Effect of aging on human muscle architecture

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Narici, M. V., C. N. Maganaris, N. D. Reeves, and P. Capodaglio. Effect of aging on human muscle architecture. J Appl Physiol 95: 2229–2234, 2003. First published July 3, 2003; 10.1152/japplphysiol.00433.2003.—The effect of aging on human gastrocnemius medialis (GM) muscle architecture was evaluated by comparing morphometric measurements on 14 young (aged 27–42 yr) and on 16 older (aged 70–81 yr) physically active men, matched for height, body mass, and physical activity. GM muscle anatomic cross-sectional area (ACSA) and volume (Vol) were measured by computerized tomography, and GM fascicle length (Lf) and pennation angle (θ) were assessed by ultrasonography. GM physiological cross-sectional area (PCSA) was calculated as the ratio of Vol/Lf. In the elderly, ACSA and Vol were, respectively, 19.1% (P < 0.005) and 28.4% (P < 0.001) smaller than in the young adults. Also, Lf and θ were found to be smaller in the elderly group by 10.2% (P < 0.01) and 13.2% (P < 0.01), respectively. When the data for the young and elderly adults were pooled together, θ significantly correlated with ACSA (P < 0.05). Because of the reduced Vol and Lf in the elderly group, the resulting PCSA was found to be 15.2% (P < 0.05) smaller. In conclusion, this study demonstrates that aging significantly affects human skeletal muscle architecture. These structural alterations are expected to have implications for muscle function in old age.

skeletal muscle; muscle fiber; sarcopenia; muscle strength

AGING IS KNOWN TO BE ASSOCIATED with a reduction in muscle mass (sarcopenia). Cross-sectional studies suggest that this phenomenon starts toward the end of the fifth decade of life (19), which also corresponds with the onset of force decline (34). This loss of muscle mass is greater for the muscles of the lower limbs than for the upper limbs, and from 20 to 70 yr of age, lower limb muscle mass decreases by ∼25% (19). When the cross-sectional area (CSA) rather than muscle mass (or volume) of essential muscles of locomotion is considered, a 25–33% difference in quadriceps CSA is found between young (20–29 yr) and elderly (70–81 yr) adults (27, 46). However, several investigators (20, 23, 29, 40, 46), but not some others (9), have observed a greater reduction in strength than of muscle CSA so that force, or torque, expressed per unit of muscle CSA, has been found to be reduced in older individuals.

MATERIALS AND METHODS

Subjects. The investigation was conducted on 16 elderly men aged 70–81 yr (height, 1.72 ± 0.03 m; body mass, 74.5 ± 8.3 kg) and, for comparison, on 14 younger men aged 27–42 yr (height, 1.73 ± 0.09 m; body mass, 73.2 ± 10.4 kg). The investigation was approved by the Ethics Committee of the Salvatore Maugeri Foundation, and each individual gave written, informed consent to the investigation after being advised about the nature and purpose of the study. Most of the elderly participants were members of the University of the Third Age of the town of Pavia, whereas the younger participants were recruited from among friends and col-

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leagues. Exclusion criteria for subject participation in the study included known muscular, neurological, metabolic, and inflammatory disease; uncontrolled hypertension; or angina. Particular care was taken in recruiting young and elderly individuals with similar activity levels and body stature. The individuals selected for this investigation (both young and elderly) were recreationally active, some belonging to walking clubs, some to aerobic and flexibility classes, some practicing ballroom dancing or using bicycle for transport, but none was engaged in sporting activities at competitive level. The number of hours spent in recreational activities was assessed by use of the Saint-Etienne Physical Activity Questionnaire (3) validated in young and elderly individuals (5). The participation in recreational activities expressed as number of hours per week was similar in the two groups, 8.6 ± 2.6 (range 5.3–12.8) in the young adults and 7.9 ± 3.1 (range 3.7–14.0) in the older individuals, with no significant differences between the two groups (P > 0.05, Mann-Whitney rank-sum test).

Muscle ACSA and volume measurements. GM ACSA was measured by computerized tomography. The subjects were positioned in a General Electric 38 mm long (Prospeed S5 power) operating at 120 kV peak, with the legs relaxed. The ACSAs of 40 contiguous 10-mm slices (50-cm field of view, matrix 512 × 512 pixels), with 0-mm interslice gap, were obtained starting from the knee space (slice 1). For each axial slice, ACSA computation (mean of three consecutive morphometric measurements on the same MRI slice) was carried out on the GM muscle. Calculation of muscle ACSA was performed by digitizing throughout the muscle contour by use of an image-analysis program (NIH Image version 1.61/ppc, National Institutes of Health, Bethesda, MD). The maximum ACSA of the GM, normally corresponding to the fifth or sixth axial scan distal to the knee space, was selected for data analysis. The error in this technique, evaluated by digitizing shapes of known areas, was estimated to be 2.1%, whereas the coefficient of variation for three ACSA measurements, repeated on the same subject on 6 different days, was 2.6%.

For GM muscle volume (Vol) calculation, all slices were fitted with a spline algorithm to interpolate for missing slices between the minimum measurable CSA at the proximal and distal ends of the muscle and the theoretical slice where the proximal and distal CSAs of the GM would be equal to zero. The total Vol was then calculated by adding the individual ACSA of each image and multiplying the sum by the slice thickness (10 mm) (36).

Muscle architecture. The participants were asked to rest prone on an examination table with legs relaxed and both feet hanging outside the table. A plastic cast shaped to the sole of the foot and calcaneus and extending 10 cm above the malleolus was taped to the dominant foot to standardize measurements at the same resting ankle joint angle. The tibial-talar joint angle chosen for this investigation was 115° (90° being the angle when the foot is perpendicular to the tibia). In both young and older participants, the ankle joint was at the spontaneous resting angle of the tibial-talar joint; hence the positioning of the foot in the cast did not require any active force by the operator. Resting fascicle length (Lf) and pennation angle (θ) were measured by real-time ultrasound (HDI-3000, ATL, Bothell). Images were obtained at midbelly of the dominant GM muscle by using a 7.5-MHz linear-array probe, 38 mm long. The probe was positioned perpendicular to the dermal surface of the GM muscle and oriented along the median longitudinal plane of the muscle. Midbelly was defined as the point along the median longitudinal axis of the muscle at 50% of the distance between the proximal and distal apexes of the myotendinous junctions. The center of the probe was aligned to this position. The probe was coated with a water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. Three images at rest were obtained within the same experimental session in each individual. The θ was measured as the angle of insertion of muscle fiber fascicles into the deep aponeurosis, and Lf was defined as the length of the fascicular path between the insertions of the fascicle into the superrior and deep aponeuroses. In cases in which the fascicle extended off the acquired ultrasound image, the length of the missing portion of the fascicle was estimated by extrapolating linearly both the fascicular path, visible in the image, and the aponeurosis. The error introduced by this technique depends primarily on the degree of curvature of the fascicle. We recently showed in the tibialis anterior muscle that, during contraction, when the curvature of the fascicles is greater than at rest, our linear extrapolation approach results in an error of only 2.4% (41). The error made in the present study would be even smaller because resting fascicles present negligible curvature (32, 33). PCSA (cm²) was calculated as the ratio between Vol (cm³) and Lf (cm) (1, 8, 16, 31, 42).

The accuracy of the ultrasound method in measuring the architectural features of the human GM muscle has been previously tested against direct anatomic measurement on a cadaver and found to be in good agreement (36): in the central region of the muscle, Lf and θ differed by an average of 1.2 mm and 1.5°, respectively, between the two techniques.

Images were captured with a video-capture card (Capsure, iREZ Research) interfaced with a Macintosh Powerbook G3 computer. They were then frozen and saved on the hard disk of the computer. Data analysis was performed with the same digitizing software used for the ACSA determination, mentioned above. The mean of three consecutive morphometric analyses of each image was used for data analysis. The architectural measurements were performed by an investigator blinded to subject identity.

Statistics. Data are presented as means ± SD. Age-related differences for all measurements were analyzed with the independent-samples Student’s t-test. In those cases (hours spent in recreational activities) in which the data did not meet the criteria of normality (Shapiro-Wilk W test, P < 0.05), a nonparametric Mann-Whitney rank-sum test was applied. Linear regression analysis (Pearson’s product-moment correlation) was used to compare the degree of association between variables. The critical level for statistical significance was set at 5%.

RESULTS

GM muscle’s maximum ACSA (ACSAmax), Vol, Lf, θ, and PCSA of the elderly and young adult populations are presented in Table 1. All the investigated muscle architectural parameters were reduced in the elderly compared with the younger adults.

The differences between the elderly and younger groups were 19.1% (P < 0.005) for ACAmax, 25.3% (P < 0.001) for Vol, 10.2% (P < 0.01) for Lf, 13.2% (P < 0.01) for θ, and 15.2% (P < 0.05) for PCSA. When θ was plotted against ACSA (Fig. 1), and the experimental data points were fitted with a linear function, a significant correlation (r = 0.432, P < 0.05) was found between the two variables, indicating that θ scales with ACSAmax. Also, it is noteworthy that in most elderly individuals values of both ACSAmax and θ were smaller than in the younger adults.
Table 1. Summary of gastrocnemius medialis architectural data in young and elderly individuals

<table>
<thead>
<tr>
<th></th>
<th>ACSA&lt;sub&gt;max&lt;/sub&gt;, cm&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Vol, cm&lt;sup&gt;3&lt;/sup&gt;</th>
<th>(L_f), cm</th>
<th>(\theta^\circ)</th>
<th>PCSA (Vol/(L_f)), cm&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Elderly</td>
<td>14.0 ± 3.6</td>
<td>208.7 ± 48.5</td>
<td>4.29 ± 0.67</td>
<td>23.6 ± 3.0</td>
<td>50.1 ± 12.6</td>
</tr>
<tr>
<td>Young</td>
<td>17.4 ± 2.8</td>
<td>279.3 ± 59.3</td>
<td>4.78 ± 0.55</td>
<td>27.2 ± 4.3</td>
<td>59.1 ± 14.4</td>
</tr>
<tr>
<td>Difference</td>
<td>19.1%</td>
<td>25.3%</td>
<td>12.6%</td>
<td>13.2%</td>
<td>15.2%</td>
</tr>
<tr>
<td>(P)</td>
<td>values (t-test)</td>
<td></td>
<td>0.005</td>
<td>0.01</td>
<td>0.05</td>
</tr>
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</table>

Values are means ± SD; \(n = 14\) for the young (27–42 yr) group and \(n = 16\) for the elderly (70–81 yr) group. ACSA<sub>max</sub>, maximum anatomic cross-sectional area; Vol, muscle volume; \(L_f\), fascicle length; \(\theta\), pennation angle; PCSA, physiological cross-sectional area.

Although the difference in ACSA<sub>max</sub> seemed greater than that of PCSA (Table 1), no significant difference was found between the ratios of ACSA<sub>max</sub> to PCSA of the young (0.30 ± 0.04) and those of the elderly subjects (0.29 ± 0.06, not significant). When the values of ACSA<sub>max</sub> and PCSAs of the young and elderly subjects were pooled together, a significant correlation \((r = 0.759, P < 0.01)\) was found between ACSA<sub>max</sub> and PCSA (see Fig. 2).

**DISCUSSION**

The present study demonstrates for the first time that human GM muscle architecture is significantly altered in old age. Because the elderly individuals specifically selected for this study were physically active and had daily energy expenditures similar to those of the younger adult group, the possibility that these alterations in muscle architecture were due to disuse seems quite unlikely. Hence, we trust that these changes are mostly attributable to the effect of aging per se rather than to disuse. Furthermore, in this study, particular care was taken in recruiting elderly and young individuals matched for height to avoid differences in muscle architecture due to a simple scaling phenomenon. A different approach was, instead, followed by Kubo et al. (24, 25), who recently investigated muscle architecture in sedentary young and elderly men and women not matched for height and physical activity status. Interestingly, after normalizing of the measurement to limb segment length, Kubo et al. (24) found differences in \(L_f\) and \(\theta\) between young and elderly subjects for the vastus lateralis muscle but not for the GM and triceps brachii muscles. The lack of physical activity matching between subjects makes it difficult to ascertain whether the above differences were due to aging per se or the combined effect of aging and disuse. The absence of architectural changes in the GM was attributed by Kubo et al. (24) to a greater use of the plantarflexors compared with the knee extensors in locomotor activities or to a different plasticity of these two muscles in response to aging. However, our results demonstrate that, when the influence of disuse is controlled for by matching individuals for physical activity level, significant alterations in the architecture of the GM muscle are observed; that is to say, changes in plantarflexor muscle architecture do occur in old age, even in active elderly individuals. Besides, the approach followed by Kubo et al. (24) to scale \(L_f\) to limb length does not fully eliminate the effect of different body dimension on muscle architecture. This is because taller individuals will have a greater body mass that would place a greater mechanical load on weight-bearing muscles such as the vastus lateralis and GM. This mechanical stimulus is likely to affect \(\theta\) and \(L_f\), a factor that is accounted for by the approach we employed to match the subjects for body height and mass.

In the present study, muscle Vol, ACSA<sub>max</sub>, \(L_f\), and \(\theta\) were all found to be significantly reduced in the older individuals compared with the younger adults. As a result of the decrease in muscle Vol (−25.3%) and in fiber \(L_f\) (−10.2%), a reduction in PCSA was also found (−15.2%). This decrease in PCSA was affected more by the reduction in muscle Vol than by that in fiber fascicle length. Nevertheless, the fact that both of these decreases strongly suggest that sarcopenia involves a loss of sarcomeres not only in parallel but also in series. Hence, the decrease in PCSA is likely to be a primary factor for the well-documented decrease in contractile force-generating potential in old age (20, 23, 29, 40, 46). This major role of the reduction in PCSA in the loss of muscle strength in old age was also shown in the arm muscles by Klein et al. (22). However, in this case, PCSA was estimated by dividing muscle Vol,
determined by MRI, by $L_f$ estimated from the ratio of fiber length to muscle length published in the literature, thereby assuming that the ratio of fiber length to muscle length does not change with age. A secondary factor could be the decreased tensile stiffness of the in-series tendon in old age (30, 39), which would result in a leftward shift of the length-tension relation (48). However, it could be argued that this secondary deteriorating effect might be partly cancelled out by the decrease in the total number of serial sarcomeres, as indicated by the present results, which theoretically would shift rightward the length-tension relation of the muscle. Other factors accounting for the reduced force-generating potential in old age would be a decrease in single fiber-specific tension (26), an increased antagonist muscle coactivation (11, 17, 29), and in some cases a reduced muscle activation capacity (4, 12, 47), not always found (6, 7, 18, 22, 43).

Assuming a linear relationship between in-series sarcomere number and $L_f$ (14), it follows from previous reports on human cadaver GM architecture measurements (16) that the number of sarcomeres in series would be ~14,540 in the elderly (for a $L_f$ of 4.29 cm) and ~16,200 in the younger adults (for a $L_f$ of 4.78 cm). This difference in sarcomere number predicts that, in these elderly individuals, the maximum shortening velocity of the GM fascicles should be at least 10% lower than in the younger adults. Nevertheless, the actual reduction in maximum shortening velocity in the elderly is likely to be greater given that 1) the intrinsic maximum speed of shortening of (the most abundant) type I myosin heavy chain isoform is significantly lower in old age (15), 2) antagonist muscle coactivation is greater in old age (22, 29), and 3) tendon stiffness decreases in old age (30, 39). Because of the reduction in PCSA (and thus of maximum isometric force) and in fiber $L_f$ (and thus of maximum shortening velocity), the maximum muscle power is also expected to be reduced in the elderly. However, considering that in elderly subjects PCSA and $L_f$ were, respectively, 85 and 90% of those found in the young adults, the data predict a greater reduction in isometric force than in maximum shortening velocity. As a result, the optimum velocity for peak power generation is also expected to be lower in the elderly than in the young adults.

At present, without the use of labeling with radioactive markers such as $[^3]H$adenosine injected into the muscle, it is difficult to speculate on the mechanisms leading to this decrease in $L_f$ in aged muscle. However, it seems plausible that the removal of sarcomeres in series occurs at the distal and proximal ends of the fascicles, through mechanisms similar to those mediating the loss of sarcomeres in series in disuse due to immobilization (44).

In the elderly, fascicles were found to be not only shorter but also less pennate than in the younger adults. This effect is likely due to the decrease in contractile tissue packed along the tendon aponeuroses and is similar to that observed in disuse atrophy (38). For both sarcopenia and disuse atrophy, this phenomenon is probably due to the decrease in fiber size; however, in sarcopenia an additional decrease in $\theta$ should be expected owing to the reduction in fiber number (27). It seems that both sarcopenia and disuse atrophy involve changes in $\theta$ that are diametrically opposite to those found in hypertrophy, which is characterized by an increase in $\theta$ (21). A decrease in $\theta$ with muscle atrophy was predicted as early as in 1952 by Benninghoff and Rollhäuser (2) and was thought to give fibers a slight mechanical advantage due to a more effective force transmission to the tendon during contraction (10). Given that the resting $\theta$ is one of the factors determining the $\theta$ during contraction, quantifying architectural differences between subjects in the resting state is important to identify the origin of the respective differences in architectural changes on contraction.

Although in this study a significant correlation was found between ACSA and PCSA, the coefficient of determination ($R^2$) was 0.576, indicating that <60% of the variance in ACSA is explained by the variance in PCSA. This suggests that ACSA and PCSA should not be used interchangeably, particularly when normalizing force per CSA for estimating specific force, known to strictly depend on PCSA.

In conclusion, this study demonstrates that human GM architecture is significantly altered by aging. The
findings suggest that sarcopenia not only involves a loss of sarcomeres in parallel but also in series. These architectural changes are believed to play a significant role in the loss of muscle function in old age because they are likely to affect the length-tension as well as the force-velocity and power-velocity relations of this muscle on which common daily functions such as walking and stair negotiation depend.

The authors appreciate the collaboration of Dr. Edda Capodaglio in the screening of subjects for physical activity. We are also indebted to the participants of this study, particularly the senior volunteers, for the commitment and time given to this project.

REFERENCES