Fiber Typing in Aging Muscle

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PURVES-SMITH, F.M., N. SGARIOTO, and R.T. HEPPLE. Fiber typing in aging muscle. Exerc. Sport Sci. Rev., Vol. 42, No. 2, pp. 45–52, 2014. It is accepted widely that fast-twitch muscle fibers are preferentially impacted in aging muscle, yet we hypothesize that this is not valid when aging muscle atrophy becomes severe. In this review, we summarize the evidence of fiber type-specific effect in aging muscle and the potential confounding roles of fibers coexpressing multiple myosin heavy-chain isoforms and their histochemical identification.

Key Words: sarcopenia, aging, muscle atrophy, fiber type, denervation, myosin heavy-chain coexpression, skeletal muscle

INTRODUCTION

A preferential fast-twitch (Type II) fiber involvement is among the most widely accepted features of aging muscle and informs many of the therapeutic approaches to this problem of aging, yet the rigor of this view should be closely scrutinized because of the complexity of classifying fiber type in advanced age when many individual muscle fibers express multiple myosin heavy-chain (MHC) isoforms simultaneously (MHC coexpressing fibers) (1,10,26,30,33). As will be discussed, denervation is the primary event responsible for producing these MHC coexpressing fibers in aging muscle (30) and not only do they pose a challenge in terms of their histological identification, but understanding whether they are derived from formerly pure slow (Type I) or pure fast (Type II) fibers has a critical role in accurately assessing the degree to which fast versus slow fibers are affected in aging muscle (Fig. 1). It also is important to stress that the abundance of these MHC coexpressing fibers increases dramatically at more advanced ages (≥80 yr) (1) where denervated fibers are common (30,31) (Fig. 2) and aging muscle atrophy is most likely to have clinical impact (13). Thus, the impact of the MHC coexpressing phenomenon is greatest at clinically relevant ages (≥80 yr), underscoring the importance of understanding how this phenomenon may obscure identification of fiber type-specific behavior in aging muscle.

To illustrate how these issues can confound our understanding of fiber type-specific effect in aging muscle, consider a hypothetical situation where MHC slow-fast coexpressing fibers originate from formerly pure slow fibers and that their identity is misclassified as fast fibers. In this situation, the atrophy behavior of what were formerly pure slow fibers is incorrectly ascribed to the fast fiber population. Although it is not possible to determine the degree to which this situation has occurred in previous assessments of fiber type changes in aging muscle, we hypothesize that this situation has high potential to adversely impact our understanding of fiber type-specific changes in aging muscle at clinically relevant ages (i.e., when muscle atrophy becomes severe enough to cause an increase in falls and impair mobility). Consistent with this hypothesis, as will be shown in this review, recent reassessment of the atrophy behavior of slow fibers in aging muscle using methods well suited to identification of MHC coexpressing fibers, and which used a strategy for tracking the origins of these slow-fast coexpressing fibers, has revealed a marked slow fiber atrophy potential in very advanced age that has not been described previously (26,30). In view of this behavior, we also address in this review whether there are circumstances where formerly pure slow fibers that transition to slow-fast coexpressing fibers in aging muscle could be misclassified as fast fibers. In this latter respect, we show that use of myofibrillar adenosine triphosphatase (mATPase) histochemistry to classify fiber types in aging muscle is a method particularly vulnerable to this problem. Thus, the purpose of this review is to revisit the evidence for preferential fast fiber involvement with aging, to account for how the phenomenon of MHC slow-fast coexpression can obscure our understanding, and finally to evaluate the potential for mATPase histochemistry to cause misclassification of MHC coexpressing fibers in aging muscle. The most important outcome of this review is that we conclude, at ages where muscle atrophy severity is most likely to have clinical impact, the pursuit of treatments for aging muscle atrophy needs to
fibers are frequently extremely atrophied. The types is contingent upon understanding from which parent fiber population.

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Consider the involvement of both slow and fast fibers because both are impacted significantly at advanced age.

What Is the Evidence for Preferential Fast Fiber Involvement in Aging Muscle?

There is widespread perception that aging muscle is characterized by a progressive shift toward a greater abundance of slow fibers and that fiber atrophy principally affects fast fibers. However, scrutiny of even the early studies (where use of mATPase histochemistry was most common) reveals a remarkable disparity on the fiber type proportion issue in particular. For example, focusing first on studies in aging rats, whereas some studies found a reduction in fast fiber abundance (14,21), other studies reported no change in fiber type proportion (3,17), and others reported an increase in fast fiber abundance in some muscles (7,8). The human literature does not paint a picture that is any clearer, with some studies reporting a decrease in fast fiber abundance (22), others reporting no change (2,12,23), and one longitudinal study even reporting an increase in fast fiber abundance during a 12-yr period (18). There also is a study reporting that the changes depended on the muscle examined, with the masseter exhibiting increased fast fiber abundance and the biceps brachii exhibiting a small decrease in fast fiber abundance with aging (24).

It is also important to mention that the physical activity status of the subjects is another factor that can influence the direction and magnitude of shift in fiber type with aging and likely contributes to the inconsistent findings in human literature. Thus, even based on these early studies, it is unclear why the idea of reduced fast fiber abundance in aging muscle has become so widely accepted as fact. It is most certainly not based on a consistent body of evidence. It also should be appreciated here that there are very little data pertaining to ages where muscle atrophy becomes severe in these early studies. In particular, most human studies focused on the ages of 65 to 75 yr (ages not typically associated with high fall incidence, mobility impairment, and other consequences of severe muscle atrophy). Furthermore, most rodent studies examined ages that approximate a similar point in the aging muscle trajectory (e.g., using survival rate and severity of muscle atrophy as points of comparison) as these human studies and, thus, the same caveat applies: with very rare exception, these early studies do not address ages when muscle atrophy becomes most severe and most likely to have clinical impact.

Another aspect of fiber type-specific effect in aging muscle that is not widely mentioned is that different muscles behave differently with aging (7,10,24) and this intermuscle difference alone should make us question the accuracy of a statement that generalizes fiber type shifting to occur in a fast-to-slow direction with aging. To reinforce this latter point, the rat soleus (Sol) muscle, a muscle that is almost exclusively composed of slow fibers in young-adult animals (5,10), exhibits an increase in fast MHC-positive fiber abundance with aging (8,15,33), yet for the most part, this observation seems to have been overlooked in drawing conclusions about the direction of fiber type changes in aging muscle. Under scoring the potential for mATPase histochemistry to have contributed to confusion in this respect, a previous study using mATPase histochemistry suggested that there were essentially no fast fibers remaining after the age of 12 months in the rat Sol muscle (7), a point that is profoundly at odds with studies examining the same muscle in the same rat strain at the same ages using antibodies to label fast MHC (10,33). The issue of potential misclassification of fibers using mATPase histochemistry will be examined in greater detail in the section, Classification of MHC Coexpressing Fibers Using mATPase Labeling.

We have argued previously that the direction of shift (from fast to slow or from slow to fast) depends on the initial fiber type proportions. This is particularly the case at ages when muscle atrophy becomes severe where the largely fast twitch rat gastrocnemius (Gas) muscle exhibits an increased slow fiber abundance with aging, whereas the largely slow twitch rat Sol muscle exhibits an increased fast fiber abundance with aging (10). Importantly, the increase in MHC slow in the Gas and the increase in fast MHC in Sol muscle in very old rats are both caused by a dramatic increase in MHC slow-fast...
coexpressing fibers (29), most of which are denervated (30). Thus, on the basis of the above review of evidence, it must be concluded that a reduction in fast fiber abundance is not a consistent feature of aging muscle and, in advanced age, some muscles can show the polar opposite change — a pronounced increase in fibers positive for fast MHC.

Factors Driving MHC Coexpression in Aging Muscle

As mentioned from the outset of this review, when muscle atrophy becomes severe, MHC coexpression is a common occurrence in both aging rat (15,29,33) and aging human (1) skeletal muscle. In the case of human muscle, Andersen (1) has suggested previously that MHC coexpression may manifest in two distinct forms. In one form, the MHC expression within a given fiber differs between individual myonuclear domains, such that there is a transition in MHC expression along the length of the myofibers (Fig. 3A). In another form, there are multiple MHC expressed within a given myonuclear domain (1) (Fig. 3B). We are not aware of any literature reporting the relative occurrence of these distinct forms of MHC coexpression in aging muscle, but it is relevant to point out that the majority of studies reporting MHC coexpression in aging muscle do so with multiple MHC antibody labeling in a single muscle cross section (15,26,29,30,33), and thus the MHC coexpression phenomenon identified by this approach is occurring within a single myonuclear domain.

MHC coexpression can occur for a variety of reasons, but some are more relevant for aging muscle than others. In particular, denervation (30,31) and regeneration (15) are both scenarios that occur in aging muscle and could contribute to this phenomenon. For a normal healthy muscle in the absence of overt injury, denervation is the most likely culprit because the proportion of fibers that become MHC coexpressers in advanced age can be very dramatic. Indeed, it seems highly likely that the majority of fibers judged as showing evidence of regeneration (noting that these fibers most often expressed multiple MHC isoforms) in one previous study of aging rat SOL muscle (15) were in fact denervated on the basis of their highly angular nature (6).
The basis for the view that denervation is the most important contributor to MHC coexpression in aging muscle comes in part from the many reports showing that muscle exhibits a dramatic increase in the abundance of MHC coexpressing fibers after experimental denervation (4,35). Furthermore, the direction of shift in MHC expression (i.e., away from slow toward fast, away from fast toward slow) after denervation depends on the basal fiber type composition of the muscle (25), a feature also seen in aging muscle (10). Indeed, it is likely that, on denervation, an individual myocyte reverts to the MHC expression profile it exhibited in the immediate postnatal period before the influence of activation patterning becomes evident (i.e., it recapitulates its embryonic origins). For example, Rana et al. have proposed a model wherein the fiber type properties (in this case, modeled with troponin I fast expression) between prototypical fast (mouse extensor digitorum longus (EDL)) and slow (mouse Sol) muscles are very similar in the early postnatal period and consist of a mixture of fast and slow isoforms. Thus, the contrasting adult fiber type of these distinct muscles is achieved postnatally largely through the influence of neural recruitment patterns (28). In this model, if the neural activation patterning is lost secondary to denervation, the muscle fiber type reverts to its original postembryonic state, meaning that fast muscles become slower and slow muscles become faster. This point is particularly interesting because it likely explains the disparate

Figure 4. Slow fibers exhibit marked atrophy behavior in advanced age. (A) Photomicrographs of rat soleus (Sol) muscle immunolabeled with myosin heavy-chain (MHC) fast or MHC slow. Solid arrow indicates a pure MHC fast fiber, striped arrow indicates a pure MHC slow fiber, and dashed arrow indicates MHC slow-fast coexpressing fibers. Note that very old rat Sol muscle is characterized by a dramatic increase in number of fibers that are coexpressing both slow and fast MHC isoforms (bar = 150 μm). (B) Mean fiber type proportions in young adult and very old. (C) Mean fiber size in young adult and very old. (Reprinted from (26). Copyright © 2012 Elsevier. Used with permission.)

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direction of change in MHC expression seen with aging between the largely slow rat Sol muscle and the largely fast rat Gas muscle. Specifically, as noted in the section, What Is the Evidence for Preferential Fast Fiber Involvement in Aging Muscle?, we have shown previously that whereas the Gas muscle exhibits an increase in slow MHC expression with aging (largely through an increase in slow-fast MHC coexpressing fibers), the Sol muscle exhibits an increase in fast MHC expression with aging (again, largely through an increase in slow-fast MHC coexpressing fibers) (29). This exactly is what is seen with surgical denervation where denervated fast muscle exhibits an increase in MHC slow expression (27,35), whereas denervated slow muscle exhibits an increase in fast MHC expression (4,25). Thus, the available evidence is strongly indicative that denervation is the primary cause of MHC coexpression in aging muscle. Indeed, we recently showed that more than 70% of MHC slow-fast coexpressing fibers in aging Gas muscle are positive for a sodium channel isoform that only is seen in adult muscle after denervation, Nav\textsubscript{1.5} (30).

Notwithstanding the established primary role of denervation in driving MHC coexpression in muscle at very advanced age (30), the physical activity status of the subjects also can play a role in this phenomenon. In particular, a previous study of 74-yr-old untrained men showed that progressive resistance training markedly reduced the abundance of MHC coexpressing fibers (36), a finding that resonates with studies of younger adult subjects where muscle from trained individuals contain very few MHC coexpressing fibers compared with those from less active subjects (19).

Illustrating the Problem of MHC Coexpression in Assessing Atrophy Susceptibility of Slow Versus Fast Fibers in Aging Muscle

We recently conducted a study to evaluate the hypothesis that slow fibers exhibit substantial atrophy at very advanced age, when muscle atrophy is severe and most likely to have clinical impact, but that this behavior is obscured by MHC coexpression (26). In this study, one of two muscles we examined was the rat Sol muscle because of its composition of more than 90% slow Type I fibers in young adulthood (33). We reasoned that the large predominance of slow fibers in this muscle would allow us to ascribe the appearance of MHC slow-fast coexpressing fibers with aging largely to the transition of formerly pure slow fibers in young adulthood. The alternative that MHC coexpressing fibers are de novo generated fibers in the process of differentiation is inconsistent with the limited regenerative capacity of aging muscle (9,11,20). As such, the atrophy behavior of slow fibers on their transition to MHC slow-fast coexpressing fibers could be evaluated.

As shown in Figure 4, the rat Sol exhibited a marked increase in the proportion of MHC slow-fast coexpressing fibers in very old age (Fig. 4A, B). Significantly, these slow-fast coexpressing fibers in very old rat Sol muscle were 58% smaller than pure slow fibers in young adulthood (Fig. 4C). Because these coexpressing fibers are most likely to derive from formerly pure slow fibers, our observations clearly demonstrate that slow fibers exhibit marked atrophy behavior in advanced age, but this behavior is obscured by MHC coexpression. It follows that the assumption of preferential fast fiber atrophy in aging muscle is inaccurate, particularly at ages that are most relevant in terms of clinical impact. Thus, we conclude that one cannot ignore the atrophy of slow fibers when seeking appropriate remedies to aging muscle atrophy.

Classification of MHC Coexpressing Fibers Using mATPase Labeling

The mATPase method remains one of the most widely used approaches for identifying fiber type in skeletal muscle. Indeed, this approach was used in the majority of the initial studies of fiber type in aging muscle. More recently, other commonly used approaches for fiber typing in aging muscle include MHC antibody labeling in tissue cross sections and single fiber sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). MHC antibody labeling has greater specificity than mATPase but is similar in this respect to SDS-PAGE. SDS-PAGE permits accurate identification of MHC expression patterns within single fibers with aging (36) and may offer a broader appreciation of MHC coexpression in muscle because a longer segment of the fiber is represented in the analysis than in the usual 7- to 10-μm-thick cross sections used for mATPase histochemistry or MHC antibody labeling. On the other hand, it remains unclear whether the single-fiber SDS-PAGE approach yields a representative sampling of all the muscle fibers present in very advanced age. For example, whereas studies of octogenarian subjects using single-fiber SDS-PAGE often report no difference in fiber size compared with younger subjects (32), this contrasts dramatically from what is seen at this age in tissue cross sections where there is a substantial reduction in mean fiber size because of the accumulation of severely atrophic fibers among fibers of relatively normal size (e.g., Fig. 2C). Comparison of the fiber size and type distributions obtained by single-fiber SDS-PAGE...
and MHC antibody labeling in tissue cross sections of the same elderly subjects is warranted to resolve this issue.

As we stated in the Introduction, we believe that there is strong potential for mATPase histochemistry to confound accurate fiber type classification at ages where muscle atrophy becomes severe because these ages are characterized by a high abundance of MHC coexpressing fibers. To understand the potential problem of using mATPase histochemistry for fiber typing in aging muscle, it is helpful to understand some fundamentals of this method. Specifically, the mATPase method produces discrete gray shading intensities between individual fiber types based on fiber type-specific inhibition of mATPase activity after incubation at acid versus basic pH (Fig. 5A). Careful application of method also can permit identification of MHC coexpressing fibers (34) (Fig. 5B) and indeed the first reports of MHC coexpressing fibers in aging muscle used this approach (16). However, the mATPase staining patterns become more complex in advanced age where the gray staining becomes a continuum at both acidic and basic pH preincubations (Fig. 6). Thus, although the mATPase staining pattern permits accurate fiber type categorization by the trained eye in healthy adult muscle, in very advanced age, the continuum of gray shade intensities poses a significant challenge in fiber type classification to even the most meticulous and patient individual. Indeed, as shown here, when using MHC antibodies to identify MHC slow-fast coexpressing fibers definitively, it is striking that the mATPase staining pattern for many MHC coexpressing fibers in the Sol muscle of very old rats (remembering that these fibers originate from pure MHC slow fibers in young adulthood; see section 3) is frequently indistinguishable from the staining pattern of MHC fast fibers (Fig. 7). As a result, these MHC coexpressing fibers have a high probability of being misclassified as fast fibers. Because, in this example, the MHC coexpressing fibers originate from the slow fiber population, ascribing the atrophy behavior of these MHC coexpressing fibers to the fast fiber population would lead one to conclude incorrectly that the atrophy is occurring primarily in the fast fibers. On this basis,

![Figure 6](https://example.com/image6.png)

*Figure 6.* Myofibrillar ATPase histochemistry to identify fiber types in young adult versus very old rat soleus muscle. Note the marked increase in variation in gray staining intensities at both basic (10.4) and acidic (4.6 and 4.2) pH preincubation in very old age. This results in a high probability of misclassifying slow-fast MHC coexpressing fibers as fast fibers, particularly under basic preincubation. See Figure 5 for classification scheme.
we conclude that the use of mATPase histochemistry to examine fiber type at ages where muscle atrophy is severe can lead to erroneous conclusions about age-related changes in both fiber type proportions and fiber type-specific atrophy.

CONCLUSIONS

The purpose of this review was to reevaluate the notion of preferential fast fiber involvement in aging muscle, particularly at ages where muscle atrophy becomes severe and most likely to have clinical impact. We hypothesized that the phenomenon of MHC coexpression, wherein individual muscle fibers express more than MHC isoform, has high potential to obscure our understanding of fiber type-specific effect in aging muscle because of difficulty in classifying these fibers and assigning their atrophy behavior to the correct originating fiber type. Furthermore, we argued that this problem would be of particular relevance when muscle atrophy is most likely to have clinical consequence because this is when MHC coexpressors are in high abundance. Consistent with our hypothesis, we reviewed recent evidence from our laboratory showing that, using methods well-suited to identifying MHC coexpressing fibers and studying a muscle where the origins of these fibers was primarily from slow muscle fibers in young adulthood, slow fibers exhibit a marked degree of atrophy. We also showed that the use of mATPase histochemistry for fiber typing has a high probability of confounding accurate assessment of fiber type-specific effect in aging muscle because of difficulty classifying the MHC coexpressing fibers in very old muscle, where mATPase staining patterns become a continuum of gray staining intensities. Thus, we conclude that previous assertions of a preferential fast fiber effect in aging muscle are not valid when muscle atrophy becomes most severe. The implications of these findings for elderly individuals who have severe muscle atrophy and resulting weakness are that the therapeutic strategies for treating this problem need to consider the atrophy of both slow and fast fibers to have optimal impact.

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