

Muscle Senescence in Short-Lived Wild Mammals, the Soricine Shrews *Blarina brevicauda* and *Sorex palustris*

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ABSTRACT Red-toothed (soricine) shrews are consummate predators exhibiting the highest energy turnovers and shortest life spans (ca. 18 months) of any mammal, yet virtually nothing is known regarding their physiological aging. We assessed the emerging pattern of skeletal muscle senescence (contractile/connective tissue components) in sympatric species, the semi-aquatic water shrew (WS), *Sorex palustris*, and the terrestrial short-tailed shrew (STS), *Blarina brevicauda*, to determine if muscle aging occurs in wild, short-lived mammals (H_0 : shrews do not survive to an age where senescence occurs), and if so, whether these alterations are species-specific. Gracilis muscles were collected from first-year ($n = 17$) and second-year ($n = 17$) field-caught shrews. Consistent with typical mammalian aging, collagen content (% area) increased with age in both species (*S. palustris*: ~50%; *B. brevicauda*: ~60%). Muscle was dominated by stiffer Type I collagen, and the ratio of collagen Type I:Type III more than doubled with age. The area ratio of muscle:collagen decreased with age in both species, but was considerably lower in adult STS, suggesting species-specificity of senescence. Extracellular space was age-elevated in *B. brevicauda*, but was preserved in *S. palustris* (~50 vs. 10% elevation). Though juvenile interspecific comparisons revealed no significance, adult WS myocytes had 68% larger cross-sectional area and occurred at 28% lower fibers/area than those of adult STS. We demonstrate that age-related muscle senescence does occur in wild-caught, short-lived mammals, and we therefore reject this classic aging theory tenet. Our findings moreover illustrate that differential age adjustments in contractile/connective tissue components of muscle occur in the two species of wild-caught shrews. *J. Exp. Zool.* 311A:358–367, 2009. © 2009 Wiley-Liss, Inc.

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Impairment of skeletal muscle health and function is a key component of aging, and much is known regarding mammalian muscle aging in domestic (laboratory) settings. The typical aging response of muscle is characterized by a loss of force-generating capacity, or “sarcopenia,” resulting from myofiber loss, a decline in muscle cross-sectional area and force per cross-sectional area (Evans, '95; Clark and Manini, 2008). In humans, and domestic and laboratory animals, lost muscle functionality generally limits performance and mobility and increases the likelihood of injuries

with advancing age. In wild animals, however, muscle performance can be the difference between

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life and death, as foraging ability and elusion of predators rely critically on both the endurance and the power output of the skeletal muscle (Arnold, '83; Johnson et al., 2008). Despite the potentially profound impact of aging phenomena on foraging capacity and predator avoidance (i.e., survivorship), muscular senescence has not been comprehensively investigated in free-ranging mammals.

Classic aging theories predict the absence of observable senescence in wild populations, postulating that death will occur prior to apparent manifestations of aging (Medawar, '52; Williams, '57; Hamilton, '66; Comfort, '79). Such classic tenets have retained modern applicability and use (e.g., Charlesworth, 2000; Kirkwood and Austad, 2000; Bronikowski and Promislow, 2005; Williams et al., 2006), making empirical investigations valuable. As large-bodied, long-lived mammals are a demonstrated exception to this tenet (Promislow, '91), small, short-lived mammals represent a logical choice to test this classic prediction. Shrews are a particularly attractive natural aging model system as they exhibit the shortest life span and highest mass-specific energy turnover rates of any mammal (Hindle et al., 2003; Gusztak et al., 2005). Indeed, oxidative stress theories of aging suggest that accelerated rates of tissue-level senescence may result from elevated production of reactive oxygen species associated with heightened throughput via the respiratory chain (Sohal and Weindruch, '96).

Further, investigations of shrew muscles (fiber-type patterns, myosin composition and enzyme activities) suggest that they are composed exclusively of fast-twitch-type fibers (Savolainen and Vornanen, '95a; Peters et al., '99), which are preferentially targeted by apoptosis and atrophy during aging (Holloszy et al., '91). Exercise hypoxia, as commonly incurred during apneustic foraging in some air-breathing vertebrates, should further elevate oxidative stress (Elsner et al., '98) and may accelerate cellular dysfunction and aging (Sohal and Weindruch, '96; Hulbert et al., 2007), including remodeling of the skeletal muscle. Thus, we might envisage that differing lifestyles (i.e., the exercise requirements of exploiting species-specific ecological niches) could modulate the pattern of senescence, if it does occur, in individual species. We considered two sympatric species of soricine shrews with differing lifestyles for this study. The short-tailed shrew (STS) (*Blarina brevicauda*) and the water shrew (WS) (*Sorex palustris*) often cohabit in moist riparian environments. The

primary demarcation between them is the specialization of the WS for aquatic foraging (Gusztak et al., 2005; Catania et al., 2008), whereas the STS exploits an entirely terrestrial, often subterranean, home range (Getz, '61).

Skeletal muscle form and function rely on both contractile and connective tissue components. The predominantly collagenous extracellular matrix (ECM) provides an overall three-dimensional framework for the muscles' connective tissue, and is important in defining both its passive and active mechanical properties (Alnaqeeb et al., '84; Kovanen et al., '84). Higher collagen content, for example, correlates with increased muscle stiffness and increased internal work (Alnaqeeb et al., '84) as the elastic components provide resistance to movement (contraction or relaxation).

Remodeling of this ECM can have a significant influence on muscle function. Some type of ECM adjustment is observed with virtually all examples of muscle stress, including exercise, injury and disease (e.g., Marshall et al., '89; Williams et al., '99; Miller et al., 2001; Mackey et al., 2004). Similarly, as skeletal muscle ages in laboratory animals, changes occur in both the abundance and the composition of the ECM. For instance, the total collagen content of the muscle increases with age (Mays et al., '88; Kovanen and Suominen, '89; Gosselin et al., '98) as it becomes more resistant to collagen-degrading enzymes (Mohan and Radha, '80). An altered ratio between collagen isoforms has also been documented with aging in laboratory rats (Mays et al., '88; Kovanen and Suominen, '89). Both increased total collagen and an increased proportion of the stiffer Type I collagen isoform are hypothesized to accelerate the decline in skeletal muscle contractile function seen with advancing age (Mays et al., '88; Kovanen and Suominen, '89).

Contractile tissue components have been examined with muscle aging in wild-caught common shrews, *S. araneus* (Savolainen and Vornanen, '95a). Myosin heavy chain isoforms appear to follow the "slowing" trend documented for human and laboratory animal skeletal muscles, whereby fiber-type and myosin isoforms shift toward a greater proportion of slow fibers. Age-associated remodeling of the ECM, however, has not previously been considered in these animals. Controlled laboratory exercise training appears protective against age-related ECM remodeling in rats (Kovanen, '89; Kovanen and Suominen, '89; Gosselin et al., '98), and although exercise does not strictly reduce the total collagen content

of old muscle, it does reduce the overall muscle stiffness (Gosselin et al., '98). It is possible that the foraging activities of many wild animals, especially shrews given their near-continuous activity cycles (Gusztak et al., 2005), are comparable to lifelong exercise training in otherwise sedentary laboratory populations, which may reduce the impact of age-related connective tissue remodeling (Kim et al., 2008).

This study aims to quantify the age-related morphological changes of a selected skeletal muscle in two species of wild-caught soricine shrews. Old and young field-caught shrews were examined to address these specific questions: (1) Does muscular senescence in contractile and connective tissue components, as we understand it for humans, domesticated and laboratory species, also occur in wild mammal populations?, (2) does senescence occur in short-lived mammalian taxa with high energy turnover rates? and (3) is there any evidence that differing locomotor activities likely associated with foraging behavior and ultimately niche separation of these sympatric species modulate aging effects? We specifically tested the null hypothesis (based on classic aging theory) that wild-caught shrews do not survive to a time in which age-associated remodeling of myofibers and the ECM is detectable.

MATERIALS AND METHODS

Capture, animal care and sampling

Two sympatric species of red-toothed shrew (Family: Soricidae) of similar mass and occupying similar habitat were utilized for this study. These are the only two species of comparable size that inhabit the study region. The maximum life span for both species in the wild is about 18 months (George et al., '86; Beneski and Stinson, '87), and both species can breed in the season or year in which they were born (George et al., '86; Beneski and Stinson, '87).

Adult (second summer; ca. 13–15 months of age) and young-of-the-year (1–3 months) shrews of both species were captured in Whiteshell and Nopoming Provincial Parks (49°49'N, 95°16'W; 50°67'N, 95°28'W, respectively) and at the Fort Whyte Centre, Winnipeg, Manitoba (49°50'N, 97°10'W), during July and August of 2005 and 2006. Animals were transported to the Animal Holding Facility, University of Manitoba within 8 hr of capture, and placed in a controlled-environment room held at $20 \pm 1^\circ\text{C}$ and with a

12L:12D photoperiod. Here they were housed separately in either 72-L plastic terraria fitted with screen lids (STS) or modified 264-L glass aquaria with terrestrial and aquatic compartments (WS), as previously described (Hindle et al., 2003; Gusztak and Campbell, 2004; Gusztak et al., 2005).

During their brief period in captivity (ca. 1 week), shrews were provided numerous opportunities for natural exercise/behavior. This included substrate for burrowing and nest construction within the terraria, access to an aquatic enclosure for WS and daily diet supplementation with live invertebrates (Hindle et al., 2003; Gusztak and Campbell, 2004). All study animals were captured under Manitoba Conservation permits WPB 20407 and WPB 21706, and cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care (University of Manitoba Animal Use Protocol # F05-014).

Shrews were euthanized with an overdose of isoflurane inhalant anesthetic. The gracilis muscle was immediately dissected free, frozen in liquid nitrogen-cooled isopentane for subsequent histochemical and immunohistochemical analyses and stored at -80°C .

Age determination

Following tissue collection, the lower jaws were removed and processed for age determinations. Mandibles were cleaned of tissue and placed in a detergent solution at 60°C for 24 hr to remove all remaining skeletal muscle. Following digestion, mandibles were decalcified (RDO, Apex Engineering, Aurora, IL) at room temperature (RT) for 1 hr (WS) or 1.5 hr (STS). Decalcified bone was rinsed in phosphate-buffered saline (PBS) (138 mM NaCl, 2.7 mM KCl, pH = 7.4), embedded in paraffin and 20 μm sections were cut on a microtome. Sections were mounted on glass slides, treated with the general protein stain, hematoxylin (Fisher Scientific, Waltham, MA), and coverslipped. The presence or absence of a growth ring in the mandible and teeth was diagnostic for the individual's status as young (first year) or old (second year; see Fig. 1).

Muscle morphology

For all histochemical analyses of muscle, 7–9 μm cross-sections were first cut on a cryostat at -20°C and transverse orientation verified using a standard light microscope. Slides were air dried, rinsed in PBS and treated with hematoxylin (1 min) for

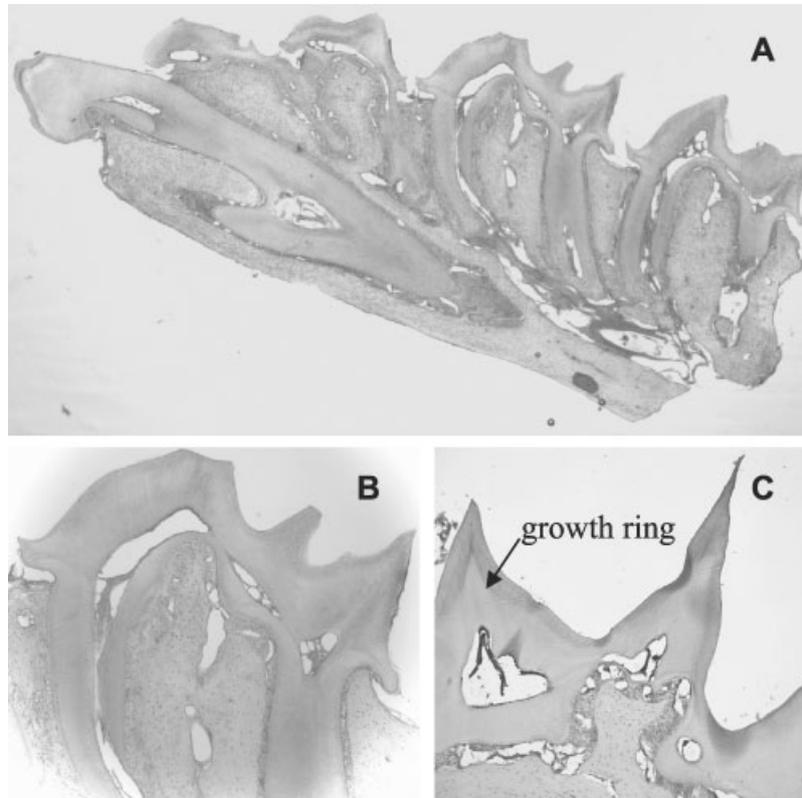


Fig. 1. Sectioned short-tailed shrew lower jaws and teeth (20 μ m), stained with hematoxylin, were used for age determination. Image (A) represents an entire jaw (20 \times magnification) of a young short-tailed shrew. Tooth crowns (100 \times magnification) were examined for the absence (B, young) or presence (C, old) of a growth ring.

myocyte cross-sectional area and density determinations. Extracellular space (ECS) was calculated from these variables as the remaining slide area not occupied by myocytes (myocyte density \times average cross-sectional area).

Collagen

Picrosirius red histochemical staining (Sweat et al., '64) was used to visually label collagen. Staining proceeded with the modifications described previously (Dolber and Spach, '87) to minimize cytoplasmic stain uptake. Briefly, the modified method was as follows: fixation in Bouin's solution (30 min), distilled water rinse (1 min), phosphomolybdic acid treatment (0.2%, 5 min) and picrosirius red staining (0.1%, 90 min). Slides were given final rinses to preserve picrosirius red (acidified water, picric alcohol), dehydrated in 70, 95 and 100% ethanol, cleared with xylene and mounted.

Collagen Types I and III were identified via immunohistochemistry (IHC) as previously described (Mackey et al., 2004). In summary, frozen sections were fixed directly in acetone at -20°C

and blocked with goat serum (60 min, RT). After washing, sections were incubated individually with rabbit 1 $^{\circ}$ antibody (40 min, RT, Rockland Immunochemicals, Gilbertsville, PA) for both collagen forms. Slides were washed and incubated in horseradish peroxidase (HRP)-conjugated goat 2 $^{\circ}$ antibody (30 min, RT, Rockland Immunochemicals) and then washed again. Collagen isoform amount was detected and localized using diaminobenzidine substrate chromogen (DAKO, Carpinteria, CA). Slides were rinsed in dH $_2$ O and then dehydrated in 70, 95 and 100% ethanol, cleared with xylene and mounted.

Microscopy and image analysis

All images were collected using a Spot Pursuit Slider CCD camera (Diagnostic Instruments, Inc., Sterling Heights, MI) and a Nikon E400 microscope (Nikon, Inc., Melville, NY). Images were analyzed using ImageJ software (version 1.37s, National Institutes of Health, Bethesda, MD). Muscle morphology (myocyte density and cross-sectional area) was counted or calculated directly from images calibrated (to the nearest 1 μ m) using

a stage micrometer, though cells directly adjacent to the edge of the section were excluded from the analysis. A minimum of 50 myocytes were examined for each animal.

Percent of image area occupied by intercellular collagen was quantified using the ImageJ thresholding feature. Regions identified as Type I or III collagen based on immunohistochemistry were similarly isolated and quantified.

Statistical analyses

Results were analyzed using SPSS statistical software (version 11.5.1). Data were tested for normality using the Shapiro–Wilkes statistic and homogeneity of variance was confirmed using a modified Levene test. Data were natural log-transformed when necessary to meet assumptions for parametric tests. Results were compared using nested linear mixed model procedures (i.e., fixed factor “AGE” nested within fixed factor “SPECIES”). Overall significance was further explored using simultaneous least significant difference (LSD) pairwise comparisons. Significance was set at the 5% level and means are presented ± 1 SEM.

RESULTS

Study animals

A total of 19 WS and 15 STS were examined. There was no statistical difference between the masses recorded for old ($n = 9$ WS; $n = 8$ STS) vs. young ($n = 10$ WS; $n = 7$ STS) animals from either species ($t = 1.057$, $df = 14$, $P = 0.308$ for WS; $t = 1.317$, $df = 13$, $P = 0.210$ for STS). Pooled mass for the two age groups for wild-caught WS and STS was 14.12 ± 0.29 and 22.36 ± 0.47 g, respectively.

Myocyte morphology

Although the mean myocyte cross-sectional area was different between young and old WS (829 ± 62 vs. $1,059 \pm 134$; Table 1), the distribution was

noticeably right skewed, with two outlying high values recorded for second-year WS. These data were natural log-transformed to regain a normal distribution, and the subsequent nested analyses did not reveal any age-related differences in cross-sectional area for either species ($F_{2,26} = 2.773$, $P = 0.081$). Although a similar statistical trend was apparent, myocyte density was likewise not different between age groups for either species ($F_{2,26} = 2.74$, $P = 0.083$). ECS, which is calculated from both previous morphological variables, was significantly elevated in the older age class ($F_{2,26} = 4.431$, $P = 0.022$), and no significant difference was detected between species ($F_{1,26} = 0.161$, $P = 0.692$). The overall age elevation of ECS was primarily driven by the large (ca. 50%) age increase for this variable in the STS ($P = 0.007$) vs. a nonsignificant change in the WS (10% increase; $P = 0.537$).

WS myocytes in the gracilis muscle were on average 30% larger in cross-sectional area (ln-transformed; $F_{1,26} = 7.533$, $P = 0.011$) and occurred at 18% lower density ($F_{1,26} = 7.771$, $P = 0.01$) than did those of STS (Table 1; Fig. 2). In both cases this significance was owing to differences between older adults of the two species (area $P = 0.002$; density $P = 0.011$) and not because of differences in younger adults (area $P = 0.737$; density $P = 0.270$).

Collagen content and isotypes

A considerable increase in the intercellular collagen (~50–60%) was noted in second-year shrews of both species ($18.7 \pm 2.3\%$ area vs. $12.3 \pm 1.3\%$ area in WS; overall $F_{1,25} = 7.406$, $P = 0.003$; Table 2). No significant difference between species was noted ($F_{2,25} = 1.099$, $P = 0.305$). The area ratio between muscle and collagen within the sections followed a similar aging trend (age effect: $F_{2,25} = 14.752$, $P < 0.001$). However, age effects were more pronounced in STS (147% increase) compared with the WS (58% increase; Table 2), leading to a significant species

TABLE 1. Morphological characteristics of gracilis muscle sampled from two species of wild-caught shrew

| | Water shrew (<i>Sorex palustris</i>) | | Short-tailed shrew (<i>Blarina brevicauda</i>) | |
|---|--|-----------------------|--|------------------|
| | Young ($n = 10$) | Old ($n = 9$) | Young ($n = 7$) | Old ($n = 8$) |
| Cross-sectional area (μm^2) | 829 ± 62 | $1,059 \pm 134^{**}$ | 780 ± 35 | 632 ± 52 |
| Myocyte density (cells mm^{-2}) | 247.5 ± 15.6 | $195.3 \pm 16.4^{**}$ | 275.8 ± 14.0 | 270.1 ± 28.1 |
| Extracellular space (%) | 16.2 ± 2.5 | 18.7 ± 3.3 | $12.0 \pm 3.7^*$ | 26.4 ± 2.9 |

Asterisks denote significant differences ($\alpha = 0.05$) between age classes (single) and species (double). Values are mean \pm SEM.

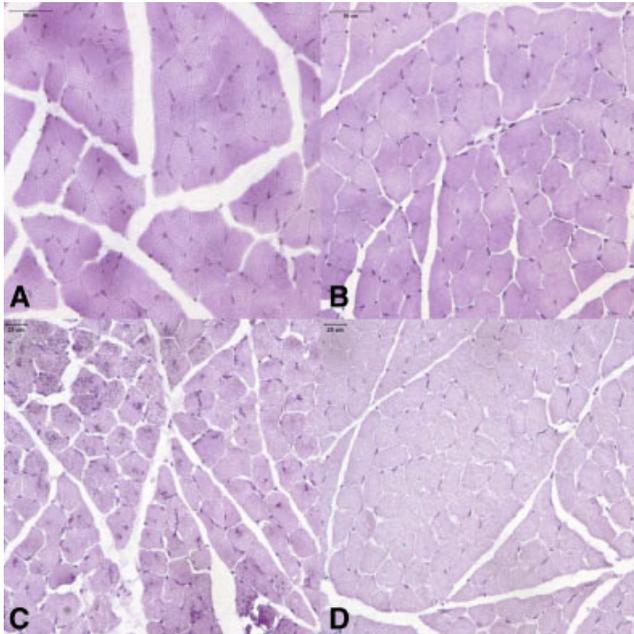


Fig. 2. Gracilis muscle (7–9 μm) stained with hematoxylin for contrast (400 \times magnification). Samples were collected from water shrews, *Sorex palustris* (A: old; B: young) and short-tailed shrews, *Blarina brevicauda* (C: old; D: young).

difference among older adults ($P = 0.02$; Table 2). Type I was the dominant form of collagen in all samples analyzed (Fig. 3A). There was a greater than two-fold increase in the ratio of Type I: Type III collagen (ln-transformed: $F_{2,24} = 8.745$, $P = 0.001$), and no species difference in this increase occurred (ln-transformed: $F_{1,24} = 0.57$, $P = 0.458$; Table 2; Fig. 3).

DISCUSSION

Evidence for muscular senescence in small, short-lived wild mammals

Our data indicate that muscular senescence does occur in contractile and connective tissue of wild and even short-lived mammals. Most notably, age-related ECM remodeling such as increased collagen deposition and a shift toward a higher ratio of Type I to III collagen isoforms occurred with advancing age in both species.

Myocyte dimensions

Myocyte cross-sectional area did not change significantly with advancing age in either shrew species. For WS, a trend for increased myofiber

TABLE 2. Summary of collagen distribution within gracilis muscle from first- and second-year shrews

| | Water shrew (<i>Sorex palustris</i>) | | Short-tailed shrew (<i>Blarina brevicauda</i>) | |
|--------------------------------|--|------------------|--|-----------------|
| | Young ($n = 10$) | Old ($n = 9$) | Young ($n = 7$) | Old ($n = 8$) |
| Total collagen (% area) | 12.3 \pm 1.3* | 18.7 \pm 2.0** | 13.4 \pm 2.1* | 21.7 \pm 1.9 |
| Muscle/collagen ratio | 7.0 \pm 0.7* | 4.4 \pm 0.4 | 7.7 \pm 1.6* | 3.1 \pm 0.2 |
| Type I/Type III collagen ratio | 1.9 \pm 0.1* | 4.3 \pm 0.8 | 2.3 \pm 0.5* | 4.9 \pm 1.1 |

Asterisks denote significant differences ($\alpha = 0.05$) between age classes (single) and species (double). Values are mean \pm SEM.

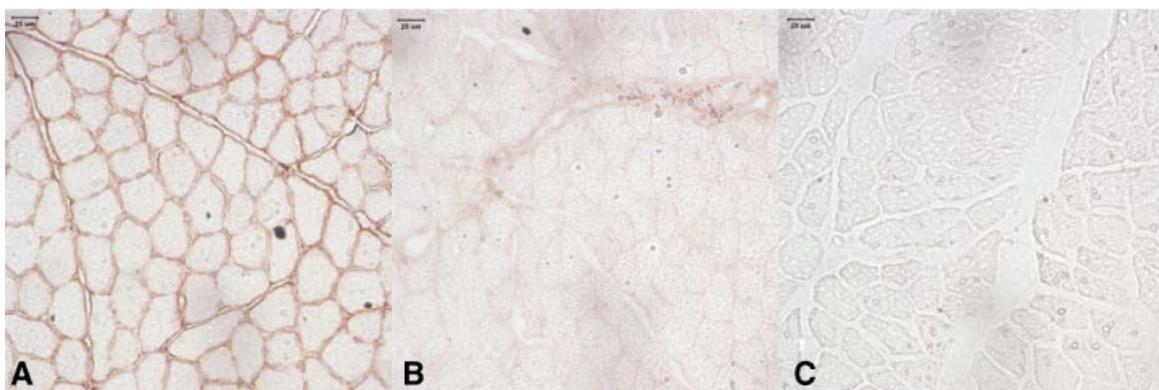


Fig. 3. Immunohistochemical staining for collagen subtypes in water shrew gracilis muscle (7–9 μm ; 400 \times magnification). Type I is shown in (A), Type III in (B) and the negative control in (C).

size (a 28% increase; Table 1) with age occurred despite the lack of detectable mass differences between age groups. Although this trend parallels age-related increases in myocyte cross-sectional area observed in horses (Rivero et al., '93), it contrasts with the typical mammalian response to aging—namely muscle atrophy and fiber loss (e.g., Zhong et al., 2007). Perhaps the loss of neighboring motor units, or those in nearby muscles (which is supported by the trend for reduced myocyte densities; Table 1), coupled with the sustained exercise requirements necessary to fuel their relatively high metabolic rates (Hindle et al., 2003; Gusztak et al., 2005) could result in an exercise-induced hypertrophy of remaining fibers, accounting for their increased size.

Extracellular space

A significant increase in ECS was the prevailing age-related change to muscle morphology in *B. brevicauda*. Indeed, STS demonstrated a ~50% increase in this parameter, whereas this component increased by only 10% in the WS. This difference largely arises from an increased myocyte cross-sectional area (CSA) (possibly activity-driven hypertrophy) in second-year WS, which compensates for the decreased ECS observed in this species. This highlights the potential for species-specificity in age remodeling of the skeletal muscle in wild animals. An elevated ECS may contribute to a reduction in muscle-specific force (force per muscle CSA) with age as a smaller proportion of the entire muscle area is composed of contractile tissue. One mechanism underlying the age-related increase in ECS is the loss of motor units via apoptosis and denervation, and the incomplete reinnervation by slower neighboring motor neurons. The ECS increase could also arise from increased collagen deposition (increased synthesis or reduced degradation) mediated by transforming growth factor- β following tissue exposure to cytokine, mechanical or oxidative stress (e.g., Border and Noble, '94; Cannon and St. Pierre, '98).

Collagen

An increase in extracellular collagen within the endomysium was noted in mature shrews of both species. As expected, this was accompanied by a decrease in the area ratio of muscle to collagen. Increases in total collagen, collagen stability and cross-linking and resulting passive muscle stiffness have each been widely documented in human and domestic models (Mohan and Radha, '80;

Kovanen and Suominen, '89; Gosselin et al., '98). Biosynthesis of collagen is not affected by age, indicating that the build-up of connective tissue in the ECS is the product of reduced degradation (Kovanen and Suominen, '89; Gosselin et al., '98). Extracellular degradation of collagens is handled by matrix metalloproteases, primarily by the collagenases MMP-1 and MMP-8 (Kovanen, 2002). Maturation of collagen in the ECS involves the development of hydroxylysylpyridinoline, or nonreducible cross-linking, as well as increased glycation (Kjaer, 2004), which serves to increase collagen stability. This not only impedes collagen breakdown and turnover in senescent muscle but also increases the muscle's passive stiffness (Alnaqeeb et al., '84). In fact, generally speaking, total muscle collagen is positively correlated to tissue stiffness (Alnaqeeb et al., '84; Kovanen et al., '84; Gosselin et al., '94, '98).

Type I collagen tends to be oriented in parallel, conferring both much of the rigidity required for isometric contractions and the storage of elastic energy captured upon lengthening to increase fatigue resistance (Kovanen, 2002). In contrast, Type III collagen fibers exist as a loose meshwork, and are generally thinner in structure than the Type I isoform. This confers compliance to muscle, allowing the muscle to change size and contract more quickly (Kovanen, 2002). Type I was the dominant form of collagen in all samples analyzed. Consistent with muscle aging described in laboratory/domesticated animals, the area ratio of collagen Type I to III more than doubled with age in both species of wild-caught shrew (Table 2; vs. collagen I:III ratio at 1 month old = 2.1; at 2 years old = 4.0 in laboratory rats; Kovanen and Suominen, '89). No difference was detected between the two species examined. Although this is the first study to demonstrate that this widely acknowledged structural change also occurs in wild populations, its ecological significance remains undetermined.

On the one hand, the combined effect of the elevated total collagen and the Type I:Type III collagen ratio with aging increases muscle tensile strength, at least when stretched (Alnaqeeb et al., '84; Kovanen et al., '84), thereby conferring some benefits for stability, injury prevention and fatigue resistance to slower-twitch muscles. Conversely, muscle stiffness affects its ability to store and release elastic energy during contraction (e.g., Alnaqeeb et al., '84). In shrews, which are thought to exclusively possess Type II fibers (Savolainen and Vornanen, '95a,b; Peters et al., '99; Jürgens,

2002), this heightened stability may occur at the expense of contraction speed and force generation, which may compromise stride frequency, and in turn locomotion. A decrease in the passive compliance of older muscle improves load resistance, but decreases its ability to adjust to altered loading, which can lead to injury. How the interplay of these factors effects muscle function remains unclear.

Although these changes in collagen content and type ratios could compromise shortening or lengthening abilities and work output, specific laboratory or field investigations are required to unravel the functional and ecological significance of such structural changes. For example, stiffness, shortening ability and contractile efficiency of isolated skeletal muscle collected from different age classes of shrews could be examined. Work output, fatigue resistance, metabolic cost of exercise as well as locomotor performance including endurance, sprint and maximal acceleration can be assessed through captive animal exercise studies or cost of transport manipulation experiments on telemetered wild animals.

Comparative aspects of muscular senescence in two sympatric shrew species that occupy differing ecological niches

Although both species exhibited clear evidence of muscular senescence, patterns emerged that suggest a species-specific modulation of aging responses possibly related to exercise requirements of the particular ecological niches occupied. The average cross-sectional area of gracilis muscle fibers was $938 \mu\text{m}^2$ for WS and $713 \mu\text{m}^2$ for STS. These data are in general consensus with those from hindlimb muscles (ca. $550\text{--}625 \mu\text{m}^2$; Savolainen and Vornanen, '98) previously published for the 7.9 g common shrew. The high value for the intermediate-sized (14.1 g) WS is notable as it contrasts with the finding that skeletal muscle fiber area tends to decrease in concert with body size in small aerial and terrestrial mammals (Pietschmann et al., '82). Contrary to the expected age reduction in myocyte numbers and CSA, gracilis myofibers increased in average size for WS, and changed little in density for STS. We suggest that the elevated fiber size within WS gracilis indicates that their primarily hindlimb-propelled aquatic locomotion is powered to some degree by this muscle. The relatively elevated muscle myoglobin contents of WS, compared with STS (Stewart et al., 2005; Gusztak, 2008), may

permit larger fiber CSA by compensating for increased diffusion distances (Kanatous et al., 2002). This aggressive predator often leaps into shallow water to ambush aquatic prey (Lampman, '47; A. G. Hindle and K. L. Campbell, unpublished observation) and rapidly lunges in response to detection of water disturbances while immersed (Catania et al., 2008). By comparison, the venomous STS target larger and less-agile invertebrate and vertebrate prey (Getz, '61) and, being more fossorial in nature, would be expected to rely more heavily on forelimb-driven digging.

The STS showed a marked (ca. 50%) elevation of ECS with age, whereas only a modest change was detected in the WS. The species difference for this parameter suggests that aging WS may suffer less from such reduced specific force of contractions than do mature STS. Such attenuation of power loss may have important fitness considerations during the second-year breeding season, when energy requirements are presumably at their highest for this aquatic predator. The lack of age-related myofiber hypertrophy (a reduction of $\sim 20\%$) in STS occurred concurrently with an increase in the collagen to muscle ratio with age that was approximately double of that seen in the WS, suggesting greater muscle stiffening with age and more rapid senescence in the STS than in the WS. Taken together, these data suggest that WS are subject to a lesser degree of age-related skeletal muscle remodeling, which is possibly related to lifestyle (i.e., adaptation to repeated hypoxia and reoxygenation). Considering that younger mammals appear to have a better response to exercise- (or stretch-) induced hypertrophy (e.g., Lee and Alway, '96) this is strongly indicative of the reliance on the gracilis muscle for locomotion and foraging by WS. A concomitant increase in fiber area was not seen with age in STS, consistent with a reduced dependence on this muscle group in this species.

Our data suggest that biochemical specializations associated with an apneustic breath-hold foraging mode in the amphibious WS may confer a benefit against skeletal muscle senescence. Underwater foraging results in cyclic episodes of tissue hypoxia and reoxygenation (Elsner et al., '98), an occurrence that may elevate periodic and lifelong tissue oxidative stress (Vanden Hoek et al., '97; Elsner et al., '98). The oxidative stress theory of aging couples this occurrence with accelerated cellular aging and dysfunction (Sohal and Weindruch, '96; Hulbert et al., 2007), possibly affecting remodeling of skeletal muscle. As a

greater degree of remodeling was documented in the terrestrial STS, the protective adaptations possessed by WS merit further consideration.

CONCLUSIONS

Using the example of two small, sympatric, short-lived species with high energy turnover rates we demonstrate that age-related muscular senescence does occur in contractile and connective tissue components of wild-caught mammals. We therefore reject the classic aging theory tenet that in wild animals mortality occurs prior to observable senescence. Although our results suggest that patterns of muscular senescence may be modulated by niche-specific adaptations, parallel findings of such senescence in the two species with differing lifestyles strengthen our rejection of the classic tenet. Increased total and relative collagen content of muscle as well as a shift toward a collagen component containing a larger proportion of the stiffer Type I isoform was documented. If a given force output is required of muscle to maintain stride frequency and locomotor speed, these occurrences as a consequence of age could be detrimental to individual survival and fitness. Even small-scale changes in force-generating capacity would be of particular concern for a small predatory animal such as a shrew, which relies solely on fast-twitch muscle contraction.

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