Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr

IAN JANSSEN,1 STEVEN B. HEYMSPIELD,2 ZIMIAN WANG,2 and ROBERT ROSS1
1School of Physical and Health Education, Queen’s University, Kingston, Ontario, Canada K7L 3N6; and 2Obesity Research Center, St. Luke’s/Roosevelt Hospital, Columbia University, College of Physicians and Surgeons, New York, New York 10025

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Janssen, Ian, Steven B. Heymsfield, ZiMian Wang, and Robert Ross. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. J Appl Physiol 89: 81–88, 2000.—We employed a whole body magnetic resonance imaging protocol to examine the influence of age, gender, body weight, and height on skeletal muscle (SM) mass and distribution in a large and heterogeneous sample of 468 men and women. Men had significantly ($P < 0.001$) more SM in comparison to women in both absolute terms (33.0 vs. 21.0 kg) and relative to body mass (38.4 vs. 30.6%). The gender differences were greater in the upper (40%) than lower (33%) body ($P < 0.01$). We observed a reduction in relative SM mass starting in the third decade; however, a noticeable decrease in absolute SM mass was not observed until the end of the fifth decade. This decrease was primarily attributed to a decrease in lower body SM. Weight and height explained ~50% of the variance in SM mass in men and women. Although a linear relationship existed between SM and height, the relationship between SM and body weight was curvilinear because the contribution of SM to weight gain decreased with increasing body weight. These findings indicate that men have more SM than women and that these gender differences are greater in the upper body. Independent of gender, aging is associated with a decrease in SM mass that is explained, in large measure, by a decrease in lower body SM occurring after the fifth decade.

magnetic resonance imaging; aging; human skeletal muscle

There is growing awareness of the importance of skeletal muscle (SM) in many physiological and disease processes, including the influence of aging on muscle wasting (2, 19, 20, 21, 27, 30, 34) and the anabolic effects of physical training on muscle size (19, 36, 41). Identification of individuals with low or high quantities of muscle mass requires normative data based on large and heterogeneous sample sizes wherein SM is measured using a criterion method. Absent from the literature are studies reporting values for SM mass that meet these criteria.

Although several studies have assessed the influence of age and gender on SM (10, 16, 20, 21, 26, 42), few have examined the influence of these variables on the distribution of SM (20, 21). In addition, these studies are generally characterized by relatively small sample sizes and/or the use of an indirect method (i.e., total body potassium) for estimating muscle tissue. Given the importance of SM in both clinical and applied medicine (13, 14), there is a need to establish reference values on the basis of age and gender. Moreover, understanding the independent influence of age and gender on SM mass may be useful in the development of therapeutic strategies designed to preserve SM, improve functional capacity, and decrease health risks, particularly for elderly men and women.

There is now solid evidence to support the observation that magnetic resonance imaging (MRI) provides precise and reliable measurements of SM (5, 12, 32) and thus can be considered a criterion method for measuring SM in vivo (23, 28). We have previously reported that the correlation coefficient between corresponding MRI and cadaver sections of human appendicular SM approached unity and that the relative difference between MRI and cadaver SM measurements was 1.3% (32). This observation is consistent with others who report that MRI provides valid estimates of SM by comparison to cadaver data (5, 12).

The specific aim of this study was twofold: first, to establish reference data for total and regional SM mass in men and women; and, second, to examine the influence of age, gender, and simple anthropometric measurements on total and regional SM distribution. To accomplish this, we measured SM in a heterogeneous sample of 468 women and men using a whole body MRI protocol.

METHODS

Subjects consisted of healthy adult men ($n = 268$) and women ($n = 200$) who had participated in body composition studies at Queen’s University (Kingston, ON) and St. Luke’s/Roosevelt Hospital (New York, NY). The subjects varied in age (18–88 yr) and adiposity [body mass index (BMI) 16–48 kg/m²]. None of the subjects was taking medications (i.e., hormone replacement therapy) known to affect the study variables. One hundred and ninety-nine subjects were studied in Kingston, and 269 subjects were studied in New York.

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Subjects were recruited from among hospital employees, students at local universities, and the general public through posted fliers and local media. All participants gave informed consent before participation in accordance with the ethical guidelines of the respective institutional review boards.

**SM Measurement by MRI**

The MRI images were obtained with a General Electric 1.5-T scanner (Milwaukee, WI). A T1-weighted, spin-echo sequence with a 210-ms repetition time and a 17-ms echo time was used to obtain the MRI data. The MRI protocol is described in detail elsewhere (36). Briefly, the subjects lay in the magnet in a prone position with their arms placed straight overhead. With the use of the intervertebral space between the fourth and fifth lumbar vertebrae (L₄-L₅) as the point of origin, transverse images (10-mm slice thickness) were obtained every 40 mm from hand to foot, resulting in a total of ~41 images for each subject (6 data sets of 7 images). The total time required to acquire all of the MRI data for each subject was ~25 min. All MRI data was transferred to a computer workstation (Silicon Graphics, Mountain View, CA) for analysis by specially designed image-analysis software (Tomovision, Montreal, PQ).

**Segmentation and SM Area, Volume, and Mass**

The model used to segment the various tissues is fully described and illustrated elsewhere (32, 36). Briefly, a multiple-step procedure was used to identify tissue area (cm²) for a given MRI image. In the first step, one of two techniques was used. Either a threshold was selected for adipose tissue and lean tissue on the basis of the gray-level histograms of the images (36), or a filter distinguished between different gray-level regions on the images, and lines were drawn around the different regions using a watershed algorithm (32). Next, the observer then labeled the different tissues by assigning them different codes. Each image was then reviewed by an interactive slice-editor program that allowed for verification and, where necessary, correction of the segmented results. The original gray-level image was superimposed on the binary segmented image by using a transparency mode to facilitate the corrections. The area (cm²) of SM in each image was computed automatically by summing the SM pixels and multiplying by the individual pixel surface area. The volume (cm³) of SM in each slice was calculated by multiplying tissue area (cm²) by the slice thickness (10 mm). The volume of SM for the space between two consecutive slices was calculated with the use of a mathematical algorithm given elsewhere (32). SM volume units (liters) were converted to mass units (kg) by multiplying the volumes by the assumed constant density for adipose tissue-free SM (1.04 kg/l) (40).

Whole body SM mass was determined using all 41 images. To determine whether regional differences existed, the body was divided into different anatomic regions. Lower body SM mass was calculated using the images extending from one image below L₄-L₅ to the foot, whereas upper body SM mass was calculated using the images extending from L₄-L₅ to the hand.

We determined whether the relationships between age, gender, and whole body SM differed if SM was measured using either a single image [i.e., area (cm²)] or a single series of images [i.e., 7 images obtained simultaneously, partial volume (cm³)] obtained in the appendicular regions. SM area (cm²) was measured in the midthigh at a level 20 cm below the femoral head. The partial volume (cm³) of SM in the thigh region was calculated using a series of seven images extending from the femoral head to 30 cm below. The partial volume for SM in the arm region was calculated using a series of seven images extending from the humeral head to 30 cm above. These regions were chosen because identification of the humeral and femoral heads facilitated the use of a common landmark and because studies that measure SM using a single computerized tomography (CT) image typically measure SM in these regions (12, 29, 31, 35).

**Anthropometric Variables**

Body mass was measured to the nearest 0.1 kg with the subjects dressed in light clothing. Barefoot, standing height was measured to the nearest 0.5 cm with a wall-mounted stadiometer.

**Reliability of MRI Measurements**

Our laboratory has recently determined the reproducibility of MRI-SM measurements by comparing the intra- and interobserver estimates for MRI measurements (1 series of 7 images taken in the legs) obtained in three male and three female subjects (32). The interobserver difference was 1.8 ± 0.6%, and the intraobserver difference was 0.34 ± 1.1% (32). The intraobserver difference was calculated by comparing the analysis of two separate MRI acquisitions in a single observer, whereas the interobserver difference was determined by comparing two observers’ analysis of the same images. The reproducibility of MRI-SM measurements across the laboratories was determined by comparing the analysis from two laboratories of the same images (whole body) for five subjects. The interlaboratory difference was 2.0 ± 1.2%.

**Statistical Analysis**

The differences between men and women were tested for significance by a paired t-test. An analysis of covariance was used to compare SM in the men and women when it was necessary to adjust for other gender differences (i.e., height and body weight).

Pearson correlations and multiple-regression analyses were performed to determine the relationship between SM mass and age, height, and body weight within each gender. Multiple-regression analysis and analysis of variance were used to determine the equality of the slopes and intercepts for the regression lines. In addition, potential interaction terms and nonlinear relationships were explored for selected variables.

Data are expressed as group means ± SD. The 0.05 level of significance was used for all data analysis. Data were analyzed using SYSTAT (Evanston, IL).

**RESULTS**

**Subject Characteristics**

The anthropometric characteristics for the men and women are listed in Table 1. The subjects varied in age (18–88 yr) and adiposity (BMI 16–48 kg/m²). Sixty-seven percent of the subjects were Caucasian, 17% were African-American, 8% were Asian, and 7% were Hispanic. The men (40 ± 14 yr) and women (43 ± 16 yr) were not different with respect to age (P > 0.05). However, the men were taller, heavier, and had a larger BMI in comparison to the women (P < 0.01; Table 1).
Table 1. Subject characteristics

<table>
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<tr>
<th>Gender and Age</th>
<th>Range, yr</th>
<th>n</th>
<th>Weight, kg</th>
<th>Height, cm</th>
<th>BMI, kg/m²</th>
<th>Total SM, kg</th>
<th>Relative SM, %</th>
<th>Lower Body, SM, kg</th>
<th>Upper Body, SM, kg</th>
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<td>Women</td>
<td>18–29</td>
<td>40</td>
<td>65.6 ± 16.8</td>
<td>164 ± 6</td>
<td>24.1 ± 5.3</td>
<td>21.8 ± 4.6</td>
<td>34.1 ± 5.7</td>
<td>12.5 ± 2.6</td>
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<td></td>
<td>30–39</td>
<td>63</td>
<td>73.6 ± 21.3</td>
<td>165 ± 7</td>
<td>27.0 ± 7.3</td>
<td>21.6 ± 3.7</td>
<td>30.6 ± 5.6</td>
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<td>40–49</td>
<td>46</td>
<td>75.6 ± 17.1</td>
<td>162 ± 7</td>
<td>28.9 ± 6.0</td>
<td>21.4 ± 3.4</td>
<td>29.2 ± 5.0</td>
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<td></td>
<td>50–59</td>
<td>21</td>
<td>72.7 ± 17.1</td>
<td>165 ± 8</td>
<td>26.8 ± 4.3</td>
<td>20.9 ± 3.4</td>
<td>29.1 ± 4.4</td>
<td>12.0 ± 2.0</td>
<td>8.3 ± 1.5</td>
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<tr>
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<td>60–69</td>
<td>11</td>
<td>69.7 ± 16.8</td>
<td>162 ± 8</td>
<td>26.4 ± 5.6</td>
<td>18.4 ± 2.2</td>
<td>27.3 ± 4.6</td>
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<tr>
<td></td>
<td>70+</td>
<td>19</td>
<td>60.8 ± 12.2</td>
<td>157 ± 6</td>
<td>24.6 ± 4.9</td>
<td>18.0 ± 2.5</td>
<td>30.2 ± 4.7</td>
<td>9.7 ± 2.0</td>
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<td>All women</td>
<td>200</td>
<td>70.9 ± 18.2</td>
<td>163 ± 7</td>
<td>26.6 ± 6.2</td>
<td>21.0 ± 3.8</td>
<td>30.6 ± 5.5</td>
<td>12.2 ± 2.5</td>
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<td>Men</td>
<td>18–29</td>
<td>66</td>
<td>79.9 ± 15.4</td>
<td>178 ± 7</td>
<td>25.3 ± 4.5</td>
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<td>40–49</td>
<td>64</td>
<td>90.9 ± 16.6</td>
<td>177 ± 7</td>
<td>28.9 ± 4.5</td>
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<td>31.4 ± 4.8</td>
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<td>60–69</td>
<td>14</td>
<td>90.1 ± 11.5</td>
<td>177 ± 5</td>
<td>28.6 ± 3.5</td>
<td>30.2 ± 3.1</td>
<td>33.8 ± 3.9</td>
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<tr>
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<td>11</td>
<td>78.8 ± 12.1</td>
<td>173 ± 8</td>
<td>26.5 ± 4.5</td>
<td>27.8 ± 3.4</td>
<td>36.0 ± 7.3</td>
<td>13.8 ± 2.9</td>
<td>13.5 ± 2.8</td>
</tr>
<tr>
<td>All men</td>
<td>268</td>
<td>87.1 ± 16.2*</td>
<td>177 ± 7*</td>
<td>27.7 ± 4.7*</td>
<td>33.0 ± 5.3*</td>
<td>38.4 ± 5.1*</td>
<td>18.1 ± 3.1*</td>
<td>14.1 ± 2.6*</td>
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</table>

Values are group means ± SD; n, no. of subjects. BMI, body mass index; SM, skeletal muscle; relative SM, body mass/SM mass. For determination of lower and upper body SM see METHODS. *Men significantly greater than women, P < 0.01.

**Effects of Gender on SM Mass and Distribution**

Gender- and age-specific mean values for SM mass and distribution are shown in Table 1. The men had significantly (P < 0.001) more SM in comparison to the women in both absolute terms (Fig. 1) and relative to body mass (Table 1). The men had significantly (P < 0.001) more SM in both the upper and lower body (Table 1). These differences remained significant (P < 0.001) after controlling for height and body mass. In comparison to women, men had a significantly (P < 0.01) greater percentage of total SM mass in the upper body and a lower percentage of total SM mass in the lower body (Fig. 1).

**Effects of Age on SM Mass and Distribution**

Age was negatively correlated (P < 0.05) to total (men r = −0.24, women r = −0.29), lower body (men r = −0.27, women r = −0.31), and upper body (men r = −0.15, women r = −0.17) SM mass in men and women. When height was included with age in multiple-regression analysis, age remained a significant (P < 0.001) correlate of SM mass in men (r = −0.23) and women (r = −0.24). Visual inspection of Fig. 2A suggests there was a curvilinear relationship between age and SM mass, with a change in the slope of the regression line occurring at ~45 yr for both men and women. Indeed, within both genders, age² contributed significantly (P < 0.01) to the multiple-regression model, which implies there was a nonlinear relationship between age and SM mass. Age was also negatively correlated (P < 0.01) to relative SM mass (body mass/SM mass) within the men (r = −0.50) and women (r = −0.24; Fig. 2B). This was a linear relationship because age² did not contribute significantly to the multiple-regression model. The slope of the regression line between age and relative SM mass was significantly (P < 0.01) greater in men (−0.19 ± 0.02) than in women (−0.08 ± 0.02), indicating that the age-associated decrease in SM is greater in men.

To further examine the effects of age on absolute SM mass, the subjects were empirically separated into two age categories: 18–44 and 45+ yr. Independent of gender, within the 18–44 yr age category, total, lower, and upper body SM values were not related to age (P > 0.3). Within the 45+ yr age category, SM mass was significantly (P < 0.01) related to age in both men (r = −0.27) and women (r = −0.27). Within the men, age was significantly (P < 0.05) related to lower body (r = −0.48) but not upper body SM (Fig. 3A). Within the women, age was related (P < 0.05) to both lower body (r = −0.48) and upper body SM (r = −0.26; Fig. 3B). For the women, the slope of the regression line between age and lower body SM mass (−0.09 ± 0.02) was significantly (P < 0.01) greater than the slope of the regression line between age and upper body SM (−0.02 ± 0.02). These results suggest that the reduction in SM with advancing age was greater in the lower body.
body for both men and women, and thus age influenced muscle distribution.

**Relationship Between Body Weight and Height With SM Mass and Distribution**

**Height.** Height was significantly ($P < 0.001$) correlated with SM in both men ($r = 0.48$) and women ($r = 0.53$). In multiple-regression analysis, height$^2$ failed to contribute to the model beyond the effects of height alone, indicating that there was a linear relationship between height and SM mass. There was a very low, but significant ($P = 0.05$), correlation between the percentage of total muscle contained in the lower body and height within women ($r = 0.14$). A greater height favored a proportionately larger increase in lower body muscle. Height was not related to muscle distribution in men ($P > 0.05$).

**Body weight.** Body weight was significantly ($P < 0.001$) correlated with SM mass in both men ($r = 0.69$) and women ($r = 0.65$). Independent of gender, weight$^2$ contributed to the model beyond the effects of weight alone, indicating a nonlinear relationship between SM mass and body weight. Indeed, the larger the body weight, the smaller the increase in SM mass (Fig. 4A). In other words, when expressed as a percentage of total body weight, there was a negative relationship between SM mass and body weight (Fig. 4B). There was a significant ($P < 0.01$) correlation between the percentage of total muscle contained in the lower body and body weight in women ($r = 0.23$). Increased weight favored a proportionately larger increase in lower body muscle. Body weight was not related to muscle distribution in men ($P > 0.05$).

**Comparison of Regional and Whole Body SM Measurements**

The observation that a noticeable decrease in SM begins at ~45 yr in men and women and that men have more SM mass than women in both absolute terms and
relative to body weight remained true whether whole body or appendicular (SM area in the thigh, partial volume of SM in the thigh and arm) SM measurements are examined. This is illustrated for the relationship between age and whole body SM mass (Fig. 2A), the partial volume of SM in the thigh (Fig. 5A), and the partial volume of SM in the arm (Fig. 5B).

**DISCUSSION**

**Reference Values for SM Mass**

We used a whole body MRI protocol to measure SM in a large and heterogeneous sample of men and women. This allowed us to make unique observations with respect to the quantity and distribution of SM and to present reference values for SM mass. These values may be used as comparative standards in future studies assessing, for example, the influence of aging and disease on muscle wasting and the anabolic effects of physical training on muscle mass.

To test the generalizability of our sample, we compared the BMI of our sample to a large population database from the United States (43). The mean BMI in the US population for both men and women is ~26.6 kg/m², which is similar to the mean BMI for our subjects (men 27.7 kg/m², women 26.6 kg/m²). Furthermore, our sample consisted of an ethnically mixed group, varying in age from 18 to 88 yr. Thus our sample is typical of the North American adult population. Therefore, the data for muscle mass presented in the present study, which is based on the most comprehensive data set of SM to date and which used a gold-standard method for estimating muscle mass, can be used as normative values.

**Effect of Gender on SM**

Muscle mass. The findings of this study extend and strengthen the results of previous studies that report that men have more appendicular muscle than women, as estimated by dual-energy X-ray absorptiometry (DEXA) (20, 21) and with a single CT image (31). Our findings indicate that there are gender differences for regional and whole body muscle mass. On average, SM...
mass in men was 36% greater than in women. This gender difference remained after controlling for gender differences in body weight and height because SM mass relative to body weight was 38% in men and only 31% in women. Given the strong influence SM has on muscle mass, the increased prevalence of osteoporosis in women may be explained, in part, by their lower SM mass. These findings highlight the importance of weight-bearing and/or resistance exercise in women for building and maintaining muscle mass, strength, and bone mineral density.

**Muscle distribution.** Whereas the women in the present study had 40% less muscle than men in the upper body, in the lower body gender differences in muscle mass were only 33%. This agrees with the findings of Gallagher and Heymsfield (20), who report that women have a larger proportion of their total appendicular SM in their lower extremities in comparison to men, as estimated by DEXA. Miller et al. (31) also report that relative gender differences in CT-measured muscle cross-sectional area are larger in the upper arm than the thigh. Together, these findings are consistent with the observation that gender differences in lower body strength (~30%) are smaller than those observed for upper body strength (~50%) (31, 33).

**Effect of Age on SM**

**Muscle mass.** Inspection of Table 1 reveals that the substantial increase in body weight observed between 18 and 40 yr is not associated with a corresponding increase in SM. Thus, for both genders, the composition of weight gain before age 40 yr is predominantly fat, and, consequently, SM expressed as a percent of body weight is reduced relatively early in life. That SM relative to body mass is reduced in the third decade differs from our finding that the absolute quantity of SM is preserved until the fifth decade, with noticeable losses thereafter. The age-associated decrease in MRI-measured SM confirms previous observations wherein SM was measured by elemental analysis (total body potassium and/or nitrogen) (10), urinary creatinine excretion (42), DEXA (20, 21), muscle biopsy (27), and CT (7, 35). As reported previously with appendicular muscle (20), the loss in whole body muscle mass was independent of change in stature and was greater in men than in women, both on an absolute basis and relative to body mass. The finding that the decline in the quantity of SM is noticeable after ~45 yr of age agrees with others who report that muscle fiber cross-sectional area (i.e., contractile muscle) (27), body cell mass (16, 26), and isometric (4, 9, 25) and isokinetic (25, 41) strength do not change substantially until ~45 yr of age. These observations contrast with a single report wherein the reduction in the absolute quantity of DEXA-measured appendicular muscle began in the third decade (21). We are unaware of studies that compare measurements of MRI- and DEXA-measured muscle. Combined with our observation that appendicular measurements of muscle were similar to those observed for whole body MRI data, the discrepant findings are unexplained.

**Muscle distribution.** The findings here indicate that the loss of SM mass with age was greater in the lower body in both men and women. In earlier studies using animal and human subjects, muscle atrophy was also reported to be greater in the lower compared with upper extremities (30), a finding consistent with the observation that the loss of muscular strength tends to occur earlier in the lower compared with upper extremity (4). The age-associated reduction in physical activity (43) may be at least partially responsible for the change in muscle distribution with age. It is reasonable to assume that a reduction in physical activity would primarily be associated with a decreased use of lower body muscles, but not upper body muscles, given that the muscles in the lower body are required for most common activities (i.e., walking, stair climbing).

The causes and consequences of the age-related loss in muscle mass are only partly understood. Because we eliminated subjects who were bedridden, had physical disabilities, and/or had chronic illness, it is unlikely that these factors influenced our findings. However, given that there is an increased prevalence of physical inactivity in the elderly (43), a sedentary lifestyle may be an important cause of muscle atrophy in aging. Another plausible explanation is reduced levels of and responsiveness to trophic hormones with aging, including growth hormone (24, 37, 38), androgens (3, 37), and insulin-like growth factor I (3, 37). Data indicate that the age-related reduction in muscle mass may be associated with an increased prevalence of disability (2), as well as decreased functional capacity (14, 15, 25), basal metabolic rate (34, 42), and bone mineral density (6, 39). Although the mechanisms that would explain the age-related loss of muscle remain to be determined, the findings of this study and others (16, 26, 27) support the notion that intervention strategies designed to preserve SM mass be initiated no later than the fifth decade. As a result, it is recommended that resistance exercise, which can attenuate (41, 44) or reverse (14, 19) the age-associated decrease in muscle strength and mass, be a fundamental component of the treatment program in both men and women.

**Effects of Body Mass and Height on SM**

Our finding that height and weight explained ~50% of the variance in SM within both genders confirms a previous report based on measurements of appendicular muscle (20). This is reasonable given that taller subjects have longer bones and muscles and would be expected to have a greater muscle mass. Similarly, heavier subjects require greater muscle mass for movement and would be expected to have more muscle than their lean counterparts. Although we observed a linear relationship between muscle mass and height, the relationship between muscle mass and body mass was curvilinear. The larger the increase in body weight, the smaller the relative contribution of SM to the weight gain. The curvilinear relationship between muscle
mass and body mass in our study is similar to the relationship observed between lean body mass and body weight (17). Lean body mass rises with increased degrees of obesity; however, the heavier the individual, the smaller the relative contribution of lean body mass to the weight gain (17, 18).

Comparison of Regional and Whole Body SM Measurements

In general, the observations based on limited MRI data from the thigh and arm regions paralleled those derived using whole body measurements. This has important implications for future studies because routine access to and the high cost associated with whole body MRI measurements remain a problem. However, if appendicular measures are used to assess the effects of age or gender on muscle distribution, both upper and lower body measurements should be included to ensure that, as noted in this study (Figs. 1 and 3), regional differences in muscle distribution are identified.

Study Limitations

The observations of this study are tempered by the limitations inherent to cross-sectional studies. In addition, our cohort included subjects who were self-selected, healthy, and primarily younger than 70 yr. In comparison to longitudinal studies, it is reported that cross-sectional studies underestimate the age-associated loss in muscular strength (1, 9, 25). When combined with the observation that the decrease in muscular strength with aging is predominantly due to a corresponding decrease in muscle size (14, 19), it is possible that we have underestimated the true effect of aging on muscle mass and distribution. Studies wherein muscle mass is longitudinally studied are required to confirm the findings reported here.

Conclusions

The present study presents reference values for whole body SM mass for a healthy, multiethnic sample of 468 men and women who ranged in age from 18 to 88 yr. These values may be used as comparative standards for future studies assessing SM status in aging and disease. Although relative (%) muscle mass decreased starting in the third decade, a noticeable decrease in absolute SM mass was not observed until the end of the fifth decade. The decrease in SM approximated 1.9 and 1.1 kg/decade in the men and women, respectively. Aging was associated with a preferential decrease in muscular strength with aging is predominantly due to a corresponding decrease in muscle size (14, 19), it is possible that we have underestimated the true effect of aging on muscle mass and distribution. Studies wherein muscle mass is longitudinally studied are required to confirm the findings reported here.

REFERENCES