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Fiber Content and Myosin Heavy Chain Composition of Muscle Spindles in Aged Human Biceps Brachii

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SUMMARY The present study investigated potential age-related changes in human muscle spindles with respect to the intrafusal fiber-type content and myosin heavy chain (MyHC) composition in biceps brachii muscle. The total number of intrafusal fibers per spindle decreased significantly with aging, due to a significant reduction in the number of nuclear chain fibers. Nuclear chain fibers in old spindles were short and some showed novel expression of MyHC α -cardiac. The expression of MyHC α -cardiac in bag₁ and bag₂ fibers was greatly decreased in the A region. The expression of slow MyHC was increased in nuclear bag₁ fibers and that of fetal MyHC decreased in bag₂ fibers whereas the patterns of distribution of the remaining MyHC isoforms were generally not affected by aging. We conclude that aging appears to have an important impact on muscle spindle composition. These changes in muscle spindle phenotype may reflect an age-related deterioration in sensory and motor innervation and are likely to have an impact in motor control in the elderly. (*J Histochem Cytochem* 53:445–454, 2005)

KEY WORDS

aging
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SKELETAL MUSCLE AGING is characterized by a decline in muscle mass, deterioration in muscle function, changes in muscle structure and a slowing in the rate of synthesis of muscle proteins (for review see Basu et al. 2002; Carmeli et al. 2002). Aging also causes alterations in the neuromuscular junction and a reduction in the conduction velocity of motor nerve fibers (for review see Lexell 1997; Vandervoort 2002). The decline in muscle mass is mainly due to the loss and atrophy of muscle fibers (Porter et al. 1995; Roos et al. 1997), whereas the decrease in muscle strength is associated with a reduction in the cross-sectional area of the muscle fibers and in capillary bed density as well as in a reduction in the number of muscle fibers (Lexell 1995; Porter et al. 1995; Frontera et al. 2000).

Skeletal muscle fibers are characterized as fast or slow on the basis of their physiological, histochemical

and biochemical properties, including their myosin heavy chain (MyHC) content. MyHC isoforms, key contractile muscle proteins, are the major determinants of the maximum shortening velocity in muscle cells, and a close relationship between the maximum shortening velocity and the MyHC composition has been documented in different species (Larsson and Moss 1993; Moss et al. 1995; Bottinelli et al. 1996; Schiaffino and Reggiani 1996; Bottinelli and Reggiani 2000; Pellegrino et al. 2003). MyHCs are therefore considered the best markers of the functional properties of skeletal muscle fibers. The pattern of expression of MyHC isoforms is rather plastic and subject to a number of influences such as developmental cues, neural activity, and hormonal input as well as mechanical activity (for review see Pette and Staron 1997, 2001). Numerous studies have shown that human skeletal muscles undergo age-related changes in fiber type and MyHC composition (for review see Lexell 1995; Monemi et al. 1999a; Andersen 2003; Thornell et al. 2003). In human limb and trunk muscles there is an increase of type I fibers and slow MyHC isoform, concomitant with a decrease of type II fibers and fast MyHC isoforms during aging (Klitgaard et al. 1990; Harridge et

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al. 1995; Larsson et al. 1997; Monemi et al. 1999a). Furthermore, there is accumulating evidence showing that different muscles behave differently upon aging (for review see Thornell et al. 2003). For instance, the age-related changes in the human masseter, a jaw-closing muscle, and the lateral pterygoid, a jaw-stabilizing muscle, are opposite to those reported for limb and trunk muscles (Monemi et al. 1998; Monemi et al. 1999b, c). On the contrary, changes in the anterior and posterior bellies of the digastricus, a jaw-opening muscle, resemble those of limb and trunk muscles (Monemi et al. 1999c; Monemi et al. 2000).

Advanced age is also associated with significant changes in the physiological properties of the muscle spindle, the mechanoreceptor sensing muscle tension and length. Muscle spindles have been considered to play an important role in voluntary and reflex movements (Miwa et al. 1995) and to contribute to position and velocity sense (Matthews 1981). Both dynamic and static position sense deteriorate with advanced aging (Ferrell et al. 1992; Meeuwssen et al. 1993; Robbins et al. 1995; Verschueren et al. 2002) and movement detection thresholds appear to increase in the elderly (Skinner et al. 1984; Gilding et al. 1995; Thelen et al. 1998). A decrease in the dynamic sensitivity of muscle spindle primary endings has also been described in aged rats (Miwa et al. 1995).

Morphological changes in the innervation and structure have also been reported in aged muscle spindles. Changes in the fine structure of the muscle spindle innervation consisting of spherical axonal swellings and expanded, abnormal motor end-plates have been described in some old muscles (Swash and Fox 1972). Additionally, an increase in the spindle capsular thickness and a decrease in the number of intrafusal fibers per spindle have also been observed in aging muscles (Swash and Fox 1972).

Muscle spindles contain three types of intrafusal fibers: nuclear bag₁, nuclear bag₂ and nuclear chain fibers (Barker and Banks 1994). Each type of human intrafusal fiber contains a particular combination of MyHC isoforms (Thornell et al. 1988; Thornell et al. 1989; Pedrosa-Domellöf et al. 1993; Eriksson et al. 1994; Liu et al. 2002; Liu et al. 2003). We have previously shown very complex patterns of co-expression of several MyHC isoforms for each intrafusal fiber type in muscle spindles of biceps brachii from young human adults (Liu et al. 2002). Both nuclear bag₁ and bag₂ fibers contained slow tonic MyHC (MyHC_{sto}) along their entire fiber length and slow MyHC (MyHC₁), α -cardiac MyHC (MyHC α -c), fetal MyHC (MyHC_{fet}) and embryonic (MyHC_{cemb}) with regional variations. Nuclear chain fibers had a homogeneous content of MyHC_{fet} and MyHC_{cemb}, and they contained fast MyHC (MyHC_{IIa}) along a variable part of their length. However, no data have thus far been reported on the

fiber type and MyHC composition of muscle spindles in the elderly. To investigate whether there are age-related changes in human muscle spindles with respect to intrafusal fiber types and MyHC composition, we examined muscle spindles of biceps brachii taken from subjects aged 69 to 83 years old. Our results show an altered fiber type composition and pattern of MyHC expression in the muscle spindles of the elderly.

Materials and Methods

Muscle Samples

The muscle samples used in the current study were collected postmortem from the biceps brachii muscles of five subjects following the Swedish Transplantation Law and with the approval of the Medical Ethical Committee, Umeå University. None of the subjects was known to suffer from neuromuscular disease. A total of 16 muscle samples were obtained: 2 samples containing 5 muscle spindles from an 83-year-old male; 6 samples containing 12 muscle spindles from an 82-year-old male; 4 samples containing 9 muscle spindles from a 79-year-old male; one sample containing 4 muscle spindles from a 78-year-old male; 3 samples containing 8 muscle spindles from a 69-year-old female. The muscle specimens were mounted, rapidly frozen in propane chilled with liquid nitrogen and stored at -80°C until analysis. Serial transverse sections, 7 or 8 μm , were cut in a Reichert Jung cryostat (Leica; Nussloch, Germany) at -23°C . Four muscle blocks containing 1–5 muscle spindles each and 13 spindles in total were consecutively sectioned for ~ 2 –2.8 mm. The remaining 12 specimens were sectioned for ~ 0.6 mm. The sections were processed for demonstration of mATPase activity after alkaline (pH 10.4) and acid (pH 4.6 and 4.3) preincubations (Dubowitz 1985) and for immunohistochemistry, with the exception of five samples (0.6 mm) that were only processed for enzyme histochemistry.

Enzyme Histochemical Classification of Fiber Types

The muscle spindles were analyzed in three regions: the A region, including equator and juxtaequatorial parts, containing the periaxial fluid space; the B region, extending from the end of the periaxial fluid space to the end of the capsule, and the C region corresponding to the extracapsular portion of the spindle (Barker and Banks 1994). Each region was further subdivided into an inner (proximal to the equator) and outer (distal to the equator) portion.

The intrafusal fibers were classified as nuclear bag₁, bag₂ and chain fibers according to their alkaline and acid mATPase activity (Ovalle and Smith 1972). In brief, fibers having low staining intensity at pH 4.3 and 10.4 in the outer A and B regions were classified as nuclear bag₁ fibers whereas fibers showing high mATPase activity at pH 4.3 and moderate mATPase activity at pH 10.4 in the outer A and B regions were identified as nuclear bag₂ fibers. The nuclear chain fibers could be recognized by their high mATPase activity at pH 10.4 and low activity at pH 4.3. In the C region, the distinction between bag₁ and bag₂ fibers could be carried out at pH 4.6. Fibers with very high mATPase activity were

Table 1 Monoclonal antibodies used to detect different MyHC isoforms

Antibody	Specificity	Short name	Reference
ALD19 ^a	MyHCslow-tonic	Anti-MyHCsto	Sawchak et al. 1985; Thornell et al. 1989
A4.840 ^b	First developmental MyHCI	Anti-MyHCI/1 st	Hughes et al. 1993
A4.951 ^b	Second developmental MyHCI	Anti-MyHCI/2 nd	Hughes et al. 1993
N2.261 ^b	Third developmental MyHCI, MyHCIIa ⁹	Anti-MyHCI/3 rd +IIa*	Hughes et al. 1993
A4.74 ^b	MyHCIIa	Anti-MyHCIIa	Hughes et al. 1993
BF35 ^c	MyHCI, MyHCIIa ^h	Anti-MyHC "all except IIx"	Schiaffino et al. 1989
2B6 ^d	MyHCembryonic	Anti-MyHCemb	Gambke and Rubinstein 1984
NCL-MHCn ^e	MyHCfetal	Anti-MyHCfet	Ecob-Prince et al. 1989
F88 ^f	MyHC α -cardiac	Anti-MyHC α -c	Leger et al. 1985

^aGift from Dr. Donald A. Fischman (Cornell University; New York, NY).

^bPurchased from Developmental Studies Hybridoma Bank (Department of Biological Sciences, University of Iowa, Iowa City, IA).

^cGift from Prof. S. Schiaffino (University of Padova, 35100 Padova, Italy).

^dGift from Prof. A. Kelly (University of Pennsylvania School of Medicine, Philadelphia, PA).

^ePurchased from Novocastra Laboratories Ltd. (24 Claremont Place, Newcastle upon Tyne NE2 4AA, UK).

^fGift from Dr. Jean J. Leger (Institut National de la Sante et de la Recherche Medicale, Unite 249; Montpellier, France).

^hOriginally reported specificity, yet it also reacts with MyHCeom and MyHC α -c in human extraocular and heart muscles, respectively (Liu et al. 2002).

⁹Originally reported to react with MyHCI, MyHCIIa and MyHCIIb in rat muscle (Schiaffino et al. 1989). Liu et al. (2002) reported that it also reacts with human MyHCI, MyHCIIa, MyHCeom, MyHC α -c, MyHCfet, MyHCemb, and with MyHCsto in chicken anterior latissimus dorsi muscle.

classified as bag₂ fibers and those with moderate or low activity as bag₁ fibers.

Analysis of MyHC Isoforms of Intrafusal Fibers

Monoclonal antibodies (MAbs) specific for various MyHC isoforms were used (Table 1). In addition, MAb NCL-MEROSIN against laminin α_2 chain (Sewry et al. 1997) was used to double label the contours of the muscle fibers in some sections. All antibodies were diluted in 0.01 M PBS containing 0.1% bovine serum albumin (BSA) and used at their optimal dilution (Liu et al. 2002).

Cross-sections, serial to those used for mATPase staining, were processed for immunocytochemistry by using standard indirect peroxidase-antiperoxidase (PAP) technique [Sternberger 1979, as previously described in detail (Liu et al. 2002)].

The sections were examined using a Nikon microscope (Eclipse, E800; Tokyo, Japan) or a Zeiss microscope (Zeiss; Oberkochen, Germany). Computer-generated images were processed using the Adobe Photoshop software (Adobe System Inc.; Mountain View, CA).

Young Adult Subjects

To determine whether there was a difference in the number of intrafusal fibers between muscle spindles from old and young subjects, we used our previous data on muscle spindles from young adults (Liu et al. 2002). In brief, a total of 36 muscle spindles (25 from females, 11 from males) were examined in 12 muscle samples collected at autopsy from the biceps brachii muscle of four females (ages 48, 38, 19 and 15) and six males (ages 38, 37, 27, 25, 22 and 29).

Statistical Analysis

The null hypothesis that there was no difference in the number of intrafusal fibers between muscle spindles from old and young subjects was rejected at the 0.05 level of significance. Unpaired *t*-test and the StatView software (3rd edition, SAS Institute Inc., Cary, NC) were used for statistical analysis.

Results

The study comprised a total of 38 muscle spindles containing 182 intrafusal fibers, of which 57 fibers were classified as nuclear bag₁, 54 as nuclear bag₂, and 71 as nuclear chain fibers.

Fiber-type Composition

The intrafusal fiber-type composition was studied in 21 muscle spindles (16 from males, 5 from females) encountered in either the A or inner B region, where

Table 2 Content of intrafusal fiber types in individual muscle spindles from biceps brachii in old subjects*

	n ^a	Bag ₁	Bag ₂	Chain	n ^b
	2	1	0	1	1
	3	1	2	0	1
	4	1	1	2	1
	4	2	2	0	1
	4	3	1	0	1
	5	1	1	3	2
	5	2	2	1	1
	6	1	1	4	1
	6	1	3	2	1
	6	2	1	3	1
	6	3	1	2	1
	7	1	2	4	1
	7	2	2	3	3
	8	3	1	4	1
	9	2	3	4	1
	9	3	2	4	1
	10	1	5	4	1
	11	2	1	8	1
Elderly, mean (SD)	6.2 (2.3) ^c	1.8 (0.8)	1.7 (1.1)	2.8 (1.8) ^c	
Young adults ^d , mean (SD)	9.1 (2.6)	2.2 (0.7)	1.3 (0.9)	5.7 (2.3)	

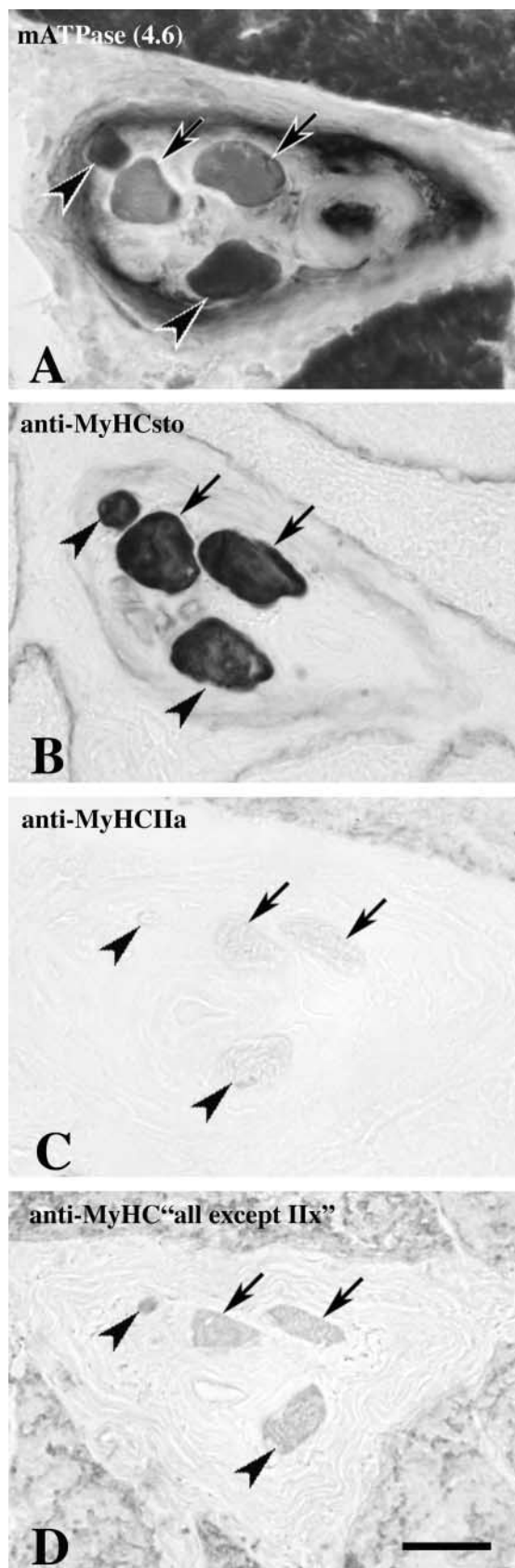
*For comparison, data from young adults are included.

^aTotal number of intrafusal fibers in each muscle spindle.

^bNumber of muscle spindles with identical fiber type content.

^cStatistically significant difference from young adults.

^dFrom Liu et al. 2002 (36 muscle spindles, 25 from females and 11 from males).



the full complement of intrafusal fibers is present. On average, there were 6.2 intrafusal fibers per spindle, including 1.8 (range from 1 to 3) bag₁, 1.7 (0 to 5) bag₂, and 2.8 (0 to 8) chain fibers (Table 2). Nineteen percent of the muscle spindles contained one bag₁, one bag₂, and two to four chain fibers. The remaining spindles contained three or more bag fibers except for one muscle spindle that contained only one bag₁ and one chain fiber (Figure 7E). Of 21 muscle spindles, 16 had a unique allotment of numbers of nuclear bag₁, bag₂, and chain fibers (Table 2).

Strikingly, 3 out of 21 muscle spindles examined in the A or inner B region did not contain any nuclear chain fibers (Table 2; Figure 1). Moreover, we found no nuclear chain fibers extending into the outer B or the C region, and very few chain fibers were observed in the inner B region. A number of nuclear chain fibers transiently appeared and ended in the A or even in the inner A region. The muscle spindles in the elderly had a lower total number of intrafusal fibers ($p = 0.0004$) and a lower number of chain fibers ($p < 0.0001$) per spindle in comparison with the muscle spindles from young adults (Table 2).

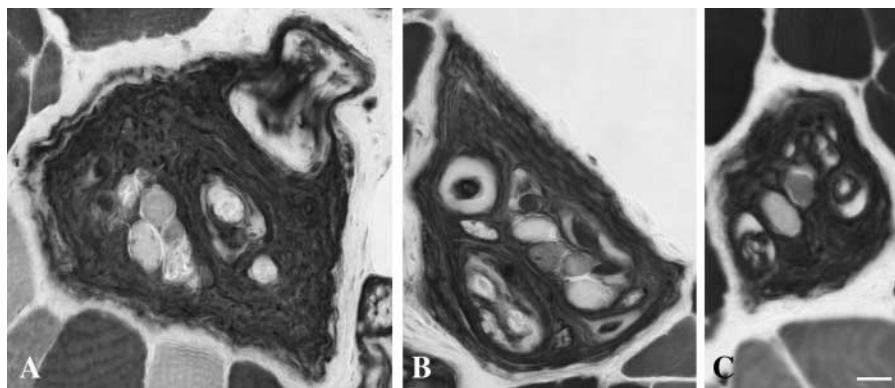
The thickness of the spindle capsule varied between spindles. Although the thickness of the capsule in aged biceps muscle spindles was generally similar to that of spindles in young biceps, the capsule of some spindles was extremely thick (Figure 2). In addition, the capsule of a spindle enclosed three type I and two type II extrafusal fibers (Figure 3).

MyHC Isoforms

Nuclear Bag₁ Fibers. All nuclear bag₁ fibers were strongly and evenly stained along their entire length with the mAb ALD 19 against MyHCsto (Figures 4B, 5B, and 6D) and unstained with MAb NCL-MHCn against MyHCfet (Figures 4H, 5G, and 6B). The other MABs stained the nuclear bag₁ fibers with variable intensities. The MABs A4.840 and A4.951 against MyHCI did not stain the bag₁ fibers in the equator and stained them weakly in the inner A region (Figures 4C and 4D). However, the staining intensity gradually increased throughout the outer A and the inner B regions and was moderate to strong in the outer B and the C regions (Figure 5C). Both antibodies stained the bag₁ fibers similarly. Anti-MyHCI+IIa* generally did not stain the bag₁ fibers in the A and the inner B regions (Figure 4E) whereas it stained them weakly in

Figure 1 Muscle spindle lacking nuclear chain fibers. Consecutive sections through the A region stained to show mATPase activity after preincubation at pH 4.6 (A), and treated with anti-MyHCsto (B), anti-MyHCIIa (C) and anti-MyHC "all except IIx" (D). Bag₁ fibers: arrows; bag₂ fibers, arrowheads. Bar = 20 μ m.

Figure 2 Three different muscle spindles with thick capsules. The sections were treated to show mATPase activity after preincubation at pH 4.6. Compare the thickness of the capsules with those in Figures 1A, 4A, and 5A. Bar = 20 μ m.



the outer B and the C regions (Figure 5D). The bag₁ fibers were usually unstained with anti-MyHCIIa, although a few fibers exhibited very low staining in the B region (Figure 5E). The MAb BF35 (anti-MyHC “all except IIx”) did not stain the bag₁ fibers in the equatorial region and, in some cases, not in the inner A region. The staining intensity increased from weak to moderate toward the end of the fibers (Figures 4G and 5F). The majority of the bag₁ fibers showed weak staining with anti-MyHCemb in the A region (Figure 4I), yet moderate stainings were also occasionally seen in the B region (Figure 5H). Thirty-nine percent of the bag₁ fibers were unstained with anti-MyHCemb in the A region and 33% in the B region. Very few bag₁ fibers were stained with anti-MyHCemb in the C region (Figure 6C). Seventy-one percent of nuclear bag₁ fibers in the A region and 94% in the B region were unstained with anti-MyHC α -c (Figures 7A and 7B). All bag₁ fibers were unstained with anti-MyHC α -c in the C region (Figure 7C). The bag₁ fibers labeled with anti-MyHC α -c showed variable staining intensities from strong to weak (Figures 7D and 7E).

Nuclear Bag₂ Fibers. Nuclear bag₂ fibers generally showed very strong staining intensity with anti-MyHCsto along their entire length (Figures 4B, 5B, 6D, and 6F). Yet, one bag₂ fiber was weakly stained

and two were unstained near the ends (not shown). The nuclear bag₂ fibers were strongly stained with both anti-MyHCI/1st and anti-MyHCI/2nd along the whole fiber length, except for a short segment in the central part of the fibers (Figures 4C, 4D, 5C, and 6G). The anti-MyHCI+IIa* usually stained the bag₂ fibers only in the C and the outer B regions, whereas the remaining parts of the fibers were unstained (Figures 4E and 5D). MAb A4.74, specific for MyHCIIa, did not label the bag₂ fibers throughout their length (Figures 4F, 5E, and 6H), except for two bag₂ fibers encountered in the A region that were weakly and strongly stained, respectively. The bag₂ fibers were weakly to strongly stained with anti-MyHC “all except IIx” and the staining intensity increased from the A to the C region (Figures 4G, 5F, and 6I). The bag₂ fibers were not stained with anti-MyHCfet regardless of the region (Figures 4H and 5G), except for two bag₂ fibers which were weakly stained in the A region and in the C region, respectively (Figure 6B). The staining intensity of the bag₂ fibers with anti-MyHCemb varied both among spindles and along their length (Figures 4I, 5H, and 6C). Fifty percent of the bag₂ fibers in the A region were unlabeled and the others were weakly labeled. The majority of bag₂ fibers were unstained with anti-MyHC α -c either in the A region (79%) or in the B region (86%), and no bag₂ fibers

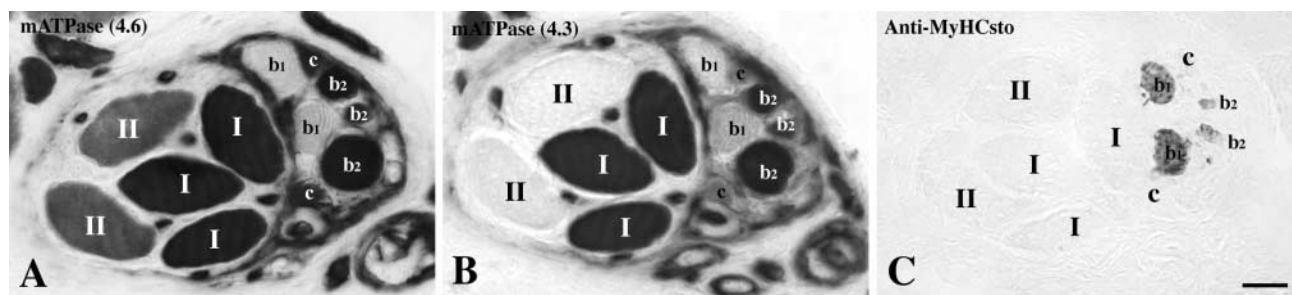


Figure 3 One spindle associated with a group of slow (I) and fast (II) extrafusar fibers. Serial sections treated to show mATPase activity after preincubation at pH 4.6 (A), pH 4.3 (B) and stained with anti-MyHCsto (C). b₁, bag₁ fibers; b₂, bag₂ fibers; c, chain fibers. Bar = 20 μ m.

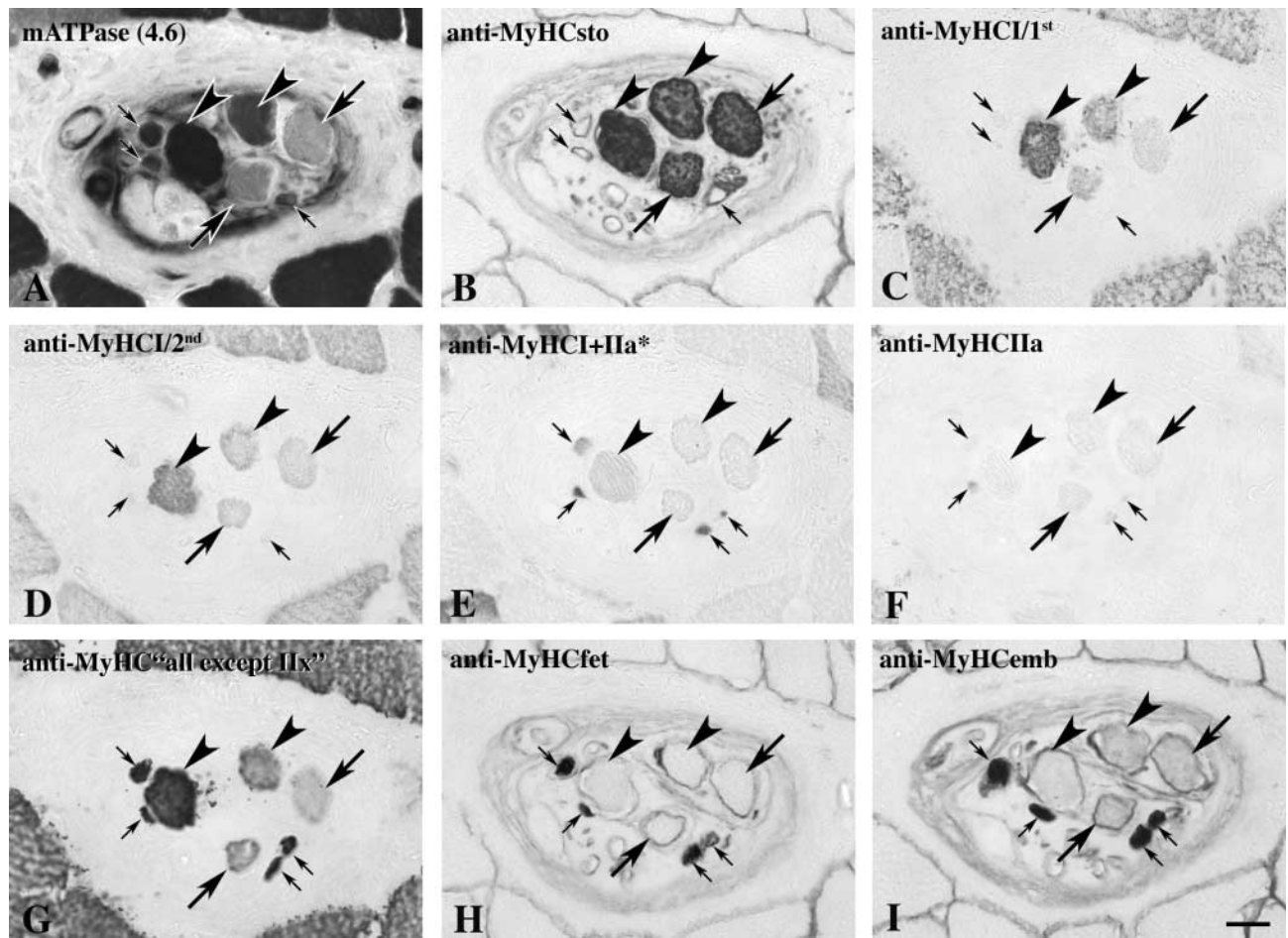


Figure 4 Staining patterns of muscle spindles in the A region. Serial transverse sections stained to show mATPase activity after preincubation at pH 4.6 (A), and stained with antibodies against MyHCsto (B), MyHCI/1st (C), MyHCI/2nd (D), MyHCI+IIa* (E), MyHCIIa (F), MyHC "all except IIx" (G), MyHCfet (H) and MyHCemb (I). bag₁ fibers, thick arrows; bag₂ fibers, arrowheads; chain fibers, thin arrows. Bar = 20 μ m.

were stained in the C region (Figures 7A–7C). The staining intensities of bag₂ fibers labeled with anti-MyHC α -c varied from strong to weak (Figure 7D).

Nuclear Chain Fibers. The nuclear chain fibers were unstained with anti-MyHCsto (Figure 4B), anti-MyHCI/1st, or anti-MyHCI/2nd (Figures 4C and 4D), whereas they were strongly and uniformly stained with anti-MyHC "all except IIx" (Figure 4G). In the B region, all chain fibers were stained strongly with anti-MyHCI+IIa* and anti-MyHCIIa, whereas in the A region 10 and 12% of the nuclear chain fibers were unstained with anti-MyHCI+IIa* (Figure 4E) and anti-MyHCIIa (Figure 4F), respectively. These two antibodies stained the remaining chain fibers strongly to weakly in the A region (Figures 4E and 4F). The vast majority of the chain fibers were strongly or moderately stained with anti-MyHCfet (Figure 4H) and only a few chain fibers were unstained (1%) in the A region. Likewise, anti-MyHCemb stained all chain fibers strongly and evenly

along their entire length (Figure 4I), except for three chain fibers that were transiently unstained in the A region. Nuclear chain fibers were unstained with anti-MyHC α -c in the A or the inner B regions (Figures 7A and 7E). However, six chain fibers belonging to two muscle spindles showed weak staining in the A or inner B regions (Figure 7F).

Discussion

The present study showed a significant reduction in the number of nuclear chain fibers and altered patterns of MyHC expression in the three intrafusal fiber types, suggesting that aging has a potential effect on the composition of human muscle spindles.

Alterations in Intrafusal Fiber Composition

A decrease in the number of intrafusal fibers and increased capsule thickness with increasing age have

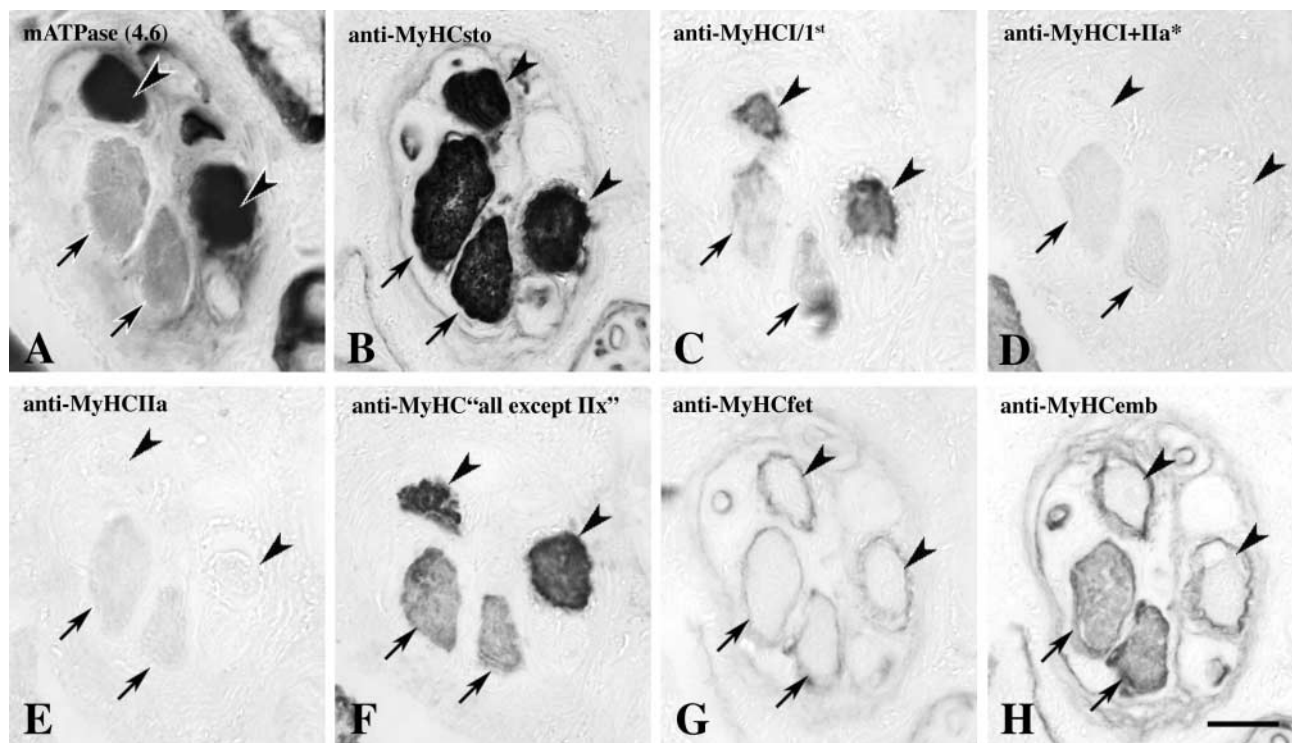


Figure 5 Staining patterns of muscle spindle in the B region. Serial transverse sections stained for mATPase at pH 4.6 (A), and stained with antibodies against MyHCsto (B), MyHCI/1st (C), MyHCI+IIa* (D), MyHCIIa (E), MyHC "all except IIx" (F), MyHCfet (G) and MyHCemb (H). bag₁ fibers, thick arrows; bag₂ fibers, arrowheads. Note that no chain fibers are present here in the B region. Bar = 20 μm.

previously been reported in human muscle spindles (Swash and Fox 1972). However, no data were available on the fiber-type composition of the old muscle spindles. In the current study we showed that the average number of both nuclear bag₁ and bag₂ fibers per spindle did not change with increasing age whereas the nuclear chain fibers decreased significantly in number or were completely absent in some muscle spin-

dles. Our data also showed that the chain fibers became much shorter in the aged spindles as they were seldom seen in the inner B region and were not found in the outer B or C regions. The factors regulating the decline in the number of chain fibers remain to be determined. However, deafferentation in the neonatal period is known to influence intrafusal fiber number (Soukup et al. 1993; Zelena and Soukup 1993); there-

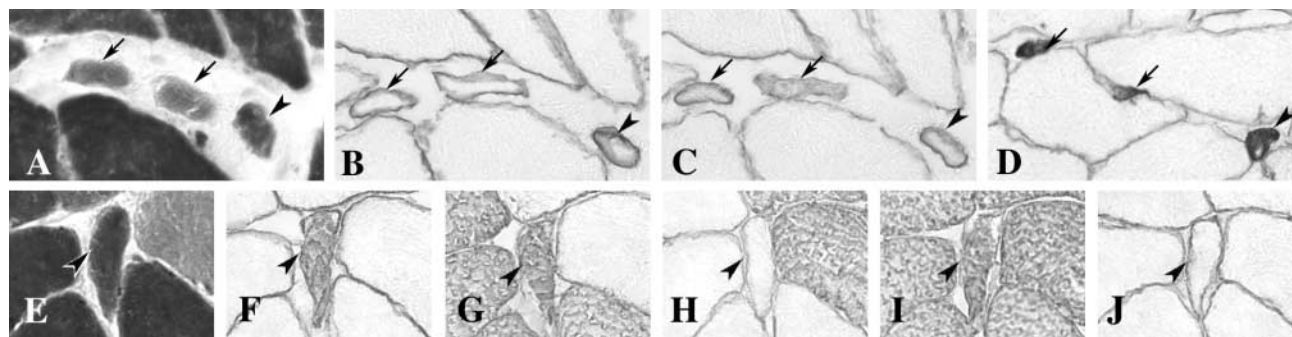


Figure 6 Staining patterns of two muscle spindles in the C region. One spindle (A–D) contained two bag₁ fibers (arrows) and one bag₂ fiber (arrowhead), and the other (E–J) contained one bag₂ fiber (arrowhead) only. The cross-sections were stained for mATPase activity after pre-incubation at pH 4.6 (A,E), and stained with anti-MyHCfet (B,J), anti-MyHCemb (C), anti-MyHCsto (D,F), anti-MyHCI/2nd (G), anti-MyHCIIa (H), anti-MyHC "all except IIx" (I). In (B–D) and (F–J), in addition, antibodies against laminin α₂ chain to label the contours of the muscle fibers have been used. Bar = 20 μm.

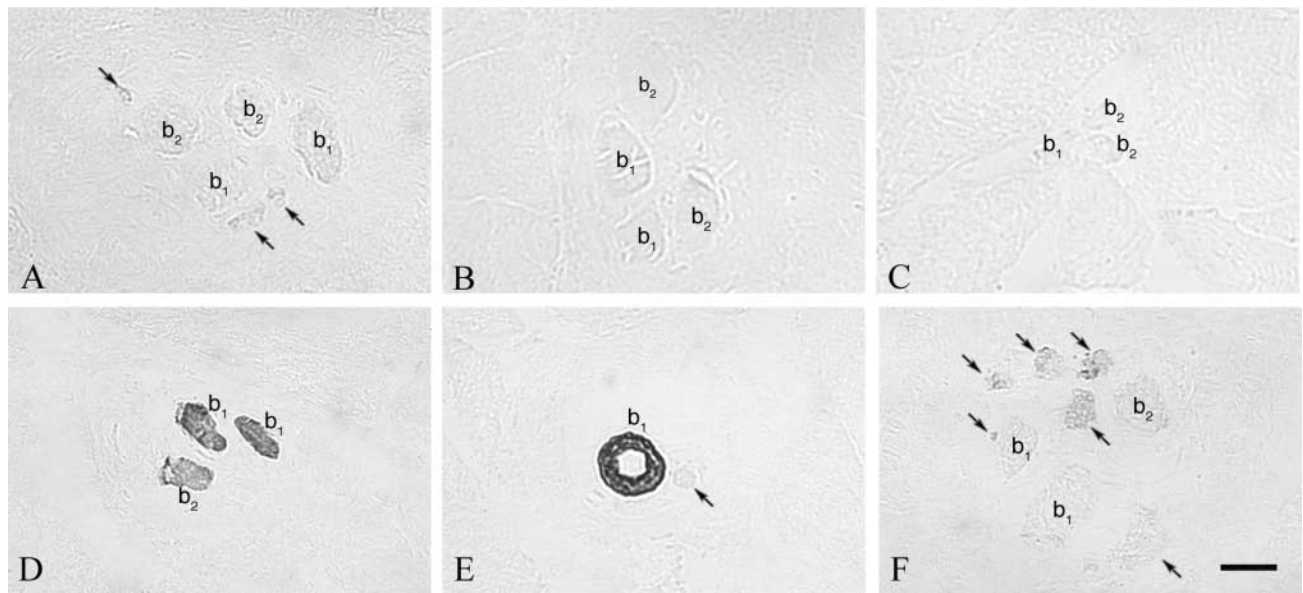


Figure 7 MyHC α -c expression in intrafusal fibers of old muscle spindles in the A region (A,D,E,F), B region (B) and C region (C). Bar = 20 μ m.

fore, changes in innervation are likely candidates influencing the number of chain fibers in the aged muscle spindles.

The functional significance of muscle spindles lacking or having fewer nuclear chain fibers remains to be elucidated. One would expect those muscle spindles to have a relatively less static sensitivity compared with the muscle spindles from young adults. This assumption is supported by previous physiological data that revealed a decline in static position sense at the ankle joint in the elderly (Meeuwssen et al. 1993; Robbins et al. 1995). A deterioration in the dynamic position sense has also been observed with aging (Verschueren et al. 2002), but it was 6-fold smaller than the deterioration observed in static position sense (Robbins et al. 1995; Verschueren et al. 2002). The question whether the diminution of the static sensitivity is related to the reduction in the number of nuclear chain fibers or whether it is due to alterations of the biomechanical properties of the aged muscle still remains to be determined.

Alterations in MyHC Composition

The present study showed that aging caused significant changes in the patterns of expression of MyHC α -c, MyHCfct and MyHCI, although the typical profiles of MyHC composition of intrafusal fibers observed in old subjects were generally similar to those observed in young adults. Nuclear bag₁ and bag₂ fibers from aged muscle spindles were not as frequently and strongly stained with anti-MyHC α -c in the A region as those from young adults. Instead, they were mostly

unstained or occasionally stained weakly to moderately, indicating a significant reduction in the MyHC α -c content in the aged nuclear bag₁ and bag₂ fibers. A similar reduction has also been observed in rat nuclear bag₁ fibers after neonatal and adult deafferentation (Pedrosa et al. 1990; Walro et al. 1997; Wang et al. 1997) and in both nuclear bag₁ and bag₂ fibers after a 14-day period of hypogravity (De-Doncker et al. 2002). In contrast to the nuclear chain fibers in young adult biceps, some nuclear chain fibers in old muscle spindles showed novel expression of MyHC α -c isoform. Novel expression of MyHC α -c has also been observed over a short distance in the A region in nuclear chain fibers of rat soleus muscle after 14 days of hindlimb unloading (De-Doncker et al. 2002).

The expression of MyHCfct was downregulated in nuclear bag₂ fibers, and the presence of MyHCI extended into the B and A regions of nuclear bag₁ fibers. The downregulation of the expression of MyHCfct in bag₂ fibers and upregulation of MyHCI in bag₁ fibers were also reported in nuclear bag fibers following deafferentation in adult rat muscle spindles (Walro et al. 1997; Wang et al. 1997). It is possible that the parallel changes in MyHC composition found in the present study of aging spindles and those reported following deafferentation in adult rat spindles (Walro et al. 1997; Wang et al. 1997) might be caused by similar neural factors. However, the number of intrafusal fibers does not change following deafferentation in the rat (Walro et al. 1997; Wang et al. 1997), indicating that the changes seen with human aging are more complex and may be multifactorial. We suggest that

deterioration in the motor and sensory innervation might cause the phenotypic changes observed in aged muscle spindles and are likely to have an impact in motor control in the elderly, as the muscle spindles play important roles in voluntary and reflex movements and, together with skin and joint sensory organs, also contribute to position and velocity sense.

In conclusion, the present study suggests an age-related loss of intrafusal fibers and changes in MyHC expression. Loss of fibers was found for the chain group and changes in MyHC expression for MyHC α -c, MyHCI and MyHCfet isoforms. Notably, the results imply that aging does not affect all muscle spindles or each intrafusal fiber in the same way, further supporting the concept that each muscle spindle is particularly adapted to its functional context.

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