EFFECT OF AGING ON HUMAN MUSCLE ARCHITECTURE

M.V. Narici¹, C.N. Maganaris¹, N.D. Reeves¹, and P. Capodaglio²

¹Centre for Biophysical and Clinical Research into Human Movement (CRM), Manchester Metropolitan University, Alsager Campus, Alsager, ST7 2HL, Cheshire, United Kingdom
²Centro Studi Attivita’ Motorie, Fondazione Salvatore Maugeri, via Ferrata 8, 2100 Pavia, Italy

Running title: Aging and human muscle architecture

Address for correspondence: Prof. M.V. Narici Centre for Biophysical and Clinical Research into Human Movement (CRM), Manchester Metropolitan University, Alsager Campus, Alsager, ST7 2HL, Cheshire, United Kingdom
E-mail: m.narici@mmu.ac.uk
Tel:+44-161-247 5659, Fax: :+44-161-247 6375
Abstract

The effect of aging on human gastrocnemius medialis (GM) muscle architecture was evaluated by comparing morphometric measurements on 14 young (aged 27-42 years) and on 16 older (aged 70-81 years), physically active males, matched for height, body mass and physical activity. GM muscle anatomical cross sectional area (ACSA) and volume (VOL) were measured by computerised tomography, while 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 25.4% (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.

Key words: aging, skeletal muscle, architecture, muscle fibre
Introduction

Aging is known to be associated with a reduction in muscle mass (sarcopenia). Cross-sectional studies suggest that this phenomenon starts towards 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+ years of age, lower limb muscle mass decreases by about 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 has been found between young (20-29 years) and elderly (70-81 years) 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.

These studies suggest that sarcopenia alone cannot fully account for the observed loss of muscle strength and other factors, such as a reduction in motor unit activation capacity (12, 45), an increased antagonists muscles co-activation (22, 29), and a decrease in single fibre specific tension (26) have been shown to play a role, the contribution of changes in muscle architecture has seldom been considered. As a matter of fact, most investigators comparing differences in strength and muscle size of young and elderly individuals, have related force (or torque) to the anatomical CSA (ACSA) of the muscle, which is at right angles to the muscle belly. However, in pennate muscles (as is the case for most locomotor muscles) in which muscle fibres run at an angle to the axis of traction of the muscle, the ACSA does not represent the cross-section perpendicular to all fibres in the muscle, i.e., the physiological cross-sectional area (PCSA) (28, 35, 42). In fact, the maximum force of a muscle depends on its PCSA rather than its ACSA (1, 10, 13, 28, 35). Nevertheless, little is known on how aging affects muscle architecture, this is despite the fact that changes in muscle architecture could potentially modify not only the maximum force generating potential, dependent on the maximum number of sarcomeres placed in-parallel, but also the maximum shortening velocity, which depends on the number of sarcomeres placed in-series. The present study aims to
demonstrate that sarcopenia, not only involves a decrease in muscle mass, but also entails changes in muscle architecture. The study also addresses the functional significance of these changes. Preliminary data of this work have been presented elsewhere (37).
Material and Methods

Subjects
The investigation was conducted on sixteen elderly males aged 70-81 years (height, 1.72 ±0.03 m; body mass, 74.5 ± 8.3 kg) and for comparison, on fourteen younger males aged 27-42 years (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, while the younger participants were recruited amongst friends and colleagues. 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 practising ball-room dancing or used to move around by bicycle, but none was engaged in sporting activities at competitive level. The number of hours spent in recreational activities was assessed using the Saint-Etienne Physical Activity Questionnaire (QAPSE) (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).

Measurements

Muscle cross-sectional area and volume. Gastrocnemius medialis (GM) CSA was measured by computerised tomography (CT). The subjects were positioned supine in a General Electric scanner (ProSpeed Sx power) operating at 120kV peak, with the legs relaxed. The CSAs of 40 contiguous 10-mm slices (50 cm field of view, matrix 512x512 pixels), with 0 mm interslice gap, were
obtained starting from the knee space (slice 1). For each axial slice, CSA computation (mean of three consecutive morphometric measurements on the same MRI slice) was carried out on the GM muscle. Calculation of muscle CSA was performed by digitising throughout the muscle contour using an image analysis programme (NIH image version 1.61/ppc, National Institute of Health, Bethesda, MD). The maximum CSA of the GM, normally corresponding to the 5th or 6th axial scans distal to the knee space, was selected for data analysis. The error in this technique, evaluated by digitising shapes of known areas, was estimated to be 2.1% while the coefficient of variation (CV) for three CSA measurements, repeated on the same subject on 6 different days, was 2.6%.

For GM muscle volume calculation, all slices were fitted with a spline algorithm in order 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 volume (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 their legs relaxed and both feet hanging outside the couch. A plastic cast shaped to the sole of the foot and calcaneous and extending 10 cm above the malleolus was taped to the dominant foot to standardise measurements at the same resting ankle joint angle. The tibiotalar joint angle chosen for this investigation was 115 deg (90 deg being the angle when the foot is perpendicular to the tibia), since in most subjects this was found to correspond to the resting ankle joint angle with the foot hanging freely from the examination table when lying prone. In both young and older participants, the above angle corresponded to the spontaneous resting angle of the tibiotalar 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, USA). Images were obtained at mid-belly of the dominant GM muscle 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. Mid-belly was defined as the point along the median longitudinal axis of the muscle at 50% of the distance between the proximal and distal apices of the myotendinous junctions, and the centre 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. Pennation angle was measured as the angle of insertion of muscle fibre fascicles into the deep aponeurosis and fascicle length was defined as the length of the fascicular path between the insertions of the fascicle into the superior and deep aponeuroses. In cases where 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, where 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 since resting fascicles present negligible curvature (32, 33). Physiological cross-sectional area (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 anatomical measurement on a cadaver and found to be in good agreement (36), as, in the central region of the muscle, fascicle length and pennation angle differed by an average of 1.2 mm and 1.5 deg respectively, between the two techniques.

Images were captured with a video-capture card (Capsure, iREZ Research Corporation, USA) 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 CSA 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 analysed 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-Wilks' W test, $P<0.05$) a non-parametric Mann-Whitney rank-sum test was applied. Linear regression analysis (Pearson product-moment correlation) was used to compare the degree of association between variables. The critical level for statistical significance was set at 5%.
Results

Gastrocnemius medialis muscle’s maximum ACSA_max, 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 to the younger adults.

The differences between the elderly and younger groups were 19.1% (P<0.005) for ACSA_max, 25.3% (P<0.001) for VOL, 10.2% (P<0.01) for Lf, 13.2% (P<0.01) θ, and 15.2% (P<0.05) for PCSA. When pennation angle 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 pennation angle scales with ACSA_max. Also, it is noteworthy that in most elderly individuals both values of ACSA_max and θ were smaller than in the younger adults.

Although the difference in mean ACSA seemed greater than that of mean PCSA (Table 1), no significant difference was found between the ratios of ACSA/PCSA of the young (0.30±0.04) and those of the elderly subjects (0.29±0.06, n.s.). When the values of ACSAs and PCSAs of the young and elderly subjects were pooled together, a significant correlation (r=0.759, P<0.01) was found between ACSA and PCSA.
Discussion

The present study demonstrates for the first time that human gastrocnemius medialis muscle architecture is significantly altered in old age. Since 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 normalization of the measurement to limb segment length, Kubo et al. (24) found differences in fascicle length and pennation angle between young and elderly subjects for the vastus lateralis muscle, but not for the gastrocnemius medialis and triceps brachii muscles. The lack of physical activity matching between subjects makes it difficult to ascertain whether the above muscle architecture differences found were due to aging per se or the combined effect of aging and disuse. The absence of architectural changes in the gastrocnemius medialis was attributed by Kubo et al. (24) to a greater use of the plantarflexors compared to the knee extensors in locomotor activities, or to a different plasticity of these two muscles in response to ageing. However, our results demonstrate that when the influence of disuse is controlled for by matching individuals for physical activity level, significant alterations in gastrocnemius medialis muscle architecture 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 fascicle length 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 gastrocnemius muscles. This mechanical stimulus is
likely to affect pennation angle and fascicle length, a factor that is accounted for by the approach we employed to match the subjects studied for body height and mass.

In the present study, muscle volume, maximum anatomical cross-sectional area, fibre length and pennation angle, were all found to be significantly reduced in the older individuals compared to the younger adults. As a result of the decrease in muscle volume (-25.3%) and in fibre fascicle length (-10.2%), a reduction in PCSA was also found (-15.2%). This decrease in PCSA was affected more by the reduction in muscle volume than in fibre length. Nevertheless, the fact that both of these decrease, strongly suggests that sarcopenia involves not only a loss of sarcomeres 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 volume, determined by MRI, to fascicle length estimated from the ratio of fibre length/muscle length published in the literature, thereby assuming that the ratio of fibre length/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 presents results which theoretically would shift rightwards 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 fibre specific tension (26), an increased antagonist muscle co-activation (11, 17, 29), and in some cases a reduced muscle activation capacity (4, 12, 47); however see also refs (6, 7, 18, 22, 43).

Assuming a linear relationship between in-series sarcomere number and fascicle length (14), it follows from previously reports on human cadaver GM architecture measurements (16) that the number of sarcomeres in series would be ~14540 in the elderly (for a fascicle length of 4.29 cm), and ~16200 in the younger adults (for a fascicle length 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 muscles co-activation is greater in old age (22, 29), and 3) decreased tendon stiffness in old age (30, 39). Due to the reduction in PCSA (and thus of maximum isometric force) and in fibre fascicle length (and thus of maximum shortening velocity), also the maximum muscle power is expected to be reduced in the elderly. However, considering that in elderly PCSA and fascicle length were respectively 85% and 90% those found in the young adults, the data predicts a greater reduction in isometric force than in maximum shortening velocity. As a result, also the optimum velocity for peak power generation is expected to be lower in the elderly than in the young adults.

At present, without the use of labelling 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 fascicle length 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 immobilisation (44).

In the elderly, fascicles were not only found to be 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 fibre size, however, in sarcopenia an additional decrease in pennation angle should be expected due to the reduction in fibre number (27). It seems that both sarcopenia and disuse atrophy involve changes in pennation angle are diametrically opposite to those found in hypertrophy which is characterised by an increase in pennation angle (21). A decrease in pennation angle with muscle atrophy was predicted as early as in 1952 by Benninghoff and Rollhäuser (2) and was thought to give fibres a slight mechanical
advantage due to a more effective force transmission to the tendon during contraction (10). Given that the resting pennation angle is one of the factors determining the pennation angle during contraction, quantifying architectural differences between subjects in the resting state is important to identify the origin of the respective differences in architectural changes upon 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 less than 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 normalising force per cross-sectional area for estimating specific force, known to strictly depend on PCSA.

In conclusion, this study demonstrates that human gastrocnemius muscle architecture is significantly altered by ageing. 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 since 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.

**Acknowledgements**

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

Table 1: Summary of gastrocnemius medialis muscle architectural data in young (aged 27-42 years, \(n=14\)) and in elderly individuals (aged 70-81 years, \(n=16\)). Data are means±SD. \((ACSA_{\text{max}}):\) maximum anatomical cross-sectional area; \(VOL: \) muscle volume, \(Lf: \) fascicle length; \(\theta: \) pennation angle, \(PCSA: \) physiological cross-sectional area).

Figure 1: Individual data of maximum anatomical cross-sectional area \((ACSA_{\text{max}})\) plotted against pennation angle for the young adults (filled circles) and elderly individuals (open circles). Data are fitted with a linear regression function after pooling the values for the young an elderly subjects together.

Figure 2: Typical sonographs of the gastrocnemius medialis muscle of an elderly (EM) and of a young male (YM).
Table 1

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<th>ACSA&lt;sub&gt;max&lt;/sub&gt;</th>
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<td>4.29 ±0.67</td>
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<td>50.1 ±12.6</td>
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<td>4.78 ±0.55</td>
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<td>59.1 ±14.4</td>
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(𝑡-𝑡𝑒𝑠𝑡)
y = 0.4406x + 18.422
r = 0.432, P<0.05
Fig. 2

EM

YM

10 mm
References


