THE ANATOMICAL RECORD PART A 286A:866–873 (2005)

Adult and Developmental Myosin Heavy Chain Isoforms in Soleus Muscle of Aging Fischer Brown Norway Rat

LEANN M. SNOW,[1,2] LINDA K. MCLOON,[3,4] AND LADORA V. THOMPSON[1]*

[1]Department of Physical Medicine and Rehabilitation, University of Minnesota Medical School, Minneapolis, Minnesota

[2]School of Kinesiology, Laboratory of Physiological Hygiene and Exercise Science, University of Minnesota, Minneapolis, Minnesota

[3]Department of Ophthalmology, University of Minnesota, Minneapolis, Minnesota

[4]Department of Neuroscience, University of Minnesota, Minneapolis, Minnesota

ABSTRACT

Fiber type shifts in aging skeletal muscle have been studied with myofibrillar ATPase histochemistry and gel electrophoresis, but less commonly with immunohistochemistry. Immunohistochemical study of myosin heavy chains (MHCs) in single myofibers yields additional information about aged skeletal muscle. Furthermore, many studies of aging rodent skeletal muscle have been performed on fast-MHC-predominant muscle and in several different strains. The aim of this study was to evaluate immunohistochemically MHC characteristics in the slow-MHC-predominant soleus muscle in the Fischer Brown Norway F1 hybrid aging rat (FBN). Three age groups of FBN rats were studied: 12 months, 30 months, and 36 months. Soleus muscles were excised, quick-frozen, and stained immunohistochemically for slow, fast, developmental, and neonatal MHC isoforms. Cross-sections were evaluated for the number and cross-sectional areas of fibers expressing each isoform. Single myofibers in soleus muscles of the aged rats showed significantly greater amounts of coexpression of slow and fast MHC than did younger animals. This change began by 30 months of age, but did not reach statistical significance until 36 months of age. The soleus from 36-month-old rats also expressed greater amounts of developmental MHC than did the other groups. These developmental MHC-positive myofibers also coexpressed either slow or slow and fast MHC. The age-related increase in MHC coexpression of slow with fast isoforms may indicate a fiber type shift suggestive of denervation that outpaces reinnervation. The developmental MHC-positive fibers provide evidence of ongoing myofiber remodeling in the oldest rats in the midst of the fiber degeneration of aging.

Key words: immunohistochemistry; aging; Fischer 344/Brown-Norway F1 hybrid; developmental myosin; myosin heavy chain coexpression

Skeletal muscle exhibits structural and functional changes with aging. Some of the changes identified include muscle and fiber atrophy, shifts in fiber type composition, decreases in contractile force, and a slowing of contraction velocity (Thompson, 1994, 2002; Cartee, 1995; Larsson and Ansved, 1995; Wineinger et al., 1995; Carlson, 1997; Lexell, 1997). There is also loss of spinal cord motor neurons with concomitant muscle fiber denervation and motor unit enlargement with aging (Larsson and Ansved, 1995; Lexell, 1997). The denervation is thought to be progressive with age, and it is accompanied by at least partial reinnervation (Larsson and Ansved, 1995; Lexell, 1997). Many of these characteristics with aging are similar between humans and experimental animals (Thompson 1994; Cartee, 1995).

Aging-related fiber type shifts have been demonstrated using myofibrillar ATPase histochemistry or gel electrophoresis (Florini and Ewton, 1989; Sugiura et al., 1992; Larsson et al., 1993; Danieli-Betto et al., 1995; Sullivan et al., 1995), but few immunohistochemical studies of myosin

Grant sponsor: Center for Muscular Dystrophy, University of Minnesota; Grant sponsor: Minnesota Training Grant in Aging; Grant sponsor: National Institute on Aging; Grant number: NIA5T32AG00198, NIAAG17768, NIAAG21626.

*Correspondence to: LaDora V. Thompson, Physical Medicine and Rehabilitation, University of Minnesota, MMC 388, 420 Delaware Street SE, Minneapolis, MN 55455. Fax: 612-625-4274. E-mail: thomp067@umn.edu

Received 31 January 2005; Accepted 2 May 2005

DOI 10.1002/ar.a.20218

Published online 5 August 2005 in Wiley InterScience (www.interscience.wiley.com).

heavy chain (MHC) composition in muscle of aged humans and rodents have been performed (Larsson and Ansved, 1995; Carlson et al., 2001; Nikolić et al., 2001). These reports have evaluated adult MHC isoforms and in rat have focused on muscles of predominantly fast MHC composition (Armstrong and Phelps, 1984; Carlson et al., 2001; Nikolić et al., 2001). Less is known about muscles of predominantly slow MHC composition, such as the soleus (Armstrong and Phelps, 1984). Also, immunohistochemical study of developmental and neonatal MHC isoforms has not been performed in the aging rat model.

The use of immunohistochemical techniques is important because, with use of standard myofibrillar ATPase histochemistry, fibers coexpressing several MHCs may be incompletely or incorrectly identified (Sawchak et al., 1989; Staron, 1997). Appearance of coexpression of MHC isoforms in single myofibers is unequivocal in serial sections, where MHC isoforms have been visualized immunohistochemically. Additionally, it is not always possible to detect denervation-reinnervation-related fiber type grouping with myofibrillar ATPase techniques (Sawchak et al., 1989), but detection of grouping is enhanced with immunohistochemical techniques.

The purpose of this study was to evaluate the expression of fast, slow, developmental, and neonatal MHC composition of single myofibers within the soleus (SOL) muscle of aging rats using immunohistochemical techniques. Serial sections of soleus muscle were examined for coexpression of these four MHC isoforms. The presence of myofibers positive for developmental or neonatal MHC was also evaluated to assess myofiber remodeling or regeneration. Other age-associated characteristics of the soleus muscle were determined as well and included fiber cross-sectional area, fiber shape, and fiber type grouping. It was hypothesized that the soleus muscle from aged animals would show shifts in MHC isoform expression compared to the muscle from younger animals, and that increased MHC coexpression would be seen with increasing age. It was also hypothesized that developmental MHC isoforms would be present in increasing numbers with increasing age.

MATERIALS AND METHODS

Animal Protocol and Muscle Procurement

Fifteen male (virgin) pathogen-free Fischer Brown Norway F1 hybrid rats were purchased from the aging rodent colony sponsored by the National Institute on Aging (NIA; Bethesda, MD). Three age groups were studied: young (12 months; n = 5), mature adult (30 months, n = 5), and old (36 months; n = 5). All animals were fed NIA-approved rat chow and water ad libitum and were exposed to the same activity patterns. They were housed in standard cages in the same room with inverted 12-hr dark-light cycles at the University of Minnesota for approximately 7 days prior to experimentation. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Minnesota and followed the NIH guidelines for use of animals in research.

Prior to termination, the animals were weighed. The animals were deeply anesthetized with sodium pentobarbital (60 mg/kg) for the excision of the SOL muscles. After excision, the animals were euthanized, the muscles were weighed, and muscle weight/body weight ratios were calculated. The muscles were quick-frozen in isopentane over liquid nitrogen and stored at −80°C until processed.

Tissue Processing

Cryostat sections, 10 microns thick, were cut from the mid-belly of the muscles and placed on gelatin-coated slides. Sections were stained for routine hematoxylin and eosin for morphological assessment. Immunohistochemical staining for MHC isoforms was performed on serial sections (McLoon, 1998). Monoclonal antibodies to slow, fast, developmental, and neonatal MHCs were used (Novocastra Laboratories, Newcastle upon Tyne, U.K.). All references to developmental MHC in the text will be based on the Novocastra nomenclature. Primary antibody concentrations used were as follows: slow, 1.25 μg/ml (1:20 dilution); fast, 1.7 μg/ml (1:10 dilution); developmental, 3.7 μg/ml (1:10 dilution); neonatal, 0.7 μg/ml (1:10 dilution). The antifast MHC antibody does not differentiate between MHC types IIa, IIx, and IIb. Controls were included that omitted the primary MHC antibody incubation step. Serial sections were analyzed to determine the patterns of coexpression of the four MHC isoforms.

Tissue Analysis

Three to four areas were randomly selected from each muscle cross-section as visualized at 225× magnification. Within each of these areas, 100–150 fibers were analyzed for fiber type and cross-sectional area. Thus, 300–400 individual fibers were evaluated from each rat. The proportion of each fiber type was expressed as a percentage of the total number of cells counted per rat. Cross-sectional areas were grouped by fiber type and means were calculated per rat. Cross-sectional areas were determined by using Scion Image for Windows computer program (Scion, Frederick, MD). Qualitative evaluation of anatomic features was performed using the H&E stain.

Statistical Analysis

All data are presented as mean ± standard error of the means (SEM). Differences between groups were determined using one-way ANOVA. If a significant overall F-test was obtained, posthoc comparisons were performed with the Tukey HSD test. If the data did not meet the assumptions of ANOVA, Kruskal-Wallis one-way ANOVA on ranks or two-sample *t*-tests were used. Statistical analyses were performed using NCSS (Kaysville, UT) and SPSS (Chicago, IL) statistical programs. Significance level was set at $P \leq 0.05$.

RESULTS

Subject Characteristics

Animal weights, soleus muscle weights, and muscle weight per body weight ratios are presented in Table 1. Muscle weights were significantly decreased in the 36-month-old rats compared with the 12- and 30-month-old rats.

Fiber Types

As expected, the predominant MHC isoform in the soleus was the slow MHC (Fig. 1). The percentage of fibers expressing the slow MHC isoform (alone or in coexpression) did not differ statistically between age groups (Fig. 1). The percentage of fibers expressing the fast MHC isoform, however, was approximately three times greater in the 36-month-old animals than in the 30-month group and approximately five times greater than in the 12-month

TABLE 1. Animal characteristics of Fischer 344 Brown Norway F1 Hybrid Rats

Group	Body weight, g	Soleus muscle weight, g	Soleus muscle wt/body wt $\times 10^{-3}$
12-month-old (5)[c]	469 ± 31	0.214 ± 0.037	0.45 ± 0.05
30-month-old (5)	578 ± 8	0.206 ± 0.007	0.36 ± 0.01
36-month-old (5)	422 ± 23	0.123 ± 0.010[b]	0.29 ± 0.03[a]

[a]Significantly different from 12-month group ($P = 0.02$).
[b]Significantly different from 12- and 30-month groups ($P = 0.04$ vs. 12 months; $P = 0.05$ vs. 30 months).
[c]The number of animals in each age group is identified in parentheses.

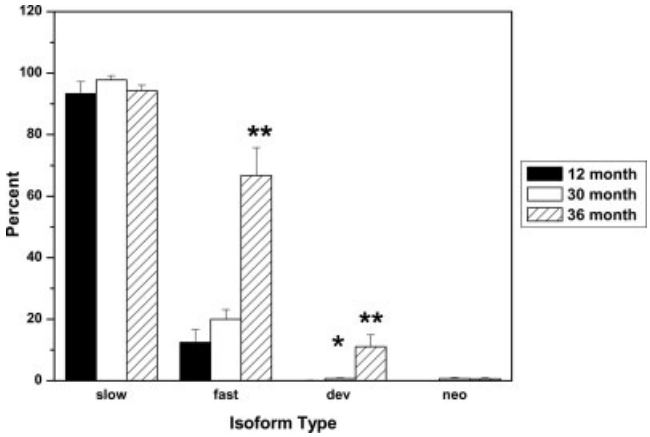

Fig. 1. Immunohistochemically determined MHC fiber types by age group, expressed alone or in coexpression. Double asterisk indicates significant difference from both 12- and 30-month groups ($P < 0.001$ for both fast MHC comparisons, $P = 0.02$ for developmental MHC 12 months, and $P = 0.03$ for developmental MHC 30 months). Asterisk indicates significant difference from 12 months ($P = 0.02$). No significant difference was seen between age groups in the proportion of fibers expressing the slow MHC isoform. No difference was noted across age groups in neonatal isoform expression. The developmental isoform was expressed only in fibers that expressed either slow, fast, or slow and fast isoforms.

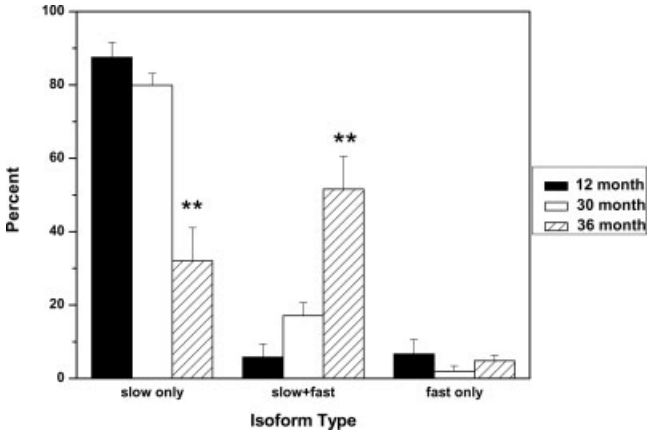

Fig. 2. Immunohistochemically determined adult MHC isoform coexpression by age group. Increased age was accompanied by increase in slow + fast MHC coexpression and concomitant decrease in slow-only expression. Double asterisk indicates significant difference from 12-month ($P < 0.001$ for slow only and slow and fast) and 30-month group ($P < 0.001$ for slow only and $P = 0.004$ for slow and fast). No significant difference was noted with fibers expressing the fast isoform alone.

group (Fig. 1). The percentage of fibers expressing the developmental MHC isoform was approximately 7 times greater at 30 months than at 12 months, and more than 100 times greater at 36 months than at 12 months (Fig. 1). Expression of this MHC isoform was also approximately 15 times greater at 36 months than at 30 months. Neonatal MHC isoform expression did not differ between age groups (Fig. 1).

Coexpression of fast and slow MHC within single myofibers increased with age. This increase was significant at 30 months and became even more pronounced at 36 months (Fig. 2). When compared to the 12-month-old group, the 36-month-old rats exhibited nearly a ninefold increase in the number of myofibers that coexpressed both slow and fast MHCs. When compared to the 30-month-old rats, the aged rats showed approximately a threefold increase in the coexpression of these two isoforms (slow and fast MHCs). The majority of the increase in myofibers positive for fast MHC expression appeared to be the result of coexpression with slow MHC, since the expression of myofibers positive for the fast-only MHC did not differ between age groups (Figs. 1 and 2). This pattern of slow and fast MHC coexpression correlated with the decrease in the proportion of slow-only MHC with age (Fig. 2).

Developmental MHC isoform expression in the oldest rats occurred only in coexpression with slow MHC or with both slow and fast MHC isoforms. Of the total number of fibers expressing the developmental MHC isoform in the 36-month-old rats, the majority (81.3%) coexpressed developmental MHC with both slow and fast MHCs. Developmental MHC was localized in single fibers in the soleus muscle from the 30-month-old group, and these fibers always coexpressed either slow MHC, fast MHC, or neonatal MHC. Developmental MHC-positive fibers were very rare in the soleus muscles from the 12-month-old group, and these fibers also coexpressed either the slow or fast MHC isoforms.

Cross-Sectional Area

Mean cross-sectional area decreased significantly with age across all major fiber types that expressed adult MHC isoforms (Fig. 3). Fibers expressing only slow MHC were 25% smaller at 36 months than those in the 12-month group. The mean cross-sectional area of myofibers expressing both slow and fast MHC was 51% smaller in the 36-month-old rats than the corresponding fibers in 12-month-old rats and 42% smaller than the associated fibers from 30-month-old rats. For fibers expressing fast MHC only, mean cross-sectional areas from the 36-month animals were 72% smaller than in the 12-month-old animals

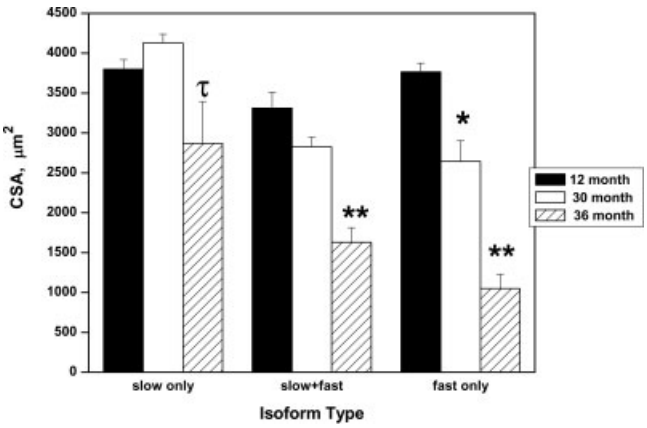

Fig. 3. Cross-sectional areas of fibers expressing adult MHC isoforms determined by immunohistochemistry. At 36 months, aging was associated with muscle fiber atrophy in all major fiber type groups. At 30 months, atrophy was noted in fibers expressing fast MHC, but not in fibers expressing only slow MHC. τ indicates significantly different from 30-month group ($P = 0.04$). Double asterisk indicates significantly different from 12 and 30 months ($P \leq 0.001$ for all comparisons). Asterisk indicates significantly different from 12 months ($P = 0.007$).

and 60% smaller than fibers from the 30-month-old animals. In the myofibers expressing the developmental MHC isoforms, no significant differences in cross-sectional areas were noted between age groups. These myofibers were small, with a mean cross-sectional area of 1,457 μm^2.

Evidence for Denervation and Regeneration and/or Myofiber Remodeling

Analysis of the H&E sections (Fig. 4) revealed several characteristics known to be associated with skeletal muscle aging (Larsson and Ansved, 1995; Brown and Hasser, 1996; Carslon et al., 2001; Nikolić et al., 2001). The muscles from the oldest rats showed many atrophic fibers interspersed with relatively large fibers. There was a large increase in the variability of the cross-sectional areas forming the muscles from the 36-month-old rats (Figs. 4 and 5). There was also an increase in connective tissue between and within fascicles (Fig. 4). Several muscles from the oldest animals exhibited inflammatory infiltrates between fibers or fascicles (data not shown).

Microscopic examination of the myofibers revealed the appearance of small angulated fibers, which is considered to be evidence of denervation, beginning at 30 months (Fig. 6); this characteristic was most frequently observed in fibers expressing fast MHC alone or in myofibers coexpressing both fast and slow MHCs. Fiber type grouping, which is considered to be evidence of reinnervation, was evident in at least two muscles in the 36-month-age group, and the groups most often were composed of fibers expressing both slow and fast MHCs (Fig. 7). Of greater note was the large shift in MHC fiber type from slow only to slow and fast coexpression in the oldest animals (Fig. 6). Small clusters of fibers expressing both slow and fast MHCs were present in two of the five muscles in the 30-month group, but were not seen in muscles from 12-month-old rats. Groups of fibers expressing developmental MHC were present in two of five muscles of the 36-month group. No groups of developmental MHC-positive fibers were seen in the 12- or 30-month groups.

Centrally located nuclei, considered evidence of regeneration, were present more frequently in the soleus muscles from the oldest group than in the soleus of the middle- or young-age groups (36 months = 19 ± 3 per mm^2; 30 months = 5 ± 1 per mm^2; 12 months = 1 ± 0.1 per mm^2). It is interesting that 90% of the fibers with central nuclei did not express either developmental or neonatal MHC (data not shown). This finding suggests that the mechanisms that control these two processes are distinct.

DISCUSSION

In this study, soleus muscle from young adult, mature adult, and aged rats was evaluated using immunohistochemical techniques for expression of adult and developmental MHC isoforms. There was a pronounced increase in the number of myofibers expressing fast and developmental MHC isoforms in the oldest rats. In the muscles from the oldest rats, the myofibers that were positive for fast MHC also coexpressed slow MHC and were accompanied by a decrease in the proportion of fibers expressing only slow MHC. Those myofibers that expressed developmental MHC in the soleus from the oldest rats also coexpressed slow and fast MHCs. Fiber atrophy occurred across all major fiber type groups in the oldest rats, but was greatest in myofibers expressing fast MHC, particularly fast MHC only. The atrophy of the fast-only myofibers also occurred at an earlier age than did atrophy in the other fiber groups. Fiber type grouping, angulated myofibers, and centrally located nuclei were present in the soleus muscles by 30 months, and these changes were even more apparent in the 36-month group.

Slow MHC and Cross-Sectional Areas

The data on cross-sectional areas are consistent with prior reports on age-related muscle atrophy, where type II (fast MHC) fibers atrophy earlier and to a greater extent than type I (slow MHC) fibers (Cartee, 1995; Brown and Hasser, 1996). In contrast to the present study, several studies have reported a general fast-to-slow fiber type transformation with age in the soleus muscle (Sugiura et al., 1992; Danieli-Betto et al., 1995; Larsson and Ansved, 1995; Sullivan et al., 1995; Thompson, 1999). The muscles in these studies showed a relative increase in the proportion of type I myofibers with age, with or without a decrease in the percentage of type IIa fibers, as evidenced by myofibrillar ATPase histochemistry and/or gel electrophoresis. Other studies showed no change in the percentage of type I fibers with age in Fischer 344 rats (Florini and Ewton, 1989; Deschene et al., 2001). Our study, however, used a different strain of rats and examined animals significantly older than those used in previous studies. The most significant differences in fiber types appeared in the soleus muscle in the very old rats, 36 months of age.

Fast MHC

One unique result of the current study is the increase in the proportion of single myofibers that express the fast MHC isoform in the oldest rats. Increased proportion of myofibers expressing the fast MHC isoform and increased coexpression of other MHC isoforms in single myofibers have been reported in the literature before, but it occurred in the denervated soleus muscle following spinal cord transection (Talmadge et al., 1995) and after sciatic nerve transection (Bodine and Pierotti, 1996). These reports pos-

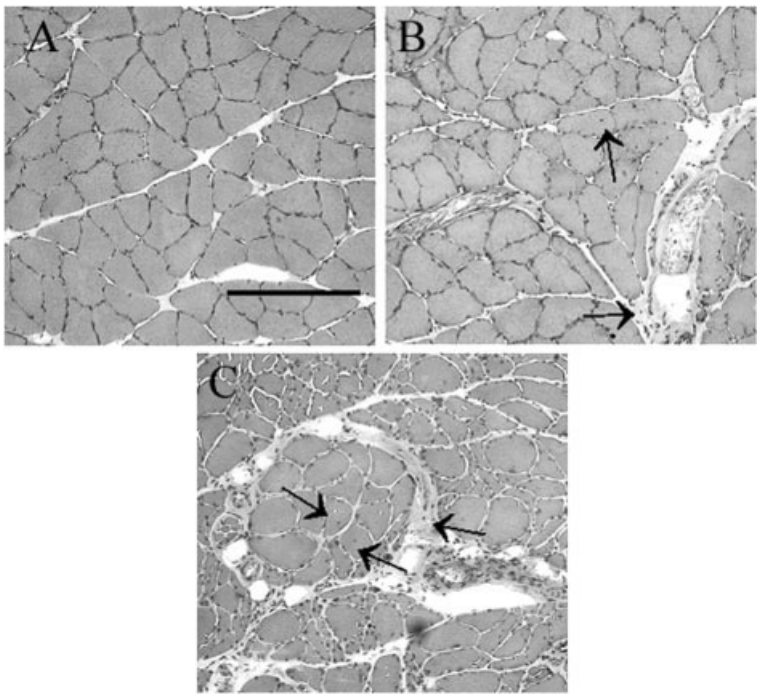

Fig. 4. Histologic manifestations in aging skeletal muscle, hematoxylin and eosin. **A:** 12 months. **B:** 30 months. **C:** 36 months. With increasing age, central nuclei and connective tissue become more prominent (arrows). Scale bar = 100 microns.

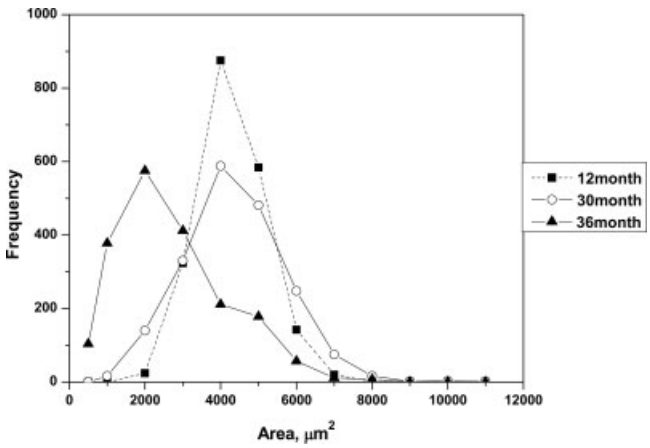

Fig. 5. Histogram of muscle fiber cross-sectional areas. Fiber atrophy occurs with increasing age and variability of fiber cross-sectional area increases with age.

tulated that the slow-to-fast isoform shift was related to the change in neuromuscular activity due to muscle denervation. The skeletal muscle changes shown in the very oldest animals in the present study mimic those reported in the denervated soleus muscles from the transection studies. Thus, the increase in the number of myofibers positive for fast MHC may suggest that denervation occurs with aging.

Interestingly, reduced neuromuscular activity from mechanical unloading, without nerve transection or denervation, induces slow-to-fast isoform transitions (Pette and Staron, 2000; Baldwin and Haddad, 2001; Deschene et al., 2001). It would appear that the myofiber populations in skeletal muscles encompass a continuum of pure and hybrid fiber types that are continually adjusting to altered functional demands. It is therefore possible that the increased expression of fast MHC in the oldest animals is related to decreased neuromuscular activity due to altered mechanical loading of the muscle. The effects of denervation and decreased hindlimb activity may be additive in their influence on MHC composition in aged postural muscles. Further study is needed to tease out the influence of denervation and altered neuromuscular activity (secondary to altered muscle loading) on skeletal muscle aging and expression of MHC isoforms.

Adult MHC Coexpression

Increased MHC coexpression in single myofibers has been described in various studies (Sawchak et al., 1989; Andersen et al., 1999; D'Antona et al., 2003). Increased coexpression of adult MHC isoforms was reported in muscle of persons with neuropathy (Sawchak et al., 1989). This coexpression took the form of both slow and fast MHCs in single fibers and was hypothesized to be the result of reinnervation of denervated fibers. Significant (52.6%) MHC coexpression in single myofibers was seen in the vastus lateralis muscles of frail elderly individuals (mean age, 88 years) (Andersen et al., 1999), where the most common MHC combination was of type I and IIa, with the slow isoform comprising the major component. Again, this fiber type shift was hypothesized to be the result of the reinnervation of denervated fibers. Coexpression of type IIa and type IIx MHC isoforms was seen in

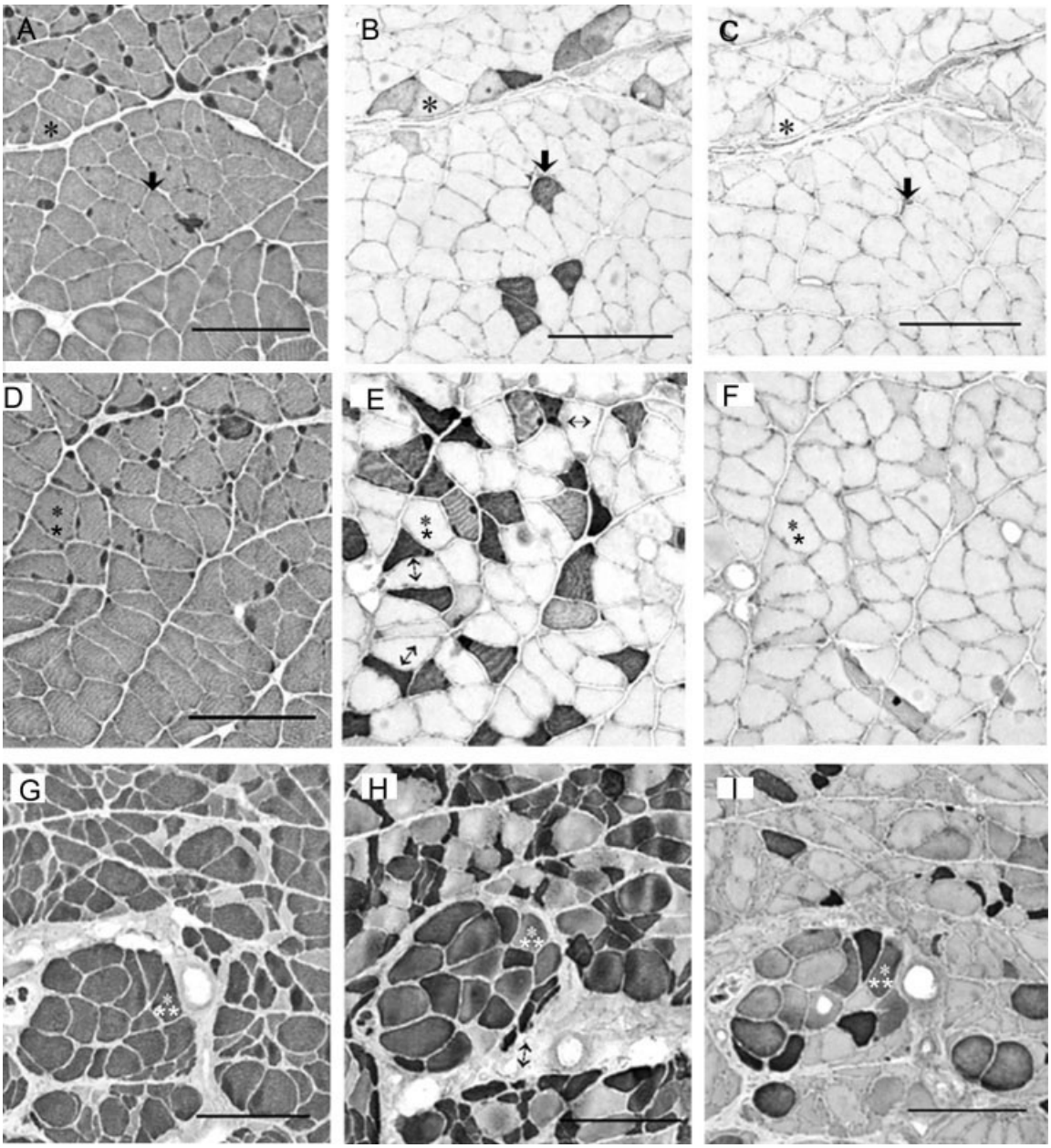

Fig. 6. Immunohistochemical staining characteristics in serial sections of soleus in 12-month-old rats (top row), 30-month-old rats (middle row), and 36-month-old rats (bottom row). **A**, **D**, and **G:** Dark cells indicate positive staining for slow MHC. **B**, **E**, and **H:** Dark cells indicate positive staining for fast MHC. **C**, **F**, **I:** Dark cells indicate positive staining for developmental MHC. The soleus muscle is composed predominantly of fibers expressing the slow MHC isoform. The soleus from the 36-month animals exhibits an increased expression of the fast MHC isoform. Note also the presence of angular and atrophic fibers in the older animal groups (double-headed arrows). Asterisk, arrow, double asterisk, and triple asterisk denote the same cell in respective sections. Scale bar =100 microns.

single myofibers from the tibialis anterior of aging rats (Larsson et al., 1993). Our results also document an aging-related increase in MHC coexpression in rat single myofibers, but this coexpression was of both slow and fast MHCs in single fibers within the soleus rather than of isoforms of fast MHC. The composition of the coexpressing myofibers may be related to the difference in predominant fiber type of the soleus (slow) as compared to the tibialis anterior (fast) (Armstrong and Phelps, 1984). Our findings in the aged rats indicate that this process may be universal in mammalian skeletal muscle.

It is possible that the small numbers of myofibers that show MHC coexpression in aging muscle may not be physiologically significant (Larsson and Ansved, 1995). However, if this process increases exponentially in muscles of extremely aged animals, as seen by the 52% rate of coexpression of fast and slow MHC isoforms present in this study, it is likely to be physiologically significant in the oldest rats.

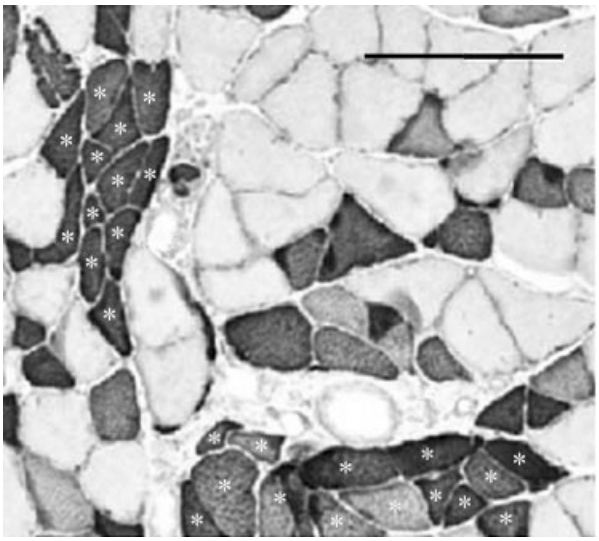

Fig. 7. Fiber type grouping in the soleus of a 36-month-old rat, stained immunohistochemically for fast MHC. Fibers in group indicated by asterisk. Scale bar = 100 microns.

Developmental MHC

In addition to changes normally indicative of denervation, there is evidence of apparent fiber regeneration and/or reinnervation seen in the oldest muscles. Such evidence includes the presence of centrally located nuclei and fiber type grouping. In addition, the oldest soleus muscles in this study showed an increased expression of developmental MHC. Developmental and neonatal MHCs have been identified not only in developing skeletal muscle, but also in denervated or regenerating muscle (Schiaffino et al., 1988). A small percentage of immunohistochemically defined developmental and neonatal MHC-positive myofibers appeared in the soleus of young rats after spinal cord transection (Talmadge et al., 1995). Myofibers positive for developmental and neonatal MHCs were seen also in muscles of persons with denervating neurologic conditions (Sawchak et al., 1989). Myofibers positive for these developmental and neonatal MHCs coexpressed with slow or fast MHC isoforms and were not seen in control muscle. The authors concluded that the expression of developmental and neonatal MHC isoforms was consistent with regenerating muscle fibers within neuropathic, partially denervated muscle. Thus, the relatively large proportion of small developmental MHC-positive fibers present in the 36-month-old rats of the present study is consistent with other observations in conditions of denervation with subsequent reinnervation and/or fiber regeneration.

Additionally, developmental and neonatal MHC isoforms have been observed in the soleus of young adult rats in the absence of any apparent denervating condition (Wanek and Snow, 1995). This result was thought to indicate fiber remodeling or regeneration in response to overload or increased recruitment encountered in routine use of lower extremity postural muscles. Therefore, another possible explanation for the increased developmental MHC in the soleus of the aged rats is that the amount of routine soleus damage, and subsequent remodeling and regeneration, is increased compared with young animals due to age-related muscle atrophy and weakness. Further study is needed on the question of functional significance of these findings.

This immunohistochemical study has shown that aging is associated with diffuse fiber atrophy and an increase in the proportion of skeletal muscle fibers that coexpress adult MHC isoforms. This finding in rodents parallels similar findings in frail elderly persons, but the functional significance of such changes is not clear. There is also evidence that is suggestive of muscle denervation, reinnervation, and regeneration in senescent rodent muscle as evidenced by the presence of developmental MHC isoforms, fiber type grouping, and centralized myonuclei.

The functional significance of these findings has yet to be determined. Greater MHC coexpression within single myofibers in aged muscle may influence its ability to respond to daily demands. Such influences may need to be considered in the design and implementation of exercise programs for the elderly, whether used for health maintenance or for rehabilitation.

ACKNOWLEDGMENTS

The authors thank Janice Shoeman, Jeff Doughty, Shari Fujan, Holly Gappert, Debbie Nelson, and Jenny Pennaz for their assistance.

LITERATURE CITED

Andersen JL, Terzis G, Kryger A. 1999. Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. Muscle Nerve 22:449–454.

Armstrong RB, Phelps RO. 1984. Muscle fiber type composition of the rat hindlimb. Am J Anat 171:259–272.

Baldwin KM, Haddad F. 2001. Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle. J Appl Physiol 90:345–357.

Bodine SC, Pierotti DJ. 1996. Myosin heavy chain mRNA and protein expression in single fibers of the rat soleus following reinnervation. Neurosci Lett 215:13–16.

Brown M, Hasser EM. 1996. Complexity of age-related change in skeletal muscle. J Gerontol 51A:B117–B123.

Carlson BM. 1997. Skeletal muscle regeneration during aging and after long-term denervation. Tsitologiia 39:965–968.

Carlson BM, Dedkov EI, Borisov AB, Faulkner JA. 2001. Skeletal muscle regeneration in very old rats. J Gerontol 56A:B224–B233.

Cartee GD. 1995. What insights into age-related changes in skeletal muscle are provided by animal models? J Gerontol A Biol Sci Med Sci 50A:137–141.

Danieli-Betto D, Betto R, Megighian A, Midrio G, Salviati G, Larsson L. 1995. Effects of age on sarcoplasmic reticulum properties and histochemical composition of fast- and slow-twitch rat muscles. Acta Physiol Scand 154:59–64.

D'Antona G, Pellegrino M, Adami R, Rossi R, Carlizzi C, Canepari M, Saltin B, Bottinelli R. 2003. The effects of ageing and immobilization on structure and function of human skeletal muscle fibres. J Physiol 552:499–511.

Deschene MR, Britt AA, Chandler WC. 2001. A comparison of the effects of unloading in young adult and aged skeletal muscle. Med Sci Sports Exerc 33:1477–1483.

Florini JR, Ewton DZ. 1989. Skeletal muscle fiber types and myosin ATPase activity do not change with age or growth hormone administration. J Gerontol 44:B110–B117.

Larsson L, Biral D, Campione M, Schiaffino S. 1993. An age-related type IIB to IIX myosin heavy chain switching in rat skeletal muscle. Acta Physiol Scand 147:227–234.

Larsson L, Ansved T. 1995. Effects of ageing on the motor unit. Prog Neurobiol 45:397–458.

Lexell J. 1997. Evidence for nervous system degeneration with advancing age. J Nutr 127:1011S–1013S.

McLoon LK. 1998. Muscle fiber type compartmentalization and expression of an immature myosin isoform in the sternocleidomastoid muscle of rabbits and primates. J Neurol Sci 156:3–11.

Nikolić M, Malnar-Dragojević D, Bobinac D, Bajek S, Jerković R, Šoić-Vranić T. 2001. Age-related skeletal muscle atrophy in humans: an immunohistochemical and morphometric study. Coll Antropol 25:545–553.

Pette D, Staron RS. 2000. Myosin isoforms, muscle fiber types, and transitions. Microsc Res Tech 50:500–509.

Sawchak JA, Lewis S, Shafiq SA. 1989. Coexpression of myosin isoforms in muscle of patients with neurogenic disease. Muscle Nerve 12:679–689.

Schiaffino S, Gorza L, Pitton G, Saggin L, Ausoni S, Sartore S, Lømo T. 1988. Embryonic and neonatal myosin heavy chain in denervated and paralyzed rat skeletal muscle. Dev Biol 127:1–11.

Staron RS. 1997. Human skeletal muscle fiber types: delineation, development, and distribution. Can J Appl Physiol 22:307–327.

Sugiura T, Matoba H, Miyata H, Kawai Y, Murakami N. 1992. Myosin heavy chain isoform transition in ageing fast and slow muscles of the rat. Acta Physiol Scand 144:419–423.

Sullivan VK, Powers SK, Criswell DS, Tumer N, Larochelle JS, Lowenthal D. 1995. Myosin heavy chain composition in young and old rat skeletal muscle: effects of endurance exercise. J Appl Physiol 78:2115–2120.

Talmadge RJ, Roy RR, Edgerton VE. 1995. Prominence of myosin heavy chain hybrid fibers in soleus muscle of spinal cord-transected rats. J Appl Physiol 78:1256–1265.

Thompson LV. 1994. Effects of age and training on skeletal muscle physiology and performance. Phys Ther 74:71–81.

Thompson LV. 1999. Contractile properties and protein isoforms of single skeletal muscle fibers from 12- and 30-month-old Fischer 344 Brown Norway F1 hybrid rats. Aging Clin Exp Res 11:109–118.

Thompson LV. 2002. Skeletal muscle adaptations with age, inactivity, and therapeutic exercise. J Orthop Sports Phys Ther 32:44–57.

Wanek LJ, Snow MH. 1995. Presence of embryonic myosin in normal postural muscles of the adult rat. Cell Tissue Res 280:541–548.

Wineinger MA, Sharman RB, Stevenson TR, Carlsen RC, McDonald RB. 1995. Peripheral nerve and muscle function in the aging Fischer 344/Brown-Norway rat. Growth Dev Aging 59:107–119.