Slow twitch soleus muscle is not protected from sarcopenia in senescent rats

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Abstract

Although most literature suggests a relative protection of slow twitch muscle with aging, there is limited data in senescence when muscle atrophy and functional decline markedly accelerate. To address this issue we examined age-related changes in muscle mass, contractile function, mitochondrial enzyme activities, and myosin heavy chain (MHC) expression in the slow twitch soleus (Sol) and fast twitch gastrocnemius (Gas) muscle of young adult (YA) and senescent (SEN) rats. Muscle mass declined between YA and SEN in the Sol by 35% compared to 55% in the Gas muscle. After normalizing for muscle mass, tetanic force per g of muscle declined by 58% in Sol and by 36% in Gas muscle. Time-to-peak tension was increased only in the Gas (30%), whereas time-to-half relaxation was increased by 70% in Sol and 51% in Gas. Citrate synthase and complex IV activity declined in homogenates of Sol (30–36%) and red oxidative region of Gas (46–51%), but not white glycolytic region of Gas. Strikingly, the shift away from the dominant adult MHC isoform with aging was much greater in Sol (fibers positive for MHC fast: 11±2% in YA versus 36±3% in SEN) than in Gas (fibers positive for MHC slow: 12±1% in YA versus 26±3% in SEN) muscle. Collectively, these results show that the slow twitch Sol muscle undergoes large phenotypic alterations in very old age and for several measures (tetanic tension per g, time-to-half relaxation and shift in adult MHC expression) that is of greater magnitude than fast twitch muscle, underscoring the importance of including age-related changes in slow twitch muscle in seeking potential treatments for sarcopenia.

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1. Introduction

The age-related loss of muscle mass and function known as sarcopenia is widely considered to preferentially impact fast twitch muscle and to be characterized by an increase in slow twitch or type I fiber abundance with aging (Dirks et al., 2006; Snijders et al., 2009). On the other hand, as researchers have adopted newer models of aging (e.g., the Fischer 344×Brown Norway F1-hybrid [F344BN] rat) and examined more advanced ages where muscle atrophy becomes severe (regardless of model employed), there has been an increasing number of publications documenting significant atrophy even in muscles that are largely slow twitch in character (Edstrom and Ulfhake, 2005; Rice and Blough, 2006; Seo et al., 2008; Snow et al., 2005). Not only this, there is evidence that age-related changes in myosin heavy chain (MHC) expression in the slow twitch soleus (Sol) muscle of the rat exhibit significant shifts in the opposing direction (towards more fast MHC) (Edstrom and Ulfhake, 2005; Snow et al., 2005) compared to more glycolytic or fast twitch muscles like the gastrocnemius (Gas) muscle. This latter finding challenges the notion that aging generally results in an increase in slow twitch fiber abundance. Despite this emerging evidence, there is a scarcity of data documenting the magnitude of phenotype changes in slow twitch muscle in comparison to fast twitch muscle at more advanced stages of sarcopenia. Given that the degree of sarcopenia is most severe for the oldest old and that it is this age in particular where sarcopenia is most likely to lead to frailty (Cruz-Jentoft et al., 2010; VanItallie, 2003), a broader understanding of the impact of aging on both slow twitch and fast twitch muscles at very advanced age is required to guide development of the most effective treatments.

The F344BN rat has emerged as a powerful model for examining the causes and consequences of sarcopenia. Previous work by our group and others has demonstrated in this model that muscle atrophy and functional decline are modest and primarily impact fast twitch muscle between young adulthood (YA) and late middle age (LMA; defined here as a survival rate of 70–80%) (Brown and Hassler, 1996; Hagen et al., 2004; Lushaj et al., 2008). However, there is a marked acceleration in muscle atrophy (Brown and Hassler, 1996; Hagen et al., 2004; Lushaj et al., 2008) and functional decline (Hagen et al., 2004; Hepple et al., 2004a) between late middle age and senescence (SEN; defined here as a survival rate ≤ 50%), when even the slow twitch Sol muscle exhibits a marked degree of atrophy and contractile dysfunction (Hagen et al., 2004; Hepple et al., 2004a; Thompson and Brown, 1999). As noted above, to date there has been no
systematic comparison of phenotypic changes in the slow twitch Sol muscle versus the fast twitch Gas muscle at advanced stages of sarcopenia, and the established trajectory of sarcopenia in the F344BN rat makes this a useful model to address this issue. To provide a broad basis on which to make our comparisons, we examined muscle mass, contractile function, mitochondrial enzyme activities, and myosin heavy chain (MHC) expression in situ of the largely slow twitch Sol muscle and the largely fast twitch Gas muscle in YA and SEN male F344BN rats. We hypothesized that there would be large phenotypic alterations in the Sol muscle between YA and SEN that would rival those seen in fast twitch Gas muscle, contradicting the notion of a relative protection of slow twitch muscle with aging. Our results reveal that there are large phenotypic shifts with aging in both slow and fast twitch muscles, and that for several of these features the changes in the slow twitch Sol muscle are even greater than those in the fast twitch Gas muscle, underscoring the importance of accounting for changes in both slow and fast twitch muscles in seeking effective treatments for sarcopenia.

2. Methods

2.1. Animals

Young adult (YA: 7–10 mo old) and senescent (SEN: 35 mo old) male F344BN rats were obtained from the colony supported by the National Institute on Aging (Bethesda, USA). Two groups of animals at each age were studied in the current investigation. Specifically, 9 YA and 9 SEN rats were studied for muscle contractile function, whereas 8 YA and 8 SEN rats were studied for the biochemical and MHC in situ expression analyses. Upon arrival at the University of Calgary Biomedical Sciences Vivarium, all animals were housed in pairs of a given age in cages with filter bonnets (12:12 light dark cycle; 22 °C). All procedures were conducted with approval from the University of Calgary Animal Care Committee. Rats were anesthetized with sodium-Pentobarbital (i.p.) at a dose of 75 mg kg$^{-1}$. For rats in the contractile function studies the left gastrocnemius–plantaris–soleus muscle group was surgically exposed, and the Achilles tendon was severed with a piece of the calcaneus attached. The Achilles tendon was then dissected longitudinally to separate the soleus muscle (Sol) from the gastrocnemius–plantaris (Gas/Plan) muscles, while being careful not to disrupt the vascular circulation or neural supply to either muscle compartment. The sciatic nerve was located in the avascular space between the hamstrings, and cut proximal to its insertion into the gastrocnemius–plantaris–soleus muscle group in preparation for induction of electrically stimulated muscle contractions. The rat was transferred to a heating pad on a stereotaxic plate where the left hindlimb was secured by a bone clip around the proximal portion of the femur (through an incision near the hip) and by a bone clip around the distal tibia to prevent movement during muscle contractions. The Sol and Gas/Plan muscles were then individually attached to force transducers (Grass Instruments, FT-10) by non-compliant silk thread tied to their respective portions of the Achilles tendon, such that tension could be measured in the Sol independently from the Gas/Plantaris muscles. The distal hindlimb was then wrapped in warm saline-soaked gauze and plastic wrap, with a thermostatic probe inside the wrappings to permit the temperature of the Sol and Gas/Plan muscles to be maintained at 37 °C via an external heat lamp.

For rats used for the biochemical and MHC in situ expression studies the right Sol and Gas muscle were surgically removed, dissected free of fat and connective tissue and weighed. A cross-section through the midbelly of each muscle was mounted in optimal cutting temperature compound on cork and frozen in liquid isopentane cooled in liquid nitrogen for subsequent immunolabeling studies. Remaining portions of the Sol, and the red (Gr) and white (Gw) regions of the Gas were frozen individually in liquid nitrogen for subsequent biochemical analyses.

2.2. Surgical procedures

All procedures were conducted with approval from the University of Calgary Animal Care Committee. Rats were anesthetized with sodium-Pentobarbital (i.p.) at a dose of 75 mg kg$^{-1}$. For rats in the contractile function studies the left gastrocnemius–plantaris–soleus muscle group was surgically exposed, and the Achilles tendon was severed with a piece of the calcaneus attached. The Achilles tendon was then dissected longitudinally to separate the soleus muscle (Sol) from the gastrocnemius–plantaris (Gas/Plan) muscles, while being careful not to disrupt the vascular circulation or neural supply to either muscle compartment. The sciatic nerve was located in the avascular space between the hamstrings, and cut proximal to its insertion into the gastrocnemius–plantaris–soleus muscle group in preparation for induction of electrically stimulated muscle contractions. The rat was transferred to a heating pad on a stereotaxic plate where the left hindlimb was secured by a bone clip around the proximal portion of the femur (through an incision near the hip) and by a bone clip around the distal tibia to prevent movement during muscle contractions. The Sol and Gas/Plan muscles were then individually attached to force transducers (Grass Instruments, FT-10) by non-compliant silk thread tied to their respective portions of the Achilles tendon, such that tension could be measured in the Sol independently from the Gas/Plan muscles. The distal hindlimb was then wrapped in warm saline-soaked gauze and plastic wrap, with a thermostatic probe inside the wrappings to permit the temperature of the Sol and Gas/Plan muscles to be maintained at 37 °C via an external heat lamp.

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2.3. Muscle contractile studies

Muscle twitch contractions were induced by electrical stimulation via a platinum hook electrode placed under the sciatic nerve. Twitch contractions were induced by supramaximal square wave pulses of 50 µs in duration (Grass S48, Grass Instruments). Muscle length was set at a reference length that produced the largest active tension during a single supramaximal pulse. Twitch contraction tension and time data were collected using WinDaq software (DATAQ DI-720, DATAQ Instruments). Active tension was measured as the peak tension less the passive tension immediately prior to the contraction. Time-to-peak tension was measured as the time from tension development to peak tension, and half relaxation time was measured as the time from peak tension to the time at 50% peak tension.

2.4. Biochemistry

To obtain representation of age-related changes in the activity of mitochondrial enzymes involved in the Krebs Cycle and electron transport chain, respectively, citrate synthase activity and complex IV activity were assayed in homogenates of Sol, Gr and Gw muscle, according to methods described by our lab previously (Baker et al., 2006; Hepple et al., 2005; Hepple et al., 2006).

2.5. MHC expression in situ

Ten-micron thick cross-sections of the Sol and Gas muscle were cut on a cryostat (–18 °C) and mounted on lysine-coated slides for immunolabeling experiments that employed methods similar to those we have reported previously (Wong et al., 2009). Cross-sections mounted on the slides were kept at –80 °C until use in immunolabeling experiments. In one experiment, cross-sections were incubated with primary antibodies against laminin (rabbit anti-laminin, 1:500 dilution, Sigma) and either fast MHC (mouse anti-fast MHC in Sol only, 1:10 dilution; Novocastra) or slow MHC (mouse anti-slow MHC in Gas only, 1:10 dilution; Novocastra). In a second experiment, serial cross-sections to those used in experiment 1 were incubated with a primary antibody against developmental MHC (mouse anti-developmental MHC, 1:10 dilution; Novocastra). These two experiments allowed us to examine the expression of the non-dominant adult MHC isoform in each muscle (fast MHC in Sol, slow MHC in Gas) and a developmental MHC isoform previously shown to be elevated in senescent muscle (Edstrom and Ulfhake, 2005; Snow et al., 2005). Sections labeled with laminin and either slow MHC or fast MHC were subsequently incubated with secondary antibodies according to the host of each of the primary antibodies used (goat anti-mouse AlexaFluor 688, goat anti-rabbit AlexaFluor 546, both at 1:200 dilution; Invitrogen Molecular Probes). Following labeling, these cross-sections were imaged via confocal fluorescence microscopy (Olympus) and photomicrographs taken at a magnification of 200×. Sections labeled for developmental MHC were subsequently treated with the Vectastatin ABC kit (Vector Laboratories) to visualize the primary antibody, and imaged by brightfield microscopy, with photomicrographs taken at a magnification of 200× (Nikon Coolpix 990).

2.6. Morphometry

Images were acquired by systematically randomized (Sol and Gas) and region-specific sampling (Gr and Gw) techniques, as done
previously (Hepple et al., 2004b; Wong et al., 2009). This approach permitted us to obtain representation of MHC expression in the whole muscles as well as in regions that are predominantly slow twitch (Sol), fast glycolytic (Gw), and fast oxidative (Gr) in YA animals, based on the fiber type distribution in the rat (Armstrong and Phelps, 1984). Note that in pilot experiments with YA muscle we confirmed that fibers negative for fast MHC in the Sol were positive for slow MHC, whereas fibers negative for slow MHC in the Gas were positive for fast MHC (S.L. Rowan and R.T. Hepple, unpublished results). As such, fibers which were positive for fast MHC in the Sol were classed as type Ila positive fibers, based on the absence of other adult fast MHC isoforms in the rat Sol (Armstrong and Phelps, 1984), and fibers that were negative for fast MHC were classed as type I fibers. In Gas, fibers that were negative for slow MHC in the Gw region were classed as type Ib/x because we were unable to distinguish between Ib and Ix via our method. Conversely, fibers that were negative for slow MHC in the Gr region were classed as type Ila, based on the established adult MHC expression patterns in these regions of the Gas muscle in the rat (Armstrong and Phelps, 1984). Fibers that were positive for slow MHC in Gas were classed as type I positive fibers. Morphometric analyses of fiber size and MHC expression were conducted on an average of 367±15 (mean±SE) fibers per muscle in Sol and 457±40 fibers per muscle in Gas.

2.7. Statistics

Comparisons of muscle mass, peak twitch tension, peak tetanic tension, time-to-peak tension, time-to-half relaxation and between age groups were made with t-tests due to the unequal variance between muscles for these parameters (precluded assessment by ANOVA). Comparisons of tetanic tension per g of muscle, biochemical enzyme activity, fiber size, and non-dominant MHC abundance between age groups were made by Two-way ANOVA (age×muscle), with a Holm-Sidak post-hoc test. Comparisons of developmental MHC abundance were compared between muscle regions (Sol, Gr and Gw) by an ANOVA on Ranks (failed normality test during One-way ANOVA). Values are presented as means±standard error (SE).

3. Results

3.1. Body mass and muscle mass

The body mass of the SEN animals (478±20 g) was not different from that of the YA animals (454±11 g). In contrast, the mass of the Sol muscle was reduced by 35% and that of the Gas muscle by 55% in SEN compared to YA animals (Fig. 1), demonstrating marked atrophy in both slow (Sol) and fast (Gas) muscles at this advanced age.

3.2. Contractile function

Peak twitch and tetanic tension measured in the Sol versus Gas/Plan muscles with aging are presented in Fig. 2. In the largely fast twitch Gas/Plan muscles the peak twitch tension was reduced with aging from 4.96±0.48 N in YA to 2.46±0.31 N in SEN (50% decline), whereas in Sol the peak twitch tension was reduced with aging from 0.41±0.02 N in YA to 0.25±0.04 N in SEN (38% decline). Peak tetanic tension was reduced from 30.8±2.2 N to 12.1±1.8 N in the fast twitch Gas/Plan (−61%), whereas it was reduced from 2.56±0.14 N to 0.85±0.12 N in slow twitch Sol muscle (−67%). Peak tetanic...
tension normalized for muscle mass was reduced from 11.7 ± 0.8 N g⁻¹ to 7.5 ± 1.0 N g⁻¹ in Gas/Plan (−36%), whereas it was reduced from 14.9 ± 0.9 N g⁻¹ to 6.3 ± 0.8 N g⁻¹ in Sol muscle (−58%). Fig. 3 depicts the contraction times in Gas/Plan versus soleus muscle. Time-to-peak tension was reduced only in the Gas/Plan with aging, going from 18 ± 1 ms in YA to 24 ± 1 ms in SEN. On the other hand, the time-to-half relaxation was increased in both muscles with aging, with the Gas/Plan increasing from 17 ± 2 ms in YA to 26 ± 3 ms in SEN (51% increase with aging), and the Sol increasing from 72 ± 9 ms in YA to 122 ± 10 ms in SEN (70% increase with aging).

### 3.3. Biochemistry

Mitochondrial enzyme activities declined with aging only in the slow twitch Sol and fast oxidative Gr muscle regions, with no change in the fast glycolytic Gw region (Fig. 3). Specifically, in Sol citrate synthase activity declined from 22.7 ± 1.3 µmol min⁻¹ g⁻¹ wet mass in YA to 16.8 ± 2.1 µmol min⁻¹ g⁻¹ wet mass in SEN (26% decline), whereas in Gr citrate synthase activity declined from 29.5 ± 1.1 µmol min⁻¹ g⁻¹ wet mass in YA to 15.8 ± 0.8 µmol min⁻¹ g⁻¹ wet mass in SEN (46% decline). Similarly, in Sol complex IV activity declined from 7.6 ± 0.7 µmol min⁻¹ g⁻¹ wet mass in YA to 5.3 ± 1.2 µmol min⁻¹ g⁻¹ wet mass in SEN (30% decline), whereas in Gr complex IV activity declined from 8.8 ± 0.8 µmol min⁻¹ g⁻¹ wet mass in YA to 4.2 ± 0.2 µmol min⁻¹ g⁻¹ wet mass in SEN (52% decline).

### 3.4. MHC expression in situ

Photomicrographs of muscle cross-sections immunolabeled for fast (Sol) or slow (Gas) adult MHC isoforms are shown in Fig. 4. Generally, these images show an increase in the non-dominant adult MHC isoform expression with aging in both Sol and Gas muscles. Morphometric analyses of the proportion of MHC fast (Sol) or MHC slow (Gas) showed that the abundance of fibers positive for MHC fast in Sol increased from 11.4 ± 2.0% in YA to 36.4 ± 3.4% in SEN (more than a tripling with aging), whereas in Gas the abundance of fibers positive for MHC slow increased from 12.4 ± 1.1% in YA to 25.9 ± 2.9% in SEN (doubling with aging) (Fig. 5). In a sub-sample of two Sol and two Gas muscles, we determined that the increase in fibers positive for the non-dominant adult MHC isoform in SEN animals was largely due to the presence of hybrid fibers. Specifically, we observed that 73 ± 5% of MHC fast positive fibers were also positive for MHC slow in Sol muscle, and similarly, 70 ± 8% of MHC slow positive fibers were also positive for MHC fast in Gr muscle.

Whereas the adult MHC isoform shifts with aging were greater in Sol, the overall reduction of mean fiber size with aging was greater in Gas than Sol (Fig. 6). Within the Sol muscle, type I fibers exhibited the least atrophy with aging (25% reduction in size), and this was similar to Gas muscle where type I fibers exhibited the least atrophy compared to the other fiber types in this muscle (38% reduction in size). In both Sol and Gas, it was the type Ila fibers which had the greatest atrophy (53% and 58% reduction in size, respectively). Note that there were no MHCs positive fibers in the Gw muscle region at either age, consistent with the established fiber type composition of this region in the rat (Armstrong and Phelps, 1984). dMHC expression was virtually non-existent in all muscles examined in YA animals, but was observed with aging in slow twitch Sol, fast oxidative Gr, and fast glycolytic Gw muscle regions (Fig. 7A). Interestingly, the abundance of dMHC positive fibers in SEN animals was lower in Gw than in Gr (Figs. 7B, 8).

### 4. Discussion

The purpose of our study was to compare and contrast the nature and magnitude of phenotypic alterations in the slow twitch Sol muscle to those of the fast twitch Gas muscle in senescence muscle. The underlying hypothesis was that the relative protection of slow twitch muscle generally seen with aging may not apply at more advanced ages where the magnitude of sarcopenia is severe. Our results support this hypothesis in that they show large phenotype alterations in both the slow twitch Sol and fast twitch Gas with aging, and that in some instances the direction of changes are not only opposite to those of the Gas (i.e., the Sol exhibits increased abundance of MHC fast positive fibers with aging), but also of larger magnitude (i.e., tetanic tension per g, time-to-half relaxation for a twitch contraction, and the increase in non-dominant adult MHC were all greater in Sol than Gas with aging). On the other hand, our results show that in both the Sol and Gas muscles the type I fibers exhibit the least atrophy, consistent with the prevailing view that type II fibers exhibit more atrophy with aging. Collectively, therefore, our results suggest that in addition to continuing to identify the basis for the greater atrophy seen in type II fibers with aging, development of treatments for sarcopenia in its more advanced stages should address changes in both slow twitch and fast twitch muscles.

### 4.1. Impact of aging on fast twitch versus slow twitch muscle

It is generally considered that fast twitch muscle is more susceptible to atrophy and dysfunction with aging. As a result, many studies have focused exclusively upon age-related changes in fast twitch muscles (e.g., Chabi et al., 2008; Clavel et al., 2006; Dirks and Leeuwenburgh, 2002; Hepple et al., 2008). On the other hand, there
Fig. 4. Citrate synthase (left) and complex IV (right) activity in homogenates of the soleus muscle, and red (Gr) and white (Gw) regions of the gastrocnemius muscle in young adult versus senescent rats. *P<0.05 versus young adult.

Fig. 5. Immunofluorescent photomicrographs of cross-sections of soleus muscle (Sol) and the red (Gr) and white (Gw) regions of the gastrocnemius muscle labeled for laminin (soleus: blue; Gr and Gw: red), fast MHC (soleus: grey) or slow MHC (Gr and Gw: blue) in young adult versus senescent rats. Bar = 50 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
are several reports of significant atrophy (Brown and Hasser, 1996; Hagen et al., 2004), myofiber loss (McKiernan et al., 2004), force decline (Thompson, 1999; Thompson and Brown, 1999), reduced mitochondrial enzyme activities (Ansved and Larsson, 1989; Varovaya et al., 2002), and altered MHC expression (Ansved and Larsson, 1989; Edstrom and Ulfhake, 2005; Snow et al., 2005) in aged rat Sol muscle, which is largely slow twitch in its composition (Armstrong and Phelps, 1984). Therefore, it is unclear why age-related changes in slow twitch muscle have been largely overlooked in seeking treatments to sarcopenia. We conducted the present study on the premise that one reason contributing to the relative lack of inclusion of slow twitch muscle changes in seeking treatments for sarcopenia is a paucity of information regarding the nature and magnitude of phenotypic alterations in slow twitch versus fast twitch muscle, particularly at very advanced age where sarcopenia is severe and its consequences more likely to impact frailty. To provide a broad context for our comparisons, we examined changes in muscle mass, contractile function, mitochondrial enzyme activities, and MHC isoform expression in situ.

The approximately one third reduction in Sol muscle mass we observed is similar to that reported previously for male F344BN rats in SEN, as is the greater than 50% decline in mass of the Gas for F344BN rats in SEN (Brown and Hasser, 1996; Hagen et al., 2004). Although this would appear to indicate a greater rate of atrophy with aging in the fast twitch Gas muscle, we note that our previous data (Hagen et al., 2004) demonstrates the relative protection of the slow twitch Sol muscle from age-related atrophy is only present up to late middle age, with similar rates of atrophy in each muscle thereafter. Specifically, whereas the Gas muscle lost approximately 20% of its mass between YA and late middle age, the Sol muscle mass was preserved across this period. In contrast, the amount of muscle lost between late middle age and SEN was strikingly similar in both muscles with the Gas losing 40% of its mass and the Sol losing 37% of its mass (Hagen et al., 2004). Our morphometric measures of fiber size further underscore this point because if we compare the reduction in fiber size between late middle age from our previous study (Hepple et al., 2004b) and SEN from our current study we see a very similar 50% decline in Sol and 54% decline in Gas muscle. Thus, the relative protection of slow twitch muscle from age-related atrophy is only seen at the early stages of sarcopenia and as it advances from this point both slow twitch Sol and fast twitch Gas muscles atrophy at strikingly similar rates.

We are not aware of any prior data comparing age-related changes in the peak twitch tension, tetanic tension or contraction times in Sol versus Gas muscle of senescent F344BN rats (although there are data available for other rat strains at younger ages than reported here; e.g., see Narayan et al., 1996). Our results show smaller changes in peak twitch tension in Sol versus Gas/Plan, a greater reduction in both peak tetanic tension in absolute terms and peak tetanic tension per g of muscle in Sol versus Gas/Plan, an increase in time-to-peak tension only in the Gas/Plan, and a greater increase in time-to-half relaxation between YA and SEN in Sol versus Gas/Plan muscle. The greater decline in peak tetanic tension in Sol with aging reported here (−58% versus −36%) could indicate greater perturbation of Ca\(^{2+}\) release in the Sol versus Gas/Plan muscles. Although we are not aware of any data comparing Ca\(^{2+}\) release in slow twitch versus fast twitch muscles in senescence, a reduced maximal Ca\(^{2+}\) release has been observed in aged muscle previously (Delbono et al., 1995). The lack of increase in time-to-peak tension in the Sol versus the increase observed in Gas/Plan with aging has not been reported previously, but is likely due in part to their disparate shifts in adult MHC expression with aging, where the Sol exhibited an increase in fast MHC expression whereas the Gas exhibited an increased slow MHC expression. The much greater increase in time-to-half relaxation with aging in the Sol muscle could reflect a greater impairment in Ca\(^{2+}\) reuptake with aging in the slow twitch Sol, a point that has been suggested previously in the F344 rat in a comparison of the fast twitch extensor digitorum longus muscle and slow twitch soleus muscle (Viner et al., 1999).
In regard to the nature of mitochondrial enzyme activity changes in slow versus fast twitch muscle with aging, one might intuitively expect greater reductions in those muscles with the greatest reduction in recruitment. Since the Sol is largely postural and since body mass is not lower in SEN animals, the postural load on Sol muscle is not reduced with aging. In contrast, the Gas is a locomotor muscle and locomotor activity declines markedly at advanced age in F344BN rats (Hagen et al., 2004), meaning recruitment of Gas muscle would be reduced with aging. Based upon this reasoning, one might logically expect the greatest declines in mitochondrial enzyme activities in the locomotor Gas muscle, and particularly the fast oxidative Gr region. Our results show declines in the activity of representative enzymes of the Krebs Cycle (citrate synthase activity) and the electron transport chain (complex IV) in both the Sol and Gr muscle regions, but not the Gw region (a region normally recruited for very fast and/or powerful locomotor movements). As such, it seems unlikely that the declines in mitochondrial enzyme activities observed in Sol and Gr relate exclusively to differences in the degree of declining muscle activation with aging (otherwise the Sol muscle would have maintained activities), and these results further underscore the susceptibility of the slow twitch Sol muscle to aging. Although we did not have sufficient tissue to perform these analyses in the current study, another comparison of interest between these fast and slow muscles would be glycolytic enzyme activities.

4.2. Impact of aging on fiber myosin heavy chain expression in situ

A popular view in the literature is that aging is associated with a progressive shift towards increased abundance of slow twitch muscle fibers in skeletal muscle (Larkin et al., 1998; Lushaj et al., 2008). While there are many studies that generally support this view, it is not a universal finding. Some of the strongest evidence contrary to this view comes from the landmark study by Lexell et al. (1988) who showed that in whole human vastus lateralis muscles obtained from cadaver specimens aged 15 to 83 years there was no change in the proportion of type I (slow twitch) fibers with aging. Equally striking were the observations of Monemi et al. (1999) who showed that whereas there was a decrease in type II (fast twitch) MHC expression with aging in the biceps brachi of human subjects, there was an increase in type II MHC with aging in the masseter muscle of the same subjects. Finally, Frontera et al. (2000) showed that the abundance of type I fibers actually declined in the vastus lateralis muscle of subjects studied longitudinally between the ages of 65 and 77 years of age. For additional commentary on this topic in the human literature, Andersen reviews their experiences in examining the oldest of the old in humans with similar conclusions to those noted above (Andersen, 2003). In the rodent literature, previous results from the F344BN rat show that fiber type changes with aging vary considerably between muscles comprised of different fiber types (Brown and Hasser, 1996), and that the pattern of change is not consistent between muscles with increasing age (Lushaj et al., 2008). As such, both human and rat studies suggest that the direction of change in slow versus fast MHC expression with aging is not uniformly in the direction of an increase in slow twitch fiber abundance with aging. On the other hand, these prior studies provide little basis for understanding why age-related changes in MHC expression should vary between muscles.

One of the factors that may be involved in explaining variance in direction of MHC isoform shifts with aging is the proportion of fast versus slow MHC within a given muscle in YA animals. If this premise
is true, another reason for the general notion of an increase in type I or slow twitch fiber abundance with aging (despite the aforementioned human studies providing evidence to the contrary) could relate to the large number of studies examining rodent muscles that are largely comprised of fast twitch fibers, something that is particularly the case in murine muscles where even the soleus muscle exhibits ≤50% slow twitch fibers in YA animals (Grange et al., 2001; Hegedus et al., 2007; Hughes et al., 1999). In the current study we directly compared changes in both adult and developmental MHC expression in the largely slow twitch Sol muscle to changes in the largely fast twitch Gas muscle of the F344BN rat. These muscles in the rat provide for a very effective comparison because the Sol muscle of YA F344BN rats exhibits about 11% of fibers that are positive for fast MHC, whereas the Gas muscle of YA F344BN rats exhibits about 12% of fibers that are positive for slow MHC (similar to previous studies in Sprague–Dawley rats (Edstrom and Phelps, 1984)), allowing us to take into account the role that extremes of fast versus slow MHC expression in YA plays in the direction of change with aging. Strikingly, our data shows that whereas the Gas muscle exhibits an increase in proportion of MHC slow positive fibers with aging, the Sol muscle exhibits not only a greater magnitude of shift in MHC expression with aging, but also shifts in the opposite direction to the Gas muscle by exhibiting a large increase in proportion of fibers that are positive for fast MHC with aging. As such, our results suggest that the prevailing fiber type does influence the direction of shift with aging and that generally the shift is away from the dominant adult MHC isoform. Note that this dramatic increase in fast MHC expression in senescent soleus muscle has been noted previously in the F344BN rat (Snow et al., 2005) and Sprague–Dawley rat (Edstrom and Ulhake, 2005), although these prior observations appear to have had little impact on the general perception of preferential fast twitch fiber loss with aging. It was also interesting in the current study that the difference between YA and SEN muscles in the number of fibers positive for the lesser abundant MHC in each muscle was accounted for by fibers that were co-expressing both fast and slow adult MHC isoforms in the SEN muscles. An increase in fast MHC expression is a well-established consequence of denervation following injury (Smith et al., 1999) and in response to aging. As such, our results underscore the point that the slow twitch Sol muscle is not protected from sarcopenia at the advanced ages where the risk of frailty is greatest, and that future development of treatments for sarcopenia need to account for decrements in both slow and fast twitch muscle.

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