Functional and Morphological Adaptations to Aging in Knee Extensor Muscles of Physically Active Men

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It is not known if a physically active lifestyle, without systematic training, is sufficient to combat age-related muscle and strength loss. Therefore, the purpose of this study was to evaluate if the maintenance of a physically active lifestyle prevents muscle impairments due to aging. To address this issue, we evaluated 33 healthy men with similar physical activity levels (IPAQ = 2) across a large range of ages. Functional (torque-angle and torque-velocity relations) and morphological (vastus lateralis muscle architecture) properties of the knee extensor muscles were assessed and compared between three age groups: young adults (30 ± 6 y), middle-aged subjects (50 ± 7 y) and elderly subjects (69 ± 5 y). Isometric peak torques were significantly lower (30% to 36%) in elderly group subjects compared with the young adults. Concentric peak torques were significantly lower in the middle aged (18% to 32%) and elderly group (40% to 53%) compared with the young adults. Vastus lateralis thickness and fascicles lengths were significantly smaller in the elderly group subjects (15.8 ± 3.9 mm; 99.1 ± 25.8 mm) compared with the young adults (19.8 ± 3.6 mm; 152.1 ± 42.0 mm). These findings suggest that a physically active lifestyle, without systematic training, is not sufficient to avoid loss of strength and muscle mass with aging.

Keywords: aging, muscular adaptation, strength, muscle architecture

Aging is associated with a progressive decrease in quantity, quality, and functionality of muscle tissue. Muscle degeneration is evident in the fifth decade of life, and affects especially the lower limbs of men and women.1–4 The loss of muscle mass and strength with aging is an important aspect of the loss of functionality and independence, and thus, the quality of life, morbidity, and mortality in the elderly.5–7 Therefore, great attention has been paid to the socioeconomic impact of elderly health care in developed countries.8

Skeletal muscle force production is known to depend, among several other factors, on the length9 and velocity10 of contraction. In human studies, these properties are typically evaluated in vivo using isokinetic dynamometers to measure the torque-angle (T-A) and torque-velocity (T-V) relationships. Knee extensor strength has been found to be a good predictor of independence, and an estimator of lifespan in the elderly;11 thus, knowledge of the changes in knee extensor strength has important clinical and social implications. Cross-sectional studies of knee extensor strength showed that subjects in the seventh and eighth decades of life are 20–40% weaker than young adults.5,6 Although not exclusively, the elderly lose strength primarily because of a loss in muscle mass, called sarcopenia.3,12 Sarcopenia is related to neural, hormonal, nutritional, metabolic, immunologic, and lifestyle factors;2 is more pronounced in men than women;2,3 and is more predominant in lower than upper limb muscles.2,3,11 Sarcopenia is associated with a reduction in sarcomeres arranged in series and in parallel in muscles, which can be assessed using muscle thickness, pennation angle, and fascicle length measurements12,13 and affect the T-A and T-V relationships.

The most reliable data on muscle adaptations with aging are obtained through longitudinal studies, as performed by Winegard et al14 and Frontera et al.11 However, because of the difficulties in performing longitudinal studies, strength loss and muscle mass decrease have been documented primarily through cross-sectional studies.1–4,15–22 It has been argued that degeneration of muscles with aging is related to natural biological processes and to reductions in physical activity. Exercise programs, specifically resistance training, have been shown to maintain and/or restore strength and muscle mass in the elderly.7,23 However, it is not clear if a physically active lifestyle (without systematic training) helps in reducing muscle impairments associated with aging.
Comparisons of muscle strength and function have been made using young and elderly trained subjects\textsuperscript{20,21} and recreationally active subjects.\textsuperscript{24} However, in these studies, physical activity levels between the young and elderly group subjects were not matched. Here, we compare knee extensor muscle morphology and function in anthropometrically similar male subjects aged 21–80 who have similar physical activity levels, thereby eliminating physical activity as a confounding variable. We hypothesized that an active lifestyle prevents the deleterious effects of aging on muscle structure and function in the elderly group.

**Methods**

**Participants**

Healthy men, between 21 and 80 years old, participated in this study. All volunteers completed the long form of the *International Physical Activity Questionnaire* (IPAQ)\textsuperscript{25} and only subjects classified as moderately active (ie, subjects that reached level 2 in IPAQ) were included in this study. Based on an interview with the researchers and evaluations with a physical therapist and a cardiologist, the following exclusion criteria were used: (1) a history of lower limb musculoskeletal injuries that could interfere with maximal isokinetic dynamometry results, (2) cardiovascular disorders or contraindications to perform maximal strength tests, and (3) enrollment in a systematic physical training program.

Thirty-six volunteers gave written informed consent to participate in this study. One subject was excluded from the study because of troubles executing the tests according to instructions, and another subject was excluded because joint pain limited his ability to perform maximal strength tests. In addition, one subject’s torque values were identified as statistical outliers (more than 3 SD from the group mean value), and thus were excluded from analysis.

Therefore, 33 subjects comprised the final study and were allocated into three age groups: young adult group (YG; n = 12; 21–40 y); middle age group (MG; n = 11; 41–60 y); and elderly group (EG; n = 10; 61–80 y). Height, weight, and body mass index were similar among subjects from the YG, MG, and EG groups (Table 1).

**Procedures**

This study was approved by the university ethics in research committee (project nr. 2006611). Subjects made one visit to the laboratory for the IPAQ and interview with the researchers, followed by another visit for evaluations of muscle architecture, T-A and T-V relationships. The evaluations with the physical therapist and the cardiologist were made in the period between these two visits to the laboratory.

*International Physical Activity Questionnaire (IPAQ).*

This retrospective questionnaire was developed as a surveillance instrument to measure multiple domains of physical activity. IPAQ has been validated,\textsuperscript{25} and used as an instrument to validate other physical activity questionnaires.\textsuperscript{26} Moreover, a study with more than 50,000 subjects between the ages of 18 and 65 concluded that IPAQ is an acceptable instrument,\textsuperscript{27} and IPAQ has been widely used in Brazil (where our study was developed) for the last decade.\textsuperscript{28} Briefly, IPAQ captures the intensity of activities performed during leisure time, at work, during domestic tasks and active transport. The sum of all these activities is defined as the total physical activity. The long form of IPAQ measures 27 items of domain-specific activity levels and intensity-specific categories. It allows for classifying participants into “low,” “moderate,” and “high” activity performers.

**Muscle Architecture Assessment.** An ultrasonography machine (SSD 4000; 51 Hz; Aloka, Japan) with corresponding 38 mm linear array probe (7.5 MHz) were used to assess muscle thickness, pennation angle, and fascicle length.\textsuperscript{29,30} Muscle morphology was obtained with the volunteer at rest and seated in an isokinetic dynamometer chair (Biodex System 3 Pro, Biodex Medical System, USA) with the hip at 85° of flexion (0° = anatomical position) and the knee at 60° of flexion (0° = full knee extension). A skin marker was placed midway between the great trochanter and the lateral knee joint line to ensure placement of the ultrasound probe at the midbelly of the vastus lateralis (VL). The probe was covered in ultrasound gel for optimal contact, and was placed parallel to the VL fibers.

Muscle thickness was defined as the mean distance between deep and superficial aponeuroses, measured at five places along the ultrasound image (Figure 1).

<table>
<thead>
<tr>
<th>Table 1 Characteristics of subjects in the three age groups</th>
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<tbody>
<tr>
<td><strong>Young Adults (YG)</strong></td>
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<tr>
<td>Number of subjects</td>
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<tr>
<td>Age (y)</td>
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<tr>
<td>Height (m)</td>
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<tr>
<td>Weight (kg)</td>
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<td>BMI (m²·kg⁻¹)</td>
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The angle of pennation was defined as the mean angle between the deep aponeurosis and three fascicles in the midbelly of VL. Fascicle length was defined as the mean of three fascicle lengths in the midbelly of VL. Since fascicle lengths often exceeded the range of the ultrasound probe, fascicle lines of action were extrapolated linearly and lengths calculated from the knowledge of the muscle thickness and pennation angle of the fascicles.\textsuperscript{18,19,31–38}

**Torque Assessment.** Subjects were positioned with the dominant lower limb strapped to the isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical System, USA), following the manufacturer’s recommendations for evaluation of knee flexion-extension strengths (seated with 85° of hip flexion and fixed by the equipment straps). Subjects performed a protocol aimed at determining the T-A and T-V relationships of the knee extensor musculature. Before each evaluation, gravity correction was performed with the knee joint at an angle of 30° according to the manufacturer’s instructions. Submaximal isometric and isokinetic contractions were performed for familiarization and warming up.

For the T-A evaluation, subjects were asked to perform maximal voluntary isometric knee extensor contractions lasting 5 s at six different joint angles: 30°, 45°, 60°, 75°, 90°, and 105° provided in random order. Three contractions were performed at each knee angle, and 2 min of rest was given between contractions to minimize fatigue. Verbal encouragement was given by researchers during each muscle contraction.

Before the T-V protocol, subjects were given 5 min of rest. For the T-V testing, subjects were instructed to perform three maximal voluntary knee extensor contractions at six concentric angular velocities: 60°/s, 120°/s, 180°/s, 240°/s, 300°/s, and 360°/s in random order. Two minutes of rest was given between each test. Subjects were previously instructed and encouraged during the tests to perform maximal force throughout full range of motion.

The highest torque among the three repeat contractions for each condition was used for analysis. The first maximal voluntary contraction of the T-A test was repeated at the end of the entire protocol (ie, after the last T-V test) to determine possible fatigue. Statistical analysis revealed that there was no significant fatigue.

To determine the joint angle of maximal torque from the six discrete torque-angle measurements, we fitted a quadratic equation to the raw data and determined the maximum value, and corresponding joint angle, analytically. To estimate the maximum speed of shortening of the knee extensor muscles, we calculated the power-velocity relationship from the raw data of the T-V relationship, calculated the maximal power and corresponding angular velocity using a best fit quadratic approximation, and calculated the maximal knee extensor angular velocity as three times the angular velocity at maximal power output.\textsuperscript{39}

**Statistical Analysis**

A one-way ANOVA followed by a Holm-Sidak post hoc test was used for comparisons of anthropometric data (height, weight, and body mass index), muscle architectural parameters (muscle thickness, pennation angle, and fascicle length), and mechanical values (optimal joint angle, isometric peak torque, maximal angular velocity) between age groups.

A two-way ANOVA (group × joint angle; group × angular velocity) was used to analyze knee extensor torque for isometric and for isokinetic conditions. When group-angle or group-velocity interactions were observed, a one-way ANOVA followed by a Holm-Sidak post hoc test was applied to compare the three groups at each joint angle and for each angular velocity.

All statistical procedures were performed in Sigma-Plot 11.0 software (Systat Software Inc., Germany) using a level of significance of 0.05. Results are presented as means ± 1 SD.
Results

Muscle thickness was greater in YG subjects compared with EG subjects ($P = .034$). Fascicle lengths were greater in YG subjects compared with MG subjects ($P = .045$) and were also greater compared with EG subjects ($P = .004$) (Table 2; Figure 2). Pennation angle of the fascicles was significantly different between age groups.

Torque values in YG subjects were higher than in EG subjects for all knee angles except 30° (ie, 45° $P = .013$; 60° $P < .001$; 75° $P < .001$; 90° $P = .001$; 105° $P = .003$). The YG subjects also had greater isometric torques than MG subjects at a knee angle of 75° ($P = .034$). Torque values were higher for MG subjects than EG subjects only for a knee joint angle of 90° ($P = .034$).

In other words, MG and EG subjects had similar torques for most of the T-A relationship, but torques were lower in EG compared with YG subjects for most of the T-A relationship. Torque values in YG subjects were 10–21% and 30–36% higher compared with MG and EG subjects, respectively (Figure 3).

Maximal isokinetic torques in YG subjects across all angular velocities were 18–32% ($P < .001$) and 40–53% ($P < .002$) higher than in MG and EG subjects, respectively (Figure 4). Similarly, torques for MG subjects were higher than those observed for EG subjects at all angular velocities ($P < .002$), except at 360°/s where they were similar ($P = .063$).

The optimal angle, or the angle of peak torque occurrence, was the same ($P = .233$) for all age groups (YG = 75.6 ± 9.0°; MG = 79.1 ± 9.1°; EG = 72.4 ± 8.2°). Maximal isometric torque was higher for YG subjects (293.5 ± 34.4 N/m) compared with MG (236.4 ± 60.8 N/m) ($P = .015$) and EG subjects (185.4 ± 45.07) ($P < .001$), and was also higher for MG compared EG subjects ($P = .021$). The maximal knee angular velocity was higher in YG (1210 ± 368°/s) compared with EG subjects (894 ± 144°/s) ($P = .016$), but was similar for YG and MG subjects (970 ± 122°/s) ($P = .054$).

Figure 2 — Ultrasound images of representative subjects from the three age groups: young adult group (YG), middle age group (MG) and elderly group (EG). All images have the same scale and were aligned using the superior aponeurosis of the vastus lateralis muscle to emphasize the difference in muscle thickness.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Vastus lateralis architectural parameters from the three age groups: muscle thickness (millimeters), fascicle pennation angle (degrees) and fascicle length (millimeters)</th>
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<tbody>
<tr>
<td></td>
<td>Young Adults (YG)</td>
</tr>
<tr>
<td>Muscle thickness</td>
<td>19.8 ± 3.6</td>
</tr>
<tr>
<td>Pennation angle</td>
<td>8.2 ± 3.1</td>
</tr>
<tr>
<td>Fascicle length</td>
<td>152.1 ± 42.0</td>
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</table>

*Indicates significant difference between YG and EG.
†Indicates significant difference between YG and MG.
Discussion

The main purpose of this study was to evaluate if maintenance of a physically active lifestyle helps avoid the undesired loss in muscle structure and function associated with aging. Our results from similarly active men across all ages showed that subjects in the elderly age group lost quadriceps strength and showed changes in muscle morphology. These results do not support our initial hypothesis, and suggest that a physically active lifestyle is not sufficient to prevent degenerative changes associated with muscle aging.

A reduction in muscle mass is considered the major cause for the loss of strength in the elderly.2 Loss in muscle mass has been quantified in the past by reductions in anatomical cross-sectional area (ACSA), physiological cross-sectional area (PCSA) or muscle volume. Ultrasonography has also been used to measure structural changes in muscles with aging.18–21 While some studies reported significant changes in VL architecture with aging,18,19 others found no such changes.20,21

We found that VL thickness is reduced in the elderly compared with the young group subjects, in agreement with previous findings.18,19 An age related decline in

![Figure 3](image-url)  — Mean (± 1 SD) knee extensor torques as a function of knee flexion angles for the three experimental age groups: young adult group (YG), middle age group (MG) and elderly group (EG). *Indicates significant differences between YG and EG. #Indicates significant differences between YG and MG. &Indicates significant differences between MG and EG.

![Figure 4](image-url)  — Mean (± 1 SD) knee extensor torques as a function of angular knee extension velocity for the three experimental age groups: young adult group (YG), middle age group (MG) and elderly group (EG). *Indicates significant differences between YG and EG. #Indicates significant differences between YG and MG. &Indicates significant differences between MG and EG.
pennation angle has also been previously reported\textsuperscript{13,18,19,40} and is thought to be due to reductions in fiber number and/or fiber diameter that accompany sarcopenia.\textsuperscript{41} However, we found no evidence of an age-related decline in pennation angle, which is in agreement with other reports.\textsuperscript{20,21}

There are at least two reasons why we might not have found a difference in pennation angle between the three age groups in our study. First, since our middle aged and elderly subjects were fairly active, fiber diameter, and thus pennation angle, might have been preserved.\textsuperscript{2,23} a result that is in agreement with findings in elderly athletes.\textsuperscript{7,20,23} Second, an age-related increase in noncontractile material (ie, adipose and connective tissues) around the muscle fibers,\textsuperscript{42} might have prevented a change in pennation angle, despite the loss of contractile material. Our results cannot be used to accept or reject either idea, but it appears that the decrease in muscle thickness in our study can be completely explained with a reduction in fascicle lengths.

The decrease in fascicle lengths in the elderly is commonly associated with a reduction in the number of serially arranged sarcomeres.\textsuperscript{13,18,22,40} Functionally, a decrease in fascicle length shifts the optimal lengths to shorter muscle lengths, and results in a reduction in the maximal speed of muscle shortening velocity.\textsuperscript{43} However, we did not observe the anticipated decrease in optimal muscle lengths in our study. There are a variety of reasons why this anticipated change might not have been observed. Possibly, measuring isometric forces just at six lengths might have reduced the predictive power of the measurements. In addition, the relatively small number of subjects per group, and the variability associated with human strength testing, might have reduced the statistical power of the experiments. Furthermore, the shorter fascicle lengths in the elderly compared with the YG subjects might have been associated with shorter sarcomere lengths, thus offsetting an expected shift in the T-A relationship purely based on fascicle lengths. Finally, structural parameters other than fascicle length, for example, series compliance, tendon and aponeuroses lengths, changes in knee extensor moment arms, and so on, could have affected optimal length in an unpredictable manner.

The effects of resistance training on aging muscles are controversial. Fourteen to 16 weeks of conventional and eccentric resistance training with elderly subjects produced some changes in VL architecture in some groups but not in others.\textsuperscript{18,45,46} The reason for the discrepancy in outcomes is not clear, but might be related to differences in the details of the training protocols (magnitude of resistance training, frequency of training, duration of program), or differences in the studied populations (age, fitness level, adherence, enthusiasm for the training protocol, health status). The novelty of our study was that subjects across all age groups had a similar level of physical activity, a score of 2 (moderately active) on the IPAQ. However, this level of activity did not prevent muscle loss due to aging.

The EG subjects had a 30–36% decrease in knee extensor strength compared with the YG across the range of knee angles tested. These values are consistent with those reported in the literature (24% to 45%).\textsuperscript{5,6} Longitudinal studies suggest that strength loss with aging might even be greater than suggested by cross-sectional studies, as cross-sectional studies may tend to recruit "fitter" than average elderly populations, thereby underestimating the "true" average loss in strength.\textsuperscript{11,14}

Comparing the T-A relationships obtained here with previous works, they show the expected ascending-descending behavior expected from the knee extensor musculature.\textsuperscript{47} In detail, the T-A relationships for the MG and EG subjects showed a more pronounced plateau that YG subjects. This result might indicate that, aside from the pure strength, the shape of the T-A relationship might also be affected by age-related changes to the muscles, the neuromuscular activation, or the geometry (moment arms and excursion).

Everything else remaining the same, shorter fascicle lengths are associated with a steep ascending and descending limb of the force-length relationship, and a small plateau region.\textsuperscript{39} Here, we observed a relatively shallow ascending and descending limb, a similar optimal angle, and an extended plateau, of the T-A relationship for the EG and MG subjects, which is in contrast to what one would expect from their short fascicle lengths. Therefore, it appears that other factors than fascicle lengths (activation, calcium sensitivity, joint geometry, muscle excursion) may play a role in the shape of the T-A relationship in the elderly. Measuring the T-A relationship over a greater range of knee angles (here we restricted our analysis to 30–105°), at smaller intervals (eg, every 5° rather than 15°) might provide important information on the age-related shape changes in knee extensor T-A relationships.\textsuperscript{4}

Strengths at all speeds of shortening was smaller with increasing age, as one would expect (Figure 4), and decreased with increasing speeds of shortening, as predicted by the force-velocity relationship of skeletal muscle.\textsuperscript{30} The T-V relationships for the three age groups are similar but offset due to the smaller isometric force in the MG compared with the MG and YG subjects, and the MG compared with the YG subjects. Because of this offset, and the otherwise similar shapes in the T-V relationships, the maximal shortening velocities are predicted to be smaller for the EG than the MG, and smaller for the MG than the YG subjects.

A previous study\textsuperscript{22} has reported that almost half of the difference in maximal shortening velocity between young and older adults can be explained by the reduction in fascicle length, and the rest of the difference is likely explained by a preferred fast-twitch fiber atrophy and age-related metabolic and neural changes of muscles.\textsuperscript{4,5,7,22,44} Therefore, the smaller maximal velocities of knee extension in the elderly can be explained, in part, with the reduced fascicle lengths observed in this study. To identify the detailed mechanisms underlying the loss of maximal shortening speed in the elderly, careful analysis of the fiber type distribution in elderly compared with young people, and accurate measurement not only of fascicle lengths, but also the associated sarcomere lengths is required.

Our results for the estimated optimal angle revealed that aging did not produce a shift in the optimal length for
force production as we expected. The reason for that is not yet understood, and future studies should try to determine other parameters that may influence optimal angle for maximal force such as series elastic elements compliance. Estimated maximal isometric torque decreased with aging, similar to what was previously reported for the T-A relation. Similarly, the estimated maximal shortening (or angular) velocity decreased in the EG compared with the YG, as shown in the T-V relation. This further emphasizes that being moderately active is not enough to maintain muscle function and prevent the deleterious effects of aging on muscle function. In addition, these results suggest that training programs in elderly subjects should promote sarcomere addition in parallel and in series to increase maximal strength and shortening velocity, respectively.

Sample size for each of the age group was relatively small (n = 10–12). Nevertheless, we found statistically significant differences for most of the primary outcome variables (strength, fascicle length, maximal speed of shortening), thereby providing evidence that sample size was adequate. A greater sample size may have yielded a few more statistically significant results. However, the relevance of such findings (smaller differences with more variability) may also be more questionable.

Upon completion of the study, it became apparent that measurement of additional muscle parameters (muscle volume and physiological cross-sectional areas, fiber type distribution, and sarcomere lengths) would have helped in the interpretation of the T-A and T-V results. Future studies will take this into account.

Because of the size of the ultrasound probe, fascicle lengths were obtained by linear extrapolation, as has been done in many previous studies. Although this is by no means ideal, the error associated with this procedure has been estimated to be random and about 4.7%. Since differences in fascicle lengths between all age groups were in excess of 20%, we feel confident that our results reflect accurately the loss in fascicle length with aging. This statement is supported by the agreement of our fascicle lengths for VL with those obtained in previous studies.

Determination of the T-V relationship for knee extension exercises can be done in essentially two ways: (i) measuring the instantaneous torque at a given knee angle (eg, the optimal knee angle for isometric contractions) or (ii) measuring the maximal torque independent of the knee angle. Both approaches have advantages and disadvantages. We chose the second approach because we were interested in the functional properties of muscles across different age groups, rather than in the assessment of the force-velocity properties of the knee extensor muscles. This choice implies that the maximal torque is not measured (obtained) at the same knee angle across speeds, but it is a consistent measure that can be compared across subjects. Therefore, we feel justified in the approach.

We conclude from the results of this study, that a physically active lifestyle is not sufficient to maintain knee extensor muscle mass, strength and fascicle length with aging. Systematic exercise training may prevent muscle atrophy and sarcopenia to a greater degree than a physically active lifestyle, although our study does not allow for such comparisons.

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