Determination of skeletal muscle and fat-free mass by nuclear and dual-energy X-ray absorptiometry methods in men and women aged 51–84 y$^{1–3}$

Ross D Hansen, Chand Raja, Ali Aslani, Ross C Smith, and Barry J Allen

ABSTRACT

Background: Skeletal muscle mass (SMM) and fat-free mass (FFM) are important variables in nutritional studies. Accurate techniques for measuring these variables have not been thoroughly validated in elderly subjects.

Objectives: The objectives of this study were to 1) compare SMM values derived from dual-energy X-ray absorptiometry (DXA) with those calculated by a nuclear method from total body potassium (TBK) and total body nitrogen (TBN) measurement (both: KN) in older subjects, and 2) assess the accuracy of FFM measurement by DXA in these subjects.

Design: TBK, TBN, DXA (model XR36; Norland, Fort Atkinson, WI), bioimpedance, and anthropometric measurements were performed on healthy women ($n = 50$) and men ($n = 25$) aged 51–84 y.

Results: Mean SMM by KN was not significantly different from SMM by DXA in either sex. SMM by KN predicted SMM by DXA with an SEE of 2.1 kg ($r = 0.95$, $P < 0.0001$ for women and men together). In the men, FFM by DXA agreed well with FFM estimated by TBK, skinfold thicknesses, bioimpedance analysis, and a multicompartment model. In women, FFM by DXA was 4–5 kg less than that by the other methods ($P < 0.01$). Truncal fat was related to intermethod FFM differences ($r = 0.58$, $P < 0.0001$).

Conclusions: These data indicate that 1) either the nuclear or the DXA method can be applied to estimate SMM in healthy older subjects, and 2) the Norland DXA instrument significantly underestimates FFM in older women, in part, because of the influence of truncal adiposity.

KEY WORDS Skeletal muscle mass, dual-energy X-ray absorptiometry, sarcopenia, total body potassium, total body nitrogen, bioimpedance analysis, aging, anthropometry, humans

INTRODUCTION

Skeletal muscle mass (SMM) is an important variable to consider in nutritional studies. Skeletal muscle is metabolically active, represents a large proportion of the fat-free mass (FFM) of the body, and should be maintained in the elderly to prevent frailty and loss of independence (1, 2). Although several studies have implied that a substantial loss of SMM (sarcopenia) is an inevitable feature of human aging (3–6), some evidence suggests that sarcopenia can be considerably minimized, and even reversed, by appropriate physical activity (1, 2, 7–9).

To quantify age-associated decreases in SMM and the effects of interventions, accurate estimation of SMM is required. This has proven to be difficult because there is no direct in vivo means of measuring SMM. There are, however, several methods of indirect estimation, including anthropometric fractionation (10); creatinine excretion (11); whole-body counting and neutron activation to quantify total body potassium (TBK) and total body nitrogen (TBN), respectively (12, 13); computed tomography (14); and magnetic resonance imaging (15). These methods are all time-consuming and technically difficult to perform, and those methods often ranked the highest for accuracy (computed tomography and magnetic resonance imaging) involve considerable radiation exposure or expensive instrumentation.

Dual-energy X-ray absorptiometry (DXA) is a relatively new method of body-composition analysis that involves minimal radiation exposure (16). A whole-body DXA scan divides the body into bone, fat, and lean compartments. With appropriate definition of arm and leg regions, DXA provides an estimation of the fat-free soft tissue (FFST) in the limbs. If it is assumed that this limb FFST value closely represents limb SMM, as discussed by Heymsfield et al (17), then total SMM can be calculated for normal individuals on the basis of the proportion of limb SMM to total SMM (0.75) reported in cadaver studies (18). This method of SMM estimation was shown to agree well with computed tomography–determined SMM in 25 young to middle-aged men (13). In addition, DXA-determined limb SMM was found to correlate strongly with TBK in a sample of 148 women and 136 men aged 20–90 y (6). Because DXA can also quantify whole-body bone mineral density and bone mineral content (BMC) together with total FFM in a 5–6-min

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Table 1

<table>
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<tr>
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<th>Women (n = 50)</th>
<th>Men (n = 25)</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>63 ± 7 (54–84)</td>
<td>64 ± 7 (51–76)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162 ± 6 (153–175)</td>
<td>174 ± 7 (160–187)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68 ± 12 (47–94)</td>
<td>79 ± 10 (49–95)</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>36 ± 5 (28–48)</td>
<td>26 ± 4 (17–36)</td>
</tr>
<tr>
<td>Truncal fat:lean soft tissue</td>
<td>0.9 ± 0.24 (0.33–1.5)</td>
<td>0.41 ± 0.13 (0.19–0.69)</td>
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\[ \frac{\text{Truncal fat}}{\text{Lean tissue}} = \frac{\text{Truncal fat mass}}{\text{Truncal FFST}} \]

\[ \text{FFM}_{\text{DXA}} = (0.188 \times \text{TBK}) + (0.00183 \times \text{TBN}) \]

Subjects and Methods

Subjects

The study sample comprised white women aged 54–84 y (n = 50) and white men aged 51–76 y (n = 25). The subjects had a wide range of body sizes and adiposity (Table 1). On recruitment, they were all, by self-report, weight stable and apparently healthy. Each subject gave informed consent for the study, which was approved by the Royal North Shore Hospital Medical Research Ethics Committee and Radiation Protection Committee.

Body-composition measurements

Total body potassium and total body nitrogen

TBK was measured by supine sodium iodide counting, as described previously (22). The precision and accuracy of this method, expressed as CVs, are 1.5% and 4.5%, respectively. TBK was used to estimate FFM (FFM\textsubscript{TBK}), assuming that the potassium content of FFM is 2.26 g/kg in women and 2.52 g/kg in men (23). TBN was measured by in vivo neutron-capture analysis, as described by Allen et al (24), with a precision and accuracy of 3% and 4.5%, respectively. The radiation exposure with a TBN scan is 0.2 mSv.

Dual-energy X-ray absorptiometry

Total body and regional BMC, FFM, and FFST were estimated by whole-body DXA scan (model XR36; Norland) with a scan speed of 25 cm/s and analyzed with version 2.5.2 software. The instrument was calibrated daily with the manufacturer’s spine and soft tissue phantoms. The fat-lean phantom uses 77 combinations of acrylic and aluminum and is an advanced approach to DXA calibration (25). The software assumes a weighted linear fat distribution model that permits extrapolation of the fat content of bone-containing pixels (26).

Truncal, abdominal, arm, and leg regions were marked on the scan image by one operator before analysis, as described by Heymsfield et al (17). DXA-derived FFM (FFM\textsubscript{DXA}) was calculated from the analyzed scan output by summing total BMC and FFST. As an index of truncal adiposity, the ratio of truncal fat to lean tissue (truncal fat:lean) was calculated from the output by dividing truncal fat mass by truncal FFST. The precisions of total BMC, FFM\textsubscript{DXA}, and FFST measurements were 1.4%, 2%, and 2%, respectively.

Anthropometry

Height was measured to the nearest 0.5 cm with a wall-mounted stadiometer and body weight was measured to the nearest 0.1 kg with digital scales. Skinfold thicknesses were measured in duplicate by one researcher with constant-pressure calipers (Holtain Ltd, Crymych, United Kingdom) at the triceps, biceps, subscapular, and suprailliac sites. The skinfold-thickness measurements were used to estimate percentage body fat with the appropriate age- and sex-specific equations of Durnin and Womersley (27). The precision of percentage fat estimation was 1.1%. Skinfold thickness–derived FFM (FFM\textsubscript{SKF}) was calculated from percentage body fat and body weight measurements.

Bioimpedance analysis

A bioimpedance analysis (BIA) measurement was taken after each subject had rested supine for 5 min, with electrodes in a tetrapolar configuration, by using a swept-frequency instrument (SEAC model SFB2.3 with associated software; UniQuest, Queensland, Australia). The BIA output measures were used to derive FFM (FFM\textsubscript{BIA}) by applying the equation of Lukaski et al (28), and total body water (TBW) by using the equation of Kushner and Schoeller (29). The latter equation was derived in a group of men and women aged 20–70 y; the equation predicts deuterium oxide space from a combination of subject resistance, height, and weight. The precisions of FFM\textsubscript{BIA} and TBW measurements were 1.6% and 1.4%, respectively.

Data reduction and analysis

Skeletal muscle mass estimation

SM was estimated from TBK and TBN data (ie, SM\textsubscript{KM}) by using the equation of Wang et al (13) as follows:

\[ \text{SM}_{\text{KM}} = (0.188 \times \text{TBK}) + (0.00183 \times \text{TBN}) \]

where TBK and TBN are in grams and SM\textsubscript{KM} is in kilograms.

Because this equation was developed empirically by relating TBK and TBN to computed tomography–determined SM in a multiple regression model, it can be regarded as a surrogate measure of computed tomography–determined SM and should therefore accurately represent SM.

Dx-derived SM (SM\textsubscript{DXA}, in kg) was calculated from the sum of arm and leg FFST values (in kg), assuming that this sum represents limb SM and that limb SM represents 75% of total body SM, as discussed above:

\[ \text{SM}_{\text{DXA}} = 1.333 (\text{arm FFST} + \text{leg FFST}) \]
TABLE 2
SMM and FFM values by several methods

<table>
<thead>
<tr>
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<th>Women</th>
<th>Men</th>
</tr>
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| SMM
| kg     | kg     |
| SMM
| KN     | 19.9 ± 3.3 | 31.7 ± 4.0 |
| DXA     | 19.6 ± 3.6 | 31.4 ± 4.4 |
| TBK     | 37.4 ± 5.9 | 57.9 ± 6.7 |
| SKF     | 41.1 ± 7.1 | 58.8 ± 7.9 |
| BIA     | 41.3 ± 5.1 | 57.6 ± 6.4 |
| 4C      | 41.6 ± 5.3 | 57.3 ± 7.0 |
| 4C – 4C | 42.6 ± 4.6 | 59.4 ± 6.6 |

1SD. SMM, skeletal muscle mass; FFM, fat-free mass; KN, total body potassium and nitrogen; DXA, dual-energy X-ray absorptiometry; TBK, total body potassium; SKF, skinfold thicknesses; BIA, bioimpedance analysis; 4C, 4-compartment model.

2Significantly different from men, P < 0.001 (Student’s t test for unpaired data).

3Significantly different from FFM, P ≤ 0.01 (Student’s t test for paired data).

Fat-free mass based on a 4-compartment body-composition model

A widely used 4-compartment body-composition model assumes that the body consists of fat, protein, water, and mineral compartments (30, 31). FFM (FFM4C, in kg) was calculated from this model as follows:

\[\text{FFM}_{4C} = \text{protein} + \text{TBW} + \text{mineral}\]

where protein is total body protein [TBN (in kg) × 6.25], TBW is determined by BIA (in kg), and mineral is total body mineral [total BMC (in kg)/0.84].

As discussed by Baumgartner (30), the assumed nitrogen content of protein (1 g N = 6.25 g protein) and the ratio of osseous to nonosseous mineral (0.84) involved in these calculations are applicable to subjects in this age range.

Bone mineral and water content of fat-free mass

The mineral content of FFM was calculated by expressing BMC as a percentage of FFM. Similarly, hydration of FFM was estimated by expressing TBW as a percentage of FFM. These measures were included to assess their potential contribution to intermethod differences in body-composition measurement because variance in these FFM components is known to affect body density and can thereby reduce the accuracy of 2-compartment models (26, 30, 31).

Statistical analysis

Correlation and regression analysis, Student’s t tests, and Bland-Altman analyses (32) were used to compare the 2 methods of SMM estimation and to compare FFM DXA with the other methods (TBK, SKF, BIA, and 4C) of FFM determination. All statistical analyses were carried out with SPSS for WINDOWS (release 6.1.4; SPSS Inc, Chicago). The level of significance was set at P < 0.05 for all analyses.

RESULTS

Skeletal muscle mass estimates

Data for women and men analyzed separately

SMM DXA and SMM KN were significantly correlated in both the women \((r = 0.83)\) and men \((r = 0.87)\) \((P < 0.0001\) for both).

Regression analysis showed that the 2 SMM methods were related as follows:

For women: \(\text{SMM}_{DXA} = 0.90 \times \text{SMM}_{KN} + 1.7\) (4)

For men: \(\text{SMM}_{DXA} = 0.97 \times \text{SMM}_{KN} + 0.5\) (5)

where SMM KN predicted SMM DXA with an SEE of 2.0 kg in the women and 2.2 kg in the men. The 95% CIs for the regression slopes in equations 4 and 5 include the line of identity.

Paired t tests showed no significant differences between the 2 estimates of SMM in data from women and men (Table 2). Bland-Altman analyses (32) did not show any systematic bias, for either sex, in the differences between the 2 methods as SMM values increased.

Pooled data

Because neither the slopes nor the intercepts of equations 4 and 5 were significantly different between the sexes, the data were pooled. In this combined data set, the 2 estimates of SMM were highly correlated and in good agreement, such that SMM KN predicted SMM DXA with an SEE of 2.1 kg (Figure 1). The 95% CIs for the regression slope given in Figure 1 include the line of identity. A paired t test showed that although mean SMM KN was 0.32 kg higher than mean SMM DXA, this difference was not significant. Further analysis with the Bland-Altman method (32) revealed that there was no systematic bias in the differences between the 2 methods as SMM values increased (Figure 2).

Age effects

Both SMM estimates correlated significantly and negatively with age for women but not for men. For women, SMM KN had a more significant relation with age \((r = -0.42, P = 0.002)\) than did SMM DXA \((r = -0.3, P = 0.035)\).
Mineral and water content of fat-free mass

Mean (±SD) values for BMC as a percentage of FFM were 5.4 ± 0.6% and 6.2 ± 0.8% for men and women, respectively. Mean values for TBW as a percentage of FFM were 73.0 ± 5.2% and 73.8 ± 4.3% in men and women, respectively.

Fat-free mass estimated by dual-energy X-ray absorptiometry compared with other methods

In contrast with the close agreement between SMM estimates, there were relatively large and significant differences between FFMDXA and several other FMM estimates, particularly in the data set from women (Table 2). To determine whether these intermethod differences were related to variables such as age, adiposity, fat distribution, BMC as a percentage of FFM, or TBW as a percentage of FFM, a series of Bland-Altman analyses (32) were performed on pooled data from men and women by plotting the difference between FFM4C and FFM DXA against these variables. The difference between the FFM methods was not related to age or percentage fat, but was related to BMC as a percentage of FFM (r = 0.43, P = 0.0001) and TBW as a percentage of FFM (r = 0.26, P = 0.02). There was a highly significant relation (r = 0.58, P < 0.0001) to truncal adiposity, as reflected in the ratio of truncal fat to leg fat (Figure 3).

DISCUSSION

The average values for weight, percentage fat, and bone mineral and water contents of FFM in these subjects were in close agreement with values for older white persons reported elsewhere. Snead et al (21) found that healthy women aged 60–73 y had a mean weight of 65 kg and a mean percentage fat (by hydrodensitometry) of 39%; values for men aged 60–82 y were 77 kg and 26%, respectively. Dual-photon absorptiometry data from Mazess et al (33) give a mean BMC:FFM value of 5.9% for women aged 50–61 y. Baumgartner (30) reported FFM hydration values of 74.3% and 71.0% in elderly women and men, respectively. Because the TBW values in the current study were obtained with a BIA technique, they should be interpreted with caution. Nevertheless, given the favorable comparisons summarized above, we expect that the subjects in the current study were representative of healthy older whites.

The strength of agreement between the SMMDXA and SMMKN estimates was striking, given that these methods involve totally independent assumptions. Although neither of the methods has been extensively validated against a gold standard SMM measure in older subjects, the close agreement between the regression line and the line of identity in Figure 1 implies that both methods were in fact measuring the same variable: SMM. Furthermore, because the equation used in this study to derive SMMKN was empirically derived from computed tomography scans in subjects with a considerably wide range of SMM (13), similar results would be expected if the DXA method were to be compared directly with computed tomography–derived SMM in healthy older subjects.

This agreement between the DXA and the KN methods implies that reasonably accurate determinations of SMM in healthy elderly people are possible by either method. The speed of data acquisition (5–6 min for the Norland model XR36 whole-body scan) and the lower radiation exposure with DXA compared with a TBN measurement (TBK involves no radiation exposure) make DXA an attractive option. In our laboratory, a combined TBN and TBK assessment takes ~60 min to complete. However, the fact that we found SMMKN values to be more highly correlated with age than were SMMDXA values (in women), considered together with the lower SDs found in the SMMKN data (Table 2), could indicate advantages for the nuclear method.

Use of the nuclear techniques can yield valuable clinical information in addition to SMM estimation. Total body protein, assessed via TBN, is an important indicator of nutritional status and has been shown to reflect the severity of several illnesses (23, 24, 34). Comparison of patient values with age- and sex-matched norms from a healthy reference population can therefore be an important prognostic guide and assist in clinical decision-making.

FIGURE 2. The difference between skeletal muscle mass determined by the nuclear method (SMMKN) and that by dual-energy X-ray absorptiometry (SMMDXA) versus the mean of the 2 methods for women (○) and men (●).

FIGURE 3. The difference between fat-free mass determined by the 4-compartment–based model (FFM4C) and dual-energy X-ray absorptiometry (FFMDXA) versus the ratio of truncal fat to lean soft tissue for women (○) and men (●).
were consistently higher than hydrodensitometry-derived values, compared percentage fat values obtained by DXA (Norland) and 1.1 kg higher (P < 0.01) than FFM\textsubscript{DXA} measured by a Lunar Corporation (Madison, WI) instrument in 19 white women aged 54–68 y. Multicomponent FFM was only 0.2 kg higher (NS) than FFM\textsubscript{DXA} in 8 men in the same age group. Nord and Payne (38) compared percentage fat values obtained by DXA (Norland) and hydrodensitometry in 219 adult subjects. DXA-derived values were consistently higher than hydrodensitometry-derived values, particularly in women, implying a considerable underestimation of FFM by DXA. In contrast, analysis of percentage fat and weight data from Snead et al (21) shows that FFM\textsubscript{DXA} measurement by Hologic (Waltham, MA) equipment was greater than hydrodensitometry-derived FFM by 3.5 and 4.7 kg, respectively, in women and men aged > 60 y. These studies indicate that the difference between FFM\textsubscript{DXA} and other FFM estimates varies considerably depending on the instrument used, with the Lunar and Norland instruments underestimating and the Hologic instrument overestimating FFM relative to the criterion method.

Our finding that truncal adiposity was positively related to intermethod FFM differences suggests that the fat distribution model in the DXA system software is a critical factor in determining the accuracy of whole-body soft tissue estimates. The SMM data indicate that the software permits good separation of fat and lean tissue in regions where the boundaries between bone and soft tissue are readily defined, such as the limbs. However, the method appears to have difficulty in separating fat and lean tissue in regions such as the trunk, where bone and soft tissue boundaries are far more irregular and fat content can be more variable (20). This shortcoming in software modeling is highly likely to affect all DXA instruments. Snead et al (21) found that Hologic instrumentation markedly underestimated exogenous fat when lard was placed over the trunk of subjects, but detected the additional fat accurately when it was placed over the thighs.

In addition to fat distribution, 2 other groups of factors could potentially influence the intermethod FFM agreement. First, if the BMC and water content of FFM vary markedly from the assumed normal values used in the original hydrodensitometry analyses, the assumed density of FFM will be incorrect. This will introduce errors when methods such as skinfold-thickness measurements and BIA are used, which are highly influenced by body density analysis (20, 30, 37). The influence of these factors in the current study was reflected in the positive correlations between intermethod FFM differences and BMC:FFM and TBW:FFM. Thus, the errors involved in predicting FFM from skinfold-thickness measurements and BIA are relatively large in comparison with those associated with criterion FFM methods such as hydrodensitometry and isotope dilution (30, 31). Similarly, because the potassium content of FFM has been shown to decline with age (36), the use of an assumed value for TBK:FFM introduces an error into the extrapolation of FFM from TBK (30, 36). Second, technical factors—including instrument make and model, calibration technique, scan speed, and software—are highly likely to affect the accuracy of body-composition measurement by DXA (20, 39). In consideration of these multiple confounding factors, DXA should be used cautiously in the measurement of FFM and fat mass in older subjects and results from different instruments should be interpreted conservatively.

In summary, the results of this study indicated that both DXA and KN measurement can be used to estimate SMM in healthy elderly subjects with reasonable accuracy and precision. DXA has the practical advantage of convenience over the nuclear method. However, when comprehensive body-composition assessment is justified, particularly in the study of disease states in which constancy assumptions are challenged, measurements of TBK and TBN provide more detailed information for nutritional analysis. Although DXA provides FFM values that agree quite well with FFM estimates from other methods in older men, it underestimates FFM in older women, possibly because of inaccuracies in defining truncal fat. Further studies are necessary to confirm whether other DXA instrument models and software can provide accurate assessment of SMM in this age group and in patient groups. Thus, these conclusions apply only to healthy subjects. The use of nuclear techniques is recommended for thorough body-composition investigations of disease states.

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