# Adaptive stretch-shortening contractions: diminished regenerative capacity with aging

**Brent A. Baker, Melinda S. Hollander, Robert R. Mercer, Michael L. Kashon, and Robert G. Cutlip**

**Abstract:** This study determined the age-related changes in acute events responsible for initiating skeletal muscle remodeling and (or) regeneration in the tibialis anterior muscle following a bout of stretch-shortening contractions (SSCs). Changes in muscle performance and morphology were quantified in young and old rats, following an acute exposure to adaptive SSCs at 6, 24, 48, 72, and 120 h postexposure ($n$ = 6 for each age at each recovery period). Following SSC exposure, all performance measures were decreased in old rats throughout the 120 h acute phase. Estimates of edema were increased in the old vs. young exposed muscle at 120 h recovery. Both young and old rats displayed an increase in developmental myosin heavy chain ($MHC_{dev}^{+}$) labeling in the exposed muscle, indicating muscle regeneration. However, old rats displayed diminished $MHC_{dev}^{+}$ labeling, compared with young rats, suggesting limited remodeling and (or) regenerative capacity. Based on these data, diminished local muscle remodeling and (or) regeneration with aging may limit skeletal muscle adaptation following mechanical loading.

*Key words:* aging, muscle regeneration, inflammation, myosin heavy chain, stretch-shortening contractions.

**Résumé :** Cette étude se propose de déterminer les événements immédiats qui sont associés au vieillissement et qui déclenchent le remodelage et (ou) régénération du muscle jambier antérieur après une série d'actions d'étirement-contraction (SSCs). On évalue chez des rats jeunes et âgés les variations de performance musculaire et les modifications morphologiques observées 6, h, 24 h, 48 h, 72 h et 120 h après les brèves expositions aux SSCs à caractère adaptatif ($n$ = 6 par groupe d'âge et par période de récupération). Après l'exposition au SSCs, on observe durant les 120 h d'adaptation une baisse de performance chez les rats âgés. D'après des estimations, le degré de l'œdème observé 120 h après l'exposition aux SSCs est plus prononcé chez les rats âgés que chez les jeunes rats. On observe tant chez les jeunes rats que chez les plus âgés une augmentation du marquage de la chaîne lourde de myosine en croissance ($MHC_{dev}^{+}$), signe de régénération musculaire. Cependant, on observe moins de marquage de la $MHC_{dev}^{+}$ chez les rats âgés comparativement aux jeunes rats, ce qui suggère une diminution de la capacité de remodelage et (ou) régénération chez ces premiers. D'après ces observations, la diminution de la capacité de remodelage et (ou) régénération observée avec le vieillissement semble limiter l'adaptation du muscle au chargement mécanique.

*Mots-clés :* vieillissement, régénération musculaire, inflammation, chaîne lourde de myosine, actions d'étirement-contraction.

[Traduit par la Rédaction]

## Introduction

Acute exposure to shortening (concentric) or isometric muscle actions does not normally produce muscle injury (Warren et al. 1993*a*, 1999; Lieber et al. 1996). In contrast, exposure to lengthening (eccentric) movements and stretch-shortening cycles (reciprocal eccentric and concentric stretch-shortening contractions (SSCs)) has been shown to result in performance deficits (Geronilla et al. 2003; Warren et al. 1993*b*), myofiber degeneration (Baker et al. 2006*a*, 2006*b*), and inflammation (Baker et al. 2006*a*, 2007), although the animals' relative ages have a considerable impact on both performance and morphology (Blough and Linderman 2000). However, repeated mechanical exposures have been shown to result in adaptation, maladaptation, and even overt injury in both young and old age groups (Brooks et al. 2001; McBride et al. 1995; Cutlip et al. 2006). Muscle plasticity from old animals is maintained and able to adapt to increased loads up to a certain age; however, the aging process inevitably diminishes the resulting hypertrophic response in old animals (Degens and Alway 2003; Alway et al. 2002). After an injurious exposure to eccentric muscle contractions, there is an increased force deficit (Zerba et al. 1990) and slower recovery of performance (McBride et al. 1995) in whole muscles (McBride et al. 1995; Brooks and Faulkner 1990, 1996; Koh et al. 2003) and single fibers (Brooks and Faulkner 1996) of old animals, compared with their young counterparts. Thus, identifying the acute events that are responsible for initiating the adaptive and remodel-

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ing process and the differential control of these events with age in skeletal muscle is of major relevance, particularly when attempting to develop countermeasures in aging populations.

Recently, various factors (e.g., recovery kinetics, repetition number, duty cycle) that contribute to the induction of contraction-induced muscle injury have been investigated (Baker et al. 2007, 2006*a*, 2006*b*; Cutlip et al. 2004, 2005). From these studies, we were able to optimize the exposure protocol (recovery kinetics, range of motion, duty cycle, number of repetitions, velocity) to produce substantial performance gains and muscle hypertrophy, which we have defined as adaptation, in young rats, compared with their old counterparts, with as little as 4.5 weeks of training (Cutlip et al. 2006). While previous data have suggested that muscle injury (myofiber degeneration) is the customary response following exposure to muscular contractions that incorporate lengthening movements (Faulkner et al. 1989; Koh et al. 2003), it is not known if this is an absolute when increased performance and muscle hypertrophy (adaptation) is the desired outcome or, moreover, if chronic maladaptation results from an initial injurious exposure.

The purpose of this study was to quantify the acute-phase myofiber response in young and old rats exposed to an acute bout of SSCs, which were optimized to elicit performance gains and muscle hypertrophy in young rats subjected to chronic SSCs (Cutlip et al. 2006). Since previous literature has indicated that decreased performance and muscle injury are customary following lengthening-type movements, and that aging exacerbates this response, we sought to determine whether aging affects the ability of skeletal muscle to respond functionally and morphologically to this acute, adaptive bout of SSCs. Identifying the underlying processes that affect the muscle's initial adaptive response (remodeling) with aging may be beneficial in optimizing current strategies for recovery and rehabilitation following loading of skeletal muscle.

## Methods

### Animals

Male Fischer Brown Norway hybrid rats (F344 × BN F1; $n = 60$) were obtained from the National Institutes on Aging colony. Thirty young adult rats (mean mass ± SD, 325 ± 29 g; age, 12 weeks) and 30 old rats (mean mass, 584 ± 43 g; age, 30 months) were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal quarters. Temperature and light:dark cycle (dark cycle from 0700 to 1900 hours) were held constant for all animals; food and water were provided ad libitum. After 1 week of acclimatization, all animals were segregated by age, and randomized to SSC groups with 6, 24, 48, 72, or 120 h recoveries ($n = 6$ for each recovery time). Each group was exposed to a standardized experimental protocol, approved by the National Institute for Health and Safety (NIOSH) Animal Care and Use Committee, and euthanized at designated recovery times.

### Experimental set-up

The dorsiflexor muscles were tested on a custom-built rodent dynamometer (Cutlip et al. 1997), as described elsewhere (Cutlip et al. 2004). Briefly, the dynamometer provides precise control over muscle length and, therefore, muscle force output parameters can be investigated. A LabVIEW-based virtual instrument was developed, which governed a National Instruments data acquisition board (PCI-MIO-16XE-10) and a Unidex 100 motion controller (Aerotech Inc, Pittsburgh, Penn.), providing precise control of a brushless DC servomotor (1410 DC, Aerotech Inc.) and muscle stimulator (Model SD9, Grass Medical Instruments, Quincy Mass.). The software also acquired and stored position, force, and velocity data in real-time, as described below.

Rats were anesthetized with 2% isoflurane gas, using a small animal anesthetic system (Surgivet Anesco Inc., Waukesha, Wis.). Isoflurane was chosen because it has been shown to be the most appealing anesthetic agent for either an acute or chronic study examining skeletal muscle function (Ingalls et al. 1996). After anesthesia, each rat was placed, supine, on the heated *x*-*y* positioning table of the rodent dynamometer, and an anesthetic mask was placed over its nose and mouth. The left knee was secured in the flexion position (at 90°) with a knee holder. The left foot was secured in the load cell fixture, using a custom-built foot holder, and the ankle axis (assumed to be between the medial and lateral malleoli) was aligned with the axis of rotation of the load cell fixture. Each animal was monitored during the protocol to ensure proper anesthetic depth and body temperature.

### Functional testing

As with the experimental set-up, the functional testing has been described in detail elsewhere (Cutlip et al. 2004). Briefly, the joint position of the animal was defined by the angle between the tibia and the plantar surface of the foot. The angular position of the load cell fixture corresponded to the angular position of the ankle. The force produced by the dorsiflexor muscles was measured at the interface of the aluminum sleeve and the dorsum of the foot. Platinum stimulating electrodes (Grass Medical Instruments) were placed subcutaneously, for each exposure session, to span the common peroneal nerve. Activation of the electrical stimulator resulted in muscle contraction of the dorsiflexor muscle group.

### Isometric and dynamic force tests

Testing for isometric and single SSCs has been detailed elsewhere (Cutlip et al. 2004). Briefly, an isometric and a single SSC were measured on the dorsiflexor muscle group, preceding (pretest isometric force) and immediately following the acute SSC protocol, and at each designated recovery point (post-test isometric force). The single SSC test was used to evaluate the muscle's ability to generate dynamic forces and to perform work during dynamic stretch-shortening (Cutlip et al. 2004, 2006, 2007). Thus, we characterized the mechanical performance of the dorsiflexor muscle group following SSC exposure. Peak force ($F_{peak}$, defined as peak eccentric force), minimum force ($F_{min}$, defined by the isometric force preceding each stretch), average force ($F_{mean}$, obtained by calculating the average of all muscle force values during the 100 ms eccentric phase of each oscillation), and cyclic force ($F_a = (F_{peak} - F_{min})$, defined by the force enhancement during each stretch), follow-

ing the SSCs, were evaluated. We also evaluated negative work (integral of the force-position plot during the eccentric phase of the cycle) and positive work (integral of the force-position plot during the concentric phase of the cycle) following the SSC exposure.

### SSC protocol

The young and old rats were exposed to 8 sets of 10 repetitions of SSCs. Within each set, there was a 2 s rest between SSCs. For each repetition, the dorsiflexor muscles were fully activated, prior to any movement, for 100 ms. The eccentric phase was then initiated, with a $60^{\circ}\cdot s^{-1}$ movement velocity of the load cell fixture over the prescribed range of motion (ankle angle of 90°–140°). The load cell fixture immediately returned in the concentric phase, at $60^{\circ}\cdot s^{-1}$, to the starting position of a 90° ankle angle. The dorsiflexor muscles were deactivated 300 ms later. Total stimulation time per repetition was 2.06 s. Although this protocol was administered only 1 time in our study, the identical protocol, when administered chronically in a recent study, resulted in increased adaptation, both functionally and morphologically, in young rats only (Cutlip et al. 2006).

### Muscle processing

At the previously described recovery times following the exposure, rats were weighed, anesthetized intraperitoneally with sodium pentobarbital (10 mg·100 $g^{-1}$ body mass), and exsanguinated. The left (exposed) and right (contralateral control) tibialis anterior (TA) muscles (LTA and RTA, respectively) were dissected, weighed, divided into 5 equal-distant transverse zones (zone 1 being most proximal and zone 5 being most distal), mounted onto cork board with OTC (VWR, West Chester, Penn.), frozen in liquid-nitrogen-cooled isopentane, and stored at –80 °C until sectioned for histology. Left and right tibias were excised and measured with a micrometer, and LTA and RTA muscles were normalized to tibia length.

### Histology and immunohistochemistry

Zone 3 was selected to obtain the largest tissue sample; this corresponds to the TA midbelly. Sequential, frozen transverse sections, 12 μm thick, were mounted on microscope slides, air dried, and stored at –80° until processing. One slide from each exposed and control limb of each rat was stained with hematoxylin and eosin, using Harris' procedure. Muscle morphology was assessed using quantitative morphometry (Underwood 1970). Immunohistochemistry for developmental myosin heavy chain ($MHC_{dev}$), a marker for muscle fiber regeneration, was performed on 1 set of sections (1 slide) from the exposed and control TA of each rat at 120 h recovery, since previous studies have shown that this recovery time is within the time period to provide the optimal labeling profile of regenerating myofibers (Smith et al. 1999). The frozen sections used for $MHC_{dev}$ immunolabeling were brought to room temperature and rinsed in PBS plus 0.1 mol·$L^{-1}$ glycine for 5 min. All slides were incubated in blocking buffer (5% normal donkey serum diluted in PBS plus Triton X-100) at room temperature for 2 h. Slides were then incubated in mouse anti-$MHC_{dev}$ antibody, diluted in blocking buffer at a ratio of 1:20 for 1 h at 4 °C. Following incubation, sections were rinsed in PBS and incubated for 1 h at room temperature in Cy2-labeled donkey anti-mouse immunoglobulin (Ig) G (1:200). Slides were then rinsed, incubated in 4′,6-diamidino-2-phenylindole (DAPI) to stain nuclei (1:1000; Sigma, St. Louis, Mo.), cover-slipped using Prolong Gold antifade reagent (Molecular Probes), and allowed to dry in a cool, dark area. The $MHC_{dev}$ antibody was obtained from Nova Castra Labs (Newcastle, U.K.), and the fluorescently tagged secondary antibody (Cy2) and normal donkey serum for blocking were obtained from Jackson Immuno-Research Laboratories Inc. (West Grove, Penn.). A number of slides were processed in the absence of the primary antibody as controls for nonspecific binding, and tissue from 120 h rat pup and 120 h SSC injured TA muscle were stained and served as positive controls.

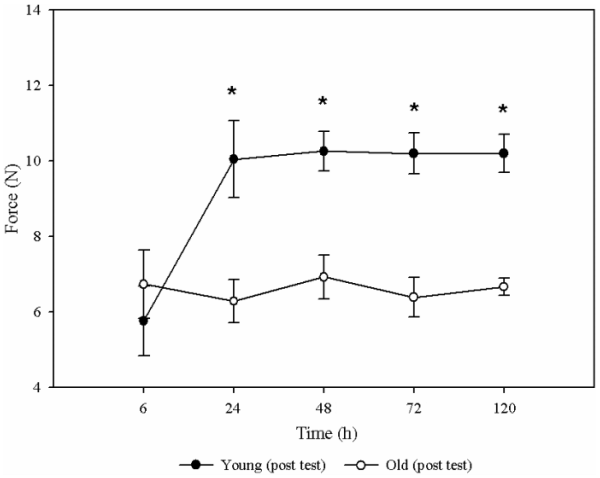
### Myofiber definitions for cross-sectional stereology

Stereology was used to quantify the degree of myofiber degeneration and the accompanying changes in the interstitial space in the TA muscle from each group. Myofibers have been defined elsewhere (Baker et al. 2006*a*). Briefly, normal myofibers demonstrated complete contact with adjacent myofibers, a smooth outer membrane, and no presence of internal inflammatory cells. Degenerative myofibers displayed a loss of contact with adjacent myofibers, the presence of internal inflammatory cells, and an outer membrane interdigitated with inflammatory cells.

### Stereology

Quantitative morphometric methods were used to measure the volume fraction, surface densities, and average thickness of normal myofibers, degenerative myofibers, and the interstitial space (Baker et al. 2006*a*). The interstitium was divided into endomysial and perimysial spaces, which included capillaries. A standardized stereological technique, as discussed elsewhere (Baker et al. 2006*a*), was used to quantify the degree of myofiber degeneration and inflammation, which was quantified as either noncellular interstitium, indicative of edema, or cellular interstitium, indicative of cellular infiltrates. Fiber volume and surface density were measured using standard morphometric analyses (Underwood 1970; Weibel 1972, 1974, 1975). Briefly, 1 of the hematoxylin-and-eosin-stained sections was taken from each animal. A stage micrometer was used to identify the midpoint of each section. Point and intercept counts, using a 121-point, 11-line overlay graticule (12.5 $mm^2$ with 100 divisions) at ×40 magnification, were taken at 5 equally spaced points across the section. This process was repeated 2 mm on either side of the midpoint of the section, for a total of 1210 points and 110 intercept lines per section. Volume density or percent volume was computed from the percentage of points over the tissue section to points over normal myofibers, degenerative myofibers, cellular interstitium, noncellular interstitium, and capillaries (Weibel 1972, 1974, 1975). Intercepts over the line overlay were counted for the perimeter of normal myofibers, degenerative myofibers, and interstitium to myofiber transitions. Points and intercepts over blood vessels greater than 25 μm in diameter were excluded. Average thickness or average distance was computed from 2 times the ratio of volume to surface density, according to standard morphometric analysis (Underwood 1970). One section per animal ($n$ = 6 young or

**Fig. 1.** Isometric force production from young and old rats exposed to 80 stretch-shortening contractions (SSCs). Pre and post isometric force production in the exposed limb of young and old rats, preceding exposure and throughout the 120 h acute phase is illustrated. Old rats were negatively influenced in their ability to produce isometric force following exposure to 80 SSCs. *, Significant at the 0.05 level. Data are reported as mean values ± standard error.




old animals in each group) was evaluated, and the results were expressed as means ± standard error (SE).

### $MHC_{dev}$ localization and quantification

Photomicrographs were captured and saved, using an Olympus photomicroscope and SimplePCI image analysis software. Positively immunolabeled $MHC_{dev}$ sections were quantified using 1 section per slide from the contralateral control RTA and exposed LTA muscles from each group. Digital color images were analyzed using standard quantitative morphometric methods (Weibel 1972, 1974, 1975; Underwood 1970) to measure the volume density (% tissue fraction) of $MHC_{dev}$ positively labeled myofibers. Briefly, each of the MHC sections was quantified independently, as 1 section per slide was analyzed, from each animal per group, and quantified using standard morphometric techniques (Underwood 1970). Volume density was computed from the percentage of points over the tissue section to points over each of the $MHC_{dev}^{+}$-labeled myofibers. MHC data are reported as means ± SE, and analyzed as described in the Data analysis section.

### Muscle quality

Pretest isometric force, measured at the last session for each respective recovery, was normalized to muscle wet-weight of the LTA of the exposed limb obtained at sacrifice, as described elsewhere (Degens and Alway 2003; Cutlip et al. 2006).

**Fig. 2.** Single stretch-shortening contraction (SSC) functional parameters obtained from the single SSC test from young and old rats exposed to 80 SSCs. (A) Peak eccentric force and (B) minimum force responded similarly, as older age negatively affected dynamic performance throughout the 120 h acute phase. (C) Older age negatively affected the ability to absorb work. (D) Older age negatively affected the ability to generate work. *, Significant at the 0.05 level. Data are reported as mean values ± standard error.

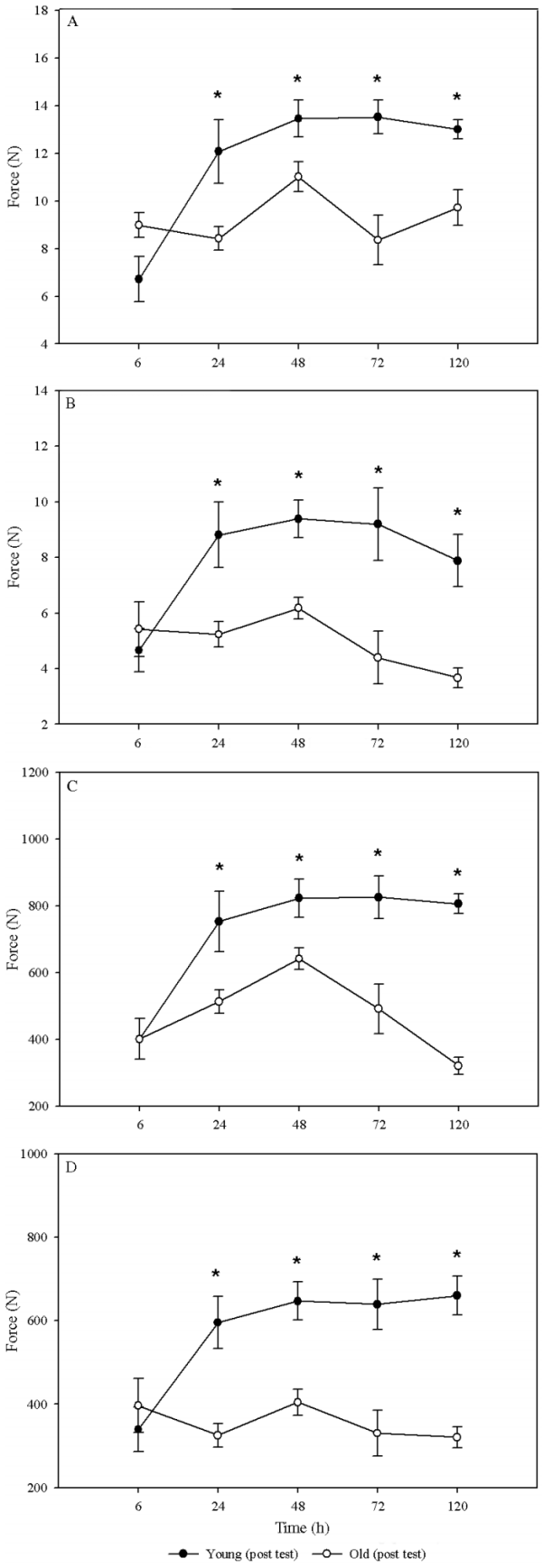


**Table 1.** Body weights and normalized right (RTA) and left tibialis anterior (LTA) muscle wet-weights.

| Recovery (h) | Body mass (g) | Normalized RTA ($mg \cdot mm^{-1}$) | Normalized LTA ($mg \cdot mm^{-1}$) | % difference |
|---|---|---|---|---|
| **Young rats** | | | | |
| 6 | 322.7±10.1 | 14.6±0.4 | 16.4±0.4*,† | 12.2±1.6 |
| 24 | 296.2±7.3 | 14.1±0.5 | 15.0±0.5 | 6.7±1.1 |
| 48 | 319.5±7.0 | 15.0±0.2 | 15.7±0.2 | 4.9±1.3 |
| 72 | 347.3±8.9 | 15.6±0.2 | 16.6±0.3*,† | 6.7±0.7 |
| 120 | 344.1±12.8 | 15.6±0.4 | 15.6±0.5† | –0.4±1.8 |
| **Old rats** | | | | |
| 6 | 592.3±18.0 | 14.2±.04 | 15.0±0.2 | 6.4±4.0 |
| 24 | 560.2±12.4 | 14.5±0.4 | 16.0±0.3* | 10.1±3.0 |
| 48 | 561.6±20.3 | 14.6±0.4 | 15.0±0.4 | 2.7±2.1 |
| 72 | 591.2±28.2 | 14.8±0.3 | 15.1±0.5 | 2.6±2.4 |
| 120 | 568.6±7.0 | 13.6±0.6 | 14.4±0.5 | 5.9±1.8 |

**Note:** All values shown are means ± standard error.
*Main effects for age significant at the 0.05 level.
†Main effects for limb significant at the 0.05 level.

### Data analysis

#### *Performance data*

Isometric forces for respective recovery points were compared using a 2-way (age × time) analysis of variance (ANOVA). Single SSC force and work data were compared similarly. All data are presented as means ± SE, unless otherwise noted, and differences with a probability of $p < 0.05$ were considered significant. All data were analyzed using JMP v. 5.1 (SAS Institute Inc., Cary, N.C.).

#### *Body masses, muscle wet-weights, and muscle quality*

Data for muscle wet-weight differences were analyzed using a 3-way mixed-model (age × limb × time) ANOVA, with animal as the random factor accounting for measures in both limbs. Muscle quality measurements were analyzed using a 2-way (age × time) ANOVA.

#### *Stereology and $MHC_{dev}$ immunohistochemistry*

Stereological measurements for volume and thickness of cellular and noncellular components and muscle quality were analyzed using a 3-way (age × limb × time) ANOVA. $MHC_{dev}$ volume density at 120 h recovery was analyzed using a 2-way mixed-model (age × limb) ANOVA.

## Results

### Functional measures

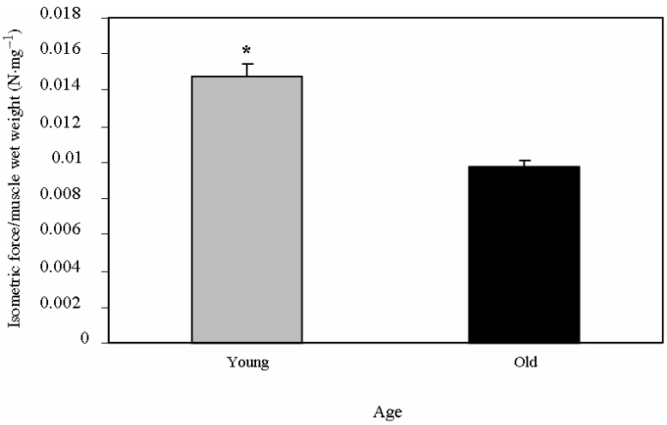
#### *Isometric performance*

Age reduced isometric force production, as old rats' post-test isometric force values were decreased, compared with young rats' post-test isometric force values, from 24 h to 120 h ($p < 0.05$; Fig. 1).

#### *Single SSC (dynamic performance)*

Age decreased the old rats' dynamic force and work production, compared with young rats, from 24 h to 120 h ($p < 0.05$; Figs. 2A–2D).

**Fig. 3.** Isometric force normalized to muscle wet-weight of the tibialis anterior, following the acute stretch-shortening contraction exposure. Young rats exhibited increased muscle quality, compared with old rats. *, Significant at the 0.001 level.



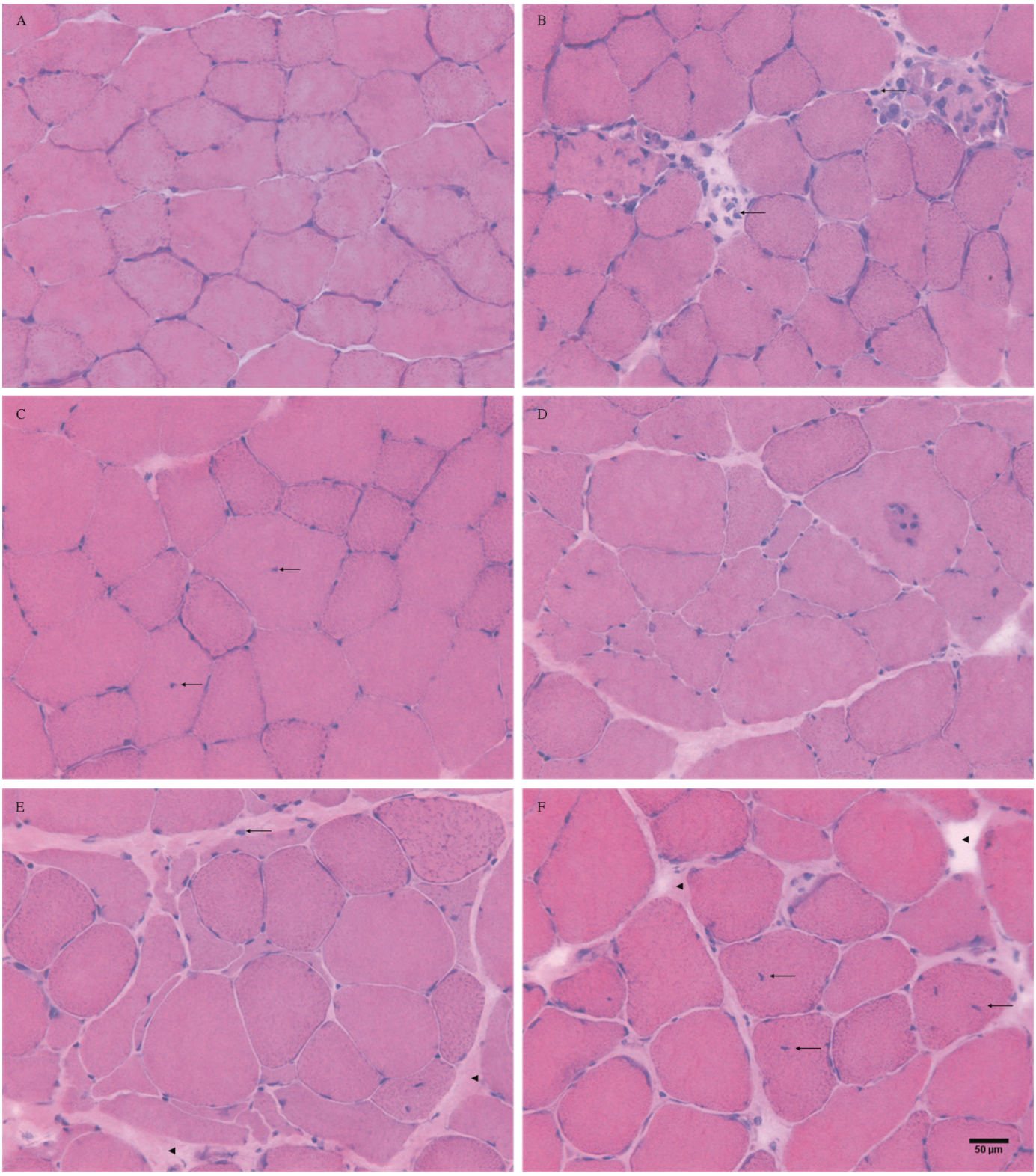

### Biological measures

#### *Body masses and muscle wet-weights*

Rat body masses and TA muscle wet-weights normalized to tibia length following the acute exposure; these are reported in Table 1. There was a significant interaction between age and time for muscle wet-weight ($p < 0.05$). As a result of exposure, the LTA muscle wet-weight was increased, compared with the RTA muscle wet-weight, in the young rats at 6 h recovery ($p < 0.05$; Table 1), and the LTA muscle wet-weight was increased, compared with the RTA muscle wet-weight, in old rats at 24 h recovery ($p < 0.05$; Table 1). Further analyses revealed significantly increased LTA muscle wet-weight in young rats, compared with old rats, at 6 h, 72 h, and 120 h ($p < 0.05$; Table 1).

#### *Muscle quality*

Age significantly affected the mean value of isometric force normalized to muscle wet-weight ($p < 0.001$), as muscle quality was 0.015 ± 0.001 $N \cdot mg^{-1}$ and 0.010 ±




0.0004 N·mg$^{-1}$ for the young and old groups, respectively (Fig. 3).

### *Stereological analyses of normal and degenerative myofibers*

Figure 4 depicts representative micrographs of TA cross sections from the exposed limb of young and old rats at 24, 72, and 120 h recoveries. No changes in normal myofibers were observed between any of the groups (unpublished data). Statistical analysis showed that degenerative myofibers were only present in TA muscle analyzed from the young rats at 72 h recovery ($p$ = 0.049; Fig. 5A), and that this response was <1% of the total tissue fraction. No significant degenerative myofibers were present in TA muscles of old rats.

**Fig. 4.** Morphological changes in muscle tissue from young and old rats, following exposure to SSCs. No myofiber or interstitial changes were observable in contralateral control right tibialis anterior (RTA) muscle from young or old rats. (A) Micrograph representing the young exposed left tibialis anterior (LTA) muscle at 24 h recovery (note, no myofiber or interstitial disruption is present). (B) Representative micrograph from the young exposed LTA muscle at 72 h recovery, displaying <1% of myofibers with a degenerative response and an increased cellular interstitial response (arrows indicate increased cellular interstitium (CI)). (C) Representative micrograph from the young exposed LTA muscle at 120 h recovery, which exhibited few fibers with central nuclei (arrows). (D) Micrograph representing the old exposed LTA muscle at 24 h recovery (note, as with young rats, no myofiber or interstitial disruption is present). (E) Representative micrograph from the old exposed LTA muscle at 72 h recovery, displaying an increased CI response (increased CI) and an increased swelling of the perimysium and endomysium (arrowheads indicate increased noncellular interstitium). (F) Representative micrograph from the old exposed LTA muscle at 120 h recovery; as observed with young rats, few fibers exhibited central nuclei (arrows). All micrographs are ×40 magnification. Scale bar = 50 μm.

### *Stereological analyses of inflammation*

Noncellular interstitium was significantly increased at 120 h recovery in the exposed LTA of old rats, compared with the LTA of young rats ($p < 0.05$; Fig. 4 (arrowheads), and Fig. 5B). Conversely, the volume density of cellular interstitium was significantly increased in the exposed muscles of old rats at 48 h and, irrespective of age, at 72 h recovery, but this value returned to baseline 120 h after exposure ($p < 0.05$; Fig. 5C).

### *Immunohistochenistry for $MHC_{dev}$*

Representative images of $MHC_{dev}^+$ myofibers at 120 h (Fig. 6A). At 120 h, the volume of $MHC_{dev}^+$ muscle cells were increased in young rats, compared with old rats, regardless of limb (Fig. 6B). Baseline values in the old rat contralateral control tissue showed a 12.5% increase in $MHC_{dev}^+$ myofibers, compared with young rats. Exposure did increase the volume of $MHC_{dev}^+$ muscle cells, compared with the contralateral control limb, irrespective of age; however, this response was diminished with aging (~2000% increase in young exposed limb vs. ~200% increase in old exposed limb) (Fig. 6B). Positive controls from SSC-injured LTA muscle at 120 h recovery (Fig. 6C) and rat-pup TA muscle at 120 h (Fig. 6D) are displayed for comparison between muscle adaption and growth versus injury. Further, the volume of $MHC_{dev}^+$ muscle cells was increased ~500% in the exposed limb of young rats, compared with old rats ($p < 0.05$; Fig. 7).

## Discussion

Recently, the current SSC protocol, when administered chronically, has been shown to lead to adaptation (defined by performance and morphological gains) in young rats, but results in maladaptation in their older counterparts (Cutlip et al. 2006). Thus, our objectives for the current study were to identify events that affect the muscle's initial adaptive response with aging; and to ascertain whether acute muscle degeneration and (or) necrosis contributes to the observed maladaptation when this SSC protocol is administered chronically in old rats (previous literature has indicated that decreased performance and muscle injury are customary following lengthening-type movements, and that aging exacerbates this response). The major findings of this study were that myofiber degeneration and (or) necrosis do not contribute to the early remodeling events following an adaptive–hypertrophic bout of SSCs and that $MHC_{dev}$ was expressed in the old rats, but to a much lesser extent than in young rats, following SSC loading.

This indicates that the signal to respond to the mechanical

**Fig. 5.** The percent volume of (A) degenerative myofibers, (B) cellular interstitium, and (C) noncellular interstitium components of inflammation from young and old rats' exposed (left tibialis anterior (TA)) and control (right TA) muscles. *, Significant at the 0.05 level. Data are reported as mean values + standard error.

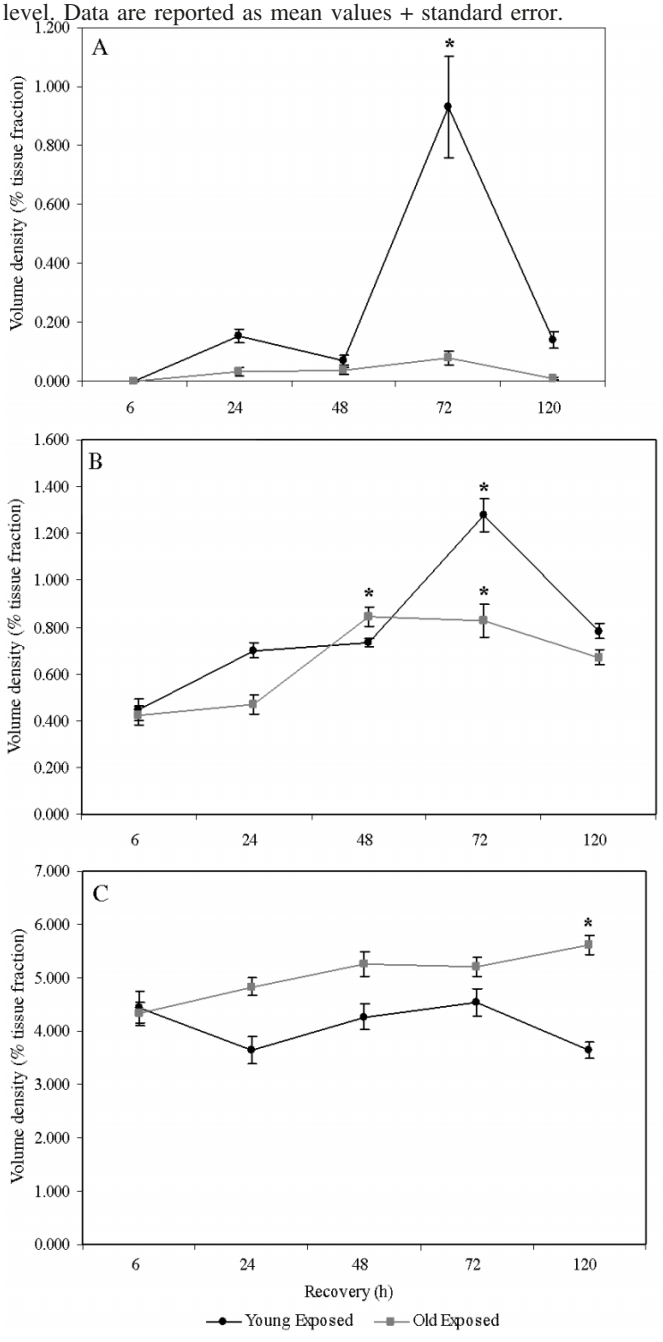
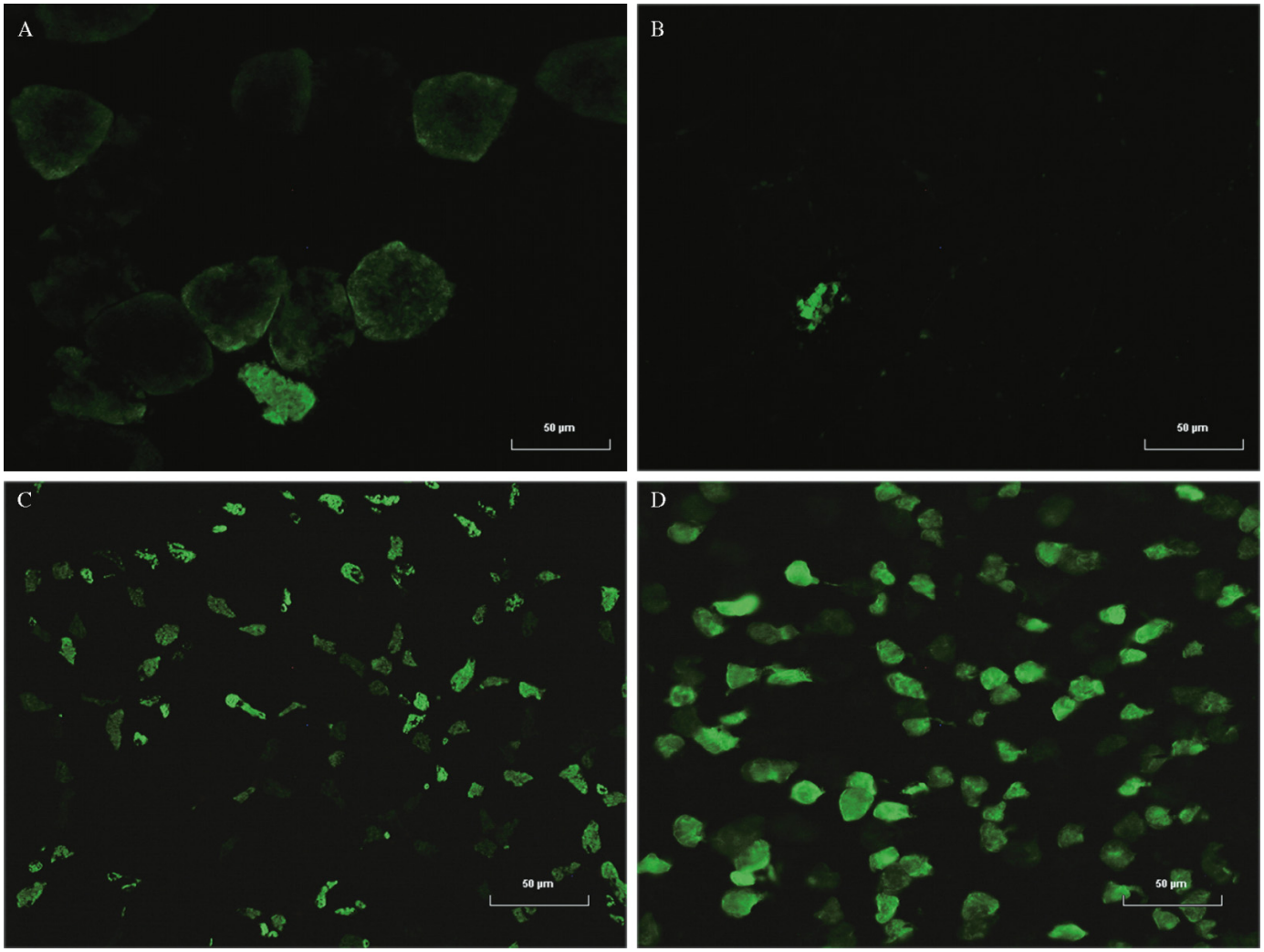
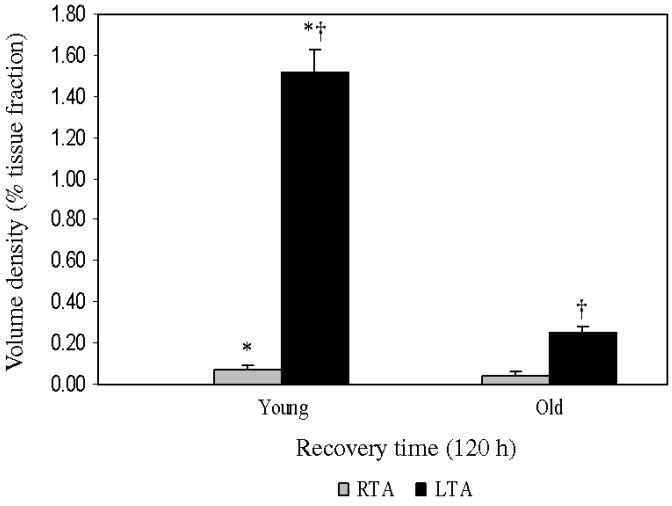


**Fig. 6.** Developmental myosin heavy chain ($MHC_{dev}$) immunohistochemistry and stereology. (A) Representative micrographs of $MHC_{dev}^{+}$-labeled muscle cells at 120 h from exposed left tibialis anterior (TA) muscle of young (A) and old (B) rats, and from positive controls from stretch-shortening contration-injured TA muscle at 120 h recovery (C) and rat pup TA muscle at 120 h (D). All micrographs are ×40 magnification. Scale bars = 50 μm.




stimulus is intact in old rats (~200% increase in $MHC_{dev}^{+}$ labeling in exposed LTA, compared with contralateral control limb), but they were unable to fully meet the demands of the exposure. Moreover, in this study, developmental myosin was expressed in rat tissue that did not exhibit signs of overt skeletal muscle injury, suggesting that $MHC_{dev}$ is indicative of remodeling events leading to muscle hypertrophy, even in the absence of muscle injury. However, this diminished signal in myofibers suggests why old rats do not have the capacity to adapt to repetitive exposures, compared with their younger counterparts. This may also explain why old rats' performance measures were decreased, compared with young rats. An alternative interpretation of the developmental myosin labeling suggests that there was decreased expression, solely because there was less damage in the old rats; however, both young and old rats had negligible percentages of degenerative myofibers, so this does not appear to be plausible. Furthermore, a study by Brown et al. (1997) concluded that adaptation of skeletal muscle following eccentric contractions may be the result of an improved ability to repair ultrastructural changes occurring in individual myofibers (not removal of necrotic myofibers). This suggestion has been substantiated by numerous studies (Friden et al. 1981, 1983; Yu et al. 2002, 2004, 2003; Yu and Thornell 2002). In the current study, dorsiflexor muscles from young and old rats exposed to the current protocol of 80 SSCs do not undergo the extent of myofiber degeneration (<1% degenerative myofibers present) that is typically reported for classical contraction-induced muscle injury (Baker et al. 2006*a,* 2007; Hesselink et al. 1996; McCully and Faulkner 1985). Thus, corroborating our current morphological findings is a collection of literature that suggests that the adaptive response of muscle following SSC loading is not dependent on myofiber degeneration (necrosis), but that adaptation occurs as a result of ultrastructural changes (Brown et al. 1997; Yu et al. 2004, 2002) and local environmental changes in the tissue (Malm 2001; Conboy et al. 2005; Cutlip et al. 2006). Accordingly, the changes in the cellular and noncellular interstitium may contribute to the collective functional and biological changes observed with

**Fig. 7.** At 120 h, the % volume of $MHC_{dev}^+$ muscle cells was increased in young rats, compared with old rats, regardless of limb. Exposure did increase the % volume of $MHC_{dev}^+$ muscle cells, compared with the contralateral control limb, irrespective of age. Further, the volume of $MHC_{dev}^+$ muscle cells was increased in the exposed LTA muscle of young rats, compared with old rats ($p < 0.05$). *, Significant at the 0.05 level for the main effect of age; †, significant at the 0.05 level for the main effect of limb. Data are reported as mean values ± standard error. RTA, right tibialis anterior; LTA, left tibialis anterior.




aging, since we observed an increase in estimates of edema at 120 h.

Acute exposure to eccentric-type exercise results in functional decreases, which have a well-documented temporal response, independent of age (Gosselin 2000; Koh et al. 2003; Zerba and Faulkner 1990), whereas aging exacerbates this response (Zerba et al. 1990; Cutlip et al. 2006; Krajnak et al. 2006). Our results indicate that, for an acute bout of SSCs, young rats do not exhibit classical signs of performance loss; following the current exposure protocol, isometric force and dynamic performance recovered as early as 24 h after the initial exposure. Yet, aging reduces functional performance, as revealed by our old rats' inability to recover throughout the 120 h acute phase. Differences in our results, compared with previous findings, may have been influenced by the species or rodent strain used, or by the exposure protocol implemented; however, it was not our intention to replicate others' findings. Whereas previous investigations have found that lengthening contractions (or contractions that included a lengthening component) result in a long-lasting isometric force deficit (Warren et al. 1999; Baker et al. 2006*a*), structural disruption at the cellular level, and cellular infiltrates as a result of the ensuing inflammatory response (Warren et al. 1999, 1993*c*; Lieber et al. 1996; Faulkner et al. 1989; McCully and Faulkner 1985; Friden et al. 1983; Baker et al. 2006*a*, 2007), we do not currently report these observations. Thus, in the absence of overt skeletal muscle injury (myofiber degeneration and heightened inflammatory response), it is our contention that performance would not be depressed at extended timepoints. Recently, it has been demonstrated that TA muscles from old rats, compared with their younger counterparts, exhibited long-lasting functional decreases following a single injurious SSC exposure, and that this is associated with increased estimates of edema and increased satellite cell apoptosis (Krajnak et al. 2006).

In the current study, there was a low level of myofiber degeneration following 24 h recovery in the exposed LTA and in the contralateral limb RTA in both the young and old rats. As suggested by others (Smith et al. 1997), this indicates that there is a low percentage of myofibers undergoing regular turnover (degeneration–regeneration). However, although this normal turnover is insignificant and does not contribute to skeletal muscle injury, acute exposure to various modes of mechanical stimuli (e.g., physical activity or exercise) has been shown to increase this response, and aging further exacerbates this effect (Baker et al. 2006*a*; Brooks and Faulkner 1996; Smith et al. 1997). This is also substantiated by the small number of myofibers labeled positively for developmental myosin in the control limb, irrespective of age.

Muscle does have a delayed recovery from a single injurious exposure, yet the capacity to adapt remains intact (Conboy et al. 2005), as evidenced by our old rats, which displayed increased $MHC_{dev}^+$ labeling in the exposed LTA muscle, compared with the nonexposed RTA. However, this response was diminished significantly, compared with exposed LTA muscles, from young rats in the current study.

Whether an acute increase in noncellular interstitium promotes an environment that aggravates long-term cellular signaling and recruitment of inflammatory cells is not known. Here, we showed that estimates of edema were increased with aging in the exposed LTA by 35% at 120 h. Thus, unresolved permeability changes may have contributed to the decreased functional performance at 120 h. Also, we demonstrated that the cellular interstitium of the exposed LTA in the old rats had returned to control values by 120 h, while noncellular interstitium remained elevated at this time. In a chronic exposure, using this protocol, an increase in cellular interstitium, with no accompanying increase in noncellular interstitium, was observed (Cutlip et al. 2006) and, importantly, under both acute and chronic conditions, there was no significant myofiber degeneration in either young or old rats following this specific SSC exposure protocol. Thus, alterations in cellular permeability present at 120 h in our old rats may contribute to long-term maladaptation, by modifying old rats' local internal environments and ultimately affecting muscle remodeling. This is plausible because alterations in the host environment with aging suggest that systemic factors may adversely affect local tissue regeneration (Conboy et al. 2005). Alternatively, it has been suggested that age-related excitation–contraction coupling, concurrent with calcium signaling and handling dysregulation, affects specific force in single muscle fibers (Gonzalez et al. 2000). However, these events would manifest as early events (24–48 h) following the mechanical exposure, which we did not observe. Thus, although the role of calcium cannot be dismissed, a more appropriate means by which to consider the impact of excitation–contraction coupling on performance with aging is the notion that mechanical perturbation causes alterations and fragility of the transverse tubules and associated receptor complexes (Payne and Delbono 2004), which may lead to decreased transmission efficiency. Furthermore, alterations in actin and myosin contractile proteins involved in cross-bridge cycling have been

shown to contribute to an age-related decline in specific force (Lowe et al. 2002). Thus, these events may also influence the age-related decline in isometric and dynamic performance we observed in the current study.

In conclusion, the results demonstrate that muscle adaptation and remodeling is not dependent on initial myofiber degeneration, and regeneration is diminished in old rats, compared with their younger counterparts. This phenomenon may underlie the capability of skeletal muscle from older animals to adapt to acute mechanical exposure. Evidently, old rats are unable to overcome this initial mechanical insult because of the diminished remodeling of the local internal environment and the decreased expression of $MHC_{dev}^{+}$ in myofibers of the exposed TA muscle.

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