Validity of fan-beam dual-energy X-ray absorptiometry for measuring fat-free mass and leg muscle mass

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1Epidemiology, Demography and Biometry Program, National Institute on Aging, Bethesda, Maryland 20892; 2Institute for Research in Extramural Medicine, Faculty of Medicine, Vrije Universiteit, 1081 BT Amsterdam, The Netherlands; 3Department of Radiology, University of California, San Francisco, California 94105; and 4Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Visser, Marjolein, Thomas Fuerst, Thomas Lang, Lorcan Salamone, and Tamara B. Harris for the Health, Aging, and Body Composition Study—Dual-Energy X-Ray Absorptiometry and Body Composition Working Group. Validity of fan-beam dual-energy X-ray absorptiometry for measuring fat-free mass and leg muscle mass. J. Appl. Physiol. 87(4): 1513–1520, 1999.—The aim of the study was to examine the accuracy of fan-beam dual-energy X-ray absorptiometry (DEXA) for measuring total body fat-free mass (FFM) and leg muscle mass (MM) in elderly persons. Participants were 60 men and women aged 70–79 yr and with a body mass index of 17.5–39.8 kg/m². FFM and MM at four leg regions were measured by using DEXA (Hologic 4500A, v8.21). A four-compartment body composition model (4C) and multislice computed tomography (CT) of the legs were used as the criterion methods for FFM and MM, respectively. FFM by DEXA was positively associated with FFM by 4C (R² = 0.98, SE of estimate = 1.6 kg). FFM by DEXA was higher [53.5 ± 12.0 (SD) kg] than FFM by 4C (51.6 ± 11.9 kg; P < 0.001). No association was observed between the difference and the mean of the two methods. MM by DEXA was positively associated with CT at all four leg regions (R² = 0.86–0.96). MM by DEXA was higher than by CT in three regions. The results of this study suggest that fan-beam DEXA offers considerable promise for the measurement of total body FFM and leg MM in elderly persons.

computed tomography; elderly; multicomartment models

DUAL-ENERGY X-ray absorptiometry (DEXA) is becoming increasingly popular for the measurement of soft tissue composition. Unlike most other body composition methods, DEXA is noninvasive and includes both regional and total body measurements as well as estimates of appendicular skeletal muscle mass (5, 7). Measurements of appendicular muscle mass by DEXA (or dual-photon absorptiometry) have previously been validated against criterion methods of the total body, including total body potassium counting (5, 7), total body nitrogen analysis (7), and computed tomography (CT) (17). However, no studies have directly compared appendicular skeletal muscle mass with regional criterion methods. Moreover, none of these studies have been carried out in elderly persons. Because of the interest in body composition in old age, especially the study of sarcopenia and the effect of intervention studies on muscle mass, accurate assessment of muscle mass by DEXA in older people is becoming more important.

In all previously mentioned DEXA validation studies, pencil-beam scanners were used. In these scanners the X-ray beam travels across the person in a rectilinear fashion, and a single detector is used to acquire the data. The newest generation of DEXA scanners uses a fan-beam X-ray source coupled with multiple detectors. These scanners have the advantage of a faster scanning speed, ~3–4 min compared with 15–25 min for a whole body scan, allowing the use of DEXA in large population studies and in subjects who have difficulty lying motionless on a flat hard surface for 20 min, such as frail older subjects and young children. However, validation studies directly comparing the newest fan-beam DEXA technology against other body composition techniques and against the pencil-beam technique are limited (4).

The aim of the present study was to examine the accuracy of the fan-beam DEXA technology for the estimation of total body fat-free mass (FFM) and leg muscle mass in men and women aged 70–79 yr. A four-compartment body composition model and multislice CT were used as the criterion methods for total body FFM and leg muscle mass, respectively.

METHODS

Subjects. A total of 60 healthy adults, 30 men and 30 women, aged 70–79 yr, were recruited through flyers and advertisements in local newspapers. Study participants were recruited to represent a large body mass index (BMI) range: 25% of the study subjects had a BMI of <25 kg/m², 50% with BMI of 25–30 kg/m², and 25% with BMI of >30 kg/m². Only Caucasians (n = 53) and African-Americans (n = 7) were included in the study. All measurements were performed in the morning after an overnight fast. After the blood draw for the determination of total body water, subjects were allowed to drink water and were provided a standard liquid meal. The experimental procedures were approved by the Human Investigation and Review Boards at the University of California at San Francisco and San Francisco State University. Written informed consent was obtained from all the subjects.

Anthropometry. Body weight was determined to the nearest 0.1 kg with subjects dressed in bathing suits, and height was measured to the nearest 0.1 cm using a stadiometer.
BMI was calculated as weight (kg) divided by height (m) squared.

DEXA. Body composition of all subjects was measured by DEXA using the fan-beam technology (model QDR 4500A, Hologic, Waltham, MA). After completion of the scans, the body composition results for the total body were provided by the system’s most recent software release, version 8.21. Total body FFM was defined as lean soft tissue mass plus total body bone mineral content (BMC). Muscle mass of the total leg, total thigh, and small subregions at the mid thigh and calf were also assessed. For the total legs a region was defined by placing a horizontal line at the lowest point of the ischial tuberosity as the upper margin for the legs and a horizontal line at the ankle joint as the lower margin for the legs. In this way the upper and lower margins of the legs were similar to the ones used in the multislice CT technique (see CT and Fig. 1). Total leg muscle mass was calculated as follows (7): leg muscle mass = total leg mass – (leg fat mass) – 1.82·leg BMC. Similarly, muscle mass of the thighs was assessed, using the ischial tuberosity and the knee joint as upper and lower margins, respectively. Additionally, muscle mass at the mid thigh (at one-half of the distance from the knee joint to the top of femur) and at the calf (at two-thirds of the distance from the ankle joint to the knee joint) was assessed by using the smallest DEXA subregion possible, which consists of 3 pixels or 3.96 cm. Triplicate scans of all 60 subjects without repositioning revealed a coefficient of variation of 0.81% for total leg muscle mass (0.81% for men and 0.83% for women). The interobserver coefficient of variation for reading the scans of 20 subjects (10 men and 10 women) was 1.4% for lean soft tissue mass of the total leg, 2.3% for the thigh, 4.3% for the mid thigh subregion, and 1.3% for the calf subregion.

Four-compartment model. To validate the DEXA results for total body FFM, a four-compartment model was used as the criterion method (9). This model requires measurements of body density, total body water, total body bone mineral mass, and body weight, and it takes into account interindividual variations in the water content and mineral content of the FFM.

Body density was measured by using underwater weighing. Subjects wore a bathing suit. Water temperature was set at 32–35°C. When possible, 10 submersions with maximal exhilation were performed. The average of the five most consistent trials (difference 0.02 kg) was used. Before submersion, residual lung volume was measured by using a Collins Respirometer (model SVR/PLUS, Braintree, MA). With the mouthpiece in place, subjects were asked to breathe normally until the spirometer equilibrated for collection of data on functional residual capacity. After equilibration, subjects performed a forced inhalation followed by a forced exhalation for collection of inspiratory and expiratory reserve capacity data, respectively. Residual volume was calculated as functional residual capacity minus expiratory capacity.

Table 1. Characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>30</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Age, yr</td>
<td>73.9±2.2</td>
<td>73.6±2.3</td>
<td>73.7±2.2 (70–79)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>83.7±12.2</td>
<td>67.9±12.6†</td>
<td>75.8±14.6 (38.2–111.0)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.73±0.06</td>
<td>1.59±0.06†</td>
<td>1.66±0.09 (1.48–1.87)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.9±3.7</td>
<td>26.9±5.2</td>
<td>27.4±4.5 (17.5–39.8)</td>
</tr>
<tr>
<td>Body fat,† %</td>
<td>26.1±5.5†</td>
<td>38.9±6.7‡</td>
<td>32.5±8.8 (17.1–51.2)</td>
</tr>
</tbody>
</table>

Values are means ± SD with range in parentheses. *n = 27 Caucasian men, n = 26 Caucasian women, n = 3 African-American men, n = 4 African-American women. †By 4-compartment model: 30 men and 28 women. ‡P < 0.001 men vs. women (by Student’s t-test).
Table 2. Validation of the fan-beam dual-energy X-ray absorptiometry technique for measuring fat-free mass against a four-compartment body composition method

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>4-Compartment Model, kg</th>
<th>Fan-Beam DEXA, kg</th>
<th>Difference, kg</th>
<th>R²</th>
<th>SEE, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>30</td>
<td>61.4 ± 6.8</td>
<td>63.4 ± 6.8</td>
<td>1.8 ± 1.7*</td>
<td>0.94</td>
<td>1.8</td>
</tr>
<tr>
<td>Women</td>
<td>28</td>
<td>41.1 ± 5.3</td>
<td>43.6 ± 6.0</td>
<td>1.9 ± 1.5*</td>
<td>0.95</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>51.6 ± 11.9</td>
<td>53.5 ± 12.0</td>
<td>1.8 ± 1.6*</td>
<td>0.98</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. DEXA, dual-energy X-ray absorptiometry; R², explained variance; SEE, SE of estimate. *P < 0.001, 4-compartment vs. DEXA.

Body composition from the four-compartment model was calculated by using the following formula (9): body fat (%) = \[\frac{\text{fat mass}}{\text{total body mass}}\]·100, where D is body density from underwater weighing, W is the water fraction of the body (total body water/body weight), and M is the mineral fraction of the body (total body mineral mass/body weight). FFM was calculated as body weight – body fat. FFM was missing for two women who were not able to complete the underwater-weighing procedure.

Total body water was assessed by using deuterium dilution (13). Blood samples were collected before and at least 4 h after an exactly weighed oral dose of deuterium oxide (~ g diluted in 50 ml of water) and stored frozen at −20°C until analysis for deuterium. Corrections were made for nonaqueous hydrogen exchange and water density at body temperature. Total body water (kg) was calculated as deuterium dilution space (liters)/1.046.

Total body mineral mass was calculated from measured total body bone mineral content by the fan-beam DEXA technology, multiplied by 1.23 (9).

Body composition from the four-compartment model was calculated by using the following formula (9): body fat (%) = \[\frac{\text{fat mass}}{\text{total body mass}}\]·100, where D is body density from underwater weighing, W is the water fraction of the body (total body water/body weight), and M is the mineral fraction of the body (total body mineral mass/body weight). FFM was calculated as body weight – body fat. FFM was missing for two women who were not able to complete the underwater-weighing procedure.

Total leg muscle volume is the sum of four segments: ankle-calf, calf-knee, knee-midthigh and mid thigh-upper thigh. Total thigh muscle volume is the sum of two segments: knee-midthigh and mid thigh-upper thigh. Muscle volumes at the mid thigh and at the calf from the 10-mm slice were multiplied by 3.96 to create the same leg area used for the DEXA mid thigh and calf subregions.

All muscle volumes from CT were multiplied by 1.04 to calculate muscle mass, where 1.04 is the assumed constant density (kg/cm³) of adipose tissue-free skeletal muscle (3, 14). Similarly, all adipose tissue volumes were multiplied by 0.923 to calculate adipose tissue mass (3). The nonadipose, non-muscle volume was assumed to consist of 60% bone (of which 75% is cortical bone and 25% is trabecular bone) and 40%...
bone marrow, which were multiplied by 1.72 and 0.9 kg/cm³, respectively, to calculate bone mass (3). Total mass was calculated by adding muscle mass and adipose tissue mass and bone mass for each of the four leg regions.

The DEXA method divides soft tissue into FFM and fat mass, whereas the CT provides muscle tissue mass and adipose tissue mass. These methodological differences were taken into account when muscle mass by DEXA was compared with muscle mass by CT. Adipose tissue as measured by CT consists of 80% fat and a lean compartment of 20% water, proteins, and minerals (16). This lean compartment within adipose tissue is measured as muscle mass in the DEXA method. Therefore, the lean component within adipose tissue was added to the muscle tissue by CT, and the total amount was then compared with muscle mass as measured by DEXA.

Statistical analysis. Data were analyzed by using SAS software (SAS Institute, Cary, NC). Results are presented as means ± SD. A P value of <0.05 was considered statistically significant. Total body FFM by fan-beam DEXA was compared with total body FFM from the four-compartment model by using linear regression analyses. The SE of the estimate (SEE) reported is the root mean square error. Absolute differences were tested by using paired t-tests. The method of Bland and Altman was used to compare fan-beam DEXA with

Table 3. Validation of a fan-beam dual-energy X-ray absorptiometry technique for measuring total leg and total thigh muscle mass against multislice computed tomography

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Computed Tomography, kg</th>
<th>Fan-Beam DEXA, kg</th>
<th>Difference, kg</th>
<th>R²</th>
<th>SEE, kg</th>
</tr>
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<tbody>
<tr>
<td><strong>Total leg muscle mass</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>30</td>
<td>14.7 ± 2.3</td>
<td>16.2 ± 2.6</td>
<td>1.6 ± 0.7§</td>
<td>0.93</td>
<td>0.7</td>
</tr>
<tr>
<td>Women</td>
<td>30</td>
<td>10.6 ± 1.6</td>
<td>10.8 ± 1.6</td>
<td>0.3 ± 0.4§</td>
<td>0.92</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>12.6 ± 2.9</td>
<td>13.5 ± 3.5</td>
<td>0.9 ± 0.9§</td>
<td>0.96</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Total thigh muscle mass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>30</td>
<td>10.4 ± 1.5</td>
<td>11.4 ± 1.9</td>
<td>1.0 ± 0.7§</td>
<td>0.90</td>
<td>0.6</td>
</tr>
<tr>
<td>Women</td>
<td>30</td>
<td>7.3 ± 1.1</td>
<td>7.6 ± 1.2</td>
<td>0.3 ± 0.3§</td>
<td>0.94</td>
<td>0.3</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>8.8 ± 2.0</td>
<td>9.5 ± 2.5</td>
<td>0.7 ± 0.6§</td>
<td>0.96</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. *From ankle joint to ischial tuberosity. †From knee joint to ischial tuberosity. §P < 0.01; ‡P < 0.001, computed tomography vs. DEXA.
the criterion method (2). This method was repeated after the exclusion of outliers, defined as those subjects for whom the difference between two methods was greater or smaller than two SD from the mean (2). Similarly, leg muscle mass measured by fan-beam DEXA was compared with muscle mass measured by CT. Reported correlation coefficients \((r)\) are Pearson's product-moment correlations.

**RESULTS**

The characteristics of the study population are shown in Table 1. The mean age of the study population was 73.7 yr, and the mean BMI was 27.4 kg/m\(^2\). Study participants covered a broad range of BMI, from 17.5 to 39.8 kg/m\(^2\). Total body mass as measured by fan-beam DEXA was highly correlated with body weight from the scale in men and women \((r = 0.999, P = 0.0001)\). However, DEXA total body mass was statistically higher than body weight from the scale \((1.1 \pm 0.4 \text{ kg}, P < 0.001)\).

Validation of total body FFM. Table 2 shows the measurement of total body FFM by fan-beam DEXA vs. the criterion method. The two measurements were positively associated with an explained variance of 98% \((R^2 = 0.98, \text{Fig. 2A})\). FFM measured by DEXA was higher than the criterion method for the complete study group \(1.8 \text{ kg}\) and for men \(1.8 \text{ kg}\) and women \(1.9 \text{ kg}\) separately. With use of the Bland and Altman procedure (2), no association was observed between the mean and the difference of the two methods \((r = 0.09, P = 0.5; \text{Fig. 2B})\).

Validation of leg muscle mass and thigh muscle mass. To examine whether the CT and DEXA methods measured the same amount of total mass, estimates of total leg mass and total thigh mass were compared. The results showed a good agreement between the two methods \((\text{total leg } R^2 = 0.98, \text{difference } -0.4 \pm 0.7 \text{ kg}, P = 0.0001; \text{total thigh } R^2 = 0.98, \text{difference } +0.0 \pm 0.5 \text{ kg}, P = 0.44)\).

Fan-beam DEXA measurements of total leg muscle mass and total thigh muscle mass were positively correlated with the CT values, with \(R^2 = 0.96\) (Fig. 3, A and B). DEXA significantly overestimated total leg and total thigh muscle mass by 0.7–0.9 kg (Table 3). The Bland and Altman procedure (2) suggested that the overestimation of leg muscle mass by DEXA increased with increasing muscle mass for the total leg \((r = 0.69; P = 0.0001)\) and for the thigh \((r = 0.72; P = 0.0001)\) (Fig. 4, A and B). Exclusion of three outliers did not change these results.

Validation of muscle mass at the midthigh and calf subregions. A good agreement between the DEXA and CT method was observed for total mass at the midthigh subregion \((R^2 = 0.94, \text{difference } 0.11 \pm 0.07 \text{ kg}, P = 0.0001)\) and at the calf subregion \((R^2 = 0.86, \text{difference } 0.00 \pm 0.06 \text{ kg}, P = 0.9)\).

Muscle mass by fan-beam DEXA was positively associated with muscle mass by CT at both subregions (Fig. 3, C and D, Table 4). The explained variance was lower for the calf \((R^2 = 0.86)\) than for all other muscle mass comparisons \((R^2 = 0.94–0.96)\). At the midthigh subregion, DEXA muscle mass was significantly higher than the CT values. After the exclusion of several outliers [defined as those subjects for whom the difference between the 2 methods was greater or smaller than 2 SDs from the mean (Fig. 4C)], the overestimation was...
not associated with the level of muscle mass. Muscle mass at the calf subregion was similar between DEXA and CT, with a positive association between the overestimation and the level of muscle mass ($r = 0.28, P = 0.03, \text{Fig. 4D}$). However, after exclusion of one outlier this association became statistically nonsignificant.

**DISCUSSION**

The results of the present study show that the fan-beam DEXA technology is a promising body composition method for measuring total body FFM and leg skeletal mass in elderly men and women. The study used a four-compartment model as the criterion method for FFM, which is considered the gold standard for body composition because it takes into account individual and age-related variations in the water content and mineral content of the FFM (9). This DEXA validation study is unique in that it used multislice CT to allow direct validation of skeletal muscle mass at four different regions of the leg, showing high correlations and a good agreement between the two methods.

Absolute differences in FFM between DEXA and the four-compartment model were small but statistically significant. We compared our findings with the results of other studies in which FFM from DEXA was compared with FFM from underwater weighing (6, 15, 18), FFM from total body potassium (6, 12), or FFM from total body water (12, 18). In these studies that all used pencil-beam DEXA scanners, the absolute differences ranged from 0.1 to 2.9 kg, and reported SEEs were 1.9 and 2.3 kg. The observed mean difference of $1.8 \pm 1.6$ kg FFM and SEE of 1.6 kg FFM in our study are comparable and suggest similar performance of the fan-beam scanner compared with the pencil-beam scanners.

Because of the shape of the fan-beam X-ray, an inherent magnification of scanned structures occurs, increasing as the distance from the X-ray source decreases. This magnification error has been shown to affect measurements of bone area and parameters of hip geometry (11) and may affect the accuracy of the fan-beam DEXA technology for measuring soft tissue composition. The DEXA 4500A scanner measures the distance of each pixel to the X-ray source. The most recent software release (v8.21), which was used in the present study, uses this measured distance in its mathematical models to calculate and adjust for the magnification error of each pixel. The close agreement between the fan-beam DEXA values and the criterion methods, even on small subregions of the leg, suggest that this adjustment is successful and that the fan-beam can be used to estimate FFM and muscle mass in older subjects.

The estimation of muscle mass by DEXA and by CT, and the comparison between the two methods, requires several assumptions. Some of the assumptions necessary for the estimation of muscle mass from DEXA, the interpolation of multiple CT slices, the estimation of the nonfat content of adipose tissue, and the estimation of wet bone mass are discussed below.

Appendicular skeletal muscle mass can be obtained from DEXA by assuming that all nonfat and nonbone tissue is muscle mass (7). This assumption is likely to be valid in the arms and legs and is most likely to be valid at the regions in between joints such as the mid thigh and the calf, where the amount of tendons and cartilage is small. For this reason DEXA muscle mass was compared with CT, not only for the total leg and the total thigh but also for smaller regions at the mid thigh and the calf. Indeed, the comparisons at the

![Image](http://jap.physiology.org/)

**Fig. 5.** Lean soft tissue mass of the legs by DEXA by using subregion analysis from the level of the ischial tuberosity (region 0) down to the ankle joint in two men (○, ▲) and two women (○, △). Symbols indicate location of CT slices.
calf subregion showed a slightly better agreement than at the other sites. However, the $R^2$ for the subregions were slightly lower, which might be explained by the increased variability in placing these small subregions when reading and analyzing the DEXA scans. The assumption that all nonfat and nonbone tissue is muscle mass also implies that skin is included in the DEXA muscle mass estimates. Skin tissue was not included in the CT muscle mass estimates. This difference may partly explain the overestimation of DEXA muscle mass vs. CT muscle mass as observed in our study.

To obtain muscle mass of the total leg and the total thigh by CT, we interpolated the information of five or three slices, respectively. A linear relationship between the slices was assumed because the use of more complicated mathematical procedures has been shown to produce similar results (8). The difference between the DEXA and CT results for total leg muscle mass and total thigh muscle mass was positively associated with the mean of the DEXA and CT results. Because this association was only observed at these two sites that required the greatest interpolation, and not for muscle mass at the midthigh and calf subregions where only one CT slice was used, we tried to test the assumption of linearity between CT slices. The amount of lean soft-tissue mass of the legs by DEXA was measured in four study participants (2 men and 2 women) using 25–31 subregions with a width of 3 pixels, going from the level of the ischial tuberosity (Fig. 5, region 0) down to the ankle joint. Figure 5 suggests that the assumption of a linear relation between two adjacent CT slices in the leg seems justified. However, because of the interpolation procedure, the possibility of bias in the muscle mass estimates by CT for the total leg and the total thigh cannot be excluded. Increasing the number of CT slices may reduce this potential bias.

In this study it was assumed that 20% of adipose tissue consists of nonfat components including water, proteins and minerals. This estimate was based on chemical analyses of fat biopsies in 16 men and women (16). In the literature the nonfat fraction within adipose tissue ranges between 32 (10) and 14% (1), suggesting the value of 20% used here is a reasonable estimate. However, whether the value changes with age or adiposity, or whether the value is different between men and women, is not known.

In the approach by Heymsfield et al. (7), the bone mass is assumed to be 1.82·BMC. The amount of bone mass by DEFA for the total legs was 1.3 ± 0.3 kg in this study. However, bone mass estimated from the CT data (total mass minus adipose tissue mass minus muscle tissue mass) was 3.8 ± 0.8 kg, which was statistically significantly larger than the DEXA estimate ($P = 0.0001$). These data suggest that the value of 1.82·BMC currently used in the DEXA equations might underestimate bone mass. This underestimation of the bone component may partly explain the overestimation of DEXA muscle mass vs. CT muscle mass as observed in our study.

In conclusion, the results of this cross-sectional validation study in 60 elderly men and women suggest that the fan-beam DEXA technology offers considerable promise for the assessment of fat-free body mass and leg muscle mass among elderly subjects. The short scanning time, only 3–4 min for a whole body scan, can substantially reduce scanning and technician costs and makes DEXA a more feasible body composition method. However, whether the fan-beam DEXA technology can accurately measure change in body composition over time cannot be concluded from this study. Longitudinal validation studies are needed to provide such evidence.

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