Estimation of skeletal muscle mass by bioelectrical impedance analysis

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The purpose of this study was to develop and cross-validate predictive equations for estimating skeletal muscle (SM) mass using bioelectrical impedance analysis (BIA). Whole body SM mass, determined by magnetic resonance imaging, was compared with BIA measurements in a multiethnic sample of 388 men and women, aged 18–86 yr, at two different laboratories. Within each laboratory, equations for predicting SM mass from BIA measurements were derived using the data of the Caucasian subjects. These equations were then applied to the Caucasian subjects from the other laboratory to cross-validate the BIA method. The equations cross-validated (i.e., were not different), the data from both laboratories were pooled to generate the final regression equation

\[
\text{SM mass (kg)} = [(Ht^2/R \times 0.401) + (\text{gender} \times 3.825) + (\text{age} \times -0.071)] + 5.102
\]

where \(Ht\) is height in centimeters; \(R\) is BIA resistance in ohms; for gender, men = 1 and women = 0; and age is in years. The \(r^2\) and SE of estimate of the regression equation were 0.86 and 2.7 kg (9%), respectively. The Caucasian-derived equation was applicable to Hispanics and African-Americans, but it underestimated SM mass in Asians. These results suggest that the BIA equation provides valid estimates of SM mass in healthy adults varying in age and adiposity.

body composition; prediction equation

ACCURATE ASSESSMENT OF SKELETAL muscle (SM) mass has important applications in physiology (10, 12), nutrition (17), and clinical medicine (4, 10, 12). Geriatricians are interested in examining the influence of aging on sarcopenia and the effects of exercise on muscle growth in the elderly, clinicians seek information on the influence of catabolic diseases on muscle wasting and the effectiveness of therapeutic interventions in these diseases, and exercise scientists are interested in relating estimates of muscle mass to exercise performance and in evaluating the influence of physical training on muscle mass.

The use of bioelectrical impedance analysis (BIA) in the study of human body composition has grown rapidly in the past two decades. BIA is a noninvasive, portable, quick, and inexpensive method for measuring body composition. BIA is based on the relation between the volume of a conductor and its electrical resistance. Because SM is the largest tissue in the body (27) and because it is an electrolyte-rich tissue with a low resistance (1, 13, 28), muscle is a dominant conductor. Previous studies have shown that there is a strong correlation between BIA resistance and SM measurements in the arms (8, 25) and legs (24, 25). However, these studies were limited in that a criterion method for measuring SM was not employed, whole body muscle mass was not measured, and small sample sizes were used.

Magnetic resonance imaging (MRI) provides precise and reliable measurements of SM (6, 11, 23), is considered a reference method for measuring SM in vivo (16, 21), and is ideally suited for measuring whole body SM mass. Therefore, the aim of this study was to develop and cross-validate a predictive equation for estimating MRI-measured SM mass by using a conventional BIA analyzer in a large and heterogeneous sample of men and women.

METHODS

Experimental design. Subjects from two different laboratories completed BIA and MRI evaluations. A prediction equation for estimating MRI-measured SM mass from BIA measurements was developed from the Caucasian subjects within each laboratory. The developed equations were then applied to the Caucasian subjects from the other laboratory to cross-validate the equations. The aim was to pool all of the Caucasian subjects, if the BIA equations were successfully cross-validated, to develop the final SM prediction equation. The final SM prediction equation was then cross-validated in

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the Hispanic, African-American, and Asian subjects to determine whether the equation was valid for all races.

Subjects. Subjects consisted of healthy adult men \( (n = 230) \) and women \( (n = 158) \) who had participated in a variety of body composition studies at Queen’s University (Kingston, ON, Canada) and St. Luke’s/Roosevelt Hospital (New York, NY). The subjects varied in age \( (18–86 \text{ yr}) \), body mass index \( (16–48 \text{ kg/m}^2) \), and race \( (269 \text{ Caucasian, 53 African-American, 40 Asian, 26 Hispanic}) \). Subjects were recruited from among hospital employees, students at local universities, and the general public through posted flyers and the local media. All participants gave informed consent before participation, in accordance with the ethical guidelines of the respective institutional review boards.

SM measurement by MRI. At both institutions, the MRI images were obtained with a General Electric 1.5-T scanner (Milwaukee, WI). A T1-weighted, spin-echo sequence with a 210-ms repetition time and a 17-ms echo time was used to obtain the MRI data. The MRI protocol is described in detail elsewhere \( (26) \). Briefly, the subjects lay in the magnet in a prone position with their arms placed straight overhead. With the use of the intervertebral space between the fourth and fifth lumbar vertebrae as the point of origin, 10-mm-thick transverse images were obtained every 40 mm from hand to foot, resulting in a total of \( \sim 41 \) images for each subject. The total time required to acquire all of the MRI data for each subject was \( \sim 25 \text{ min} \). All MRI data were transferred to a computer workstation (Silicon Graphics, Mountain View, CA) for analysis with specially designed image-analysis software (Tomovision, Montreal, PQ, Canada).

Segmentation and calculation of tissue area, volume, and mass. The model used to segment the various tissues is fully described and illustrated elsewhere \( (23, 26) \). Briefly, a multiple-step procedure was used to identify tissue area for a given MRI image. In the first step, one of two techniques was used. Either a threshold was selected for adipose tissue and lean tissue on the basis of the gray-level histograms of the images \( (26) \), or a filter distinguished between different gray-level regions on the images and lines were drawn around the different regions using a Watershed algorithm \( (23) \). Next, the observer labeled the different tissues by assigning them different codes. Each image was then reviewed by an interactive slice-editor program that allowed for verification and, where necessary, correction of the segmented results. The original gray-level image was superimposed on the binary segmented image by using a transparency mode to facilitate the corrections. The area of the respective tissues in each image were computed automatically by summing the number of pixels and multiplying by the individual pixel surface area. The volume of each tissue in each slice was calculated by multiplying the tissue area by the slice thickness of 10 mm. The volume of the 40-mm space between two consecutive slices was calculated by using a mathematical algorithm given elsewhere \( (23) \). Volume units were converted to mass units by multiplying the volumes by the assumed constant density for adipose tissue-free SM \( (1.04 \text{ kg/l}) \) \( (27) \).

Bioelectrical resistance and anthropometric variables. Bioelectrical resistance \( (R) \) was measured with a model 101B BIA analyzer (RLJ Systems, Detroit, MI) with an operating frequency of 50 kHz at 800 \( \mu \text{A} \). Whole body measurements were taken with the subject in a supine position on a nonconducting surface, with the arms slightly abducted from the trunk and the legs slightly separated. Surface electrodes were placed on the right side of the body on the dorsal surface of the hands and feet proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively, and also medially between the distal prominences of the radius and ulna and between the medial and lateral malleoli at the ankle \( (22) \). All resistance measurements were adjusted for stature \( [\text{height} (\text{H})^2/R, \text{cm}^2/\text{fl}] \).

Body mass was measured to the nearest 0.1 kg, with the subjects dressed in light clothing. Barefoot standing height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer.

Reliability of MRI and BIA measurements. Our laboratory recently determined the reproducibility of MRI-determined SM measurements by comparing the intra- and interobserver estimates for MRI measurements of one series of seven images taken in the legs in three male and three female subjects \( (23) \). The interobserver difference was 1.8 ± 0.6%, and the intraobserver difference was 0.34 ± 1.1% \( (23) \). The intraobserver difference was calculated by comparing the analysis of two separate MRI acquisitions in a single observer, whereas the interobserver difference was determined by comparing two observers’ analyses of the same images. We determined the reproducibility of MRI-determined SM measurements across the laboratories by comparing the two laboratories’ analysis of the same whole body images for five subjects. The interlaboratory difference was 2.0 ± 1.2%.

Previous studies evaluating the reliability of BIA measurements indicate that the coefficients of variation range from 1.8 to 2.9% \( (18) \).

Statistical analysis. Differences between the Caucasian subject characteristics from the two laboratories were tested for significance by a paired \( t \)-test (Table 1). Racial differences were tested for significance by an analysis of variance (Table 1). Significant differences \( (P < 0.05) \) were analyzed by using a Scheffé’s post hoc comparison technique.

Multiple-regression analysis was applied to the data of the Caucasian subjects within each laboratory to derive the best-fitting regression equations to predict MRI-measured SM mass (Table 2). The regression analyses were conducted in a stepwise manner, using the independent variables of \( \text{Ht}^2/R, \text{age, gender and weight} \). Multiple-regression analysis and analysis of variance were used to determine the equality of regression slopes and intercepts for the relationships between SM mass measured by MRI and the BIA prediction equation \( (19) \) (Fig. 1).

A cross-validation of the equations for predicting SM from BIA was performed wherein the best-fitting equation derived from the Caucasian subjects within each laboratory was applied to the Caucasian subjects in the other laboratory. Multiple-regression analysis and analysis of variance were used to determine the equality of regression slopes and intercepts for the relationships between SM mass measured by MRI and the BIA prediction equation derived from the other laboratory \( (19) \) (Fig. 2).

The difference between MRI-measured and BIA-predicted SM mass within the Caucasians was tested for significance by a paired \( t \)-test. The difference between MRI-measured and BIA-predicted SM within the Caucasians was also plotted against the mean of MRI-measured and BIA-predicted SM to explore for systematic differences, as suggested by Bland and Altman (Fig. 3B) \( (7) \).

To determine whether race influenced the BIA prediction equation, the final equation derived from the Caucasian subjects was applied to the Hispanic, African-American, and Asian subjects (Fig. 5). Multiple-regression analysis and analysis of variance were used to determine the equality of regression slopes and intercepts for the relationships between SM mass measured by MRI and the BIA prediction equation derived from the Caucasians \( (19) \).
RESULTS

Subject characteristics. The characteristics for the subjects within the two laboratories are given in Table 1. With the exception of age, the Caucasian subjects in laboratory A were different (P < 0.05) from the Caucasian subjects in laboratory B for all characteristics listed in Table 1. There were also a number of racial differences for age, weight, BMI, SM mass, and Ht^2/R (Table 1).

Derivation of the BIA regression equations. The relationship between SM mass as the dependent variable and Ht^2/R, gender, age, and weight as independent variables was analyzed for the Caucasian subjects within laboratory A and laboratory B. As shown in Table 2, Ht^2/R explained 85 and 79% of the variance in SM mass in laboratories A and B, respectively. Within both laboratories, gender and age were also significant (P < 0.01) contributors to the multiple-regression model (Table 2). Although weight also contributed significantly (P < 0.05) to the model, it explained <1% of the variance in MRI-measured SM in laboratories A and B; thus weight was excluded from the final regression equations. The relationship between BIA-predicted and MRI-measured SM for each laboratory is shown in Fig. 1. In both cases, analysis indicated that the slopes and intercepts were not different (P > 0.9) from one and zero, respectively.

Cross-validation of BIA regression equations. The prediction equation derived from the Caucasian subjects in laboratory B was used to predict SM mass in the Caucasian subjects in laboratory A (Fig. 2A), and the prediction equation derived for the Caucasian subjects in laboratory A was used to predict SM mass in the Caucasian subjects in laboratory B (Fig. 2B). The r^2 and SE of estimate (SEE) values of the validation (Fig. 1) and cross-validation (Fig. 2) analysis were similar. As shown in Fig. 2, analysis revealed that the slopes and intercepts were not significantly (P > 0.05) different from one and zero and that the plotted lines were not statistically (P > 0.1) different from the lines of identity.

Because the r^2 and SEE values of the validation and cross-validation equations were similar (Figs. 1 and 2), and because the slopes and intercepts of the regression lines derived from the cross-validated equations were not different from one and zero, respectively, we pooled the Caucasian subjects (laboratories A + B, n = 269) to generate the final regression equation

\[
\text{SM mass (kg)} = [(\text{Ht}^2/R \times 0.401) + (\text{gender} \times 3.825) + (\text{age} \times -0.071)] + 5.102
\]

where Ht is in centimeters; R is in ohms; for gender, men = 1 and women = 0; and age is in years. Analysis revealed that the slope and intercept were not significantly (P > 0.9) different from one and zero, respectively (Fig. 3A). The r^2 and SEE values of the regression equation were 0.86 and 2.7 kg or 9%, respectively.

As shown in Fig. 4, when the BIA method was used to predict SM mass, the difference between BIA-predicted and MRI-measured SM mass was within 5, 10, and 15% of the MRI-measured SM mass for 43, 74, and 15% of the MRI-measured SM mass for 43, 74, and 15%, respectively.

Table 2. Multiple-regression model for predicting MRI-measured skeletal muscle mass from BIA (Ht^2/R), age, and gender within the Caucasian subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>r^2</th>
<th>SEE</th>
<th>Significance (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(X_1) Ht^2/R, cm^2/Ω</td>
<td>0.85</td>
<td>2.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(X_2) Gender, man = 1 and woman = 0</td>
<td>0.88</td>
<td>2.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(X_3) Age, yr</td>
<td>0.89</td>
<td>2.58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Laboratory B†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(X_1) Ht^2/R, cm^2/Ω</td>
<td>0.79</td>
<td>3.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(X_2) Gender, man = 1 and woman = 0</td>
<td>0.81</td>
<td>2.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(X_3) Age, yr</td>
<td>0.83</td>
<td>2.73</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MRI, magnetic resonance imaging; BIA, bioelectrical impedance analysis; SEE, SE of estimate; \(X_1–X_3\), variables of regression equation. †MRI skeletal muscle mass (kg) = [(Ht^2/R \times 0.385) + (gender \times 4.464) + (age \times -0.057)] + 3.495. ‡MRI skeletal muscle mass (kg) = [(Ht^2/R \times 0.396) + (gender \times 3.443) + (age \times -0.078)] + 6.313.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Laboratory A Caucasians (n = 83)</th>
<th>Laboratory B Caucasians (n = 186)</th>
<th>Laboratories A + B Caucasians (n = 269)</th>
<th>Laboratories A + B African-Americans (n = 53)</th>
<th>Laboratories A + B Asians (n = 40)</th>
<th>Laboratories A + B Hispanics (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>41.9 ± 14.5</td>
<td>41.9 ± 12.1</td>
<td>41.5 ± 12.8</td>
<td>36.6 ± 11.6</td>
<td>31.8 ± 9.8†</td>
<td>33.5 ± 11.1†</td>
</tr>
<tr>
<td>Gender, women/men</td>
<td>44/39*</td>
<td>55/131</td>
<td>99/170</td>
<td>29/24</td>
<td>17/23</td>
<td>13/13</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>72.1 ± 15.1*</td>
<td>93.9 ± 16.2</td>
<td>86.8 ± 18.7</td>
<td>76.1 ± 14.7†</td>
<td>62.0 ± 10.4‡</td>
<td>72.5 ± 15.9†</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.2 ± 3.5*</td>
<td>31.1 ± 4.9</td>
<td>28.9 ± 5.5</td>
<td>26.3 ± 4.4†</td>
<td>22.0 ± 2.5‡</td>
<td>26.1 ± 3.9†</td>
</tr>
<tr>
<td>Skeletal muscle mass, kg</td>
<td>26.4 ± 7.6*</td>
<td>31.1 ± 6.6</td>
<td>29.6 ± 7.2</td>
<td>28.0 ± 6.8</td>
<td>23.6 ± 5.3‡</td>
<td>27.9 ± 9.4</td>
</tr>
<tr>
<td>Resistance index (Ht/R), cm²/Ω</td>
<td>57.9 ± 13.0*</td>
<td>64.5 ± 11.9</td>
<td>62.5 ± 12.6</td>
<td>57.8 ± 12.8</td>
<td>52.5 ± 10.4†</td>
<td>56.1 ± 15.1</td>
</tr>
</tbody>
</table>

Values are as group means ± SD; n, no. of subjects. Ht, height; R, bioelectrical resistance. *Laboratory A Caucasians significantly different from laboratory B Caucasians, P < 0.05. †Significantly less than Caucasians (laboratories A + B), P < 0.05. ‡Significantly less than African-Americans, P < 0.05. §Significantly less than Hispanics, P < 0.05.
89% of the subjects, respectively. Alternatively, the difference in MRI-measured and BIA-predicted SM mass and the mean SM mass of the two methods. Thus, with increasing SM mass, BIA overpredicted SM mass, and, with decreasing SM mass, BIA underpredicted SM mass.

**Influence on non-SM tissues on R and the BIA prediction equation.** Adipose tissue mass was significantly \((P < 0.05)\) related to \(Ht^2/R\); however, the variance explained was \(<3\%\). Total lean tissue mass \((r = 0.89)\) and SM-free lean tissue (all lean tissues - SM) \((r = 0.67)\) were also significantly \((P < 0.001)\) correlated with \(Ht^2/R\); however, these correlations were not as great as that observed for SM mass alone \((r = 0.91)\). When total lean tissue was included with SM in a multiple-regression model to predict \(Ht^2/R\), the variance explained was only improved by 2% in comparison to the variance explained by SM mass alone. Conversely, when SM was included with total lean tissue in a multiple-regression model used to predict \(Ht^2/R\), the variance explained was improved by 5% in compar-

89% of the subjects, respectively. Alternatively, the difference between BIA-predicted and MRI-measured SM mass was within 1.0, 2.0, and 3.0 kg of the MRI-measured SM mass for 27, 55, and 74% of the subjects, respectively.

Systematic differences between the BIA-predicted and MRI-measured SM were explored by using a Bland-Altman Plot (Fig. 3B). Analysis revealed that the average difference between BIA-predicted and MRI-measured SM mass in the Caucasians was not different \((-0.02 \pm 2.71 \text{ kg}; P \geq 0.9)\). However, the Bland-Altman plot (Fig. 3B) shows a small but significant positive correlation \((r = 0.20, P < 0.01)\) between
To determine whether the systematic error of the BIA method (Fig. 3A) was influenced by non-SM tissues, we included adipose tissue and SM-free lean tissue mass in the multiple-regression analysis used to predict SM mass. When these tissues were included, the $r^2$ and SEE of the regression equation were not statistically ($P > 0.1$) improved. In addition, the significant correlation ($r = 0.20$, $P < 0.01$) in the Bland-Altman plot remained.

Influence of race on the BIA SM prediction equation. To determine whether the BIA prediction equation was influenced by race, the Caucasian-derived equation (Fig. 3) was used to predict SM mass within the Hispanic $n = 26$, African-American $n = 53$, and Asian $n = 40$ subjects, respectively. For the Hispanic cohort, analysis indicated that the slope and intercept of the regression line were not significantly different ($P > 0.1$) from one and zero, respectively (Fig. 5A). When the regression equation was applied to the African-American cohort, the slope was not significantly different from one ($P > 0.05$), but the intercept was significantly greater than zero ($P = 0.03$). However, inspection of Fig. 5B reveals that the difference between BIA-predicted and MRI-measured SM mass within the normal range of SM (15–45 kg) was not significant ($-0.59 \pm 2.53$ kg; $P = 0.1$) in the African-Americans.

When the BIA equation was applied to the Asian cohort, the intercept of the regression line was not significantly different from zero ($P > 0.6$); however, the slope was significantly less than one ($P < 0.01$). Thus, with increasing SM mass, the BIA equation tended to underpredict SM mass in the Asians (Fig. 5C). Indeed, the average difference between MRI-measured and BIA-predicted SM mass in the Asians was significant ($2.45 \pm 1.61$ kg; $P < 0.001$).

DISCUSSION

The present study examined the relationship between $R$ and MRI-measured SM mass in a heterogeneous sample of 230 men and 158 women. The aim was to develop and cross-validate a prediction formula for estimating SM mass from BIA measurements. Our findings indicate that MRI-measured SM mass was strongly correlated to the BIA resistance index ($Ht^2/R$) and that the BIA method was a valid method for estimating SM mass within the Caucasian, Hispanic, and African-American subjects. The error for predicting SM mass from BIA within these cohorts was $-2.7$ kg or 9%.

BIA is a body composition method that measures tissue conductivity. Because the conductivity of the body is directly proportional to the amount of electrolyte-rich fluid that is present, BIA can be used to measure fluid components such as total body water (18,
Because there is an equilibrium between total body water and fluid volume with lean body mass, there is a strong relationship between $R$ and fat-free body mass (2, 18). Limited evidence indicates that appendicular BIA measurements are also highly correlated to appendicular SM, as estimated by a single computerized tomography image (8) and dual-energy X-ray absorptiometry (24, 25). Our findings extend these observations and indicate that whole body muscle mass is highly related to whole body resistance.

When current flows through the body, it is partitioned among different tissues according to their individual resistances and volumes. Because SM has both a large volume and a low resistance, most of the BIA current flows through SM (13). Conversely, adipose tissue only influences $R$ when the volume of adipose tissue exceeds that of SM, and this influence is small (5). The influence of bone and organ on $R$ is also small because bone has an extremely high resistance (13) and because the trunk is of little concern in whole body BIA measurements (3, 5, 14). Indeed, we found that 82% of the variance in $R$ within the Caucasians was explained by SM mass and that adipose tissue, organ, and bone only explained an additional 4% of the variance in $R$.

Numerous studies have developed equations for estimating lean body mass from BIA measurements (2, 18). In a review of these studies, Houtkooper et al. (18) report that the $r^2$ values ranged from 0.73 to 0.98 and that the SEEs ranged from 1.7 to 4.1 kg. In general, these studies found BIA to be a valid method for estimating lean body mass. In this study, we demonstrate that BIA is also an effective method for estimating SM mass. The $r^2$ value for SM mass in the present study ($r^2 = 0.86$) is similar to those previously observed for lean body mass (2, 18). The magnitude of the error (SEE = 2.7 kg or 9%) in predicting SM mass from BIA within the Caucasians, African-Americans, and Hispanics is encouraging. The BIA method was within a 5% error for 43% of the subjects and within a 10% error for 74% of the subjects. These results suggest that the BIA method may provide meaningful estimates of SM mass within adult populations. Because the model development size was large, and because the subject demographics varied, the model should be applicable to a large proportion of the adult population. Because BIA is simple, noninvasive, reliable, repeatable, and relatively inexpensive, the BIA method should be practical in epidemiological and clinical settings. However, to ensure that reliable BIA measurements are obtained, several factors such as hydration status, food intake, and exercise must be controlled (18).

Although the Bland-Altman plot indicates there was a systematic error with the BIA method, this error was small. For example, the BIA method would underestimate SM mass by an average of 3.0% in an individual with a SM mass of 20 kg and overestimate SM mass by ~2% in an individual with a SM mass of 40 kg. Moreover, although MRI-measured SM mass was related to adipose tissue and lean tissues other than SM, it appears that the influence of these tissues on $R$ did not account for the systematic error of the BIA SM prediction formula.

In this study, we found that the BIA equation for predicting SM mass derived from the Caucasians underpredicted SM within the Asian cohort. This suggests there are biological differences between these races that influence the relationship between $R$ and SM mass. For example, the height and weight of the Asian subjects was significantly less than that of the Caucasian subjects in this study. Others have also shown that body composition prediction equations are influenced by differences in body build between Asians and Caucasians (9). We also observed that age and gender had a small influence on the relationship between $R$ and SM mass, a finding that may be partially explained by age and gender differences in SM mass, height, and weight.

In summary, BIA prediction equations for whole body SM mass were developed and cross-validated in Caucasian subjects in two laboratories. The cross-validation of the BIA equations for predicting SM mass was successful, and the magnitude of the error in predicting SM mass from BIA was small. These observations are encouraging and suggest that BIA can provide rapid and accurate estimates of SM in adult populations. Our results indicate that the derived equation is applicable for Caucasian, African-Ameri-
REFERENCES