Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography

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1School of Physical and Health Education, Queen's University, Kingston, Ontario, Canada K7L 3N6; 2Clinical Nutrition Program, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131; 3St-Luke's-Roosevelt Hospital, Columbia University, College of Physicians and Surgeons, New York, New York 10025; and 4Department of Anatomy, Queen's University, Kingston, Ontario, Canada K7L 3N6

Mitsiopoulos, N., R. N. Baumgartner, S. B. Heymsfield, W. Lyons, D. Gallagher, and R. Ross. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. J. Appl. Physiol. 85(1): 115–122, 1998.—Magnetic resonance imaging (MRI) and computerized tomography (CT) are promising reference methods for quantifying whole body and regional skeletal muscle mass. Earlier MRI and CT validation studies used data-acquisition techniques and data-analysis procedures now outdated, evaluated anatomic rather than adipose tissue-free skeletal muscle (ATFSM), studied only the relatively large thigh, or found unduly large estimation errors. The aim of the present study was to compare arm and leg ATFSM cross-sectional area estimates (cm²) by using standard MRI and CT acquisition and image-analysis methods with corresponding cadaver estimates. A second objective was to validate MRI and CT measurements of adipose tissue embedded within muscle (interstitial adipose tissue) and surrounding muscle (subcutaneous adipose tissue). ATFSM area (n = 119) by MRI [38.9 ± 22.3 (SD) cm²], CT (39.7 ± 22.8 cm²), and cadaver (39.5 ± 23.0 cm²) were not different (P > 0.001), and both MRI and CT estimates of ATFSM were highly correlated with corresponding cadaver values [MRI: r = 0.99, SE of estimate (SEE) 3.9 cm², P < 0.001; and CT: r = 0.99, SEE = 3.8 cm², P < 0.001]. Similarly good results were observed between MRI- and CT-measured vs. cadaver-measured interstitial and subcutaneous adipose tissue. For MRI-ATFSM the intraobserver correlation for duplicate measurements in vivo was 0.99 (SEE = 8.7 cm² (2.9%), P < 0.001). These findings strongly support the use of MRI and CT as reference methods for appendicular skeletal muscle, interstitial and subcutaneous adipose tissue measurement in vivo.

body composition; skeletal muscle mass; subcutaneous adipose tissue

SKELETAL MUSCLE is the largest non-adipose tissue body composition component (25). The need to accurately measure skeletal muscle in vivo is apparent in several disciplines. Proper interpretation of the effects of aging on muscle wasting in geriatrics, the catabolic effects of disease in clinical medicine, and the anabolic effects of physical training in exercise science all require accurate estimates of skeletal muscle mass (11). Several methods of quantifying total body and regional skeletal muscle were developed over the past few decades that rely on selected muscle properties, including creatine content, potassium concentration, and electrical conductivity (8). Most of the presently available methods rely on a series of assumptions that are difficult to validate in vivo, and much of the present knowledge related to skeletal muscle mass is based on cadaver studies (5, 8).

An important aspect of studying skeletal muscle mass in vivo is to establish an accurate reference method. The aforementioned methods based on skeletal muscle properties are not sufficiently accurate, nor have they been adequately validated in healthy children and adults. An alternative to these methods as a reference standard are two imaging methods, computerized tomography (CT) and magnetic resonance imaging (MRI) (10). The advantage of CT and MRI over earlier methods is direct visualization of images depicting skeletal muscle cross-sectional area. These images can be used per se or combined with mathematical reconstruction algorithms to estimate the mass of individual muscle groups or total body skeletal muscle mass (10).

The use of CT and MRI as reference standards is based on the main assumption that measured cross-sectional area is equivalent to actual skeletal muscle cross-sectional area. Two types of muscle can be considered, the first related to muscle tissue that includes interstitial adipose tissue (IAT), “anatomic skeletal muscle,” and the second specifically related to adipose tissue-free skeletal muscle (ATFSM). In healthy young adults, anatomic skeletal muscle is only slightly larger than ATFSM. However, IAT increases with increasing obesity and age. Some disease states, such as muscular dystrophy, are also accompanied by a relatively large mass of IAT.

A need exists to develop ATFSM reference measurement methods that are practical and can be applied on a large scale. MRI offers this opportunity because it is widely available and can be applied in male and female subjects of all ages. Moreover, recent advances in MRI hardware and software permit rapid whole body imaging in <30 min (21). An important concern, however, is the accuracy of muscle area measurements by these new approaches. The accuracy of an obtained muscle cross-sectional area estimate is related to both image-acquisition and subsequent analysis. Earlier investigators who attempted to validate MRI and CT skeletal muscle estimates used scanning and image-analysis
procedures now outdated (2, 6), evaluated anatomic rather than ATFSM (6), studied only the relatively large thigh (2, 12), or found unduly large estimation errors (6, 12).

We recently proposed a practical approach for using whole body MRI to quantify total and regional skeletal muscle volume (21). This approach has the potential of establishing MRI as a useful skeletal muscle mass reference method. The aim of the present study was to overcome earlier study limitations by comparing arm and leg ATFSM cross-sectional area estimates by our MRI acquisition and analysis approach with corresponding cadaver estimates. The study also included a similar set of analyses for CT, which is still used in estimating regional skeletal muscle. Because adipose tissue both surrounds and is embedded within skeletal muscles, the study design included an analysis of the accuracy of MRI- and CT-derived estimates of IAT and subcutaneous adipose tissue (SAT). Finally, in a separate experiment designed to determine the utility of IAT measurement in vivo, we compared skeletal muscle composition by MRI in obese and lean subjects.

METHODS

Validity

Phantoms. Validity of volume estimates by MRI were determined by using two distinctly different-shaped phantoms. Phantom 1 was adapted from a polyethylene mold used to construct a prosthetic leg. Phantom 2 was constructed by bonding together two funnels to create an object with a more severe grade from beginning to end. A volumetric flask was used to determine the true volume of the phantoms by filling each with a paramagnetic solution. To determine whether variations in image spacing influence volume calculations, an MRI protocol that used 16 images with 10-mm spacing was compared with one that used 7 images with 40-mm spacing. For both protocols the image thickness was 10 mm. Magnetic resonance (MR) images were obtained with a General Electric Signa Advantage, 1.5-T scanner using standard procedures: 120 kVp, 220 mA, 512×512 matrix, and a 345-mm² field of view. The field of view was determined by using dimensions obtained from metric rulers simultaneously photographed with each cadaver slice. The pixel size (matrix size divided by field of view) for all leg images was 0.21 mm²; for the arm images the pixel size was 0.08 mm². Thus a very high resolution was obtained for all cadaver images. The different pixel size for the arm and leg images was due to different camera positions required to obtain similar-size pictures.

To examine whether the semifrozen state of the cadaver sections during photographing would affect area measurements, circumference measurements were taken at four sites on the leg and at two sites on the arm before and immediately after freezing. The mean circumferences from each site before and after freezing were not different (P > 0.10) (data not shown).

MRI Cadaver Image Procedure

The cadaver leg and arm were imaged separately. For the leg, the crest of the femoral head was landmarked, and 8-mm-thick images (2-mm spacing) were obtained over the entire limb. Beginning at the top of the humeral head, the same methodology was used to acquire the MR images for the entire cadaver arm. The MR images were obtained by using the T1-weighted, spin-echo sequence (repetition time = 210 ms; echo time = 17 ms; 1 excitation used to acquire the phantom data with a 512×512 image matrix and a 480-mm² field of view). The CT Cadaver Image Procedure

CT data was obtained by using standard procedures: 120 kVp, 220 mA, 512×512 matrix, and a 345-mm² field of view.

Table 1. Comparison between actual and magnetic resonance imaging-derived phantom volumes by using sets of 16 and 7 images

<table>
<thead>
<tr>
<th>No. of Images</th>
<th>Equation No.</th>
<th>Phantom 1 Volume, liters</th>
<th>Difference, %</th>
<th>Phantom 2 Volume, liters</th>
<th>Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1</td>
<td>5.62*</td>
<td>3.0%</td>
<td>3.70</td>
<td>-0.2%</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>5.79</td>
<td>2.8%</td>
<td>3.70</td>
<td>0.8%</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>5.79</td>
<td>3.0%</td>
<td>3.73</td>
<td>1.0%</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>5.79</td>
<td>2.8%</td>
<td>3.74</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

Volumes were determined by using the following equations:

\[ V = \sum_{i=1}^{N} A_i t + h/3 \sum_{i=2}^{N} [(A_{i-1} + A_i) + \sqrt{(A_{i-1} A_i)}] \]  

(see Ref. 21)  

\[ V = \sum_{i=1}^{N} [(A_i + A_{i-1})/2]^h \]  

(see Ref. 14)  

where \( V \) is the total tissue volume, \( A \) is the cross-sectional tissue area, \( t \) is the slice thickness, \( h \) is the distance between consecutive slices, and \( N \) is the total no. of images (i), and * is actual measured volume of phantoms.
With the exception of slice thickness, CT procedures matched those used for MRI. For CT, acquisition of 8-mm-thick images was not possible; thus 7-mm-thick images were obtained every 3 mm from the femoral head to the ankle (leg) and from the humeral head to the wrist (arm).

Calculation of Adipose Tissue and Skeletal Muscle Area and Volume

Once obtained, all MR and CT image data was transferred via DAT tape onto the same workstation used to evaluate cadaver data and analyzed by using the same software used to analyze cadaver images (Tomovision, Montreal, PQ). The segmentation method used to determine tissue area is based on image morphology and employs a combination of edge detection filters and Watershed techniques (3). Initially, a filter is used to distinguish between different gray-level regions on the image. Once the edges are determined, lines are drawn on the image by using a Watershed algorithm (3). If the regions (i.e., a group of voxels) are too small, they can be merged by using statistical parameters inherent in the image. Once the regions representing the various tissues (i.e., skeletal muscle, IAT, and SAT) are identified, the observer uses a mouse pointer to identify each tissue by using color codes. Each image is then reviewed by using an interactive slice-editor program that allows for verification and, where necessary, correction of the segmentation result (21). This operation is facilitated by superimposing the original gray-level image on the binary segmented image by using a transparency mode (21). To calculate tissue area (cm²), the respective tissue regions in each slice are computed automatically by summing the given tissues' pixels and multiplying by the pixel surface area. Tissue volume (cm³) for each slice is calculated by multiplying the tissue area (cm²) by slice thickness.

CT-adipose tissue and -ATFSM tissue area was segmented by using standard Hounsfield unit ranges for adipose tissue (−190 to −30), skeletal muscle (−29 to +150), and bone (+152 to +1,000). Where necessary, corrections were made for beam hardening or other artifacts commonly associated with routine CT data acquisition in vivo. All corrections were made by using the same procedures described for MRI.

The resolution between skin and SAT on cadaver images did not permit discrimination between the two tissues, and thus skin was included in the measurement of cadaver-SAT. Because skin is not readily identified on MRI and CT images, it was necessary to make a correction assuming that the skin had a thickness of 1 mm (25) and that the limb was circular. Because the limbs were not circular (Fig. 2), we tested the accuracy of our assumption by comparing the measured circumference obtained for six cadaver sections (4 leg, 2 arm) to the predicted values. The predicted value underestimated skin thickness by ~3%. An error of this magnitude has a <1% influence on the measurement of cadaver-SAT.

Measurement of IAT In Vivo

In a separate experiment, the utility of measuring IAT within skeletal muscle in vivo was determined though a comparison of skeletal muscle composition in a group of 10 obese male (body mass index [BMI], 32.4 ± 2.1) and 10 lean (BMI, 21.9 ± 2.4) subjects (8 men, 2 women) who were matched for ATFSM cross-sectional area (see Table 4). The subjects' data was obtained in retrospect from a database of subjects who had participated in previous studies within our laboratory designed to determine body composition by MRI. Skeletal muscle area (cm²) was obtained from a single MR image obtained at the proximal thigh level. All subjects had provided their informed, written consent. Similar data for CT were unavailable.

Reliability

The reliability of MRI-ATFSM and -SAT area (cm²) and volume (cm³) measurements in vivo was determined by comparing intra- and interobserver estimates for repeated measurements obtained in three male (age 22.0 ± 1.4 yr; BMI 28.9 ± 6.6) and three female (age 24.3 ± 0.9 yr; BMI 20.5 ± 0.7) subjects. For all subjects, by using the crest of the femoral head as a landmark, a series of seven MR images (10-mm thickness, 40-mm spacing) were acquired on separate days by using the T1-weighted, spin-echo pulse sequence used to obtain the phantom data and described in detail previously (21). For intraobserver error (repeatability), a single observer analyzed each set of 42 images (6 subjects × 7 images) acquired on 2 separate days. Interobserver error (reproducibility) was determined by comparing the results of two observers’ analyses of the same 42 images. Although not obtained in this study, a previous investigation reported that the coefficient of variation for repeat CT-skeletal muscle area (cm²) measurements is 1.4% (12).

The intraobserver error for measurements of cadaver-skeletal muscle was determined by comparing estimates obtained from 119 cadaver images. A single observer performed two sets of analyses separated by several months.

Statistical Analyses

The reliability error for duplicate cadaver-skeletal muscle measurements for 119 images was determined by using simple regression. ANOVA by using a repeated-measures design was performed to compare corresponding skeletal muscle, SAT, and IAT area (cm²) data obtained from 119 MR, CT, and cadaver images. Regression analyses were also performed to determine the nature of the relationship among the three methods for skeletal muscle, SAT and IAT area (cm²). The slope and intercepts of the respective regression lines were compared with the line of identity by using techniques described by Kleinbaum and Kupper (13). Intra- and interobserver reliability data from duplicate MRI measurements in vivo were compared by using a paired t-test and simple regression techniques. Statistical analyses were performed by using SYSTAT (Evanston, IL).

RESULTS

Reliability

Cadaver. The reliability for cadaver-ATFSM measurements was determined by analyzing 119 images in duplicate. Regression analyses revealed a correlation of 0.99 and a SE of the estimate (SEE) of 3.38 cm² or 8.5%. Analyses of the regression line revealed that the intercept was not different from zero and that the slope was not different from 1.0 (P > 0.10). These results suggest that when either MRI- or CT-ATFSM and cadaver values are compared, differences of <8.5% may be explained by error inherent in our method of determining cadaver-ATFSM.

MRI. For intraobserver ATFSM area measurements, data from six subjects were combined, and thus duplicate analyses for two sets of 42 images were compared. The correlation coefficient between measurements was 0.99 (P < 0.001) with a SEE of 8.8 cm² or 2.9% (Fig. 1).
The interobserver correlation coefficient for duplicate (same image) MRI-ATFSM area (cm²) was 0.99 (P < 0.001) with a SEE of 7.9 cm² (2.6%). The intraobserver correlation coefficient for duplicate IAT area (cm²) was 0.96 with a SEE of 1.7 cm². For MRI-ATFSM volume (liters), the intra- and interobserver difference was 0.34 ± 1.1 and 1.8 ± 0.6%, respectively. For SAT area (cm²), the correlation coefficients for intra- and interobserver measurements were 0.99 (SEE = 6.0 cm², 2.5%) and 0.99 (SEE = 6.5 cm², 3.3%), respectively. For SAT volume (liters), the differences for intra- and interobserver analyses were 1.5 ± 1.5 and −2.9 ± 1.2%.

Validity

MRI volume comparison to phantoms. MRI volume (cm³) did not differ from the measured volume for either of the two phantoms (Table 1). This was true independent of phantom shape or number of images (7 vs. 16) used to derive volume. In addition, for both phantoms, the MRI volume derived by using either of the two mathematical algorithms was not different.

Comparison of MRI, CT, and cadaver cross-sectional area values. A typical example of an MRI, CT and cadaver image is illustrated in Fig. 2. Analysis of variance revealed that for ATFSM and SAT, CT, MRI and cadaver cross-sectional area (cm²) values were not different (P > 0.05; Table 2).

MRI and CT estimates of ATFSM and SAT area were strongly correlated with corresponding cadaver values (Fig. 3). Correlations derived from regression analyses ranged from 0.98 to 0.99 for all variables (P < 0.001). In all cases the slopes and intercepts of the respective regression lines were not different from one and zero, respectively (P > 0.10). Compared with cadaver, for both MRI and CT the SEE for all tissues were of similar magnitude (Fig. 3).

The distribution of differences for ATFSM among MRI, CT, and cadaver are given in Fig. 4. For MRI, 81% of the differences observed were within the technical error associated with repeat analysis of the cadaver images (±8.5%), while the corresponding value for CT was 68%.

Comparison of MRI, CT, and cadaver volume values. MRI and CT volume (cm³) estimates for ATFSM and SAT were derived for the arm and leg separately by using Eq. 1 given in Table 1. The results provided in Table 3 reveal that for ATFSM and SAT, the values derived by MRI and CT were in good agreement with corresponding cadaver values. By comparison to cadaver, the relative differences for both MRI- and CT-skeletal muscle were 1.3%.

Comparison of MRI, CT, and cadaver values for skeletal muscle IAT. MRI- and CT-IAT values were not different from cadaver-IAT (P > 0.05) (Table 2).
though both MRI- and CT-IAT values were strongly correlated ($P < 0.01$) with corresponding cadaver values (Fig. 3), the SEE was $\sim 30\%$. Comparison between methods revealed that anatomic skeletal muscle (skeletal muscle plus IAT) and ATFSM area ($cm^2$) values were not different ($P > 0.05$). However, for all methods, ATFSM area was significantly less than anatomic skeletal muscle ($P < 0.01$). This observation reflects the fact that IAT represented $\sim 24\%$ of the skeletal muscle area for the 119 cadaver sections.

### Table 2. Comparison among Cad, MRI, and CT measurements for ATFSM, SAT, and IAT areas

<table>
<thead>
<tr>
<th></th>
<th>Cad</th>
<th>MRI</th>
<th>CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATFSM</td>
<td>39.5 ± 23.1</td>
<td>38.9 ± 22.4</td>
<td>39.7 ± 22.8</td>
</tr>
<tr>
<td>SAT</td>
<td>44.9 ± 38.0</td>
<td>44.2 ± 36.6</td>
<td>44.6 ± 36.2</td>
</tr>
<tr>
<td>IAT</td>
<td>12.4 ± 12.5</td>
<td>12.7 ± 13.1</td>
<td>11.7 ± 13.1</td>
</tr>
</tbody>
</table>

Values are means ± SD given in $cm^2$ for 119 images. Cad, cadaver; MRI, magnetic resonance imaging; CT, computerized tomography; ATFSM, adipose tissue-free skeletal muscle; SAT, subcutaneous adipose tissue; IAT, interstitial adipose tissue. MRI and CT values for all variables are not different from Cad at $P = 0.05$.

Having demonstrated that MRI and CT provide comparable estimates of skeletal muscle composition, we performed a second experiment to determine the magnitude and distribution of IAT within skeletal muscle in vivo. To do so we analyzed skeletal muscle composition for a single MR image obtained at the proximal thigh level in 10 obese male and 10 lean subjects. Inspection of Table 4 reveals that, despite no difference between the groups for ATFSM cross-sectional area ($P > 0.10$), the mean IAT value for the obese subjects ($13.8 \pm 1.3 \, cm^2$) was significantly ($P < 0.001$) greater than the corresponding value observed in the lean subjects ($3.1 \pm 1.4 \, cm^2$). Whereas for the obese subjects IAT represented 8.0% of the total (anatomic) skeletal muscle area, IAT represented only 2.1% of the total skeletal muscle area in the lean subjects. Although we do not report similar observations using CT, the fact that both imaging methods provide estimates for IAT that are not different (Fig. 3) demonstrates that either modality may be used to determine muscle composition in vivo.

**Fig. 3.** Regression analyses among MRI, CT, and Cad for ATFSM (A), subcutaneous adipose tissue (B) cross-sectional areas (CSA), and interstitial adipose tissue (C).
DISCUSSION

The primary observation of this study is that both MRI and CT methods provide accurate estimates of appendicular ATFSM, IAT, and SAT compared with cadaver sections. For MRI a comparison to phantom data also confirm the accuracy of the technique. In addition, MRI provides reliable measurements of appendicular ATFSM, IAT, and SAT in vivo.

Phantom Studies

Validation studies can be performed by using phantoms, animals, or human cadavers. Experiments that use phantoms are useful because the geometric accuracy of the reconstructed image areas as well as volume measurements can be verified. In a previous study (19), we compared the mean cross-sectional areas of polyvinyl tubes computed from a set of 12 transverse MR images with the actual measurement. In that study the mean tube area obtained from MR images was 2.45 cm² and differed from the actual mean area (2.48 cm²) by only 0.6% (19). These observations are consistent with Stark et al. (27), who, by using an 11-cm-long Lucite phantom filled with oil, reported that MRI measurement errors for volume were less than 1%.

In this study we extend previous observations by demonstrating that MRI accurately predicts the volume of phantoms independent of shape, mathematical formula, and number of images (7 vs. 16). Phantoms of different shape were designed to test the assumption of linearity in the mathematical formulas employed to calculate volume. Although the accuracy of volume for any tissue would be improved if contiguous images were obtained (i.e., no space between images) were obtained, our findings indicate that volumes can be estimated with an error <1% when spacing between images is between 10 and 40 mm. Taken together, present evidence based on phantom data strongly supports the accuracy of MRI because the error for both area (cm²) and volume (cm³) measurements are <2%.

Cadaver Studies

The findings of this study demonstrate that MRI and CT estimates of ATFSM are in good agreement with those obtained from cadaver sections. For MRI these observations are consistent with earlier work by Engstrom et al. (6), who reported that the correlation coefficient between corresponding MRI- and cadaver-skeletal muscle obtained at the proximal thigh level approached unity (>0.99). Beneke et al. (2) compared MRI-skeletal muscle areas from two images to cadaver-skeletal muscle and reported a mean error of 1.2%. In both studies a limited number of images were obtained from a restricted anatomic region. We examined the accuracy of MRI-skeletal muscle measurement by using a large number of cadaver images varying widely in skeletal muscle cross-sectional area. Our findings clearly demonstrate that MRI provides accurate measurements of skeletal muscle cross-sectional area through

Table 3. Comparison of Cad, MRI, and CT volume estimates for ATFSM, SAT, and IAT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cad</th>
<th>MRI</th>
<th>CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATFSM</td>
<td>1.49</td>
<td>1.47</td>
<td>1.51</td>
</tr>
<tr>
<td>SAT</td>
<td>1.15</td>
<td>1.14</td>
<td>1.18</td>
</tr>
<tr>
<td>IAT</td>
<td>0.27</td>
<td>0.22</td>
<td>0.18</td>
</tr>
<tr>
<td>Leg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATFSM</td>
<td>3.17</td>
<td>3.13</td>
<td>3.18</td>
</tr>
<tr>
<td>SAT</td>
<td>4.20</td>
<td>4.08</td>
<td>4.08</td>
</tr>
<tr>
<td>IAT</td>
<td>1.20</td>
<td>1.27</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Values are given in liters. Cad, MRI, and CT volumes were derived by using Eq. 1 (Table 1).

Table 4. Comparison of skeletal muscle composition in obese and lean subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obese</th>
<th>Lean</th>
<th>Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>32.4 ± 2.1</td>
<td>21.9 ± 2.4*</td>
<td></td>
</tr>
<tr>
<td>ATFSM, cm²</td>
<td>161.4 ± 11.1</td>
<td>151.8 ± 31.9</td>
<td>6.2</td>
</tr>
<tr>
<td>IAT, cm²</td>
<td>13.8 ± 1.3</td>
<td>3.1 ± 1.4*</td>
<td>77.5</td>
</tr>
</tbody>
</table>

Values are means ± SD obtained from a single magnetic resonance image obtained at the proximal thigh level from 10 subjects in each group. BMI, body mass index. *Significantly different from obese; \( P = 0.001 \).
out a wide range of values (≈10–100 cm²) typical of the appendicular region.

In contrast to our observations, previous studies suggest that CT overestimates cadaver-skeletal muscle by ≈8 (11) to 20% (6). In those studies cross-sectional areas of individual muscles (i.e., sartorius and gracilis) were compared rather than total skeletal muscle area. Moreover, no attempt was made to identify the contribution of IAT in muscle, and, in one study, the observations were based on only four images (12). Taken together, these factors make direct comparisons with our observations difficult.

Although in the present study CT estimates of ATFSM were in good agreement with cadaver values, inspection of Fig. 4 reveals that the differences between cadaver and CT were negatively skewed. On review it was noted that the majority of these values represent axial images obtained in the lower leg region in which the absolute skeletal muscle area was <15 cm². Why CT underestimated skeletal muscle in this region is unclear; however, the findings reported in Fig. 4 indicate that, for appendicular regions in which the majority of skeletal muscle is located (i.e., thigh), CT provides accurate estimates.

In previous validation studies it is unclear whether the measurements included IAT embedded in skeletal muscle. In this study we report the accuracy of MRI and CT measurements for both skeletal muscle per se (i.e., ATFSM) and IAT. Indeed, we report for the first time strong evidence clearly demonstrating that MRI and CT provide accurate estimates of both ATFSM and IAT. The need to identify IAT within skeletal muscle in vivo is supported by our comparison of muscle from obese and lean subjects in whom, despite similar ATFSM, infiltration of IAT was ~77% greater in muscles from obese compared with lean subjects. Thus the difference in muscle quality between the groups was substantial. The measurement of IAT within skeletal muscle may have value when studying the underlying pathology in aging and metabolic diseases. IAT is increased in the elderly (4) and may serve as a marker of age-related skeletal muscle changes.

Infiltration of skeletal muscle with IAT is a hallmark of genetic disorders such as Duchenne muscular dystrophy (16). A recent observation is that CT-derived muscle attenuation values are strong correlates of insulin resistance in obese subjects (24). According to Simonneau et al. (24), CT-derived attenuation values are a biophysical characteristic reflecting muscle chemical composition (i.e., lipid concentration). Our findings suggest that, in addition to total attenuation value, IAT by MRI or CT can be accurately quantified. Taken together, these observations strongly support a role for skeletal muscle composition evaluation in future research encompassing a range of clinical topics.

To our knowledge, this is the first study to show that both MRI and CT provide valid estimates of appendicular SAT by comparison to cadaver sections. Indeed, the coefficient of variation for both methods was ~2%, and with both the estimates of SAT were precise throughout a wide range of values. These observations suggest that either method can accurately describe appendicular SAT distribution. Combined with the observations of Abate et al. (1), who report good agreement between MRI-measured abdominal SAT and visceral adipose tissue estimates by comparison to cadaver values, and those of Rössner et al. (22), who report similar observations for CT, it is suggested that both methods may serve as a criterion measure for appendicular and whole body adipose tissue distribution.

**Measurement Variation**

The reproducibility error for MRI-ATFSM and -SAT cross-sectional area (cm²) was determined by obtaining and analyzing duplicate MR images obtained on separate days. It is important to distinguish these results from those that report reliability data obtained from duplicate analyses of the same MR image. We report a coefficient of variation for intraobserver MRI-skeletal muscle ~2%, which is consistent with the studies of others in which the coefficient of variation for skeletal muscle reproducibility in the appendicular region ranges from 0.3 to 2.3% (2, 5, 15, 20). In this study errors of similar magnitude were noted for interobserver analyses of MRI-skeletal muscle and -SAT, confirming the observation that appendicular skeletal muscle and SAT can be identified on MRI images in a straightforward manner. Together, these observations suggest that the expected error of measurement for MRI-skeletal muscle and -SAT cross-sectional area in vivo is ~2%.

**Area Vs. Volume Measurements**

For both ATFSM and SAT, the relationship among MRI, CT, and cadaver improved when volume estimates derived by using multiple images were compared (Table 3). For both imaging modalities, when volume is calculated from a series of images, the variability associated with analyses of single images is reduced. Because there are no known health risks associated with MRI, this technique is well suited to multiple-image protocols. Moreover, because the acquisition time for a series of MR images (i.e., 7 images) is similar to the time required to obtain a single image, volume measurements can be obtained without increased time or expense. Therefore, estimates of tissue volume based on a series of contiguous or evenly spaced MR images may be a more effective method of describing and measuring changes in ATFSM in vivo.

**Imaging Protocols**

The CT imaging procedure employed in this study is routine and commonly used to obtain images with good contrast between various soft tissues (9). In contrast, there is more flexibility with respect to the MRI protocol. We employed a T1-weighted, spin-echo pulse sequence. Although other T1-weighted protocols (i.e., inversion recovery) produce images with good contrast between adipose tissue and skeletal muscle (23), and of comparable quality to spin-echo derived images (26), the acquisition time required to do so is substantially greater. For this reason alone T1-weighted spin-echo
has become the standard radio-frequency sequence in body composition studies. Indeed, the findings reported here support the validity and clinical utility of T1-weighted, spin-echo protocols when body composition data in vivo are being obtained.

Conclusion

In summary, the findings of this study confirm and substantially extend earlier observations that support MRI and CT as accurate methods for estimating appendicular ATFSM, IAT, and SAT in vivo. Accordingly, both MRI and CT are suitable for use as criterion methods when other body composition methods are being calibrated. Indeed, the reproducibility for the two methods in estimating ATFSM or SAT is $-$2% and suggests that both MRI and CT are capable of detecting small differences or changes in soft tissue composition. Imaging methods are thus ideally suited for quantifying skeletal muscle composition and adipose tissue distribution in both cross-sectional and longitudinal studies.

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