LONG-TERM HIGH-LEVEL EXERCISE PROMOTES MUSCLE REINNervation WITH AGE

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Abstract

The histologic features of aging muscle suggest that denervation contributes to atrophy, that immobility accelerates the process, and that routine exercise may protect against loss of motor units and muscle tissue. Here, we compared muscle biopsies from sedentary and physically active seniors and found that seniors with a long history of high-level recreational activity up to the time of muscle biopsy had 1) lower loss of muscle strength versus young men (32% loss in physically active vs 51% loss in sedentary seniors); 2) fewer small angulated (denervated) myoﬁbers; 3) a higher percentage of ﬁber-type groups (reinnervated muscle ﬁbers) that were almost exclusive of the slow type; and 4) sparse normal-size muscle ﬁbers coexpressing fast and slow myosin heavy chains, which is not compatible with exercise-driven muscle-type transformation. The biopsies from the old physically active seniors varied from sparse ﬁber-type groupings to almost fully transformed muscle, suggesting that coexpressing ﬁbers appear to ﬁll gaps. Altogether, the data show that long-term physical activity promotes reinnervation of muscle ﬁbers and suggest that decades of high-level exercise allow the body to adapt to age-related denervation by saving otherwise lost muscle ﬁbers through selective recruitment to slow motor units. These effects on size and structure of myoﬁbers may delay functional decline in late aging.

Trial registration: ClinicalTrials.gov (NCT01679977).

Key Words: Aging, Coexpression of fast and slow myosin heavy chains, Denervation and reinnervation, Fiber-type grouping, Human skeletal muscle, Recreational sport activity.

INTRODUCTION

Aging is characterized by a gradual decline that impairs cell homeostasis and functional reserves. Degeneration, apoptosis, and death (accompanied by loss of regenerative capacity) of all cell types progressively accumulate and ultimately lead to organism death (1–4). Histologic studies of skeletal muscle have shown that denervation is among the numerous mechanisms that contribute to tissue atrophy and degeneration in aging (5, 6). The term “disseminated neurogenic atrophy” was coined to describe the progressive accumulation and clustering of small angulated ﬁbers with aging (7–10); there is also evidence of progressive loss of α-motoneurons (11, 12). Electrophysiologic studies have conﬁrmed that there is a decrease in the number of motor units with a concomitant increase in their size with age. These results suggest that some reinnervation events follow muscle ﬁber denervation (13). Further evidence supporting the occurrence of rounds of denervation and reinnervation includes the increased clustering of myoﬁber types in the motor units of rodents and other mammals as they age (11, 14). In adult humans, fiber...
types appear randomly distributed across the muscle and become increasingly grouped with age (15). Therefore, it has been proposed that, in addition to axonal disorders, apoptosis of α-motoneurons in the spinal cord, with subsequent incomplete reinnervation of fibers by surviving motor neurons, may contribute to loss of muscle strength and mass as people grow older (16). These rearrangement processes are generally accompanied by a progressive increase in the proportion of slow muscle fibers, although there is some evidence to the contrary (17). Some of the discrepancies have been dispelled by comparisons of muscle from normally active and immobile older patients that show that muscle wasting in “normally active” seniors is accompanied by a shift toward a slow-twitch phenotype, whereas inactive seniors demonstrates a shift toward fast-twitch isoform expression. This latter case is common in ‘‘unloaded’’ muscle undergoing atrophy, for example, during limb suspension, immobilization, paralyzis, and spaceflight (18–22). To complicate the situation further, conflicting results regarding fast to slow myosin transition arise in endurance training studies using animal models and in clinical trials of humans involving either voluntary exercise or electrical stimulation—both directly to denervated muscle and indirectly to muscle through nerve stimulation (19, 21, 23–27). Furthermore, increased exercise that is sustained for decades (e.g., training as performed by track and field masters athletes) protects against age-related loss of motor units (28–30) and, thereby, of lean muscle mass (31). However, the degree to which denervation causes loss of myofibers is an open question because reinnervation events may compensate for motor neuron loss during aging as well as with spinal cord injury and/or axonal abnormalities of peripheral nerves (13–15, 32–34). Whether the aging-related shifts are under neural control or the result of the direct influence of use/disuse on myogenic processes remains to be clarified.

In the present study, we analyzed muscle biopsies harvested from the vastus lateralis of young men (aged 22–33 years), sedentary seniors (aged 67–77 years), and senior amateur athletes (aged 65–79 years). The latter routinely practiced sport activities usually more than 3 times per week up to the time of biopsy. In agreement with previous studies of masters athletes (35–37), we show that long-term high-level physical activity considerably increases the percentage of slow-type myofibers and the number of muscle fiber–type groupings. The latter provides direct evidence that long-term cycles of denervation/reinnervation occurred. In recent interim reports (38–40), we showed, and here confirm, that muscle properties of these senior recreational athletes are more similar to those of active young men than to those of sedentary seniors.

By analyzing coexpression of fast and slow myosin heavy-chain (MHC) isoforms in the muscle biopsies, we show for the first time that these events occur with recreational physical activity in seniors and that the changes may be related to selective reinnervation events. Our study supports the concept that long-term high-level exercise has beneficial effects on reinnervation of muscle fibers, resulting in preservation of muscle function, size, structure, and ultrastructure (41–43) and thereby delaying mobility decline and loss of independence that are commonly seen in aging.

**TABLE 1.** Denervated Fibers in Young Men and in Sedentary and Physically Active Seniors

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. Biopsies</th>
<th>&lt;30 μm</th>
<th>ANOVA</th>
<th>25 μm</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young men</td>
<td>10</td>
<td>0.4 ± 0.5*</td>
<td>Yes</td>
<td>0.2 ± 0.5*</td>
<td>Yes (vs sedentary)</td>
</tr>
<tr>
<td>Sedentary seniors</td>
<td>10</td>
<td>6.5 ± 3.8†</td>
<td>Yes</td>
<td>2.6 ± 1.9†</td>
<td>Yes (vs physically active)</td>
</tr>
<tr>
<td>Physically active</td>
<td>10</td>
<td>1.8 ± 3.9*</td>
<td>No</td>
<td>0.4 ± 1.1*</td>
<td>No (vs young men)</td>
</tr>
</tbody>
</table>

Within columns, groups with different footnote symbols are statistically different, p < 0.05. Yes and No refer to significant differences by ANOVA.

**MATERIALS AND METHODS**

**Study Subjects**

Approval from the national committee for medical ethics was obtained before the study onset (EK08-102-0608). With the exception of 2 female subjects in the sedentary group, recruited subjects were male volunteers. All subjects received detailed information on the study and gave informed consent. Three groups were enrolled: young men (n = 5; aged 22–33 years; 10 biopsies); seniors with a sedentary lifestyle (n = 6; aged 67–74 years; 10 biopsies), and seniors with a long history of high-level recreational sport activities (n = 7; aged 65 to 79 years; 10 biopsies) (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A541). All subjects were healthy and declared not to have any specific mobility impairment or disease. All of the subjects declared that they had no prescriptions for anti-inflammatory therapy related to neuropathies or myopathies. The seniors were not outpatients of any rehabilitation clinic but were located by newspaper advertisements that stressed that only healthy subjects without mobility impairment would be enrolled. Sedentary seniors were enrolled on the basis of their declaration that they had not performed any routine physical activity/training during the previous 10 years. On enrollment in the study, needle muscle biopsies were obtained from the vastus lateralis muscles through a small skin incision (6 mm); the biopsied tissue was then frozen for light microscopy or fixed for electron microscopy, as described (21, 41–43).

**Light and Immunofluorescence Microscopy**

Serial cryosections (8-μm thick) from frozen muscle biopsies were mounted on Polysine glass slides, air-dried, and

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stained with hematoxylin and eosin (42) or immunostained for either fast or slow MHC, laminin, or neural cell adhesion molecule (N-CAM), as described below.

For MHC, sections were washed with PBS and permeabilized with 0.1% Triton (Sigma-Aldrich, St. Louis, MO) in PBS for 15 minutes. After a PBS wash, nonspecific protein interactions were blocked by incubation with 10% fetal bovine serum in PBS for 30 minutes at room temperature (RT). The sections were then incubated for 1 hour at RT in primary mouse monoclonal anti-MHC fast or anti-MHC slow antibody (Novocastra, Milan, Italy) diluted 1:10 in PBS. The sections were subsequently washed in PBS and incubated with anti–mouse Cy3 secondary antibody (1:100; Sigma-Aldrich) for MHC slow and with anti–mouse FITC (1:100; Sigma-Aldrich) for MHC fast for 1 hour. The sections were washed again in PBS, and coverslips were mounted onto the glass slides using ProLong Gold antifade reagent with DAPI (Life Technologies, Carlsbad, CA). Images were acquired using a Zeiss microscope connected to a Leica DC 300F camera.

For detection of N-CAM–expressing myofibers, sections were fixed in methanol for 15 minutes at 20°C and then labeled for 1 hour at RT using rabbit polyclonal antibody directed against N-CAM (cat. no. AB5032; Chemicon, Millipore, Milan, Italy) diluted 1:200 in PBS. Sections were then incubated for 1 hour at RT with Cy3-labeled conjugate directed against rabbit IgG (Chemicon, Millipore) diluted 1:200 in 10% goat serum in PBS. Negative controls were performed by omitting the primary antibodies from sample incubations. After washes, nuclei were counterstained for 5 minutes at RT with Hoechst 33258 (Sigma-Aldrich); sections were then coverslipped using mounting medium (Dako, Glostrup, Denmark) and observed under a Zeiss microscope connected to a Leica DC 300F camera.

For immunolocalization of fast and slow MHC in single sections, the sections were washed, permeabilized, washed again, and incubated with blocking solution as previously described. They were then incubated for 1 hour at RT with mouse anti–MHC slow primary monoclonal antibody (Sigma-Aldrich) diluted 1:10 in PBS and secondarily with rabbit anti-laminin (Sigma-Aldrich) diluted 1:100 in PBS. Next, the sections were washed in PBS and incubated with the anti-mouse–Alexa 594 (1:200; Life Technologies) secondary antibody and the anti–rabbit FITC antibody (1:200; Sigma-Aldrich) for 1 hour. After a PBS wash, the sections were incubated for 1 hour with an anti–MHC fast primary monoclonal antibody produced in mouse (1:10; Novocastra). The sections were then washed with PBS and incubated for another hour with an anti–mouse–Alexa 488 secondary antibody (1:200; Life Technologies). After another wash with PBS, coverslips were mounted onto the glass slides using ProLong Gold antifade reagent with DAPI (Life Technologies).

Morphometric Analyses

Morphometric analyses of the fiber diameter and of the fiber-type distribution were performed on cryosections using Scion Image for Windows version Beta 4.0.2 (2000 Scion Corporation), as previously described (42–47).

Slow Fiber Correlations

To determine whether there was a correlation between slow fibers and the type of training undertaken by the physically active seniors (i.e. endurance, strength, or mixed training), percentages of slow muscle fibers were plotted against the probability that the value for each biopsy fell within the area under the curve. Excel equation DISTRIB.NORM(X;Mean;Dev_standard; Cumulative):

\[ f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x - \mu)^2}{2\sigma^2}} \]

Statistical Analysis

Analysis of variance (ANOVA) tests were performed with statistical algorithms of Origin (OriginLab Corp., Northampton, MA). The level of statistical significance was set at \( p < 0.05 \).

RESULTS

Demography and Clinical Characteristics Indicated That the Enrolled Subjects Were Healthy and Mobile

Detailed demographic and clinical characteristics of the enrolled subjects are described in (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A541). All subjects were healthy and declared not to have any specific mobility impairment or disease. Nonetheless, clinical and functional evaluations, in addition to electromyographic analyses, were performed in a few physically active seniors, resulting in detection of some neuropathic or myopathic features (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A541).

Amount of Weekly Physical Activity and Knee Extension Strength Are Significantly Higher in Physically Active Versus Sedentary Seniors

Amounts and types of physical exercise performed by the active subjects are detailed in Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A542. Four of the 5 young men performed strength training; the fifth did endurance activity. They declared that they exercised 4.0 to 7.5 hours per week during the previous 5 years. The physically active seniors trained 4.5 to 24.0 hours per week. Thus, a few seniors spent more time training than the young men; however, the apparent large difference between the mean times (11.7 ± 7.3 vs 5.3 ± 1.4 hours per week ± SD, respectively) was not significant. Sedentary seniors had not performed any exercise above normal everyday living activities throughout the previous 20 years.

To permit a comparison of specific muscle strength among the groups, quadriceps force was measured (Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A542). The mean (±SD) knee contraction strength in young men was 3.21 ± 0.55 Nm/kg; it was 2.17 ± 0.42 in the physically active seniors (a decrease of 32% relative to the young men); it was 1.57 ± 0.39 in sedentary seniors (a decrease of 51% vs the young men). The performances in a battery of functional mobility tests of the physically active seniors were more similar to...
those of young men than to those of the sedentary co-aged group (Kern et al, unpublished data). This is sound evidence that the enrolled physically active seniors are a highly active group and are likely comparable to masters athletes (35–37).

Small Angular Muscle Fibers in Both Young Men and Seniors (Sedentary and Sportsmen) Had the Size and Morphology of Denervated Muscle Fibers

Based on our experience with muscle biopsies from spinal cord–injured paraplegic patients with either disuse atrophy resulting from lesions of the central motoneuron or extreme atrophy caused by lack of innervation secondary to complete peripheral motoneuron lesions, we identify muscle fibers with a diameter less than 30 μm as denervated (20, 21, 43–47). Our interpretation of these myofibers as denervated is strengthened by the facts that half of these small fibers actually have diameters less than 25 μm and that several have angulated shapes. In the present study, serial sections of muscle biopsies from the young men reveal that myofibers having a diameter less than 30 μm are infrequent (0.4%; Table 1) and that those with a diameter less than 25 μm are even less abundant (0.2%; Table 1); the vast majority of the muscle fibers in these sections are predominantly round (Fig. 1A, B). The muscle sections from the sedentary seniors (Figs. 1C, D; 2, 3) reveal more abundant muscle fibers having diameters less than 30 and 25 μm (Table 1), and some of these have distinct angulation (Fig. 1, white arrowheads). In addition, Figure 2 shows that, in sedentary seniors, the small angular myofibers have appreciable expression of N-CAM, an accepted marker of denervation (48). The biopsies taken from the sedentary seniors contain the highest percentage of denervated muscle fibers having a diameter less than 30 μm (6.5%) and of those having a diameter less than 25 μm (2.6%). These percentages are significantly higher than those in the other 2 groups, whereas the percentages of denervated fibers are not significantly different between the young men and physically active seniors (Table 1). Furthermore, the N-CAM staining was much less abundant in biopsies from the active seniors and even less so in sections from the young men (not shown). Indeed, in a previous study, we observed only 1 N-CAM–positive muscle fiber among the 10,000 analyzed in the biopsies from young men (49).

Percentages of Fast and Slow Myofibers in Physically Active Seniors Showed a Significant Shift Toward Slow Fibers Relative to the Young Men and the Sedentary Seniors

Immunofluorescence revealed both fast and slow MHC proteins in serial sections of muscle biopsies from the young men (Fig. 1A, B), with the fast fibers being slightly more abundant than the slow fibers in these muscle sections (Table 2). Interestingly, this pattern of fiber-type distribution was not significantly different from that observed in the matched muscle of sedentary seniors, although the latter was slightly shifted toward the slow type relative to the young men (Fig. 1C, D; Table 2). However, in the active seniors (Fig. 1E, F), slow fibers were most numerous (68.5%; Fig. 1E, F); the increase

FIGURE 1. (A–F) Immunofluorescence staining for fast (A, C, E) and slow (B, D, F) myosin heavy chain (MHC) proteins in serial sections of biopsies from young men (A, B), sedentary seniors (C, D), and physically active seniors (E, F). White arrows point to small angulated fibers; white circles show fiber-type groups. Physically active seniors have fewer small fibers and more numerous slow fiber–type clusters than sedentary seniors. Fiber sizes in the biopsies of physically active seniors are comparable to those of the young men.
was significant versus both the young men (42% slow fibers) and the sedentary seniors (46% slow fibers) (Table 2).

Fiber-type grouping was almost absent in the young men; however, grouping was greater in the old subjects, with the physically active seniors having the greatest number; most of these were of the slow type, whereas those in sedentary seniors were mainly of the fast type. Fiber-type groupings are identified on the basis that at least 1 muscle fiber is completely surrounded by fibers of the same phenotype. Percentages of fiber-type groupings are determined by counting the number of muscle fibers in the biopsy that are surrounded by fibers of the same type and then dividing this number by the total number of fibers. To avoid problems related to the existence of many different fast MHC isoforms, we used an anti–fast MHC antibody that does not discriminate among the fast isoforms; therefore, we describe fiber-type clusters as either only “slow” or only “fast.” Muscle sections from the young men had few fiber-type groupings, and those that were detected were mainly of the fast type (1%; Fig. 1; Table 3). In the sedentary seniors, although both fast- and slow-type groupings were present, the fast type (3.0%) were more numerous than the slow type (0.5%) (Table 3). Most notable is the fact that biopsies taken from physically active seniors had the highest percentage of slow-type fiber groupings, with a mean of 7.9%, reaching almost 25% in extreme cases, in which 93% of total myofibers were of the slow type (Table 3). It is worth stressing that the physically active seniors with the most severe neuropathic or myopathic electromyographic results were not the subjects with the higher content of slow-type fibers and slow fiber–type groupings (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A541 and Table, Supplemental Digital Content 3, http://links.lww.com/NEN/A543).

**Muscle Fibers Coexpressing Fast and Slow MHC**

Muscle fibers coexpressing fast and slow MHCs were sparse in all of the analyzed biopsies, and there were no significant differences among these groups in terms of this parameter (Table 4). However, the serial sections from sedentary seniors that did show MHC coexpression were often small angular (denervated) muscle fibers (Fig. 1C, D, white arrows); therefore, we suggest that these are slow myofibers coexpressing fast isoforms of MHC by default myogenic programs. In contrast, the muscle fibers in the physically active seniors that were positive for both fast (green) and slow (red) MHC proteins were similar in size to the pure fast or pure slow myofibers (Fig. 4C). In Figure 4C, it is interesting to note that, although some fibers are green and others are red, not all of the fibers have the same color intensity. This might indicate that these fibers contain some variable combinations of fast and slow MHCs but not enough of both to produce the orange color of coexpression.

**Slow Fiber Correlations**

There was a strong correlation between the slow fiber percentages and slow fiber–type groupings in the physically active seniors ($R^2 = 0.82$; Fig. 5), but there was no correlation between the percentages of slow fibers and the prevalent kinds of training undertaken by the physically active seniors (Fig. 6). Indeed, the marks indicating whether the subjects had performed mainly strength training, endurance training, or a mixture of both strength and endurance trainings (mixed training) were randomly distributed among the severely and highly transformed muscle groupings.

The ages of the seniors were not correlated with percentages of slow fibers and slow fiber–type groupings in either group of seniors (Table 2). Interestingly, the study reveals some unique characteristics of the physically active seniors (Table 2), including the highest percentage of slow-type fiber groupings, with a mean of 7.9% in the physically active seniors (Table 3). This suggests that the physically active seniors were more likely to have slow-type fiber groupings than the sedentary seniors (46% slow fibers) (Table 2). It is worth noting that, although some fibers were found in the biopsies of the sedentary seniors, these fibers were mainly of the fast type, whereas those of the physically active seniors were of the slow type.

**FIGURE 3.** (A–D) Immunofluorescence staining for fast (A, C) and slow (B, D) myosin heavy chain (MHC) proteins in serial sections of biopsies from sedentary seniors (A, B) and physically active seniors (C, D). White arrows point to small angulated muscle fibers; white circles surround the central fibers that delineate fiber-type groupings. Note that the clustered fibers in the biopsies of the sedentary seniors are of the fast type, whereas those of the physically active seniors are of the slow type.

**TABLE 2. Percentages of Fast and Slow Myofibers in Young Men and in Sedentary and Physically Active Seniors**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. Biopsies</th>
<th>Fast (%)</th>
<th>Slow (%)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young men</td>
<td>10</td>
<td>58.1 ± 4.3*</td>
<td>42.0 ± 4.3*</td>
<td>No (vs sedentary)</td>
</tr>
<tr>
<td>Sedentary seniors</td>
<td>10</td>
<td>53.8 ± 13.2*</td>
<td>46.2 ± 13.2*</td>
<td>Yes (vs physically active)</td>
</tr>
<tr>
<td>Physically active</td>
<td>10</td>
<td>31.5 ± 14.1†</td>
<td>68.5 ± 14.1†</td>
<td>Yes (vs young men)</td>
</tr>
</tbody>
</table>

*Within columns, groups with different footnote symbols are significantly different, $p < 0.05$. Yes and No refer to significant differences by ANOVA.
High-Level Activity Promotes Muscle Reinnervation

TABLE 3. Percentages of Fast and Slow Fiber–Type Groupings in Young Men and in Sedentary and Physically Active Seniors

<table>
<thead>
<tr>
<th>Fiber-Type Groupings (% of Central Fibers in Clustered Areas vs Total Fibers)</th>
<th>Subjects</th>
<th>No. Biopsies</th>
<th>Fast</th>
<th>ANOVA</th>
<th>Slow</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young men Seniors</td>
<td>10</td>
<td>1.0 ± 2.0*</td>
<td>No</td>
<td>&lt;0.1 ± 0.1*</td>
<td>No (vs sedentary)</td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>10</td>
<td>3.0 ± 4.7*</td>
<td>No</td>
<td>0.5 ± 0.6*</td>
<td>Yes (vs physically active)</td>
<td></td>
</tr>
<tr>
<td>Physically active</td>
<td>10</td>
<td>0.1 ± 0.1*</td>
<td>No</td>
<td>7.9 ± 7.4†</td>
<td>Yes (vs young)</td>
<td></td>
</tr>
</tbody>
</table>

*Contents of reinnervated muscle fibers are expressed as the percentage of central muscle fibers surrounded by muscle fibers of the same phenotype relative to the total number of muscle fibers in each biopsy.

†Within columns, groups with different footnote symbols are significantly different, p < 0.05. Yes or No refer to significant differences by ANOVA.

The muscle of the active seniors in terms of fiber type and fiber-type groupings that suggest that denervation/reinnervation plays a role in the maintenance of muscle health.

The knee contraction strength in the active seniors was significantly greater than that of the sedentary seniors and not significantly different from that of the younger men (Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A542). Although the physically active seniors generated 32% less force than the young men on knee contraction (despite the fact that the groups had dedicated similar time to training), this is not surprising because it is well documented within the world sporting records of masters athletes that the young outperform the old (35–37, 50).

To explore the mechanisms that delayed deterioration in the muscle of the physically active seniors, we analyzed immunolabeled muscle biopsies taken from our groups and compared their relative amounts of 1) small angular myofibers (i.e. denervated muscle fibers); 2) molecular markers of fast and slow muscle fiber types (a measure of residual muscle plasticity); and 3) fiber-type grouping (representing denervated/reinnervated muscle fibers). We found that 1) biopsies from young men seldom contain denervated, reinnervated, or grouped muscle fibers; 2) biopsies from sedentary seniors contained both denervated and a few reinnervated clustered myofibers of the fast type; and 3) physically active seniors had a larger percentage of healthy slow myofibers, up to 90%, which appeared mainly clustered in slow fiber–type groups. The finding that physically active seniors have a significantly higher percentage of slow-type fibers and slow-type fiber groupings is consistent with our previous results using histochemical myosin ATPase staining in muscle biopsies from senior sportsmen (n = 15) (only 2 of those subjects are also part of the present study); 27 out of 28 biopsies had slow-type myofiber groupings (38). The increased slow-type fiber content and percentage of slow-type fiber groupings reflect the fact that immobilization drives muscle fibers toward atrophy and fast-type transformation. Because these parameters were significantly higher in the physically active versus the sedentary seniors, this is not simply a function of age. Furthermore, the lack of correlation between the kind of training and the percentages of slow-type fibers demonstrates that the type of activity is not the main determinant factor.

Interestingly, muscle fibers coexpressing fast and slow MCH proteins were seldom detected in the biopsies of any of our groups, and no statistical differences were found among the groups with respect to this parameter. It is possible that the observed coexpression is scant either because it is actually a rare event or because the denervation that occurs is promptly followed by reinnervation so that obvious coexpression of the MHCs is short lived. To our knowledge, this is the first evidence that fiber transformation cannot be the direct consequence of decades of high-level activity. It also further supports the hypothesis that these infrequent denervation events are not easily detectable with standard clinical electromyography. When these coexpressing fibers were found in the sedentary senior muscles, they were small (<30 μm) and often had the distinct angulation noted after experimental or clinical denervation; some were also positive with the anti–N-CAM antibody (Fig. 2), an accepted marker of denervation (48, 49). This type of myofiber is common in unloaded muscle (i.e. resulting from spaceflight, limb suspension, or immobilization) and with spinal cord injury and peripheral denervation (18–27, 51–53). Thus, we consider these to be denervated muscle fibers. Furthermore, because it is known that slow-type muscle fibers revert to the fast isotype when denervated during development and adulthood (18–27, 51–53),

TABLE 4. Percentages of Myofibers Coexpressing Fast and Slow MHCs in Young Men and in Sedentary or Physically Active Seniors

<table>
<thead>
<tr>
<th>Myofibers Coexpressing Fast and Slow MHCs</th>
<th>Subjects</th>
<th>No. Biopsies</th>
<th>%</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young men Seniors</td>
<td>10</td>
<td>0.5 ± 0.6*</td>
<td>No</td>
<td>No (vs sedentary)</td>
</tr>
<tr>
<td>Sedentary</td>
<td>10</td>
<td>1.8 ± 1.7*</td>
<td>No</td>
<td>No (vs physically active)</td>
</tr>
<tr>
<td>Physically active</td>
<td>10</td>
<td>0.6 ± 0.6*</td>
<td>No</td>
<td>No (vs young)</td>
</tr>
</tbody>
</table>

*Groups with different footnote symbols are significantly different, p < 0.05. No refers to not significantly different by ANOVA.

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we suggest that these fibers are denervated slow-type myofibers reexpressing fast MHC through default myogenic programs.

In contrast, when myofibers coexpressing fast and slow MHCs were detected in the muscles of physically active seniors, they were similar in size and shape to the pure-type fibers in the sections and, therefore, cannot lack innervation (Fig. 4). Furthermore, their low density in these muscle sections is not in agreement with the concept that they belong to a motor unit that is undergoing exercise-driven, slow-type transformation of MHCs, a mechanism that is well known to occur in cross-reinnervation models (19, 53) but is more presumed than demonstrated in humans performing voluntary exercise (17). It is likely that the transforming myofibers (i.e. those coexpressing fast and slow MHC proteins) contribute to the increase in slow fiber–type groupings (Fig. 4); this is supported by the positive correlation between the increasing percentages of slow-type fiber number and slow-type fiber groupings in sections from the physically active seniors. Thus, we suggest that these normal-sized coexpressing fibers are very likely previously denervated fast-type fibers that have been reinnervated by sprouts from slow axons and are now temporarily coexpressing fast and slow MHC isoforms before they will finally exclusively express slow MHC. This is supported by the law of “recruitment order,” which dictates that slow motor units (and thus, muscle fibers) are activated more frequently than the fast motor units (54). In fact, the most active of the α-motoneurons are the slow type, and it may be this higher level of activity that most likely maintains motoneurons, muscle fibers, and their MHC content. Therefore, the increase in slow-type fiber groupings is evidence that some muscle plasticity still exists in both sedentary and (especially) physically active seniors. In addition, the reinnervation process may be more extensive than is obvious here because if the temporarily denervated myofibers were of the slow type, they would continue their current gene expression when reinnervated by slow-type α-motoneuron axon terminals and, thereby, escape our detection.

It is our opinion that the lack of fast-type groupings in senior sportsmen is direct evidence of selective reinnervation of denervated myofibers from slow-type α-motoneurons. In summary, our working hypothesis is that muscle fibers coexpressing fast and slow MHCs are either denervated slow myofibers also expressing fast MHC isoforms by the default myogenic program (Fig. 3A, B) (22, 24, 27) or are denervated fast fibers reinnervated by axons sprouting from slow motor neurons (19, 52, 53).

FIGURE 4. Coimmunofluorescence staining of fast and slow myosin heavy chain (MHC) proteins in a single section of vastus lateralis from a physically active senior reveals sparse MHC-coexpressing myofibers. (A–C) Fast fiber MHC proteins and laminin are labeled in green (A); slow fiber MHC proteins are labeled in red (B). Muscle fibers coexpressing both fast and slow MHCs are labeled with various levels of yellow/orange (within white circles in C) in proportion to the predominance of either fast or slow MHCs, respectively. The amount of coexpressing muscle fibers is far below the large number of muscle fibers (in the hundreds) belonging to 1 motor unit.
This speculation needs further study, in particular, in situ MHC expression analyses, in more numerous subjects and different muscle types. Indeed, our study has many potential confounding factors. These include the use of the fiber type–heterogeneous vastus lateralis muscle, the small size of the specimens because of the sampling method (needle biopsy), the low number of study subjects, the inherently variable genetic backgrounds of the individual subjects, and differences in the type and extent of physical activities of the physically active seniors. Moreover, muscle biopsies from that group ranged from those with scarce fiber-type transformation and grouping to those with almost fully transformed muscles. Despite these limitations, the clinical significance of our observations is confirmed by the fact that the muscle properties of the physically active senior group are more similar to those of the active young men than to those of sedentary seniors. Specifically, relative to their sedentary counterparts, the physically active seniors had greater muscle maximal isometric force, along with better-preserved muscle morphology and mobility (39, 40).

Taken together, our results suggest that, beyond the direct effects of aging on the structure and function of muscle fibers, changes occurring in the muscle tissue of the sedentary group seem to be in part a result of sparse incremental denervation. In physically active seniors, the increase in the percentage of ‘slow fiber groupings’ is likely the result of the positive effect of long-term physical activity on the motoneuron pool, which, conceivably, has mainly spared the slow motoneurons from age-related lesion/death, thereby increasing the chance that peripheral reinnervation occurs because of sprouting of slow axons.

Certainly, numerous mechanisms contribute to long-term muscle health or deterioration, yet our study suggests that long-term exercise would allow the body to adapt to the consequences of age-related denervation and to preserve muscle structure and function by saving otherwise lost muscle fibers through recruitment of muscle fibers to different, mainly slow, motor units. We further speculate that high-level activity either by voluntary exercise or functional electrical stimulation may be applied at any age to save neurons from disorders secondary to inactivity (55) and to counteract muscle atrophy in other neuromuscular or metabolic diseases (56).

Although the subjects of this study were not masters athletes, the intensity of recreational training reported is something that the general population may achieve, particularly if properly motivated by specialists in the field. We show that recreational levels of activity are very effective in driving seniors toward improved functional performance and rearrangement of muscle fiber type. In particular, these levels of exercise seem to have beneficial effects on reinnervation of muscle fibers, resulting in preservation of muscle function, size, and structure, thereby delaying the functional decline and loss of independence that are common in late aging.

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REFERENCES


