Skeletal muscle mass and the reduction of $V_{O2}^{\max}$ in trained older subjects

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Proctor, David N., and Michael J. Joyner. Skeletal muscle mass and the reduction of $V_{O2}^{\max}$ in trained older subjects. J. Appl. Physiol. 82(5): 1411–1415, 1997.—The role of skeletal muscle mass in the age-associated decline in maximal O$_2$ uptake ($V_{O2}^{\max}$) is poorly defined because of confounding changes in muscle oxidative capacity and in body fat and the difficulty of quantifying active muscle mass during exercise. We attempted to clarify these issues by examining the relationship between several indexes of muscle mass, as estimated by using dual-energy X-ray absorptiometry and treadmill $V_{O2}^{\max}$ in 32 chronically endurance-trained subjects from four groups (n = 8/group): young men (20–30 yr), older men (56–72 yr), young women (19–31 yr), and older women (51–72 yr). $V_{O2}^{\max}$ per kilogram body mass was 26 and 22% lower in the older men (45.9 vs. 62.0 ml·kg$^{-1}$·min$^{-1}$) and older women (40.0 vs. 51.5 ml·kg$^{-1}$·min$^{-1}$). These age differences were reduced to 14 and 13%, respectively, when $V_{O2}^{\max}$ was expressed per kilogram of appendicular muscle. When appropriately adjusted for age and gender differences in appendicular muscle mass by analysis of covariance, whole body $V_{O2}^{\max}$ was 0.50 ± 0.09 l/min less (P < 0.001) in the older subjects. This effect was similar in both genders. These findings suggest that the reduced $V_{O2}^{\max}$ seen in highly trained older men and women relative to their younger counterparts is due, in part, to a reduced aerobic capacity per kilogram of active muscle independent of age-associated changes in body composition, i.e., replacement of muscle tissue by fat. Because skeletal muscle adaptations to endurance training can be well maintained in older subjects, the reduced aerobic capacity per kilogram of muscle likely results from age-associated reductions in maximal O$_2$ delivery (cardiac output and/or muscle blood flow).

METHODS

Subjects. Thirty-two endurance-trained men and women who had consistently trained for 5 or more consecutive years were recruited to participate in this study. Individuals were carefully screened via telephone interview for training and medical histories. It was our initial plan to study four groups of eight subjects: young (<30 yr) men and women and older (>60 yr) men and women. Because of the limited number of highly trained female athletes over 60 yr old available for study, it was necessary to include several women in their 50s. The physical and training characteristics of the 32 subjects are given in Table 1. The modes of training were similar between groups with roughly equal numbers of runners, cyclists, and cross-trained athletes (e.g., triathletes) in each group. Many of the subjects in each of the four groups also reported regular (≈2 days/wk) use of moderate resistance-training exercises for the upper and lower body. Each subject gave written informed consent before the study according to Mayo Clinic Institutional Review Board guidelines.

Muscle mass estimation. Regional muscle mass, FFM, and body fat were estimated by using a DXA instrument (Total Body Analysis, ver. 3.6y, Lunar, Madison, WI) at a medium scan speed (~25 min). Muscle mass was estimated as kilograms of bone-free lean tissue (20). Appendicular muscle (all limbs) was felt to be the most valid DXA-derived index of muscle mass on $V_{O2}^{\max}$. We studied highly trained older men and women to minimize the potential effects of age-related physical inactivity (i.e., reduced muscle oxidative capacity and capillarization) and to ensure that our older subjects were capable of reaching $V_{O2}^{\max}$.

AGING RESULTS IN A DECLINE in maximal O$_2$ uptake ($V_{O2}^{\max}$; ml·kg$^{-1}$·min$^{-1}$). This decline has been attributed primarily to reduced maximal cardiac output, increased body fat, and reduced peripheral O$_2$ extraction (11, 22). Reduced skeletal muscle mass may also contribute to the age-associated decline in $V_{O2}^{\max}$ but there are little quantitative data on the relationship between $V_{O2}^{\max}$ and muscle mass as a function of age (2, 3, 10). The available studies, which primarily consist of $V_{O2}^{\max}$ and fat-free body mass (FFM) measurements in sedentary subjects, are difficult to interpret due to the confounding effects of age-associated changes in body fat and muscle oxidative capacity (3, 8, 10). Additionally, many studies of the decline in $V_{O2}^{\max}$ with aging, particularly in trained subjects, have not statistically adjusted (covaried) $V_{O2}^{\max}$ for age or gender differences in body composition (10, 12, 22, 31). Finally, it is unclear what relevance indicators of whole body muscle mass (FFM, creatinine excretion) have as determinants of $V_{O2}^{\max}$ when most of the O$_2$ consumed during $V_{O2}^{\max}$ testing is used by the limb muscles (16, 17, 21, 29). These limitations have led to some confusion about how to best express and compare $V_{O2}^{\max}$ changes with aging (l/min, ml·kg$^{-1}$·min$^{-1}$, ml·kg FFM·min$^{-1}$). For these reasons, the role of skeletal muscle mass in the decline in $V_{O2}^{\max}$ with aging remains poorly defined.

The present study was designed to address many of the limitations noted above and to test the hypothesis that $V_{O2}^{\max}$ per kilogram of limb muscle is reduced in highly trained older subjects, independent of age-associated changes in body composition. Specifically, we examined the relationship between appendicular muscle mass, as estimated by using dual energy X-ray absorptiometry (DXA), and treadmill $V_{O2}^{\max}$ in chronically endurance-trained subjects. Appendicular muscle mass was used to provide a more specific estimate of the quantity of muscle recruited during maximal treadmill running, and analysis of covariance (ANCOVA) was performed to appropriately evaluate the impact of age and gender differences in muscle mass on $V_{O2}^{\max}$. We studied highly trained older men and women to minimize the potential effects of age-related physical inactivity (i.e., reduced muscle oxidative capacity and capillarization) and to ensure that our older subjects were capable of reaching $V_{O2}^{\max}$.

MUSCLE MASS ESTIMATION. Regional muscle mass, FFM, and body fat were estimated by using a DXA instrument (Total Body Analysis, ver. 3.6y, Lunar, Madison, WI) at a medium scan speed (~25 min). Muscle mass was estimated as kilograms of bone-free lean tissue (20). Appendicular muscle (all limbs) was felt to be the most valid DXA-derived index of muscle mass on the basis of comparisons with other in vivo techniques (13, 33). This index also provided a logical estimate of active muscle mass during maximal treadmill exercise (4, 32). Bony landmark sites described by Heymsfield et al. (13) were used to obtain the summed upper and lower
extremity (appendicular) muscle mass. Nonmuscle components included in DXA-derived bone-free lean tissue (i.e., connective tissue + adipose tissue water content, which is ~15% of adipose tissue mass), which could lead to an overestimation of muscle mass by DXA, were assumed to be minimal in the arms and legs of highly trained athletes (15). FFM was also estimated by using DXA because it has been the traditional body composition-based expression of VO2max (3, 4, 7, 22, 31).

Reproducibility (intraclass correlation) of repeated DXA estimates of appendicular muscle and FFM in a separate sample of 10 subjects (6 men, 4 women, age 25-50 yr) was 0.99 and 0.99, respectively. Mean values for the repeated measurements on 2 different days did not differ for appendicular muscle mass (23.7 vs. 23.9 kg; P = 0.66) or FFM (54.7 vs. 54.9 kg; P = 0.49). Fat mass (kg) was also found to be highly correlated (P < 0.001) with the sum of skinfolds in the present sample of trained men (5; r = 0.92) and women (25; r = 0.88) (19). The DXA instrument was calibrated monthly by using a series of beef blocks of known composition (Hormel, Austin, MN).

Treadmill testing. Most of the subjects had previous experience with treadmill running. Older subjects initially completed a Bruce treadmill test with 12-lead electrocardiograph (ECG) and blood pressure monitoring to screen for underlying cardiovascular abnormalities. VO2max was subsequently determined in all subjects by using a continuous, graded (2% every other minute) treadmill running test to exhaustion. A 5-min warm-up was used to determine the appropriate running speed for each subject. Treadmill speed was individually selected so that each subject would reach exhaustion within 8-12 min, excluding warm-up (14). O2 uptake (VO2) was measured using an automated breath-by-breath mass spectrometry system validated against the meteorological-balloon collection technique across a wide range of breathing frequencies (24).

The VO2max was defined as the average VO2 over the final 60 s of the test (14). The increase in VO2 over the final 60 s (compared with the previous percent grade) was <100 ml/min in all 16 of the older subjects and <150 ml/min in 8 of the 16 young subjects. Thus 75% of our subjects (including all of the older subjects) achieved a plateau in VO2 during the treadmill test (14, 29). The other eight young subjects had <75% of the expected increase in VO2 (1) over the last minute of the test and had respiratory exchange ratio levels <1.10. Ratings of perceived exertion ranged from 17 to 20 on the Borg 20-point scale in both the young and older groups, suggesting a similar level of effort between age groups. Maximal heart rate was determined from ECG (10-beat average) tracings and exceeded 95% of age-predicted values (23) in 30 of the 32 subjects.

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
<th>Age Effect (P Value)</th>
<th>Gender Effect (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24 ± 4</td>
<td>64 ± 4</td>
<td>26 ± 4</td>
<td>61 ± 8</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180 ± 7</td>
<td>178 ± 6</td>
<td>171 ± 5</td>
<td>164 ± 6</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.9 ± 7.8</td>
<td>75.5 ± 10.2</td>
<td>60.1 ± 5.5</td>
<td>58.1 ± 6.6</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%Fat</td>
<td>9.9 ± 2.5</td>
<td>20.0 ± 6.0</td>
<td>22.5 ± 4.4</td>
<td>28.4 ± 6.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>64.0 ± 8.2</td>
<td>60.0 ± 6.1</td>
<td>46.4 ± 2.8</td>
<td>41.3 ± 2.3</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Appendicular muscle, kg</td>
<td>27.7 ± 4.1</td>
<td>25.1 ± 3.3</td>
<td>19.6 ± 2.1</td>
<td>16.8 ± 0.9</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Training yr</td>
<td>9 ± 3</td>
<td>21 ± 5</td>
<td>9 ± 5</td>
<td>19 ± 8</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Training min/wk</td>
<td>444 ± 193</td>
<td>373 ± 184</td>
<td>328 ± 130</td>
<td>389 ± 189</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD for 8 subjects per group. %Fat, fat-free mass, and appendicular muscle mass were each estimated by dual-energy X-ray absorptiometry. NS, not significant. P values refer to significant main effects identified by 2-way (age × gender) analysis of variance.

Statistical analysis. Age, gender, and interaction (age × gender) effects on body composition variables and VO2max normalized by using ratios (e.g., ml·kg FFM⁻¹·min⁻¹) were evaluated with two-way analysis of variance. Comparisons of VO2max were also adjusted by using measures of muscle mass as a covariate (ANCOVA). This permitted the appropriate correction of VO2max data for age and gender differences in body composition (31, 32). Reproducibility of DXA-based estimates of muscle and FFM were evaluated by using intraclass correlation coefficients. All statistics were performed by using the SAS statistical package. Significance was accepted at P ≤ 0.05.

### RESULTS

Subject characteristics (Table 1). The women were shorter, weighed less, and had less FFM than the men, regardless of age. Older men and women had approximately the same body weights as did their younger counterparts (P = 0.65) but were shorter and had ~6–10% less FFM. Appendicular muscle mass was ~30% lower in the women of a given age. The age-associated reduction in appendicular muscle averaged 2.6 kg in men and 2.8 kg in women. Percent body fat by DXA was lowest in the young men (9.9%), similar between older men and young women (20–22%), and highest in the older women (28.4%). Older subjects had trained longer than had the young subjects, but average duration (min/wk) of aerobic training sessions over the previous year did not differ between groups.

VO2max (Table 2). Treadmill VO2max was ~30% higher in men than in women when expressed as liters per minute. However, this gender difference in VO2max was abolished when normalized to FFM or appendicular muscle mass. Age differences in VO2max ranged from 22 to 26% on either an absolute (l/min) or per kilogram body weight (ml·kg⁻¹·min⁻¹) basis. When age-related differences in body fat were eliminated from the calculation of VO2max, VO2max per kilogram of FFM was 16–17% lower (P < 0.001) in the older subjects. When expressed per kilogram of appendicular muscle, older men and women had VO2max values that were 13–14% lower (P < 0.001). These age differences were similar in both genders, but it was apparent that the range of muscle mass in the older women was substantially less than it was in the other groups. Maximal heart rates averaged 20–30 beats/min lower in the older subjects.
Figure 1 shows the individual V˙O2max values (l/min) plotted as a function of appendicular muscle mass with separate regression lines drawn for the young and older subjects. ANCOVA revealed that the apparent downward shift in this relationship was significant (P < 0.001) for the older groups, averaging 0.5 ± 0.09 l/min less than for the young groups at a given mass of appendicular muscle. In the analysis, all interaction terms (muscle mass × age, muscle mass × gender, age × gender) were found to be nonsignificant (P = 0.22–0.86). When these interaction terms were removed from the model, the age-associated changes in the relationship between V˙O2max and muscle mass were not significantly influenced by gender (P = 0.22). ANCOVA also revealed significant age differences for V˙O2max expressed per kilogram of leg muscle and FFM (data not shown).

DISCUSSION

The major new finding of this study is that V˙O2max per kilogram of limb muscle is reduced in highly trained older men and women. Graphically, the relationship between total body V˙O2max (l/min) and skeletal muscle mass is shifted downward in the older subjects when age and gender differences in muscle mass are considered appropriately by ANCOVA (Fig. 1). Because we studied chronically endurance-trained subjects, these findings are also unlikely to be confounded by age-associated differences in muscle quality or subject motivation. This means that there is a reduced aerobic capacity per kilogram of active skeletal muscle, which contributes to the reduced V˙O2max seen in highly trained older subjects.

Does loss of muscle contribute to the decline in V˙O2max with aging? The possibility that skeletal muscle loss plays an important role in the age-associated decline in V˙O2max was originally suggested by Fleg and Lakatta (10). They found that the age-associated decline in V˙O2max (ml·kg⁻¹·min⁻¹) of sedentary men and women (age 22–87 yr) was blunted by ~50% when normalized to urinary creatinine excretion, an index of whole body muscle mass. However, these results could be confounded in at least two ways. First, none of their subjects exercised regularly, and thus it is likely that their older subjects were less physically active than were their young subjects. Because skeletal muscle V2 capacity (i.e., oxidative enzyme activity, capillarization) and tolerance to heavy exercise are reduced in sedentary older subjects (5, 26), it is difficult to know how the use of sedentary subjects might affect the relationship between V˙O2max and muscle mass with aging. A second confounding aspect of the study of Fleg and Lakatta is that it is unclear what relevance measurements of whole body muscle mass have as determinants of V˙O2max when almost all of the O2 consumed during treadmill V˙O2max...
testing is used by the limb muscles, especially the legs (16, 17, 21, 29).

In the present study, we attempted to clarify these issues by studying chronically endurance-trained subjects and quantifying active muscle mass by using regional DXA measurements. The $\dot{V}O_2_{\text{max}}$ expressed per kilogram body weight was 22–26% lower in the older than in the young athletes, but $\dot{V}O_2_{\text{max}}$ per kilogram of appendicular muscle was only 13–14% lower (Table 2). These differences were also seen when $\dot{V}O_2_{\text{max}}$ was expressed per kilogram of leg mass (11–13% lower, data not shown). Thus it appears that nearly one-half of the age-associated decline in $\dot{V}O_2_{\text{max}}$ expressed per kilogram body weight in these highly trained subjects was due to age-associated changes in body composition, including increased body fat and loss of appendicular muscle mass.

Because adjustment of $\dot{V}O_2_{\text{max}}$ for FFM or skeletal muscle mass factors out the influence of adipose tissue, our data might be interpreted to suggest that body fat accumulation, and not muscle loss, contributed to the decline in whole body $\dot{V}O_2_{\text{max}}$ in these subjects. However, it is important to recognize that the higher body fat of the older subjects most likely represents a reciprocal loss of lean (muscle) tissue throughout life. Although losses of muscle and gains in fat with aging are generally less in highly trained subjects than in the general population (18), losses in appendicular muscle mass in these subjects (~2.7 kg) do appear to contribute importantly to the reduced whole body $\dot{V}O_2_{\text{max}}$ values of older endurance-trained men and women. The only exception might be elite older male endurance athletes, who can be very lean (8–15% body fat) and whose $\dot{V}O_2_{\text{max}}$ values expressed as liters per minute, milliliters per kilogram per minute, and milliliters per kilogram of FFM per minute are all similarly reduced (~10–15%) compared with young athletes having similar absolute training and/or performance characteristics (11, 12).

Does aerobic capacity per kilogram muscle decline in highly trained older subjects? To the best of our knowledge, there have been no studies of trained older subjects that have estimated active muscle mass (e.g., appendicular muscle mass) or applied ANCOVA to properly adjust $\dot{V}O_2_{\text{max}}$ for age- or gender-based differences in body composition (31). In this context, the present study is the first to demonstrate that there is a reduction in aerobic capacity per unit active muscle mass in highly trained older men and women (Fig. 1). This analysis showed that $\dot{V}O_2_{\text{max}}$ averaged 0.50 l/min less per kilogram of appendicular muscle in the older subjects. A significant age difference was also observed for $\dot{V}O_2_{\text{max}}$ expressed per kilogram of leg muscle (data not shown).

The reduced aerobic capacity per kilogram limb muscle could reflect reduced O$_2$ extraction by the active muscles and/or reduced O$_2$ delivery to the active muscles. Although neither muscle biopsies nor limb O$_2$ extraction measurements were obtained for the present subjects, previous studies (6, 27), including one from our laboratory (26), have shown that muscle oxidative enzyme activities and capillarization are similar to or higher in older compared with young men having physiological characteristics and training histories similar to those of the current subjects. Although data on muscle characteristics are not available for highly trained older women, the peripheral adaptations of older women to endurance training are not compromised by aging (5, 28). Therefore, it is unlikely that skeletal muscle oxidative capacity or capillarization is responsible for the age-associated reduction in aerobic capacity per kilogram muscle seen in our trained older subjects. The most likely explanation of this difference is the well-documented decline in maximal cardiac output (and presumably reduced peak muscle blood flow and O$_2$ delivery) associated with aging.

This view is consistent with earlier observations made in elite older endurance athletes who should have maximized any peripheral adaptations to training (9, 11, 27). In these studies, older athletes were matched with young athletes having similar training and/or endurance performance characteristics to examine the influence of age on $\dot{V}O_2_{\text{max}}$ and its physiological determinants. When this is done, there has tended to be little difference in FFM or body fat between the young and older groups. Consequently, $\dot{V}O_2_{\text{max}}$ in liters per minute, milliliters per kilogram per minute, and milliliters per kilogram of FFM per minute are reduced to a similar extent in the older compared with young athletes. Thus it is likely that a reduced aerobic capacity per kilogram of limb muscle also occurs in the most highly trained and genetically gifted older subjects as a result of reduced maximal O$_2$ delivery.

Are there gender differences in aerobic capacity per kilogram muscle? When gender differences in total fat mass are factored out by normalizing $\dot{V}O_2_{\text{max}}$ to kilograms of FFM, the gender difference in $\dot{V}O_2_{\text{max}}$ often disappears (3, 7). The present study appears to be the first to document this in highly trained young or older subjects by using region-specific analysis of muscle mass. Aerobic capacity per kilogram of appendicular muscle was virtually identical in this sample of highly trained men and women (Table 2), and aging caused a similar decline in both genders. These findings provide additional support for expressing $\dot{V}O_2_{\text{max}}$ per unit of fat-free tissue when comparing the performance of the cardiovascular-respiratory system of individuals who differ in body size or composition (7, 8, 30, 31).

Summary. The findings of the present study demonstrate that there is a reduced aerobic capacity per kilogram of appendicular muscle in highly trained older men and women that contributes to their reduced whole body $\dot{V}O_2_{\text{per}}$ with aging. The age-associated change in $\dot{V}O_2_{\text{max}}$ per kilogram of appendicular muscle appears to be similar in highly trained older men and women. This difference is likely to result from reduced O$_2$ delivery during maximal exercise and is independent of age-associated changes in body composition.

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