Assessment of skeletal muscle mass in men with spinal cord injury using dual-energy X-ray absorptiometry and magnetic resonance imaging

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SKELETAL MUSCLE IS THE LARGEST component of the fat-free mass, representing ~50% of the nonfat component in the total body (6). Accurate quantification of skeletal muscle is important in the assessment of nutritional status, disease risk, physical function, and atrophic effects of aging and muscle-wasting diseases (9). Moreover, understanding the accuracy of methodologies used to assess skeletal muscle allows for better interpretation of muscle status and its response to intervention. Because skeletal muscle in the thighs represents the largest proportion of the total skeletal muscle mass (10) and because the connection between body tissue composition and diseases such as osteoporosis appears to be site specific (12), accurate assessment of thigh skeletal muscle is of particular interest.

Magnetic resonance imaging (MRI) provides remarkably accurate estimates of skeletal muscle in vivo, rivaled only by quantitative computed tomography (QCT). Studies of human cadavers have demonstrated the validity of MRI and QCT in the assessment of regional skeletal muscle (14). Despite their accuracy, the expense of MRI and QCT and the high radiation exposure associated with QCT preclude their routine use in research and clinical practice. Hence, these reference methodologies are often used to assess the accuracy of less direct but more accessible techniques, such as dual-energy X-ray absorptiometry (DXA).

Some prediction models used to assess skeletal muscle from DXA assume that skeletal muscle represents a constant proportion of the fat-free soft tissue mass (FFST) (19) or virtually the entire FFST (10). The few studies that have assessed the validity of DXA using MRI or QCT as the criterion method suggest that DXA can provide valid estimates of skeletal muscle in the limbs (13, 19, 24). However, these studies were conducted primarily in healthy adults. The accuracy of skeletal muscle estimates from DXA may be compromised in disease states and conditions associated with extreme muscle atrophy, such as spinal cord injury (SCI). This notion is supported by a recent report in which the ratio of skeletal muscle to adipose-tissue-free mass was lower in men with acquired immunodeficiency syndrome (AIDS), a condition associated with extreme muscle atrophy, than in healthy controls (27). A lower ratio of muscle to adipose-tissue-free mass indicates a lower proportion of muscle in the FFST. To our knowledge, no studies have tested whether a similar phenomenon exists in the thighs of men with complete SCI. There is a dramatic reduction in the cross-sectional area of skeletal muscle in the thighs after SCI (5). If there is indeed a lower proportion of skeletal muscle in the thighs relative to FFST in individuals with SCI, muscle mass would be overestimated by prediction models that assume that muscle represents all or a certain proportion of the FFST.

The purpose of this study was to determine whether the proportion of skeletal muscle in the fat-free soft tissue mass (FFST) (19) or virtually the entire FFST (10). The few studies that have assessed the validity of DXA using MRI or QCT as the criterion method suggest that DXA can provide valid estimates of skeletal muscle in the limbs (13, 19, 24). However, these studies were conducted primarily in healthy adults. The accuracy of skeletal muscle estimates from DXA may be compromised in disease states and conditions associated with extreme muscle atrophy, such as spinal cord injury (SCI). This notion is supported by a recent report in which the ratio of skeletal muscle to adipose-tissue-free mass was lower in men with acquired immunodeficiency syndrome (AIDS), a condition associated with extreme muscle atrophy, than in healthy controls (27). A lower ratio of muscle to adipose-tissue-free mass indicates a lower proportion of muscle in the FFST. To our knowledge, no studies have tested whether a similar phenomenon exists in the thighs of men with complete SCI. There is a dramatic reduction in the cross-sectional area of skeletal muscle in the thighs after SCI (5). If there is indeed a lower proportion of skeletal muscle in the thighs relative to FFST in individuals with SCI, muscle mass would be overestimated by prediction models that assume that muscle represents all or a certain proportion of the FFST.

The purpose of this study was to determine whether the proportion of skeletal muscle in the FFST is the same in men with long-term, complete SCI and able-bodied men of similar age, weight, and height after SCI ( SCI group; n = 8). Muscle mass (1.36 ± 0.77 vs. 2.44 ± 0.47 kg) and FFST (1.70 ± 0.94 vs. 2.73 ± 0.80 kg) were lower in the SCI group than in the controls (P < 0.05), but the lower ratio of muscle to FFST in the SCI group (0.80 ± 0.09 vs. 0.91 ± 0.10, P < 0.05) suggested that they had a lower proportion of muscle in the FFST than in controls. This notion was supported by analysis of covariance, in which the mean adjusted to the mean FFST of the groups combined was lower in the SCI group. Despite the lower proportion of muscle in the FFST of the SCI group, the relation between muscle and FFST was strong in the SCI group (r = 0.99) and controls (r = 0.96). The findings suggest a disproportionate loss of muscle in the paralyzed thighs after SCI relative to other nonfat constituents, which may be accurately estimated in men with long-term SCI by dual-energy X-ray absorptiometry if the lower proportion of muscle in the FFST (~15%) is taken into account.

MATERIALS AND METHODS

Subjects. Men with complete SCI at the C6–L1 level and ≥2 yr after injury (SCI group; n = 8) were recruited for the study from the Shepherd Center. Healthy men of similar age, height, and weight to SCI participants were recruited as control subjects (controls; n = 8). Subjects were recruited from the Department of Health, Nutrition, and Exercise Sciences at the University of Delaware. The participants were all men who met the inclusion criteria and agreed to participate. The study was approved by the Institutional Review Board for Human Subjects at the University of Delaware. All participants provided informed consent before participating.

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the individuals in the SCI group, not on an athletic team and not participating in an exercise-training program \( \geq 3 \) days/wk, were recruited to serve as controls \((n = 8)\). None of the subjects had a history of chronic medication use. The study was approved by the University’s Institutional Review Board, and written consent was given before testing was initiated.

**DXA.** The total body was scanned using DXA (Delphi IIA, Hologic, Bedford, MA). After completion of the scan, femur length was determined by measuring the length of the right femur from the top of the femoral head to the bottom of the femoral condyle using the line that dissects the right femoral neck and is one of three lines that surround the pelvis in a triangular fashion. The agreement between femur length measured by DXA and MRI is excellent \((r = 0.99; \text{unpublished observations})\). To determine FFST, percent fat, and total mass in the thigh at the level of the midfemur (midthigh), the line was then moved to include the upper two-thirds of the femur, the surrounding soft tissue, and all bone and soft tissue in the leg. After the reanalysis, the line was moved to include the lower one-third of the femur, the surrounding soft tissue, and all bone and soft tissue in the leg and reanalyzed a second time. The difference in FFST from the first and second reanalysis was defined as midthigh FFST. The difference in total mass from the first and second reanalysis was defined as midthigh total mass. The difference in fat mass between the first and second reanalysis was divided by midhight total mass to calculate midthigh percent fat.

A calibration step wedge, consisting of thermoplastic resin (68% fat) and thermoplastic resin-aluminum (-10% fat) steps calibrated against stearic acid (100% fat) and water (8.6% fat; Hologic), was scanned before initiation of testing to calibrate fat mass and FFST. Quality control was checked before each test session by scanning a lumbar spine phantom consisting of calcium hydroxyapatite embedded in a cube of thermoplastic resin (model DPA/QDR-1, Hologic x-caliber anthropometric spine phantom). The coefficient of variation of the phantom areal bone mineral density during the 3-mo period of testing was 0.4%. To ensure consistency, one trained technician performed and analyzed all scans. The coefficient of variation of repeat assessment of the same images was <1.0%.

**MRI.** Approximately 25 axial T1-weighted images of one thigh (1 cm thick separated by 0.5 cm) were collected on a GE 1.5-T magnetic resonance imager using a whole body coil (500/14 TR/TE, 20–24 FOV, 1 NEX, 256 × 256 matrix). Images were downloaded to compact disk, and those at the level of the midfemur \((-10\text{ images})\) were analyzed on a personal computer with custom software (X-vessel, East Lansing, MI). The technique separates muscle from subcutaneous and intramuscular adipose tissue. As previously described \((11)\), the software used a fully automated global thresholding technique for muscle and adipose tissue segmentation. The optimal segmentation was then computed by maximizing (simplex algorithm) the correlation between the original image and the gradient images computed from the segmented image. Muscle and adipose tissue pixels were summed to determine their cross-sectional areas. Muscle and adipose tissue volumes were calculated by multiplying each slice by 1.5 to account for the 1.0-cm slice thickness of each image and the 0.5-cm gap between images. Muscle mass was determined by multiplying muscle volume by 1.04, the assumed density \((\text{kg}/\text{m}^3)\) of muscle \((20)\). Adipose tissue mass was determined by multiplying each volume by 0.923, the assumed density \((\text{kg}/\text{m}^3)\) of adipose tissue, and the lean portion of adipose tissue was estimated by assuming it represents 20% of the total adipose tissue mass \((25)\). To maintain consistency, all images were analyzed by a single investigator. The coefficient of variation of repeat assessment of the same images was <1.0%.

**Statistics.** Independent \(t\)-tests were used to determine whether groups differed in general physical characteristics, midthigh FFST, midthigh percent fat, midthigh total mass, and skeletal muscle mass by MRI. Independent \(t\)-tests were also used to determine the ratio of midthigh skeletal muscle mass to FFST. To further test whether the proportion of muscle in the FFST of the midthigh was lower in men with SCI, the differences in skeletal muscle between groups were examined while FFST was held constant with analysis of covariance (ANCOVA). Regression analysis was used to compare the relation of muscle mass to FFST in the SCI group and controls \((18)\). An alpha level of 0.05 was used for all significance tests. Unusual data points were considered outliers and excluded from the data set if Cook’s \(D\) and standardized DfBeta scores exceeded 1. The magnitude of effects was assessed with Cohen’s \(d\), with values of 0.2, 0.5, and 0.8 indicating small, medium, and large effects. All data were analyzed with SPSS, version 11.0 (Chicago, IL).

**RESULTS**

Physical characteristics of the two groups are reported in Table 1. There were no differences in age, height, weight, or femur length between the two groups. Midthigh FFST was, however, 39.7% lower and midthigh percent fat two times higher in the SCI group than in controls \((d = 1.18 \text{ and } 1.40, \text{respectively, } P < 0.05)\). Although the difference in total mass of the midfemur was not statistically different between the two groups, a moderate effect size suggested that it was lower in the SCI group than in controls \((24\%, d = 0.47, P = 0.24)\).

Similar to FFST, muscle mass from MRI was substantially lower in the SCI group \((44.0\%, d = 1.74, P < 0.05)\). The ratio of muscle mass from MRI to FFST was, however, lower in the SCI group by 12.9% \((d = 1.16, P < 0.05)\), suggesting a lower proportion of muscle in the FFST in the SCI group than in controls. This was confirmed by the 16.7% lower skeletal muscle mass from MRI in the SCI group than in controls when FFST in the two groups was held constant with ANCOVA \((1.73 \pm 0.19 \text{ vs. } 2.07 \pm 0.19 \text{ kg}, d = 1.79, P < 0.05)\).

The relation between muscle and FFST across the range of muscle mass was examined with regression analysis. None of the data points were identified as outliers, as indicated by Cook’s \(D\) and standardized DfBeta values of <1. The relation was strong in the SCI group \(r = 0.99, \text{standard error of the estimate (SEE) } = 0.14 \text{ kg} \) and controls \((r = 0.96, \text{SEE} = 0.14 \text{ kg}; \text{Fig. 1})\) and when the groups were combined \((r = 0.96, \text{SEE} = 0.23 \text{ kg})\). The slopes of the regression lines of the SCI group and controls were not significantly different \((P > 0.05)\), but the \(y\)-intercept for the regression was significantly lower for the SCI group, suggesting significantly less skeletal muscle mass in the SCI group across the range of FFST values.

**Table 1. Physical characteristics of men with SCI and controls**

<table>
<thead>
<tr>
<th>SCI</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>35±9</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.7±8.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79.6±19.9</td>
</tr>
<tr>
<td>Midthigh measurements</td>
<td></td>
</tr>
<tr>
<td>FFST, kg</td>
<td>1.70±0.04*</td>
</tr>
<tr>
<td>%Fat</td>
<td>33.8±16.4*</td>
</tr>
<tr>
<td>Total mass, kg</td>
<td>2.64±1.14*</td>
</tr>
<tr>
<td>Lean adipose tissue, kg</td>
<td>0.16±0.13</td>
</tr>
<tr>
<td>Muscle, kg</td>
<td>1.36±0.77*</td>
</tr>
<tr>
<td>Muscle/FFST</td>
<td>0.80±0.09*</td>
</tr>
</tbody>
</table>

Values are means ± SD; \(n = 8\). Midthigh fat-free soft tissue mass (FFST), total mass, and \%fat of men with spinal cord injury (SCI) and able-bodied controls were determined using dual-energy X-ray absorptiometry (DXA). Muscle mass and lean adipose tissue mass were determined using magnetic resonance imaging. *Statistically different from controls, \(P < 0.05\). †Different from controls, \(d = 0.67\).
mass from QCT to adipose-tissue-free mass from DXA in AIDS patients than in healthy middle-aged men (0.48 vs. 0.52) and women (0.45 vs. 0.48) observed by Wang et al. (27). In addition to studying a different population group, the present study is unique because the decline in skeletal muscle after SCI is rapid and limited almost exclusively to the lower limbs (21).

Although Wang et al. (26) reported a strong relation ($r = 0.95$, SEE = 0.59 kg) between thigh skeletal muscle determined by QCT and FFST corrected for nonmineral bone mass in a combined sample of 20 middle-aged healthy men and 7 men with AIDS, the independent relations for each group were not examined. In the present study, the relations between muscle and FFST were strong in the SCI group ($r = 0.99$) and controls ($r = 0.96$). The finding of no group difference in the slope of the regression lines but a lower intercept in the SCI group than in controls ($-11$ vs. $885$ g) further suggests that muscle mass represented a smaller amount of the FFST in the SCI group. Although close inspection of the slopes suggests that the SCI group actually had a higher slope than controls, which is contrary to what would be expected, the difference in slopes was not statistically significant and, with consideration of the small sample sizes, likely due to sampling variation. Studies with larger sample sizes are needed to adequately compare the relations between muscle mass from MRI and FFST from DXA in men with and without SCI.

Interestingly, percent fat of the midthigh from DXA was approximately twice as great in the SCI group (34%) as in controls (16%); however, adipose tissue and its estimated lean component were not different between groups. Thus, despite the larger percentage of fat in the midthigh of men with SCI, the absolute amount of fat was not different in the two groups. Because muscle mass was lower in the SCI group than in controls, the lean component of adipose tissue represented a greater proportion of FFST in the SCI group. The lean portion of adipose tissue, $\sim 20\%$ of adipose tissue (25), composed approximately half of the nonmuscle in the FFST. This is demonstrated by the reduction in the differences between midthigh skeletal muscle and FFST from 20% to 11% in the SCI group and from 11% to 5% in controls and the finding that 57% of the variance in the difference between muscle mass and FFST was accounted for by adipose tissue. The reason for the stronger relation between the difference in skeletal muscle and FFST and the lean portion of adipose tissue in controls than the SCI group is not clear. However, with consideration of the limited sample size, the discrepancy may be due to sampling variation. Future studies are needed to assess the influence of adipose tissue on the relation between muscle mass and FFST in individuals with and without SCI.

The relation between skeletal muscle and FFST has been studied in relatively healthy adults. Levine et al. (13) reported that thigh skeletal muscle measured by QCT and FFST were highly correlated in a large sample ($n = 207$) of middle-aged men and women ($r = 0.98$, SEE = 0.25 kg). In their study, the proportion of muscle in the FFST was $\sim 89\%$, which is higher than the SCI group (80%) but similar to controls (91%) in the present study. Visser et al. (24) reported that midthigh skeletal muscle mass measured by QCT and FFST were highly related in elderly men and women ($R^2 = 0.94$, SEE = 0.06 kg). However, they did not directly compare skeletal muscle and FFST. Instead, they adjusted skeletal muscle by adding an estimate of the lean component of adipose tissue (24). When

To assess the effect of adipose tissue on the prediction of muscle from FFST, muscle from MRI was adjusted for variation in adipose tissue by subtracting the estimated lean component of adipose tissue from FFST. The mean FFST for the SCI group and controls was reduced to 1.53 ± 0.92 and 2.57 ± 0.68 kg, respectively. The mean differences between mid thigh skeletal muscle and FFST were reduced by approximately one-half in each group: from 20% to 11% in the SCI group and from 11% to 5% in controls. The effect of the lean portion of adipose tissue on the prediction of muscle mass from FFST was also examined by regression against the difference in muscle mass and FFST. In the groups combined, a positive relation was observed, with the lean portion of adipose tissue accounting for more than half of the variance in the difference between muscle mass and FFST ($R^2 = 0.57$, $P < 0.05$). The strength of the relations was different in the two groups, with a weaker relation in the SCI group that was not statistically significant ($r = 0.37$, $P > 0.05$) and a stronger relation in controls ($r = 0.88$, $P < 0.05$).

**DISCUSSION**

To our knowledge, this is the first study to assess the proportion of skeletal muscle mass in the FFST of men with SCI. A major finding was that FFST contained $\sim 15\%$ less muscle in men with long-term, complete SCI than in able-bodied controls. This suggests that previous studies using DXA to estimate muscle mass may have underestimated the degree of muscle atrophy associated with SCI (21). Skeletal muscle mass from MRI and FFST from DXA were very strongly related, however, indicating that FFST can accurately predict thigh skeletal muscle mass in men with long-term, complete SCI if appropriate regression equations are developed.

The idea that men with long-term SCI have a lower proportion of muscle in the FFST than able-bodied men is supported by the 13% lower ratio of muscle to FFST in the SCI group than in controls. It is also supported by the 17% lower muscle mass, adjusted for FFST using ANCOVA, in the SCI group than in controls. Together, these findings suggest $\sim 15\%$ less muscle in the FFST in men with SCI than in able-bodied men. This is consistent with the lower ratio of total body muscle mass from QCT to adipose-tissue-free mass from DXA in elderly men and women ($0.48$ vs. $0.52$) observed by Wang et al. (27). A major finding was that FFST contained $\sim 15\%$ less muscle in men with long-term, complete SCI than in able-bodied controls. This suggests that previous studies using DXA to estimate muscle mass may have underestimated the degree of muscle atrophy associated with SCI (21). Skeletal muscle mass from MRI and FFST from DXA were very strongly related, however, indicating that FFST can accurately predict thigh skeletal muscle mass in men with long-term, complete SCI if appropriate regression equations are developed.

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the lean component of adipose tissue is added to muscle mass in the present study, the skeletal muscle proportion of FFST in the SCI group (90%) vs. controls (95%) was more similar to the proportion reported by Visser et al. for elderly subjects (91%). Together, these observations suggest that skeletal muscle is the primary component of the FFST that declines after SCI, and the loss may parallel the loss associated with aging.

Although the present study suggests that skeletal muscle may be accurately estimated in men with long-term, complete SCI if appropriate regression equations are used, tracking changes in skeletal muscle immediately after injury may be problematic. The skeletal muscle atrophy associated with SCI is remarkable. Using MRI, Castro et al. (5) observed an 18–46% lower cross-sectional area of the thigh muscles in men 6 wk after complete SCI than in able-bodied controls. The men with SCI lost an additional 8–15% during the next 18 wk (5).

If the disproportionate loss of muscle was experienced during the first 24 wk after SCI and if DXA, instead of MRI, was used to estimate muscle atrophy, the ~50% loss of muscle mass would have been estimated at 42%. The underprediction of muscle from DXA’s FFST may be equally problematic when the effect of different interventions on skeletal muscle is assessed. Although it is unlikely that the 20% increase in the quadriceps femoris of men with SCI observed after only 8 wk of electrical stimulation-evoked knee extension exercise (7) would have gone undetected by FFST from DXA, this has never been tested. Moreover, the effect of intervention strategies that elicit less pronounced increases in muscle mass may go unnoticed if FFST or other surrogates of muscle are assessed rather than muscle mass itself. This may explain, at least in part, why some exercise intervention studies do not yield increases in girth (2) or indirect estimates of muscle mass (8) in individuals with SCI, despite marked increases in weight lifted and torque. Studies are needed to assess the validity of DXA for tracking the skeletal muscle atrophy that follows SCI, or other muscle-wasting conditions, and the potential hyper trophy induced by different intervention strategies. If DXA can predict changes in skeletal muscle after SCI and subsequent intervention, it would prove to be a powerful, but practical, tool in research and clinical settings.

Because of the coordinating actions of muscle and bone and the positive relation between these companion tissues, there is a growing interest in determining the extent of their codetermination. If DXA is found to provide accurate assessment of skeletal muscle during the period of severe atrophy immediately after SCI and during subsequent intervention, it would allow for better study of the relation between muscle and bone parameters provided by DXA, such as arcal bone mineral density and bone mineral content. In addition to extreme atrophy of skeletal muscle, men with SCI can lose up to half of the bone mineral in the lower limbs (3, 4, 17). Moreover, substantial relative increases in arcal bone mineral density have been observed in individuals with SCI in response to electrical stimulation intervention (2). Because arcal bone mineral density is the most objective measurement used to detect osteoporosis (1), understanding its link to changes in skeletal muscle would further our understanding of skeletal muscle’s role in the maintenance of bone health.

One limitation of the present study is the application of the findings to the DXA instrument used to assess FFST. It is not known whether pencil-beam instruments produced by the same company or instruments produced by different companies would yield the same findings. Prior studies have demonstrated disagreement in total and regional body fat, FFST, bone mineral content, and arcal bone mineral density measures from different DXA instruments (15, 16, 22) or when different software versions are used (23). Although the reported differences are typically small in the general population, it is not known whether the difference in the proportion of skeletal muscle in the FFST in men with SCI and controls is consistent when different DXA models or software packages are used to estimate FFST.

In summary, skeletal muscle represents a smaller proportion of the FFST in the paralyzed thighs of men with long-term, complete SCI than in controls. This suggests that studies using FFST as a surrogate of skeletal muscle may underestimate muscle atrophy associated with SCI. However, because of the very strong relation between FFST and midthigh muscle mass, DXA holds promise as a method for assessing skeletal muscle status in individuals with extreme muscle atrophy. Whether DXA can accurately assess changes in skeletal muscle immediately after SCI and after intervention requires further investigation.

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GRANTS

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