Intermuscular adipose tissue-free skeletal muscle mass: estimation by dual-energy X-ray absorptiometry in adults

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Kim, Jaehee, Stanley Heshka, Dympna Gallagher, Donald P. Kotler, Laurel Mayer, Jeanine Albu, Wei Shen, Pamela U. Freda, and Steven B. Heymsfield. Intermuscular adipose tissue-free skeletal muscle mass: estimation by dual-energy X-ray absorptiometry in adults. J Appl Physiol 97: 655–660, 2004. First published April 16, 2004; 10.1152/japplphysiol.00260.2004.—Skeletal muscle (SM) is a large and physiologically important compartment. Adipose tissue is found interspersed between and within SM groups and is referred to as intermuscular adipose tissue (IMAT). The study objective was to develop prediction models linking appendicular lean soft tissue (ALST) estimates by dual-energy X-ray absorptiometry (DXA) with whole body IMAT-free SM quantified by magnetic resonance imaging. ALST and total-body IMAT-free SM were evaluated in 270 healthy adults [body mass index (BMI) of <35 kg/m2]. The SM prediction models were then validated by the leave-one-out method and by application in a new group of subjects who varied in SM mass prediction models were then validated by the leave-one-out method and thus provide a new opportunity for quantifying SM in physiological and epidemiological investigations. body composition; magnetic resonance imaging; anorexia nervosa; acromegaly

Recent advances now allow measurement of the nutritionally important skeletal muscle (SM) compartment in vivo (10, 14, 26). Among the available methods, the dual-energy X-ray absorptiometry (DXA) approach reported by Kim et al. (13) is relatively simple, affordable, and accurate. The method of Kim et al. is based on the observation that a relatively large fraction of total body SM is present in the appendages and that a high percentage of appendicular lean soft tissue (ALST) is SM (11). Using magnetic resonance imaging (MRI) as the reference, Kim et al. developed a whole body SM estimation formula with DXA-measured ALST as the main predictor variable.

Since the introduction of the DXA approach of Kim et al. (13), reports have emerged highlighting the metabolic signifi-

cance of inter- and intramuscular adipose tissue (IMAT) (6–8, 12). The IMAT component, defined as the adipose tissue found between and within muscle groups (22), is relatively small (~1–2 kg) and varies in magnitude with body mass, age, sex, and race (3, 9, 21). The MRI method originally used by Kim et al. included IMAT as part of total body SM. Hence, although small in quantity, the presence of IMAT within the SM compartment may bias SM estimates when subjects are studied across weight, age, sex, race, and disease groups.

The aim of the present study was to develop and validate IMAT-free total body SM prediction equations with whole body MRI as the reference and DXA-ALST as the main predictor variable. These new prediction models afford a practical means by which IMAT-free SM mass can be estimated in vivo. The availability of additional samples of subjects varying in muscularity provided the opportunity to investigate the range of validity of the developed models.

METHODS

Protocol and Design

The study consisted of two phases. In the first phase, healthy adult subjects underwent DXA evaluation for ALST and MRI for total body IMAT-free SM within a 2-day period. IMAT-free SM prediction formulas based on DXA-ALST estimates were developed and internally validated with these data.

In the second phase, we cross-validated the developed prediction models in groups of subjects varying in muscularity. Anorexia nervosa is a condition associated with extreme loss of body fat and SM (16). Athletic training in general is accompanied by loss of body fat and absolute or relative increases in SM (18). Acromegaly, with hyperscretion of growth hormone, is similarly associated with a loss of body fat and increase in SM (20). Subjects in all three of these categories were referred to our Center for evaluation and provided the opportunity to test the newly developed SM prediction models across a range of relative and absolute SM masses. The cross-validation subjects completed the same evaluation protocol as subjects in the model-development group.

Subjects

Subjects in the model-development group were healthy men and women, aged ≥18 yr with a body mass index (BMI) of <35 kg/m2. Inclusion criteria for the model-development group required that subjects be ambulatory, nonexercising, and nonsmoking. Subjects with untreated diabetes mellitus, malignant/catabolic conditions, or taking medications that could potentially influence body composition were excluded from the study.

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Subjects in the validation groups included women with anorexia nervosa who had participated in a psychiatric treatment program, men and women who had engaged in regular recreational endurance and/or resistance exercise training programs, and men and women before treatment for acromegaly. Resistance- and endurance-trained athletes were defined as individuals who performed a minimum of 6 h/wk of either resistance or endurance training for a minimum of 1 yr.

Each subject completed a medical examination before body composition evaluation. The studies were approved by the Institutional Review Boards of St. Luke’s-Roosevelt Hospital Center and Columbia University, College of Physicians and Surgeons. All subjects gave written consent before participation.

Body Composition

The subjects’ body masses and heights were measured with a digital scale (Weight Tronix, New York, NY) and stadiometer (Hol-tain, Crosswell, UK), respectively.

DXA. Whole body and regional body composition were estimated by DXA (software version 3.6, Lunar DPX, Madison, WI). The system software provided the mass of lean soft tissue, fat, and bone mineral for the whole body and specific regions. ALST mass was considered equivalent to the sum of lean soft tissue in both right and left arms and legs. Appendages were isolated from the trunk and head by using the DXA regional computer-generated default lines, with manual adjustment, on the anterior view planogram as described elsewhere (13). Repeated daily measurements over 5 days in four subjects showed a coefficient of variation of 1.7% for leg lean soft tissue, 2.0% for arm lean soft tissue, and 2.6% for ALST (4).

MRI. Total body SM and IMAT were measured by whole body multislice MRI. Throughout the remainder of the report, we define SM as the IMAT-free compartment unless otherwise noted. Subjects were placed on the 1.5-T scanner (6X Horizon, General Electric) with arms extended above their heads. The protocol involved the acquisition of ∼40 axial images across the whole body with 10-mm thickness and 40-mm intervals (19).

Images were analyzed with sliceOmatic image analysis software (TomoVision). The IMAT compartment includes intermuscular adipose tissue that is located between muscle groups and beneath the muscle fascia and intramuscular adipose tissue that is distributed within individual muscles visible on MRI images (Fig. 1), as previously described by Song et al. (24). The IMAT component does not include intramyocellular lipid, which is the lipid droplets within muscle cells. Manual editing by visual inspection was also performed where needed.

All MRI scans in the model-development group were read by the same reader. The technical errors for repeated readings of the same whole body scans by the same reader of MRI-derived SM and IMAT volumes in our laboratory are 1.4 and 5.9%, respectively. Scans in the model validation groups were read by different readers. The intraclass correlation coefficient between the analysts for the reading of whole-body MRI-derived SM from the same subject is 0.99.

MRI-SM and IMAT volume estimates were converted to mass using the assumed density of 1.04 kg/l for SM and 0.92 kg/l for IMAT (23).

Statistical Analysis

In the first study phase, total body SM prediction equations were developed in the model-development group using linear regression analysis. Sex and race were used as fixed factors, with MRI-measured total-body SM as the dependent variable and ALST, age, body weight, and total body fat included as covariates. All main effects for factors and covariates as well as all possible two-way interactions were investigated to find the best-fitting model with the lowest SE. The adjusted $R^2$ and SE values were used to evaluate model-fitting performance. The developed models were then validated by the leave-one-out method (2).

In the second phase of the study, model validation in groups of subjects differing in morphology, the value for total-body SM mass was calculated for each individual using the developed prediction equations. The observed differences between group mean-predicted and actual total body SM mass were tested for significance using Student’s t-tests, and the level of agreement was assessed according to the method of Bland and Altman (1).

Group data are expressed as means ± SD. Data were analyzed using SPSS version 10.0 (1999; SPSS, Chicago, IL), and statistical significance was set at $P < 0.05$.

RESULTS

Subject Characteristics

The characteristics of subjects in the model-development group, 96 men and 174 women, are presented in Table 1. The total sample of 270 healthy subjects was ethnically diverse (91 African Americans, 48 Asians, and 131 Caucasians) and ranged in age, BMI, and SM from 18–88 yr, 15.9–34.8 kg/m², and 11.7–46.1 kg, respectively.

The characteristics of subjects in the model validation group are presented in Table 2. There were 23 women with anorexia nervosa (1 Asian and 22 Caucasians) varying in BMI from 12.2 to 18.8 kg/m². The athlete group included 9 men and 7 women.

Fig. 1. Segmented mid thigh magnetic resonance image showing the subcutaneous adipose tissue (red), intermuscular adipose tissue (green), skeletal muscle (blue), and bone with marrow (yellow). Subject was a 71-yr-old woman.
Table 1. Characteristics of the model-development group

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>96</td>
<td>174</td>
</tr>
<tr>
<td>Age, yr</td>
<td>46.4±19.0 (20–84)</td>
<td>45.2±17.6 (18–88)</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>79.2±12.2 (55–114.5)</td>
<td>64.5±13.6 (40.8–107.6)*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176.4±7.9 (153.4–191.1)</td>
<td>162.3±7.5 (145.7–182.9)*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.4±3.0 (19.9–34.8)</td>
<td>24.4±4.4 (15.9–34.7)*</td>
</tr>
<tr>
<td>Fat, %</td>
<td>20.2±7.6 (4.3–35.3)</td>
<td>31.4±9.1 (7.8–48.5)*</td>
</tr>
<tr>
<td>ALST, kg</td>
<td>28.1±2.4 (15.6–39.1)</td>
<td>18.0±3.2 (11.6–28.7)*</td>
</tr>
<tr>
<td>Total body SM, kg</td>
<td>31.7±5.9 (15.8–46.1)</td>
<td>19.8±3.9 (11.7–31.4)*</td>
</tr>
<tr>
<td>IMAT, kg</td>
<td>0.8±0.5 (0.1–2.3)</td>
<td>1.0±0.6 (0.2–2.7)*</td>
</tr>
</tbody>
</table>

Values are means ± SD, with range in parentheses. BMI, body mass index; ALST, appendicular lean soft tissue; SM, skeletal muscle; IMAT, intermuscular adipose tissue. *P < 0.001, †P = 0.007, and ‡P = 0.04, men vs. women.

(1 African American and 15 Caucasians) ranging in BMI from 18.6 to 30.0 kg/m². Seven newly diagnosed patients with acromegaly were evaluated (4 men and 3 women, including 1 African American, 4 Caucasians, and 2 Hispanics), who varied in BMI from 25.5 to 38.3 kg/m².

The mean size of the IMAT compartment ranged from a low of 0.4 kg in subjects with anorexia nervosa to a high of 2.1 kg in women with newly diagnosed acromegaly (Table 2). As a percentage of SM, IMAT was lowest in male athletes (~1.5%) and highest (~7.9%) in women with acromegaly. On a relative basis, the percentage of IMAT in the SM of men in the model-development group (2.6 ± 2.0%) was less than that of IMAT in the women (5.0 ± 3.1%; P < 0.001).

Prediction Models

Development. ALST mass was the strongest predictor (P < 0.001) of total body SM, explaining 96.2% of the between-subject variance in MRI-measured SM with an SE of 1.46 kg (model 1; Fig. 2 and Table 3).

The inclusion of age (P < 0.001) in addition to ALST in the regression models explained an additional 0.3% of the variance in measured total body SM and reduced the SE from 1.46 to 1.38 kg (model 2; Table 3).

When categorical variables such as sex (0 = female and 1 = male) and race (AA = African American, AS = Asian, and C = Caucasian) were included in the models, the residual error variance of SM mass was not equal across groups. Therefore, SM values were transformed using base 10 logarithms to explore contributions of sex and race to the model. Sex and race along with ALST and age were significant predictors in the model, showing interactions with ALST and age and explaining 96.5% of the variance in measured logarithm-transformed total body SM (all P < 0.05) with an SE (back transformed) of 1.06 kg (model 3; Table 3). The addition of body weight and total body fat did not significantly improve the model. Because there were no Hispanic subjects in the model-development group, the Hispanic subjects in the model validation sample were coded as Caucasian (i.e., C = 0 for race) when model 3 was applied in subjects varying in masculinity.

Leave-one-out validation. The SD of the prediction error for model 1 derived by the leave-one-out method was 1.46 kg. The SD of the prediction error of the original regression model using all data points was 1.45 kg. For model 2, the SD of the prediction error by leave-one-out was 1.39 kg, and the SD of the prediction error of the regression model was 1.38 kg. The leave-one-out prediction error SD for model 3 was 1.06 kg, and the SD of the prediction error of the regression model was 1.06 kg. The small size of the difference between the two errors for all three models indicates that the developed regression equations would have a high validity when applied to samples of subjects similar to the one on which they were developed.

Evaluation in Subjects Varying in Muscularity

The mean values of MRI-measured and model-predicted SM in the cross-validation samples are presented in Table 4. Mean predicted total body SM values derived from all three models did not differ significantly from measured SM in recreational athletes and patients with acromegaly. Predicted and measured

Table 2. Characteristics of the groups differing in muscularity

<table>
<thead>
<tr>
<th>Group</th>
<th>Anorexia, Women</th>
<th>Athletes</th>
<th>Acromegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>n</td>
<td>23</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23.9±5.5</td>
<td>28.7±8.5</td>
<td>37.0±11.0</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>41.3±4.5</td>
<td>75.3±5.5</td>
<td>61.9±10.8</td>
</tr>
<tr>
<td>Height, cm</td>
<td>162.7±7.0</td>
<td>182.1±4.5</td>
<td>164.2±4.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>15.7±1.5</td>
<td>22.8±2.9</td>
<td>23.0±4.0</td>
</tr>
<tr>
<td>Fat, %</td>
<td>9.1±6.6</td>
<td>10.8±4.4</td>
<td>23.1±9.5</td>
</tr>
<tr>
<td>ALST, kg</td>
<td>13.8±1.8</td>
<td>28.9±2.1</td>
<td>19.4±2.6</td>
</tr>
<tr>
<td>Total body SM, kg</td>
<td>14.0±2.3</td>
<td>32.3±1.8</td>
<td>21.9±3.6</td>
</tr>
<tr>
<td>IMAT, kg</td>
<td>0.4±0.2</td>
<td>0.5±0.3</td>
<td>0.7±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SD.
SM values were also highly correlated (Table 4), and example results for model 3 are plotted in Fig. 3A (combined: measured, 28.1 ± 7.0 kg vs. predicted, 27.8 ± 7.3 kg, P = not significant; $R^2 = 0.96$, $P < 0.001$). Bland-Altman analysis failed to disclose any bias between predicted and measured SM values (athletes, $r = 0.27$; acromegaly, $r = 0.21$; both $P = not significant; Fig. 3B).

Predicted estimates for total-body SM derived from all three models were significantly ($P < 0.01–0.001$) greater than measured SM in the 23 patients with anorexia nervosa. As more predictor variables were added in models 1–3, the magnitude of mean SM overestimation increased in patients with anorexia nervosa from 0.7 to 2.6 kg. Although predicted total-body SM was highly correlated ($r = 0.86–0.89$, all $P < 0.001$) with measured SM for the three models in patients with anorexia nervosa, Bland-Altman analysis revealed a significant bias between predicted and measured SM values in this group ($r = 0.67$, $P < 0.001$; Fig. 3B).

Because BMI on the whole was low in patients with anorexia nervosa ($<16$ kg/m²), well below that of subjects in the model-development group (i.e., $\sim 25$ kg/m²), we divided the anorexia nervosa patients into two groups, those with BMI of $<16$ kg/m², which was the lower limit of subjects in the model-development group ($n = 12$), and those within the model-development group BMI range ($>16$ kg/m²; $n = 11$) (Table 4). Predicted SM by model 1 did not differ significantly from measured values in patients with BMI of $>16$ kg/m², although models 2 and 3 still overpredicted SM ($P < 0.05$ and 0.001, respectively). In contrast, all three prediction models overestimated measured SM in the group with BMI of $<16$ kg/m² (all $P < 0.001$).

Table 3. Developed models for predicting total-body SM mass

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Intercept</th>
<th>Adjusted $R^2$</th>
<th>SE, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>1.19†</td>
<td>(0.01)</td>
<td>-1.65†</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.18†</td>
<td>-0.03†</td>
<td>-0.14</td>
</tr>
<tr>
<td>Model 3*</td>
<td>0.0115†</td>
<td>-0.0034†</td>
<td>1.1932†</td>
</tr>
</tbody>
</table>

Values are estimates of regression coefficient, with SE in parentheses. Sex: 0 = female; 1 = male. Race: AA, African American; AS, Asian; C, Caucasian. *Logarithm-transformed total-body SM mass was used as a dependent variable. †$P < 0.001$; †$P = 0.006$, §$P < 0.05$.

DISCUSSION

In the present study, we developed IMAT-free SM prediction formulas for adults based on ALST as measured by the widely available and practical DXA method. The newly developed models were validated with the leave-one-out method and accurately predicted SM in cross-validation samples of recreational athletes and patients with acromegaly. In contrast, the prediction models overestimated SM mass in patients with anorexia nervosa, notably in those with a BMI below that of the lower limit of the model-development group (i.e., $<16$ kg/m²).

The present study extends the earlier report of Kim et al. (13) in which IMAT was included in the SM compartment. The extent to which IMAT influences SM estimates can be estimated from observations in the various study groups. When expressed relative to SM, IMAT would constitute $\sim 1$ to $\sim 8\%$ of total SM mass across the groups and could thus inflate estimates of the actual amount of IMAT-free SM present. The new equations now largely eliminate the overestimation of SM caused by inclusion of visible adipose tissue in the MRI analysis of the SM compartment. These prediction equations, therefore, should provide more reliable estimates of IMAT-free SM, which is often the compartment of interest in physiological investigations.

Our measurements showed that ALST alone explains $96\%$ of the observed between-individual variation in MRI-measured IMAT-free SM mass with a low SE (i.e., 1.46 kg), indicating high estimation accuracy. However, ALST and SM do not maintain a constant relationship to each other with age, and age entered as a small but significant predictor variable in model 2.,
further lowering the SE to 1.38 kg. For the same ALST, older subjects have less SM than younger subjects. For example, according to the model 2 equation, a woman with 18 kg of ALST at age 20 yr is estimated to have 20.5 kg of SM, but at age 70 yr SM would be estimated at 19.0 kg, a difference of ~1.5 kg or 7%. Given the low SE of model 2 and validation using the leave-one-out method, this relatively simple model should prove practical to apply in the clinical setting.

Some additional significant factors were noted during model development. For similar age and ALST, women had less SM than men. African American subjects had the most SM, Asian subjects had the least, and Caucasian subjects were intermediate. Model 3, including these associations and their interactions, had an even lower SE than the other two models, 1.06 kg. Our findings extend the results of earlier studies that showed race differences in SM, even after controlling for weight, height, age, and sex (5, 15). As with aging, these sex and race effects must arise secondary to variation in the proportion of ALST at appendicular SM and in the associations between appendicular SM and total body SM. Although this model reveals interesting and subtle sex and race effects, our sample was not sufficiently large and diverse to fully encompass all race groups. These concerns may offset the additional prediction accuracy gained by using this model, but the observations may prove useful when development of similar future SM prediction models is considered.

Both Kim et al.’s previous (13) and the new SM DXA-prediction models presented in this report have high $R^2$ values in the range of 0.96–0.97. The present models also have lower SEs (1.1–1.5 kg) than the previous models (~1.6 kg) indicating an improvement in estimation accuracy by removal of IMAT from the SM compartment. The previous DXA-prediction models would overestimate total body SM by the amount of IMAT present compared with the new models in an individual subject since the earlier models did not consider the contribution of IMAT to total body SM.

The newly developed DXA-SM method accurately predicted total body SM compared with the MRI reference method in two cross-validation groups characterized by absolute and relative enlargement of the SM compartment, recreational athletes and patients with acromegaly. Subjects in both groups differed substantially in body composition from those of the model-development group. For example, athletes had a greater SM mass even though their percent fat was one-third to one-half that of their model-development group counterparts. SM mass estimates are of interest in both athletes and patients with acromegaly and, given the constraints of a relatively small sample, predicted and measured SM values were highly correlated, and mean values agreed closely.

However, prediction models significantly overestimated SM in women with anorexia nervosa compared with MRI-measured SM. The women with anorexia nervosa weighed substantially less and had less SM than women in the model-development group. The lowest SM mass was observed in a patient with anorexia nervosa (9.6 kg), one-half that of the mean SM for healthy women. It is thus likely that the relations embodied in the average ALST vs. SM association are not found in this extreme group.

On further examination of results, we observed that model 1 provided relatively good prediction of SM in the women with anorexia nervosa whose BMI was within the range of the model-development group. In contrast, SM was significantly overpredicted in patients with anorexia nervosa who had a very low BMI (~16 kg/m²) by all three models. In patients with severe anorexia nervosa, the presence of emaciation evidently alters the relationships between ALST, age, race, and their interactions with SM compared with those observed in healthy subjects. Accordingly, it is reasonable with insights gained from our data analysis to anticipate poor performance of the more advanced models 2 and 3 compared with the simpler model 1. These observations suggest that our SM prediction models should not be applied below the lower BMI of the model-development group (i.e., 16 kg/m²).

Study Limitations

There are several possible sources of model error that should be considered. We were able to develop prediction models that directly relate ALST measured by DXA with total body SM measured by MRI and thus avoid the conventional assumption that most ALST is muscle and that approximately three-quarters of total body SM is in the extremities (23). However, our models still rely on the assumption that stable associations are present between ALST, appendicular SM, and total body SM. Therefore, our prediction models may not be appropriate for populations that differ in these relationships from the ones present in our model-development sample, such as anorexia nervosa patients with an extremely low BMI.

The DXA method does not differentiate between water and lean soft tissue, and an increase or decrease in hydration would result in overestimation or underestimation of lean soft tissue.
It is plausible that variability in ALST estimates across instruments from different manufacturers may introduce an error into the predicted SM. Properly calibrated, stable DXA instruments of the present generation are not likely to differ by more than ~5% in their readings of adult lean soft tissue (25). Consequently, the differences in ALST values and SM estimates might be expected to differ by similar amounts across instruments.

A potential limitation involves the assumed constant density of SM tissue of 1.04 kg/l used for converting SM volume to mass (23). Variability in actual SM density may introduce a small error into the MRI-estimated SM.

Additionally, the models are not appropriate for use in younger age groups because the proportions of ALST as SM may vary, and SM-relationships with other covariates such as age, sex, and race may be different from those present in an adult population. Therefore, further studies are needed for developing similar SM-prediction formulas for use in children and adolescents.

Our models were developed in a cross-sectional cohort and similarly validated in a study group evaluated at only one time. Further studies are needed to validate the models in longitudinal studies for the detection of change in SM mass in response to intervention.

In conclusion, three new DXA prediction models for IMAT-free total body SM mass were developed and then validated in a large and diverse sample of healthy adults. The models were then shown in cross-validations to be applicable in subjects who varied widely in muscularity and overall body composition, provided that BMI was not extremely low. These developed formulas provide a new opportunity for quantifying SM in small-scale physiological investigations and in field studies incorporating DXA in measurement protocols.

GRANTS

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