeletal muscle is a metabolically active tissue that represents a large proportion of the body fat-free mass and should be maintained in the elderly to prevent infirmity and, consequently, loss of independence (4, 17). There is no direct in vivo means of measuring skeletal muscle mass (SMM), although there are several methods of indirect estimation, including anthropometry (5), creatinine excretion (19), whole body counting with neutron activation (2, 18), computerized tomography (3), and MRI (6). All of these methods are time consuming and technically difficult to perform, and those methods often ranked the highest for accuracy (computerized tomography and MRI) involve considerable radiation exposure or expensive instrumentation.

Among the various techniques proposed to measure SMM, bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DXA) represent an attractive alternative to more expensive (e.g., MRI) or ionizing radiation-producing (e.g., computerized tomography) methods of muscle mass estimation (8, 9, 11, 12). The BIA measurement has many practical advantages: The instrumentation is relatively inexpensive and requires minimal maintenance and operator training; the measurements can be repeated as frequently as needed; and the results are available immediately. Also, the level of participation of the subjects being examined is relatively low.

The DXA method appears to represent a sensitive and accurate method for the assessment of regional soft-tissue components. As muscle mass of the limbs accounts for an estimated 75–80% of total body muscle mass (10, 11), appendicular muscle mass by DXA (i.e., bone-free lean tissue) has been endorsed as a simple means of quantifying total body muscle mass. In addition, DXA may be less sensitive to changes in muscle mass because this method does not differentiate between water and bone-free lean tissue (13, 15).

The aim of the present study was to evaluate the feasibility and reliability of estimates of appendicular lean soft tissue mass obtained by using BIA data from lean and obese subjects that were analyzed by a simple mathematical method proposed in a previous paper (16) against estimates provided by DXA. The proposed method also allows reconstructing from BIA data the pattern of the muscle cross-sectional area along the lower limb.
METHODS

Subjects and anthropometry. Subjects were recruited into two groups: nonobese and obese (body mass index < and ≥ 30 kg/m², respectively). Body weight was measured to the nearest 0.1 kg with a beam scale and height to the nearest 0.5 cm by using a stadiometer (Holtain, Crosswell, Wales, UK). The protocol conformed to the directives given by the Ethical Committee of the Institutional Health Review Board of the Catholic University, School of Medicine, in Rome. Informed consent was obtained in all cases. Subjects were women studied in the follicular phase of their menstrual cycle. All were in good health, as assessed by clinical and laboratory examinations, were not taking medications, and did not participate in intensive physical activity programs. Edema of the ankle was not clinically appreciable, and no sign of venous incontinence was revealed by ultrasonography in the obese population studied.

DXA analysis. Body composition was assessed by whole body DXA using Lunar DPX-L software version 3.65 (Madison, WI). The between-measurement coefficient of variation (CV) observed in our laboratory is 0.9% for lean soft tissue mass and 3.7% for total body fat mass.

Among the data obtained by specific software reconstruction, we have used information from the nondominant lower limb. Identical landmarks (i.e., inferior border of the ischial tuberosity) were selected and used in all subjects for separating lower limb from trunk. SMM was calculated by subtracting leg bone mineral mass from lean tissue mass. DXA rating lower limb from trunk. SMM was calculated by subtracting leg bone mineral mass from lean tissue mass. DXA scans were evaluated by the same trained observer (E. Capristo) to avoid between-reader measurement errors.

Bioelectrical impedance analysis. Resistances were determined by using a multifrequency BIA system (Human-IM DIP, DS-Medigroup, Milan, Italy) with a delivered current of 800 μA at a frequency of 50 kHz. The current-injection electrodes were positioned on the mid dorsum of the right hand, just proximal to the metacarpal phalangeal joint line, and on the mid dorsum of the right foot, just proximal to metatarsal phalangeal joint line (1).

To determine the resistance profile along the lower limb, one of the voltage electrodes was positioned on the mid dorsum of the right wrist. The other electrode was positioned at various contiguous levels along the lower limb at 2.5-cm intervals, starting from the mid ante rior right ankle up to the midline of the anterior surface of the right thigh at about the level of the inguinal crease. To guarantee correct spacing, the electrodes were fastened in a linear array to a strip of tissue. By subtracting the measured resistances from whole body resistance, we obtained the resistance profile along the lower limb. This profile gives the resistance between an electrode located at different levels along the lower limb from the ankle to the hip and an electrode located at the ankle. This resistance increases from zero to the total resistance of lower limb.

We also evaluated the effect on SM estimates by using a reduced number of measurement points. In particular, we considered measurement points located at distances of 10 cm or six measurement points located as follows: ankle (reference electrode), two equidistant points between the ankle and knee, knee, midpoint between knee and iliac crest, and iliac crest.

BIA measurements were made by the same trained investigator (A. Scarfone) to avoid between-observer measurement errors. A subset of subjects, three nonobese and three obese, was subjected to repeated measurements by two trained investigators (A. Scarfone and E. Capristo) to assess the reproducibility of BIA analysis.

Estimation of muscle cross-sectional area and volume. The estimation of muscle cross-sectional area and volume was performed after the approach proposed by Salinari et al. (16). Because the longitudinal conductivity of muscle is much larger than the conductivity of other tissues and the contribution of the reactance is small at 50 kHz, the current flowing in the longitudinal direction (z) through the lower limb is essentially carried by the resistive component of muscle and is equal to the total delivered current (I) = 800 μA. Thus, denoting the muscle cross-sectional area by \( S_m(z) \) and considering the component in the z direction of the real part of the current density (\( J_z \) in A/m²), we may write

\[
\bar{I} = \int_{S_m} J_z \, ds
\]

where \( ds \) is the element of cross-sectional area. By assuming that the electrical potential (\( V \)) is approximately constant over the cross section, \( V \) can be considered a function of \( z / \bar{V}(z) \) only, and we have

\[
J_z = \frac{1}{\rho_{\text{mus}}} \frac{dV}{dz}
\]

where \( \rho_{\text{mus}} \) (Ω · m) is the resistivity of skeletal muscle in the longitudinal direction. Thus, from Eqs. 1 and 2, we obtain

\[
\bar{I} = \frac{S_m}{\rho_{\text{mus}}} \frac{dV}{dz}
\]

If the potential at the ankle (\( z = 0 \)) is set to zero, the quantity \( \bar{V}(z)/\bar{I} \) can be interpreted as the resistance (\( \bar{R} \)) between a point of lower limb at distance \( z \) from the ankle and a point on the ankle itself, and from Eq. 3 we obtain

\[
S_m \frac{d\bar{R}}{dz} = \rho_{\text{mus}}
\]

An estimate of muscle cross-sectional area at the level \( z \) \( S_m(z) \) is obtained as

\[
\hat{S}_m(z) = \frac{\rho_{\text{mus}}}{d\bar{R}/dz}
\]

and the total muscle volume of lower limb (of length \( L \)) as

\[
\hat{V}_m = \int_0^L \hat{S}_m(z) \, dz
\]

To evaluate the derivative in Eq. 5, the resistance data obtained by BIA were approximated by a weighted sum of two gaussian cumulative functions plus a straight line \( \hat{R}(z) \) according to

\[
\hat{R}(z) = c_1[F(z;\mu_1,\sigma_1) - 0.5] + c_2[F(z;\mu_2,\sigma_2) + c_3z]
\]

where \( F(z;\mu,\sigma) \) denotes the cumulative function of a gaussian distribution with a mean of \( \mu \) and a standard deviation (SD) of \( \sigma \). The two cumulative gaussian distributions represent the specific increase of resistance that is observed in the regions of the ankle and the knee, respectively. The parameters \( c_1, c_2, \mu_1, \sigma_1, \mu_2, \sigma_2 \) of the fitting function were determined by minimization of a weighted least-squares index through a MATLAB routine. When a reduced number of data points were considered, we estimated only three parameters of \( \hat{R}(z) \) (i.e., \( c_3, \mu_1, \sigma_1 \)). The value of \( \mu_2 \) was fixed at the measured level of the knee (ankle-knee distance), whereas \( c_1 \) and \( c_2 \) were calculated by using the measured
Compute the derivative $dR(z)/dz$. 
Compute the profile of the muscle cross-sectional area $\hat{S}_m(z) = \rho_m dR(z)/dz$ with $\rho_m = 1.18 \Omega \cdot m$.
Compute $\hat{V}_m(z)$ as the integral over $z$ of $\hat{S}_m(z)$ (Eq. 6).

Five BIA data points:
Set $\mu_2$ at the value of the knee-ankle distance.
Express parameters $c_1$ and $c_2$ as a function of $c_3$ by using the total lower limb resistance $R_t = R(L) = 0.5c_1 + c_2 + c_3L$ and the ankle-knee resistance $R(\mu_2) = 0.5(c_1 + c_2) + c_3 \mu_2$.
Fit the experimental data points by $\hat{R}(z)$. The parameters $c_3, \sigma_1, \sigma_2$ of the fitting function are determined by minimization of a weighted least-squares index.
Compute the derivative $d\hat{R}(z)/dz$.
Compute $\hat{S}_m(z) = \rho_m d\hat{R}(z)/dz$ with $\rho_m = 1.18 \Omega \cdot m$.
Compute $\hat{V}_m(z)$ as the integral over $z$ of $\hat{S}_m(z)$.

BIA, bioimpedance analysis; $\hat{S}_m(z)$, estimate of muscle cross-sectional area at the level $z$; $\hat{R}(z; 0; \sigma_1)$, cumulative function of a gaussian distribution with a mean of 0 and a standard deviation of $\sigma_1$. See text for details.

total lower limb resistance ($R_1 = R(L) = 0.5c_1 + c_2 + c_3 \cdot L$ (with $L$ being the length of the lower limb from the ankle to the iliac spine and $R_0$ the measured resistance of the lower limb) and the measured ankle-knee resistance equals $0.5(c_1 + c_2) + c_3 \cdot \mu_2$.

After the parameters of $\hat{R}(z)$ were determined, Eq. 5 was then applied with $\hat{R}(z) = \hat{R}(z)$ and $\rho_m = 1.18 \Omega \cdot m$ (7, 16). 
Equation 6 then provided the total muscle volume of the lower limb. This volume was converted to mass by assuming a skeletal muscle density of 1.1 g/cm$^3$. The steps of this procedure are presented in Table 1.

Statistical methods. The Mann-Whitney test (20) was applied to establish the statistical significance of normal weight-obese subject differences, with $P < 0.05$ considered significant. The difference between DXA- and BIA-estimated lower limb skeletal muscle within each group was also tested.

Regression models for normal and obese subjects were developed by using as independent variable the ratio $L^2/R_0$, and the DXA muscle mass ($P_{DXA}$) as dependent variable.

RESULTS
The baseline subject data are presented in Table 2. The lower limb muscle mass estimates and total resistances in the two groups were significantly different ($P < 0.05$ and $P < 0.02$). The muscle mass obtained by DXA and estimated by BIA within each group was not significantly different.

An example of the pattern of the experimental resistance data from a single subject, as determined by BIA, is shown in Fig. 1 together with the curve $\hat{R}(z)$ estimated by weighted least squares. The fitting was performed on either data set: the original data points taken every 2.5 cm (continuous line) and five data points (black circles) located at specific sites as described in METHODS (dotted line). Also in Fig. 1, the pattern of the estimated muscle cross-sectional area $\hat{S}_m(z)$, in the two cases, is also reported. The abscissa $z = 0$ corresponds to the reference electrode at the ankle, and the regions of ankle, calf, knee, and thigh are easily recognizable from the pattern of $\hat{S}_m(z)$. Mean ± SD of the parameters of the fitting curve $\hat{R}(z)$ for the two groups of subjects were respectively: $c_1 = 201.7 ± 46.6 \Omega, c_2 = 73.9 ± 19.5 \Omega, \mu_2 = 32.6 ± 2.3 \Omega, c_3 = 1.1 ± 0.2 \Omega/cm$ for the lean group and $c_1 = 183.1 ± 60.8 \Omega, c_2 = 55.6 ± 16.1 \Omega, \mu_2 = 33.3 ± 2.7 \Omega, c_3 = 1.1 ± 0.2 \Omega/cm$ for the obese group. A significant difference between the parameters of the two groups was found only for the parameter $c_3$.

Table 3 presents, for the two groups of subjects, the mass of lower limb skeletal muscle as determined by DXA ($P_{DXA}$), the muscle mass as estimated by BIA ($P_{BIA}$) and the relative error $e = 100 \times (P_{DXA} - P_{BIA})/P_{DXA}$. $P_{BIA}$ data were also obtained by using a reduced number of measurements (see $e_1$ values). As shown in Fig. 1, the profile of muscle cross-sectional areas along the limb closely reproduces, in most cases, the profile obtained by the electrodes located at 2.5-cm intervals.

The reproducibility of $P_{BIA}$ was evaluated by computing the CV from the repeated measurements of bioimpedance. The intraobserver CV was 0.04, whereas the interobserver CV was 0.06.

Figure 2 shows the Bland-Altman plot of the two estimates of lower limb muscle mass ($P_{DXA}$ and $P_{BIA}$ estimated from the original measurements at 2.5-cm intervals). The plot shows no obvious presence of a systematic error as well as a substantial agreement of the two methods as all the points are within ± 2 SD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Mass Index, kg/m$^2$</th>
<th>Lower Limb Length, cm</th>
<th>Muscle Mass, kg</th>
<th>Lower Limb Resistance, $\Omega$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonobese</td>
<td>22.4 ± 2.7</td>
<td>83 ± 3</td>
<td>5.8 ± 1.1</td>
<td>275 ± 40</td>
</tr>
<tr>
<td>Obese</td>
<td>35.1 ± 6.4</td>
<td>82 ± 6</td>
<td>7.2 ± 1.2$^a$</td>
<td>215 ± 33$^a$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Significantly different from nonobese group: $* P < 0.05$; $\dagger P < 0.02$. 

J Appl Physiol • VOL 94 • APRIL 2003 • www.jap.org
similar result was obtained by considering the $P_{\text{BIA}}$ estimated from five data points.

We also developed the regression models that provide an additional estimate of appendicular muscle mass ($P_{\text{BIA}}'$) as a function of $L^2/R$. The regression formulas are $P_{\text{BIA}}' = 0.1 L^2/R_1 + 2.9$ for nonobese subjects and $P_{\text{BIA}}' = 0.27 L^2/R_1 - 1.30$ for obese subjects. These regression models were validated against additional data obtained from five nonobese and five obese subjects. The difference between model-predicted mass and DXA mass is larger when these new data are considered, with mean of the absolute error value equal to $14.2 \pm 16.8\%$ in the nonobese group and $17.8 \pm 10.5\%$ in the obese group. The absolute errors relative to the data used for the calculation of the regression lines were $11.7 \pm 10.8\%$ for nonobese and $6.3 \pm 3.7\%$ for obese subjects.

DISCUSSION

The present study shows that the estimates of lower limb SMM, obtained by analyzing BIA data with a mathematical model in both nonobese and obese subjects, is comparable to the values obtained by using DXA. The relative errors of the estimates by BIA were lower in obese compared with nonobese subjects, possibly because of the larger muscle mass in obese patients.

The method we propose is based on an approximation of experimental BIA data with a simple equation combining two gaussian cumulative functions with a straight line. This equation proved to adequately fit the experimental data as shown by the small value of the minimization index at the optimum (i.e., of the order of $10^{-5}$). From this equation, we derived the SMM of the lower limb as well as an estimate of the area occupied by skeletal muscle in the lower limb cross sections from the ankle to the thigh. In this way, it is possible to

<table>
<thead>
<tr>
<th>Subjects</th>
<th>$P_{\text{DXA}}, \text{kg}$</th>
<th>$P_{\text{BIA}}, \text{kg}$</th>
<th>$\varepsilon, %$</th>
<th>$\varepsilon_1, %$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonobese group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.7</td>
<td>5.2</td>
<td>-8.9</td>
<td>-3.6</td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>5.3</td>
<td>-9.4</td>
<td>7.8</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
<td>5.7</td>
<td>-6.7</td>
<td>4.1</td>
</tr>
<tr>
<td>4</td>
<td>6.6</td>
<td>6.5</td>
<td>-2.1</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>4.7</td>
<td>5.1</td>
<td>-10.5</td>
<td>-12.1</td>
</tr>
<tr>
<td>6</td>
<td>5.2</td>
<td>5.7</td>
<td>-9.9</td>
<td>-4.5</td>
</tr>
<tr>
<td>7</td>
<td>5.7</td>
<td>5.5</td>
<td>-4.8</td>
<td>0.4</td>
</tr>
<tr>
<td>8</td>
<td>4.4</td>
<td>4.3</td>
<td>-0.8</td>
<td>-13.5</td>
</tr>
<tr>
<td>9</td>
<td>7.7</td>
<td>6.9</td>
<td>9.8</td>
<td>-3.2</td>
</tr>
<tr>
<td>10</td>
<td>7.2</td>
<td>7.7</td>
<td>-6.7</td>
<td>-6.4</td>
</tr>
<tr>
<td>Mean $\pm$ SD</td>
<td>5.8 $\pm$ 1.1</td>
<td>5.8 $\pm$ 1.0</td>
<td>7.0 $\pm$ 3.4</td>
<td>5.6 $\pm$ 4.5</td>
</tr>
<tr>
<td><strong>Obese group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.2</td>
<td>6.6</td>
<td>8.9</td>
<td>11.9</td>
</tr>
<tr>
<td>2</td>
<td>6.1</td>
<td>5.8</td>
<td>4.6</td>
<td>1.1</td>
</tr>
<tr>
<td>3</td>
<td>6.9</td>
<td>5.6</td>
<td>7.5</td>
<td>-1.2</td>
</tr>
<tr>
<td>4</td>
<td>7.3</td>
<td>7.6</td>
<td>-4.3</td>
<td>-7.9</td>
</tr>
<tr>
<td>5</td>
<td>8.2</td>
<td>8.9</td>
<td>-7.7</td>
<td>-0.5</td>
</tr>
<tr>
<td>6</td>
<td>9.2</td>
<td>9.7</td>
<td>-4.7</td>
<td>-14.7</td>
</tr>
<tr>
<td>7</td>
<td>5.4</td>
<td>5.7</td>
<td>-7.0</td>
<td>-15.0</td>
</tr>
<tr>
<td>8</td>
<td>8.6</td>
<td>8.7</td>
<td>-1.9</td>
<td>-2.0</td>
</tr>
<tr>
<td>9</td>
<td>7.4</td>
<td>5.9</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>10</td>
<td>6.3</td>
<td>6.8</td>
<td>0</td>
<td>-0.7</td>
</tr>
<tr>
<td>Mean $\pm$ SD</td>
<td>7.2 $\pm$ 1.2</td>
<td>7.2 $\pm$ 1.4</td>
<td>5.9 $\pm$ 3.4</td>
<td>6.1 $\pm$ 5.9</td>
</tr>
</tbody>
</table>

$P_{\text{DXA}}$ and $P_{\text{BIA}}$, mass of appendicular lean soft tissue determined by dual-energy X-ray absorptiometry and BIA, respectively. Relative error was computed as $\varepsilon = 100 \times (P_{\text{DXA}} - P_{\text{BIA}})/P_{\text{DXA}}$. The error $\varepsilon_1$ was computed by using the mass estimated from 5 data points (this mass is not reported).
assess the status of the muscle across the entire lower limb. The reproducibility of the estimation procedure was fairly good, also because of the expedient of fixing the electrodes on a strip of tissue to reduce the variability in the positioning of the electrodes along the lower limb.

We also found that the predictions obtained by analyzing the bioimpedance data with the present mathematical model were not influenced substantially when, instead of the original measurements at 2.5-cm intervals, a smaller number of data points were used, as shown by the last column in Table 3. A reliable estimation appears to be generally provided by only five resistance data points so that the burden of the measurement procedure can be markedly reduced. Therefore, the method we are proposing in the present study allows us to obtain a good estimate of lower limb skeletal muscle by using a relatively simple and time-saving approach.

The method of estimating SMM of the lower limbs by a regression model that uses only the data of L and R, did not produce acceptable results in the present subjects. This finding may be explained as a consequence of the complex pattern of the experimental resistance profile observed in the lower limb (see Fig. 1). In fact, the muscle volume from the level 0 (ankle) to a level z is given, according to our model, by

$$\vec{V}_m (z) = \rho_m \int_0^z d\zeta / R' (\zeta)$$

(8)

where $R'$ denotes the derivative with respect to $z$.

To verify when this volume can be well represented by a linear function of $z^2/R(z)$ [e.g., $V_m (z) = a_1 z^2 / R(z) + a_2$], we can derive both expressions for $V_m (z)$ with respect to $z$, observing that the linear expression holds exactly when $R(z)$ has the following form: $R(z) = k z^2$, with $k$ and $c$ being positive constants. This is not the case for the lower limb. We note that the case of $c = 1$ represents a resistor with constant cross-sectional area and resistivity. *Equation 8* suggests that the larger slope of the regression line obtained for the obese group with respect to the nonobese group (0.27 vs. 0.10) is substantially related to the smaller value of the parameter $c_3$ of *Eq. 7* estimated in the obese subjects (0.8 vs. 1.1).

Some limitations of the present method might be related to the SMM measurement in more obese subjects and to the ability to detect changes with weight loss. This point should represent a subject of study in further investigations. In conclusion, the described BIA approach appears to be a reliable method of evaluating lower limb SMM in the clinical setting. Modeling the BIA profile provides an alternative technique to DXA that is less expensive and time consuming and does not require highly trained personnel. Furthermore, the possibility of obtaining a profile of the lower limb skeletal muscle might be useful for following both patients and trained athletes (14).

REFERENCES