# Chronic exposure to stretch–shortening contractions results in skeletal muscle adaptation in young rats and maladaptation in old rats

**Robert G. Cutlip, Brent A. Baker, Kenneth B. Geronilla, Robert R. Mercer, Michael L. Kashon, Gerald R. Miller, Zsolt Murlasits, and Stephen E. Alway**

**Abstract:** The objective of this research was to investigate skeletal muscle response to a chronic administration of stretch–shortening cycles (SSCs) in young and old rats. Dorsiflexor muscles of old (30 months, $n = 5$) and young (12 weeks, $n = 6$) rats were exposed 3 times/week for 4.5 weeks to a protocol of 80 maximal SSCs per exposure in vivo. Skeletal muscle response was characterized by isometric and dynamic performance, as well as by muscle wet mass and quantitative morphological analyses following the exposure period. The performance of the young and old groups was not statistically different at the start of the exposure. By the end of the exposure, however, a statistical difference was noted, as performance increased significantly in the young animals and decreased significantly in the old animals. Muscle wet mass of the left tibialis anterior (TA) in the treated limb was significantly greater in the youngthan in the old animals ($p < 0.001$), whereas there was no difference in the contra-lateral TA. No degenerative myofibers or changes in non-cellular interstitium were noted in either age group, but a significant increase was observed in the volume of the cellular interstitium in the exposed limb of the old animals ($p = 0.01$), which is indicative of an inflammatory response. Thus, a chronic exposure of SSCs results in significant performance increase and muscle hypertrophy in young animals, and a significant performance decrease and an increased cellular interstitial response in old animals. These findings suggest that age may impair the ability of skeletal muscle to adapt to repetitive mechanical loading, even in the absence of degeneration.

*Key words:* stretch–shortening cycles, dorsiflexor muscles, repetitive exposure, cellular interstitium.

**Résumé :** Le but de cette étude est d'analyser l'effet de l'administration chronique d'une série de cycles d'étirement–raccourcissement (SSCs) sur le muscle squelettique de jeunes et vieux rats. Les muscles dorsifléchisseurs de vieux rats (30 mois, $n = 5$) et de jeunes rats (12 semaines, $n = 6$) sont exposés in vivo durant 4,5 semaines à raison de 3 fois par semaine à une série de 80 SSCs maximaux. On évalue la réaction musculaire par des mesures de performance isométrique et dynamique de même que par la masse humide du muscle et l'analyse morphologique quantitative à la suite de la période de stimulation. Au début de la période de stimulation, les performances isométrique et dynamique des deux groupes ne diffèrent pas statistiquement; à la fin de cette période, on observe une amélioration significative de la performance chez les jeunes rats et une diminution significative chez les vieux. La masse humide du jambier antérieur gauche (TA) de la patte expérimentale est significativement plus importante chez les jeunes que chez les vieux ($p < 0001$); on n'observe aucune différence du côté controlatéral. De plus dans les deux groupes, on n'observe aucune fibre musculaire en dégénérescence ni aucun changement dans l'interstitium non cellulaire; on observe cependant chez les vieux rats une augmentation du volume de l'interstitium cellulaire de la patte expérimentale ($p = 0,01$) ce qui constitue un indice de réaction inflammatoire. En conséquence, l'exposition chronique à une série de SSCs améliore significativement la performance et favorise l'hypertrophie chez le jeune rat; cette même exposition diminue significativement la performance et augmente la réponse cellulaire interstitielle chez le vieux rat. D'après ces observations, le vieillissement représente une entrave à l'aptitude du muscle à s'adapter à une tâche mécanique répétitive même en l'absence de dégénérescence.

*Mots clés :* cycles d'étirement–raccourcissement, muscles dorsifléchisseurs, exposition répétée, interstitium cellulaire.

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## Introduction

Senescence-related changes in strength and skeletal muscle mass have been studied previously (Booth et al. 1994; Evans and Campbell 1993); however, changes in functional performance and muscle plasticity with training

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in aged populations are not fully understood. It is clear that susceptibility to contraction-induced injury increases with age (Bernard et al. 1997) in both humans (Manfredi et al. 1991) and animals (Brooks and Faulkner 1996; Zerba et al. 1990). After an injurious exposure to eccentric muscle contractions, there is an increased force deficit (Zerba et al. 1990; Brooks and Faulkner 1996; Koh et al. 2003) and slower recovery of performance (Manfredi et al. 1991; Brooks and Faulkner 1990; McBride et al. 1995; Sacco and Jones 1992) in whole muscles (Zerba et al. 1990; Brooks and Faulkner 1996; Koh et al. 2003; Brooks and Faulkner 1990; McBride et al. 1995; Sacco and Jones 1992) and single fibers (Brooks and Faulkner 1996) of old animals than in those of young animals. The ability to prevent injury in older animals is thought to be attenuated owing to higher eccentric force generation during eccentric muscle actions (Brooks and Faulkner 1994) when compared with young animals, and this difference is magnified with increased stretch velocity (Eddinger et al. 1986). Finally, aging is associated with an impaired adaptation to subsequent exposures of injurious eccentric contractions (McBride et al. 1995).

Few studies have investigated age effects on muscle adaptation and injury following a specific repetitive loading protocol designed to induce hypertrophy. In the case of aerobic training, there is evidence that 10 weeks of treadmill training attenuates eccentric muscle damage in vitro in both young and old rats (Gosselin 2000). In addition, exposure to 6 weeks of eccentric contractions provided a protective effect in mice by preventing a substantial force deficit and morphological evidence of damage in muscles from both young and old animals exposed to a protocol that typically injures non-trained muscles (Brooks et al. 2001). However, older mice adapted more slowly than younger mice (Brooks et al. 2001). Although these studies show that muscles from older animals can be protected from eccentric contraction-induced injury, the conditioning stimulus was inadequate to promote hypertrophy of the target muscles or increase performance. We have previously shown that muscles from old animals are capable of adaptation to increased loads, but aging attenuates loading-induced muscle hypertrophy in both rodents (Degens and Alway 2003; Alway et al. 2002) and birds (Lowe and Alway 1999; Carson et al. 1995). However, there are no studies to date that have determined if muscles from old animals can positively adapt to repetitive exposures of resistive muscle contractions that promote adaptation and performance increases in muscles of young animals.

Natural movement is comprised of stretch–shortening cycles (reciprocal concentric and eccentric muscle actions, SSCs), and are an effective means to introduce resistance exercise in skeletal muscle. SSCs have been studied in the context of human locomotion and athletic performance (Avela and Komi 1998) and have been shown to produce muscle injury owing to the eccentric component of the cycle (Horita et al. 1999). Since natural muscle function is comprised of SSCs, this approach provides an improved physiologically relevant exposure model over the traditional eccentric-only injury model (Komi 2000). The pattern of length changes during SSCs simulates in vivo function more accurately than the ramp stretches typically used (Stevens 1996).

The purpose of this research was to investigate if aging affects the ability of skeletal muscle to adapt to repetitive exposures of SSCs. Skeletal muscle adaptation was assessed by monitoring the changes in isometric and dynamic performance during the chronic exposure period, as well as by morphological changes in skeletal muscle after completion of the SSC protocol. Our general hypothesis is that muscles from older animals have a lower safety threshold or tolerance to repetitive mechanical loading than muscles from young animals. Specifically, we hypothesized that muscles from young animals could adapt to a repetitive mechanical loading protocol that produces hypertrophy, whereas muscles from older animals would be unable to adapt to the same repetitive loading protocol owing to a lower safety threshold or tolerance. Adaptation was defined as a maintenance or increase in contractile performance as a result of the repetitive exposures and the absence of morphological evidence of injury or inflammation. Maladaptation was defined as a decrease in contractile performance and the presence of morphological evidence of injury or inflammation as a result of the exposures.

## Materials and methods

### Animal handling

Male Fischer Brown Norway Hybrid rats ($F_{344} \times BN\ F_1$, $n = 11$) were obtained from the National Institutes on Aging colony. Young adult ($n = 6$, 330 ± 28 g, 12 weeks of age) and old ($n = 5$, 588 ± 32 g, 30 months) rats were housed in AAALAC-accredited animal quarters. Temperature and photoperiod (12 h light : 12 h dark; dark cycle from 07:00 to 19:00) were held constant for all animals and food and water were provided ad libitum. After 1 week of acclimatization, all animals were subjected to a standardized experimental protocol approved by the NIOSH Animal Care and Use Committee.

### Experimental setup

The dorsiflexor muscles were tested on a custom-built rodent dynamometer (Cutlip et al. 1997). The dynamometer provides precise control over the muscle length and muscle force output parameters to be studied. The pennate-fibered dorsiflexor muscle group was chosen in our study so that we could directly compare our data with the findings of many previous studies conducted using this muscle group in mice, rats, and rabbits (Benz et al. 1998; Brooks et al. 1995; Cutlip et al. 2004; Davis et al. 2003; Devor and Faulkner 1999; Faulkner et al. 1989; Geronilla et al. 2003; Lieber and Friden 1993; Lynch and Faulkner 1998; Macpherson et al. 1996; Warren et al. 1996).

A Labview-based virtual instrument was developed that governed a National Instruments data acquisition board (PCI-MIO-16XE-10) and Unidex 100 motion controller (Aerotech Inc., Pittsburgh, Pa.) for precise control of a brushless DC servomotor (1410 DC, Aerotech Inc., Pittsburgh, Pa.) 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., Wau-

kesha, Wis.). Isoflurane was chosen because it has no effect on in vivo force production (Ingalls et al. 1996). After anesthesia, each rat was placed supine on the heated *x–y* positioning table of the rodent dynamometer, with an anesthetic mask placed over its nose and mouth. The knee was secured in flexion (at 90°) with a knee holder. The left foot was secured in the load cell fixture using a custom-built foot holder with the ankle axis (assumed to be between the medial and lateral malleoli) 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. The animal setup was previously described by Cutlip and colleagues (Cutlip et al. 2004).

### Functional testing

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 with angular position of the ankle. Vertical forces applied to an aluminum sleeve fitted over the dorsum of the foot were transmitted to a load cell transducer (Sensotec Inc., Columbus, Ohio) in the load cell fixture. 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 each exposure session to span the peroneal nerve. Accuracy and reliability of repeated electrode placement was validated via pilot studies measuring isometric forces (coefficient of variation < 9.2% for 14 repetitive exposures, $n = 6$). Activation of the electrical stimulator resulted in muscle contraction of the dorsiflexor muscle group. Stimulator settings were optimized based on pilot studies to maximize dorsiflexor isometric force using a supramaximal stimulus. Muscle stimulation for all protocols was a 120 Hz square wave pulse at 0.2 ms pulse duration, and 4 V. To reduce the effect of excitation–contraction fatigue, all electrical stimulation times were kept to a minimum with 2 min of recovery time between stimulations (Ingalls et al. 1998).

### SSC protocol

The young and old age groups (12 week, $n = 6$; 30 month, $n = 5$) were exposed to 8 sets of 10 repetitions of SSCs with 2 min intervals between each set. Within each set, there was a 2 s rest between each stretch–shortening contraction (Fig. 1*a*). For each repetition, the dorsiflexor muscles were fully activated for 100 ms duration via the electrical stimulator, and the eccentric contraction phase was initiated with a 60°/s movement velocity of the load cell fixture over the prescribed range of motion of 90° to 140° ankle angle. The load cell fixture was then immediately returned in the concentric phase at 60°/s to the starting position of 90° ankle angle. The dorsiflexor muscles were then deactivated 300 ms later. Total stimulation time for each repetition was 2.06 s. The exposure paradigm was designed based on findings from a previous study (Geronilla et al. 2003) that indicated that 70 repetitions of SSCs at a higher velocity (500°/s) produced myofiber injury, thus the repetitions were increased with more rest time between repetitions, and the velocity decreased to 60°/s to promote hypertrophy and adaptation.

### Isometric force test

A pre-test isometric contraction (pre-test isometric force) was measured on the dorsiflexor muscle group at an ankle angle of 90° using a 300 ms stimulation duration in a similar fashion as Davis et al. (2003) and Willems and Stauber (2001). An isometric contraction was also performed immediately following the SSC protocol (post-test isometric force), and consisted of 8 sets of 10 stretch–shortening contractions.

### Single stretch–shortening cycle test

A single stretch–shortening contraction was measured on the dorsiflexor muscle group 2 min preceding and 2 min following treatment with the SSC protocol as previously described (Cutlip et al. 2004). This test was used to evaluate the muscle's ability to generate dynamic forces and to perform work during dynamic stretch–shortening. The stretch–shortening contraction was performed by activating the dorsiflexor muscles for 300 ms, then moving the load cell fixture from 70° to 140° at an angular velocity of 500°/s The load cell fixture was immediately returned to 70° at 500°/s (Fig. 1*b*). Activation was continued for 300 ms after cessation of the movement.

### Chronic exposure

The SSC protocol and performance tests were administered three times per week for a total of 14 exposures over a 4.5 week period.

### Histology

Twenty-four hours after the last exposure, rats were weighed, anesthetized with sodium pentobarbital (i.p., 10 mg/100 g body mass), and exsanguinated. The left (exposed, LTA) and right (contra-lateral control, RTA) tibialis anterior muscles were dissected and weighed. The midbelly region of the TA was mounted on cork and immersed in optimal cutting-temperature medium. Samples were allowed to rest for 3 min at room temperature before freeze fixation in isopentane cooled in liquid nitrogen and then stored at –80 °C. Transverse frozen sections (12 μm) were cut, mounted on pre-coated microscope slides, air dried, and stained using a routine procedure with Harris hemotoxylin and eosin. Tissue sections were viewed using a Leica DMLB microscope.

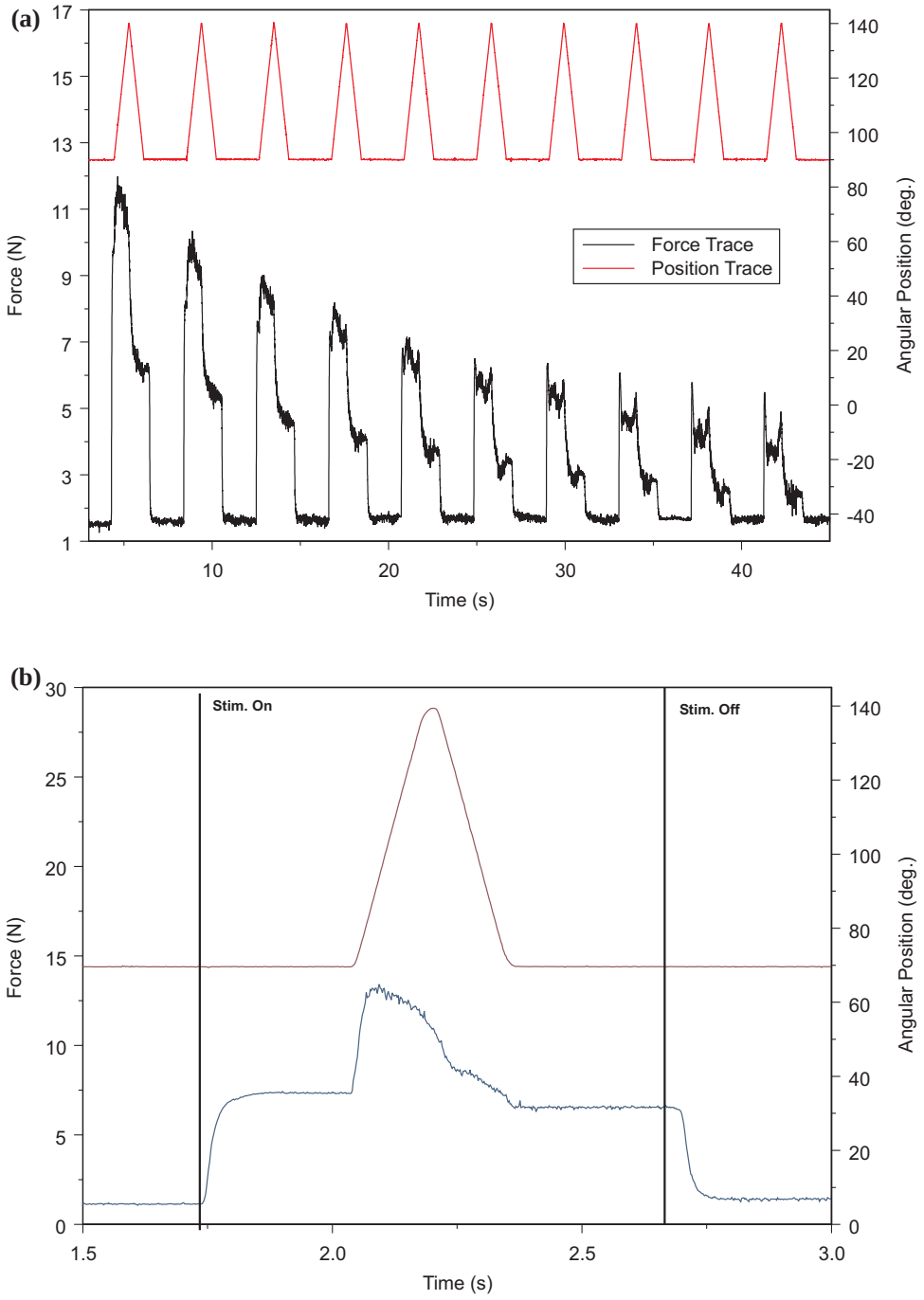
### Muscle quality

Pre-test isometric force measured at the last session of the chronic exposure period was normalized to muscle wet mass of the tibialis anterior of the exposed limb obtained at sacrifice as previously described (Degens and Alway 2003).

### Fiber cross-sectional area

For muscle fiber cross-sectional area (CSA) analysis, 10 non-overlapping digital images were obtained from hematoxylin- and eosin-stained muscle sections at 40× magnification and, subsequently, were analyzed for TA fiber CSA ($\mu m^2$) with National Institutes of Health imaging software (ImageJ). Each fiber was traced with a handheld mouse, and the number of pixels traced was calibrated to a defined area in square micrometres. Approximately 200 fibers were traced per sample. Preliminary studies have shown that a

**Fig. 1.** (A) Real-time force and position trace for 1 set of 10 intermittent SSCs. B) Real-time force and position trace for a single SSC. The displayed force is in Newtons (N) and angular position is in degrees (°) in each image.




sample size of 200 fibers is adequate because this sample number had no change in the standard deviation or the mean of the fiber CSA as compared with a higher number of fibers sampled (data not shown).

## 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). The interstitium was divided into endomysium and perimysium spaces, which included capillaries. A standardized stereological technique, as previously discussed (Baker et al. 2006), was used to quantify the degree of myofiber degeneration and inflammation, which was quantified as either non-cellular interstitium (NCI), indicative of edema, or cellular interstitium (CI), indicative of cellular infiltrates. Fiber volume and surface density were measured using standard morphometric analyses (Underwood 1970; Weibel 1972, 1974, 1975). Briefly, one of the hemotoxylin- and eosin-stained sections was taken from each animal. A stage micrometer was used to identify the mid-point of the section. Point and intercept counts us-

ing a 121-point – 11-line overlay graticule (12.5 mm square 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 mid-point 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, and non-cellular interstitium plus 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 per group, with an $n = 6$ for young and an $n = 5$ for old animals, was evaluated and the results expressed as mean ± SEM. Stereology was used to quantify the degree of myofiber degeneration, and the accompanying changes in the TA muscle from each group. Myofibers were defined using the following criteria: normal myofibers demonstrated (*i*) complete contact with adjacent myofibers, (*ii*) a smooth outer membrane, and (*iii*) no presence of internal inflammatory cells; degenerative myofibers displayed (*i*) a loss of contact with adjacent myofibers, (*ii*) presence of internal inflammatory cells, and (*iii*) an outer membrane interdigitated with inflammatory cells.

### Data analysis

#### *Single stretch–shortening cycle*

The experimental data from each single SSC was used to calculate the group means and standard error of the mean for $F_{peak}$ (peak eccentric force), $F_{min}$ (isometric pre-stretch force), negative work, and positive work. These force and work parameters were calculated for all 14 exposures over the 4.5 week exposure period. Work was calculated in the same fashion as in previous studies (Cutlip et al. 2004; Ettema 1996; Stevens and Faulkner 2000; Stevens 1996). The dynamic force parameters $F_{peak}$ and $F_{min}$ were calculated in the same fashion as previous work by Cutlip et al. (2004) and Geronilla et al. (2003). The data analysis for this study was generated using SAS/STAT software version 8.2 of the SAS System for Windows (SAS Institute Inc., Cary, N.C.). Isometric force, dynamic forces, and work measures were analyzed using the mixed model analyses of variance (age × day) with repeated measures on day using the "Proc Mixed" command. A spatial power covariance structure was used for the repeated measure aspect to appropriately model the spacing of the exposures. Stereological measures were analyzed using a factorial (age × limb) mixed-model analysis of variance with animal as the random factor to account for measures in both limbs. Post hoc comparisons between groups at each exposure were analyzed using the slice option in SAS.

#### *Muscle masses*

Data for muscle mass differences between the exposed muscle and control muscle were analyzed using a 2-sample *t* test procedure assuming unequal variances.

#### *Stereology*

Statistical analyses for stereology were conducted using SAS version 8 (SAS Institute Inc.). Stereological measurements for volume and thickness of cellular and noncellular components and muscle quality were analyzed using 2-way mixed-model (age × limb) ANOVAs with the animal as the random factor accounting for measures in both limbs.

## Results

### Functional performance

#### *Isometric force*

The pre-test isometric force response over the chronic exposure period was different between the old and young age groups ($p = 0.0003$, Fig. 2). Despite the age difference, the young and old animals generated very similar magnitudes of isometric force on the first day of exposure ($p = 0.455$, Fig. 2). However, the isometric forces between the 2 groups started to diverge by day 8 (fourth exposure) of the exposure period ($p = 0.045$, Fig. 2) and continued to diverge throughout the exposure period, resulting in a substantial difference in isometric force generated by the two age groups by the end of the 30 d exposure period (14th exposure, $p < 0.0001$, Fig. 2). The pre-isometric test showed a 25% increase in isometric force for the young group after 4.5 weeks, whereas the old group had a decrement of 34%.

#### *Peak force ($F_{peak}$)*

The peak eccentric force response over the chronic exposure period was also quite different for the 2 age groups ($p = 0.0081$, Fig. 3*a*). The young and old animals produced similar $F_{peak}$ forces during the pre-exposure SSC on the first day of exposure ($p = 0.761$, Fig. 3*a*), but began to diverge by day 15 (7th exposure, $p = 0.013$, Fig. 3*a*). The $F_{peak}$ generated by the 2 age groups continued to diverge throughout the remainder of the exposure period, resulting in an increase of 27.9% in the young group and a decrease by 22.4% in the old group by the end of the 4.5 week exposure period ($p < 0.0001$, Fig. 3*a*).

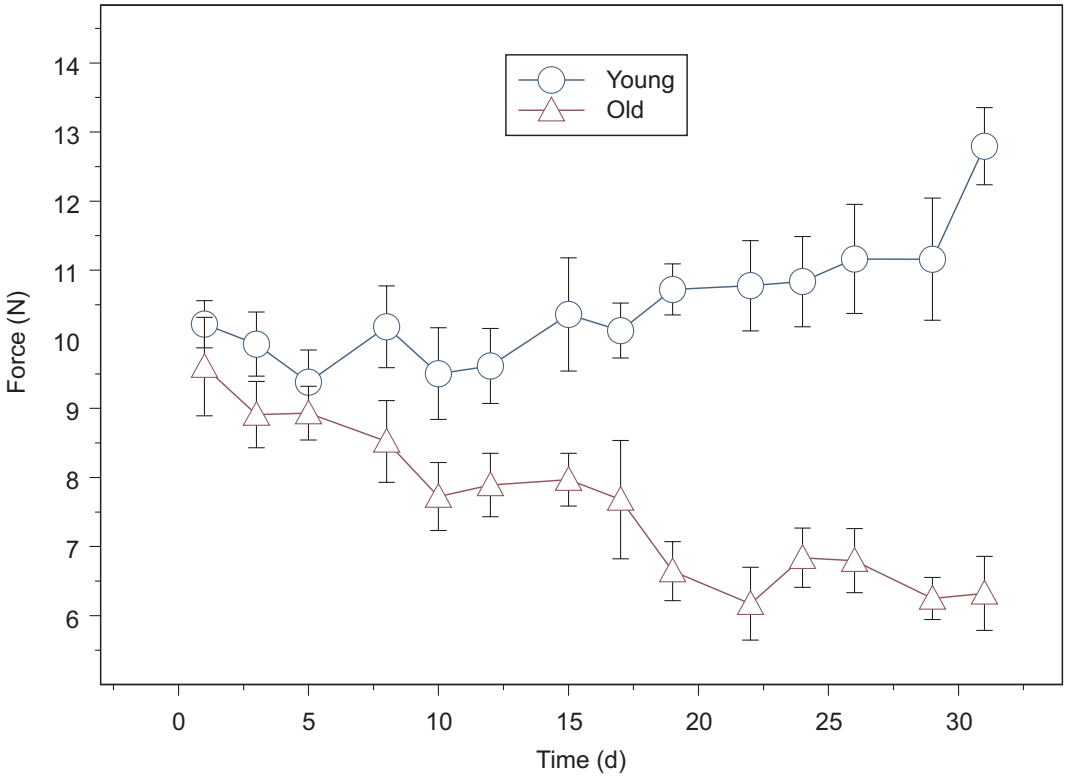
#### *Minimum force ($F_{min}$)*

The minimum force response over the chronic exposure period was similar to the peak force and isometric force responses ($p = 0.0011$, Fig. 3*b*). The 2 age groups produced similar $F_{min}$ forces during the SSC cycle on the first day of exposure ($p = 0.853$, Fig. 3*b*), but began to diverge by day 19 (9th exposure, $p = 0.002$, Fig. 3*b*). The $F_{min}$ generated by the 2 age groups continued to diverge throughout the remainder of the exposure period much like the $F_{peak}$ and isometric force, thus resulting in an increase in $F_{min}$ of 25.9% in the young group and decrease of 32.5% in the old group by the end of the 4.5 week exposure period ($p < 0.0001$, Fig. 3*b*).

### Work

The age of the animal also influenced the ability of the dorsiflexor muscles to both absorb work (negative work) and produce work (positive work). The 2 age groups exhib-

**Fig. 2.** Pre-test isometric force of the young and old groups at each of the 14 exposures during the chronic exposure period. The pre-test isometric force response over the chronic exposure period was quite different for the old and young groups ($p = 0.0003$). The old and young groups generated very similar magnitudes of isometric force on the first day of exposure ($p = 0.455$). The isometric forces between the 2 groups diverged throughout the exposure period, resulting in a substantial difference in isometric force generated by the 2 age groups by the end of the 30 d exposure period (14th exposure, $p < 0.0001$). Data are reported as mean values ± standard error.




ited the same ability to absorb work during the first exposure ($p = 0.455$, Fig. 4*a*). However, the ability to absorb work differed between age groups with subsequent exposures during the chronic exposure period ($p = 0.0044$, Fig. 4*a*). Negative work increased by 27.0% in the young group and decreased by 24.9% in the old group, resulting in a significant difference by the end of the chronic exposure period ($p < 0.0011$, Fig. 4*a*). The change in response became significantly different between the old and young groups on day 15 (exposure 7) of the chronic exposure ($p = 0.0063$, Fig. 4*a*).

The 2 age groups also exhibited the same ability to produce positive work during the first exposure ($p = 0.475$, Fig. 4*b*). However, in a fashion similar to that for negative work, the ability to produce positive work differed between age groups with subsequent exposures during the chronic exposure period ($p = 0.0011$, Fig. 4*b*). Positive work increased by 26.5% in the young group and decreased by 39.2% in the old group, resulting in a substantial difference by the end of the chronic exposure period ($p < 0.0011$, Fig. 4*b*). A significant change in the response was observed between the old and young groups on day 15 (exposure 7) of the chronic exposure ($p = 0.016$, Fig. 4*b*).

### Muscle wet masses

Masses of the exposed muscles and contralateral control muscles were normalized to the body mass at the end of the exposure period as shown in Fig. 5. Following 4.5 weeks of SSC contractions, the wet mass of the exposed muscle from young animals was significantly increased when compared with the contralateral control muscles ($p < 0.001$). However, there was no difference between the exposed muscle and control muscle wet mass in old animals after the experimental intervention. Further analyses revealed a significant increase in the wet mass of exposed muscles in young animals when compared with the exposed muscles in old animals ($p < 0.001$). In addition, there was a significant difference between the young contralateral control and the old contralateral control muscle mass ($p < 0.001$).

### Fiber cross-sectional area

Review of the histological sections is supportive of the muscle wet mass data. Representative sections from the young exposed LTA (Fig. 6*a*) depict normal morphology and larger fibers than the young non-exercised RTA (Fig. 6*b*), indicative of a hypertrophic response. However, the muscle sections from the old exercised LTA (Fig. 6*c*) depicts cellular infiltrates and central nucleated fibers that are not evident in the samples from the non-exercised limb in either the old animals (Fig. 6*d*) or the young animals (Figs. 6*a* and 6*b*).

In the contralateral control muscle (RTA), approximately 45% of the fibers from the old animals and 54% of the fibers from the young animals were ≥1500 $\mu m^2$ (Fig. 7). In the exposed limb (LTA), approximately 46% of the fibers from the old animals and 76% of the fibers from the young animals were ≥1500 $\mu m^2$ (Fig. 8). This indicates that exposure to chronic SSCs resulted in an increased percentage of

**Fig. 3.** (*a*) Peak force ($F_{peak}$) of the young and old groups at each of the 14 exposures during the chronic exposure period. The peak force response over the chronic exposure period was quite different for the old and young age groups ($p = 0.0081$). The old and young groups generated very similar magnitudes of peak force on the first day of exposure ($p = 0.761$). The peak forces between the 2 groups diverged throughout the exposure period, resulting in a substantial difference in peak force generated by the 2 age groups by the end of the 30 d exposure period (14th exposure, $p < 0.0001$). Data are reported as mean values ± standard error. (*b*) Minimum force ($F_{min}$) of the young and old groups at each of the 14 exposures during the chronic exposure period. The minimum force response over the chronic exposure period was quite different for the old and young groups ($p = 0.0011$). The old and young groups generated very similar magnitudes of minimum force on the first day of exposure ($p = 0.853$). The minimum forces between the 2 groups diverged throughout the exposure period, resulting in a substantial difference in minimum force generated by the 2 age groups by the end of the 30 d exposure period (14th exposure, $p < 0.0001$). Data are reported as mean values ± standard error.

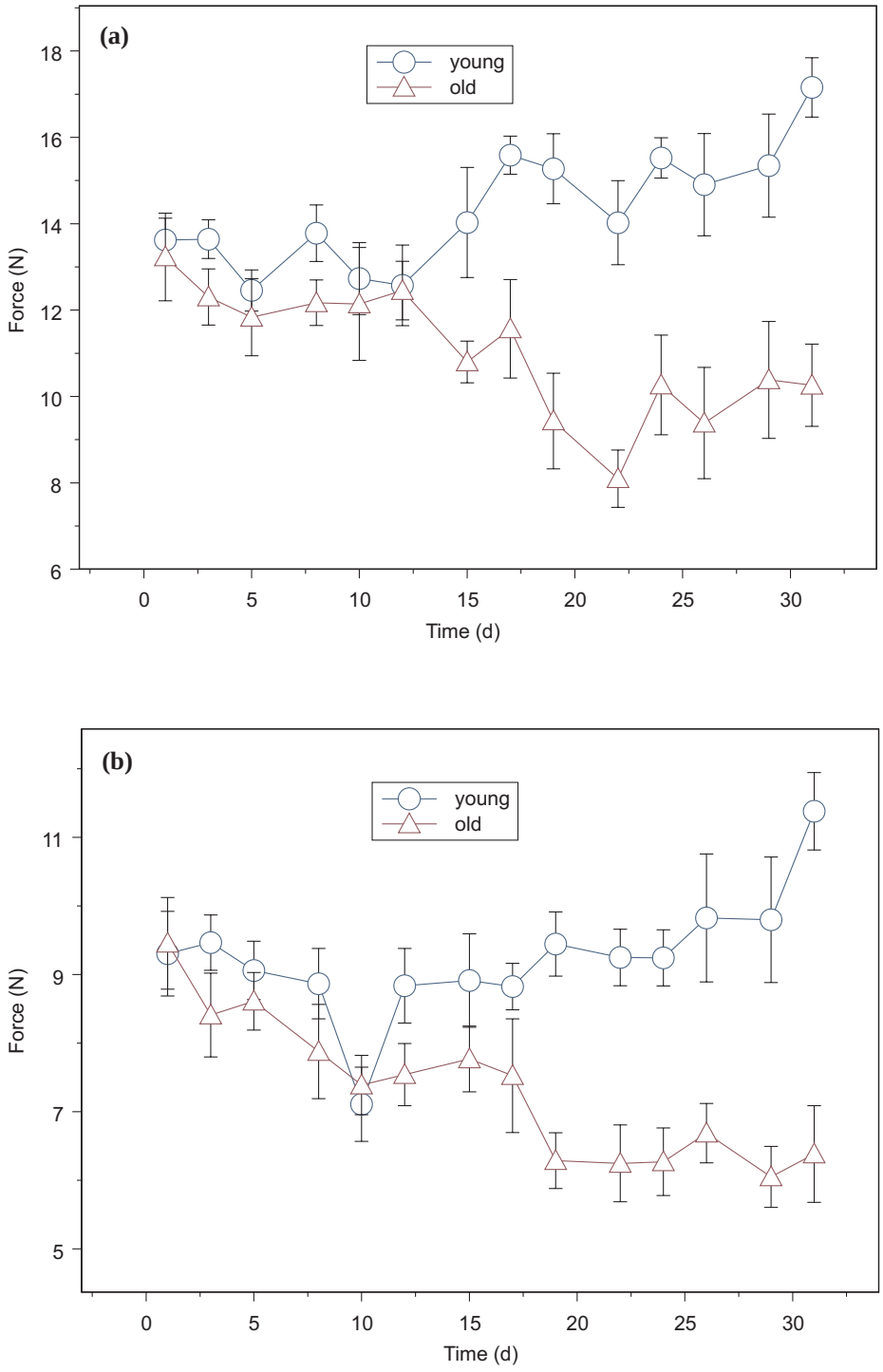


**Fig. 4.** Single SSC work parameters from the single SSCs performed before each SSC protocol. (*a*) The 2 age groups exhibited the same ability to absorb negative work during the first exposure ($p = 0.455$). The ability to absorb negative work differed between age groups with subsequent exposures during the chronic exposure period ($p = 0.0044$). The young group increased its negative work by an average of 27.0%, whereas the old groups' negative work decreased by 24.9%, resulting in a substantial difference by the end of the chronic exposure period ($p < 0.0011$). Data are reported as mean values ± standard error. (*b*) The 2 age groups exhibited the same ability to produce positive work during the first exposure ($p = 0.475$). The ability to produce positive work differed between age groups with subsequent exposures during the chronic exposure period ($p = 0.0011$). The young group increased its positive work by an average of 26.5%, whereas the old groups' positive work decreased by 39.2%, resulting in a substantial difference by the end of the chronic exposure period ($p < 0.0011$). Data are reported as mean values ± standard error.

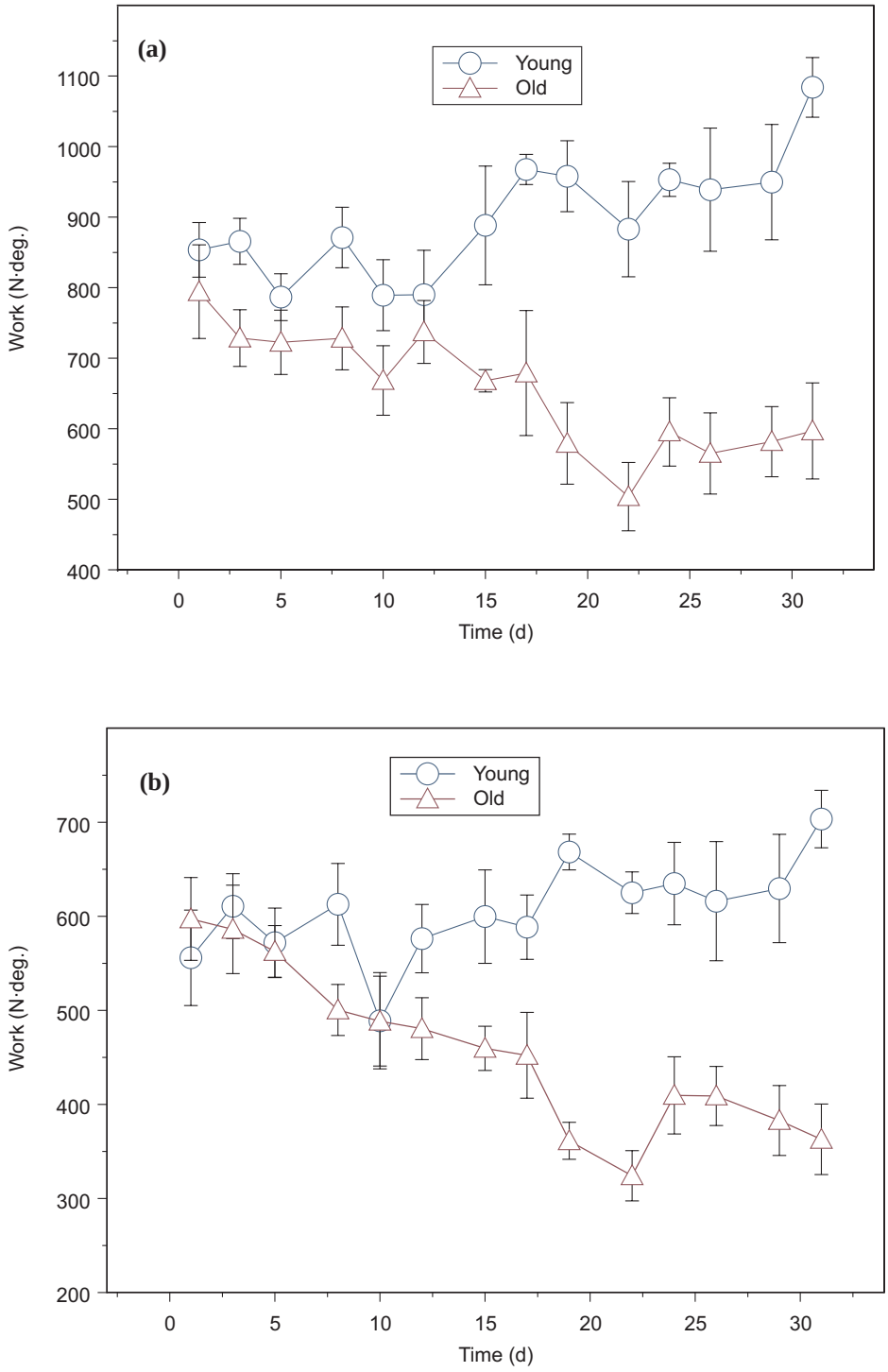
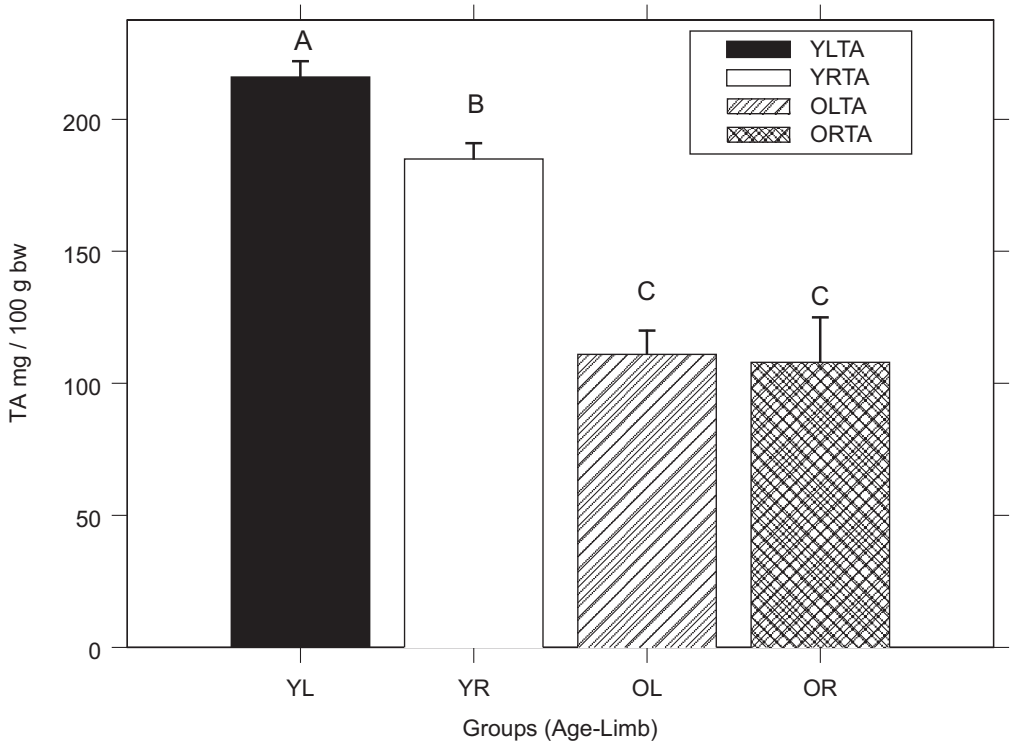


**Fig. 5.** Muscle wet masses of the left (exercised) and right (control) tibialis anterior muscle (standardized to body mass) for young and old animals after chronic exposure. Exercised muscles from the young animals are significantly larger than the control muscles from the young animals and exercised and control muscles from the old animals. Control muscles from the young animals are significantly larger than both the control and exercised muscles from the old animals. The exercised muscles from the old animals are not statistically different from the control muscles in the old animals. Different letters denote statistical significance at the 0.05 level. Data are reported as mean values ± standard error.




larger fibers in the young animals (from 54% to 76%) without a corresponding shift to larger fibers in the older animals (45%–46%).

### Body masses

The average body mass of the young group was 331 g at the start of the study and increased by an average of 3.3% over the 4.5 week protocol. The average body mass of the old group was 588 g at the start of the study and decreased by an average of 11.2% over the 4.5 week exposure.

### Muscle quality

The mean value of isometric force normalized to muscle wet mass was 0.017 ± 0.001 N/mg and 0.010 ± 0.0009 N/mg for the young and old groups, respectively (Fig. 9). The young group had a significantly greater isometric force normalized to muscle wet mass in the exercised limb after the chronic SSC protocol than the old group ($p$ = 0.001, Fig. 9).

### Stereological analyses of myofiber degeneration

Statistical analysis showed that there were no degenerative myofibers present in muscle samples analyzed from either the young or old animals.

### Stereological analyses of inflammation

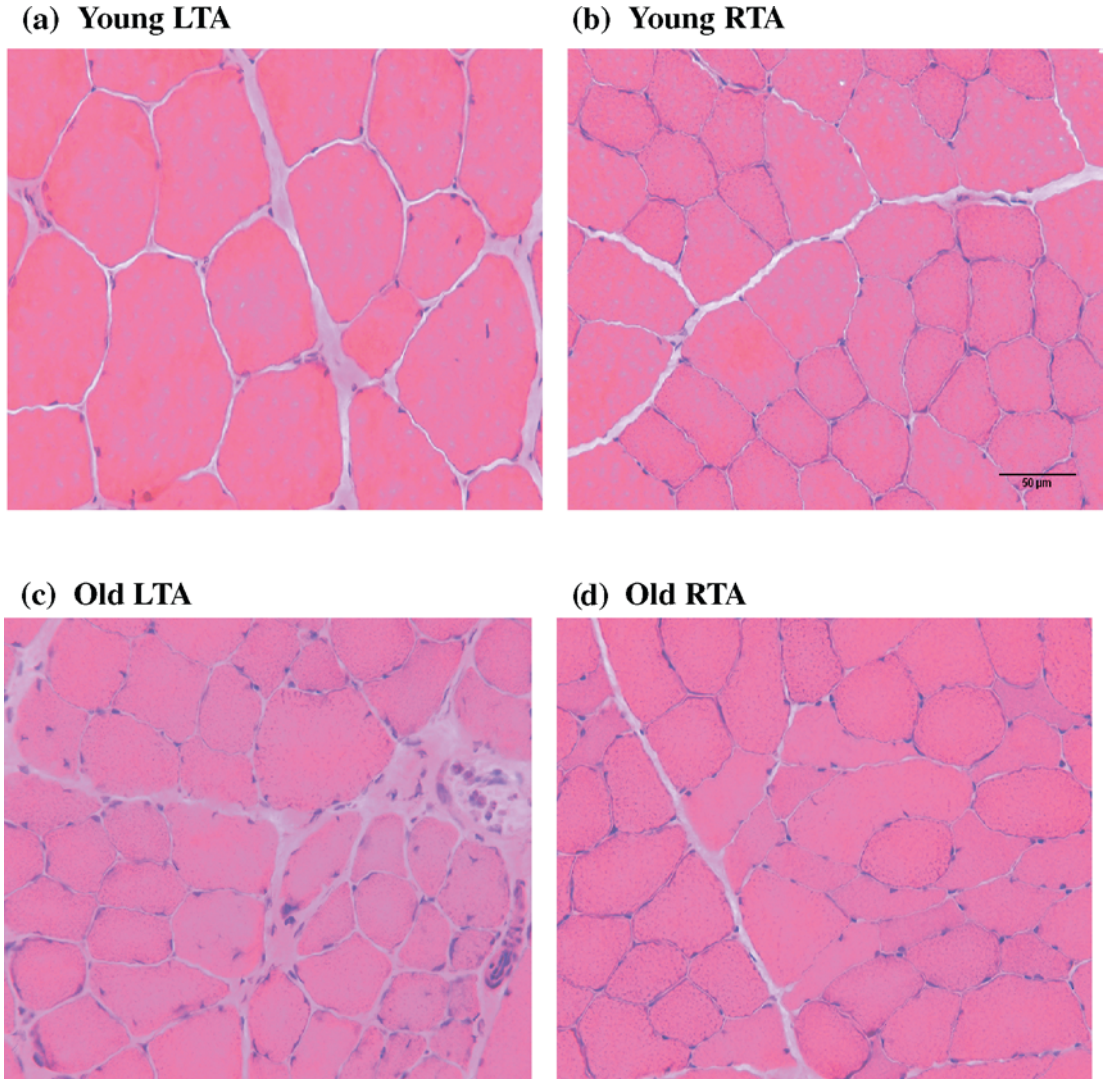
We observe no change in NCI as a result of the repetitive loading intervention in either age group, which suggests that no edema was present. Nevertheless, there was a significant effect of age ($p$ = 0.02) for the volume density of CI. The volume density of CI in the exposed muscles was significantly greater in old as compared with young animals ($p$ = 0.01, Fig. 10). Furthermore, the volume of CI was significantly higher in the exposed muscles when compared with the contralateral control muscles of old animals ($p$ = 0.05, Fig. 10). Also, volume density of CI was significantly higher in the exposed muscles of old animals when compared with the control muscles of young animals ($p$ = 0.01, Fig. 10).

## Discussion

The major finding of this study is that the dorsiflexor muscles from young animals adapt functionally to 4.5 weeks of SSC exposure, whereas the same SSC protocol results in functional maladaptation in muscles from old animals. This maladaptation in old animals does not appear to be the result of necrotic myofibers due to an absence of morphological markers of myofiber degeneration. However, the exposed muscles of old animals did exhibit an increased cellular interstitium response, indicative of inflammation, suggesting the myofibers may have been under stress, but not to the level of necrosis.

The observation that the functional performance of both the young and old animals was similar at the start of the exposure period is critical, because this establishes that age did not have an affect on the ability of untrained animals to generate force at the onset of the exposure protocol. Addition-

**Fig. 6.** Hematoxylin- and eosin-stained sections of the exercised tibialis anterior muscle (LTA) and non-exercised tibialis anterior muscle (RTA) from young rats (*a* and *b*) and old rats (*c* and *d*), respectively. Micrographs shown are representative of the overall groups. The micrograph from the LTA of the young animals (*a*) depicts normal morphology and larger fibers than the non-exercised RTA (*b*). The micrograph from the LTA of old animals (*c*) depicts cellular infiltrates, and central nucleated fibers that are not evident in the samples from the non-exercised limb in either the old animals (*d*) or young animals (*a* and *b*). Scale bar = 50 μm for all panels.




ally, isometric performance and dynamic performance, as indicated by isometric pre-stretch forces ($F_{min}$) and peak eccentric forces ($F_{peak}$) during the single SSC, as well as negative and positive work absorbed and produced during the SSC, were not different at the start of the exposure. This is consistent with Brooks and Faulkner (1994), who reported no difference in concentric performance in old and young animals or in isometric performance in soleus muscles of mice. There was, however, evidence of lower forces in the TA of older animals when normalized for muscle dry mass (Brooks and Faulkner 1988). Brooks and colleagues (Brooks et al. 2001) also showed no initial difference in isometric performance of the TA or extensor digitorum longus (EDL) muscles in adult versus old mice.

In earlier studies, a single exposure to injurious eccentric exercise resulted in similar isometric force decrements regardless of age in mice (Brooks and Faulkner 1996; Zerba and Faulkner 1990; Koh et al. 2003) and rats (Gosselin 2000), but older animals can exhibit larger force deficits with more severe exposures (Zerba et al. 1990). However, McBride et al. showed that muscle from adult animals exposed to damaging eccentric contractions in situ exhibit isometric force recovery 14 d after the exposure, whereas muscles from old animals did not (McBride et al. 1995). Our data are consistent with these findings in that old animals appear unable to recover from repeated bouts of SSCs as evidenced by their reduced force-generating capacity.

Although aging muscles have a delayed recovery from a single injurious exposure, these muscles do retain some ability to adapt. For example, when damaging eccentric muscle actions are incorporated into a repetitive exposure model, there is evidence that age does not have a deleterious effect on adaptation when this eccentric protocol is administered once per week in vivo (Brooks et al. 2001). In support of

**Fig. 7.** Mean cross-sectional area (CSA, $\mu m^2$) distribution in young and old contralateral, non-exercised tibialis anterior muscles (RTA). The frequency histogram depicts the frequency of fibers that lie above or below our arbitrary cut-off of 1500 $\mu m^2$. Approximately 45% of the fibers from the older animals and 54% of the fibers from the younger animals were ≥1500 $\mu m^2$.

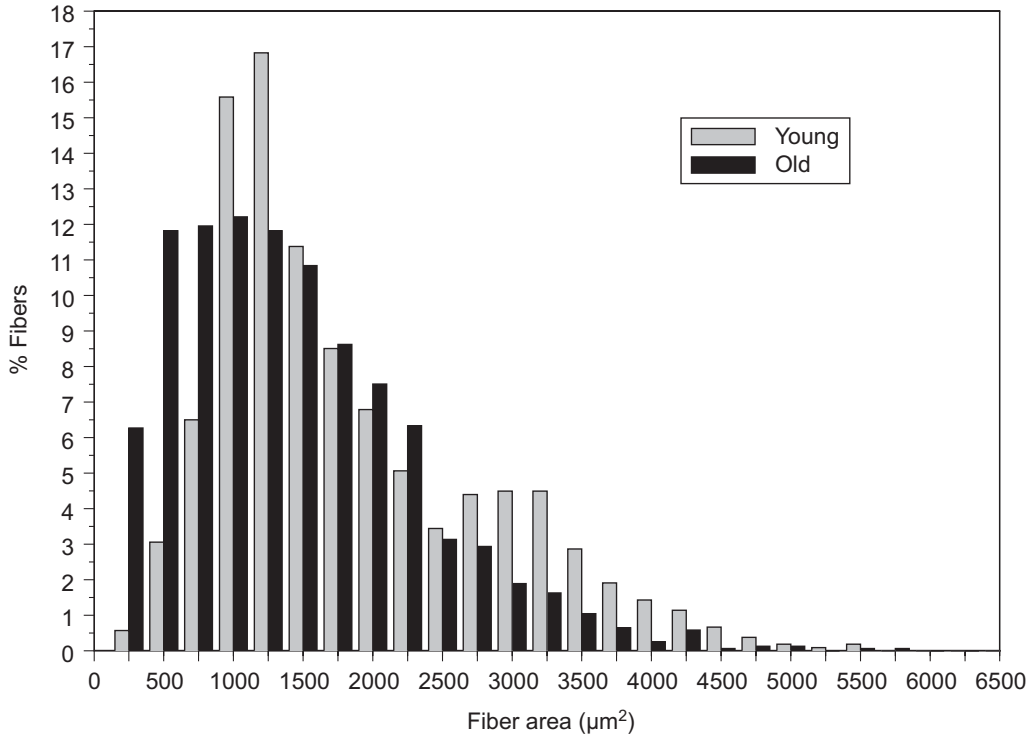



**Fig. 8.** Mean cross-sectional area (CSA, $\mu m^2$) distribution in young and old exercised tibialis anterior muscles (LTA). The frequency histogram depicts the frequency of fibers that lie above or below our arbitrary cut-off of 1500 $\mu m^2$. Approximately 46% of the fibers from the older animals and 76% of the fibers from the younger animals were ≥1500 $\mu m^2$ . Note the occurrence of small fibers (≤1500 $\mu m^2$) in the old, exercised tibialis anterior muscle.

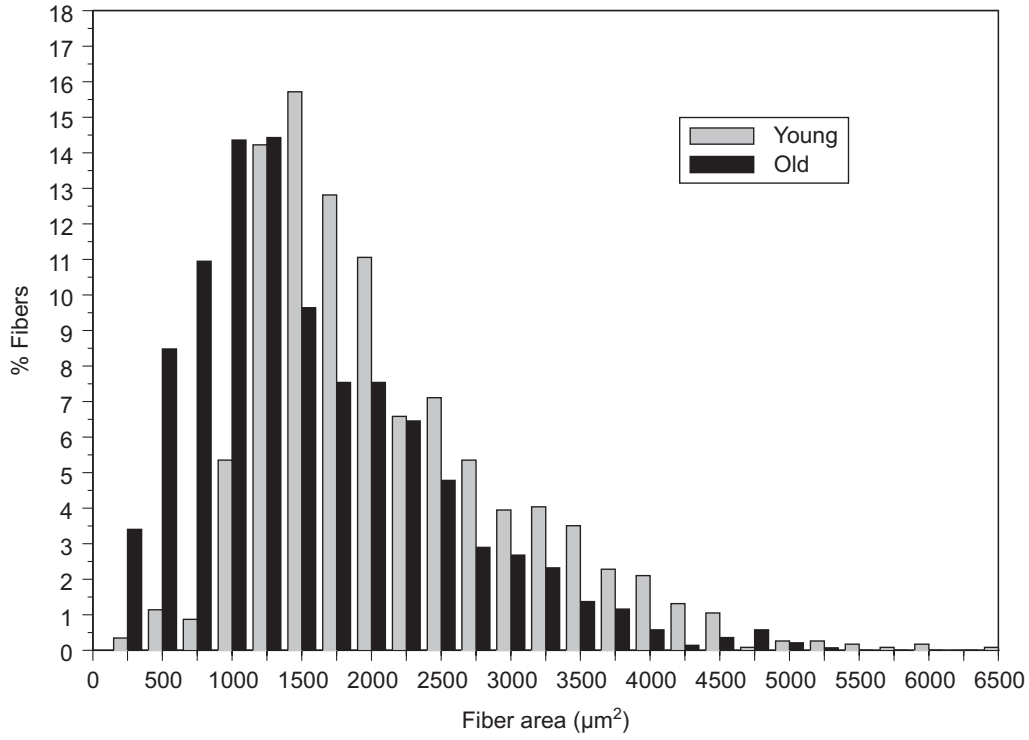


**Fig. 9.** Isometric force normalized to muscle wet mass of the tibialis anterior after the chronic exposure period (N/mg). The young group exhibited values that are significantly greater than the old group. Significance is reported at the 0.05 level. Data are reported as mean values ± standard error.

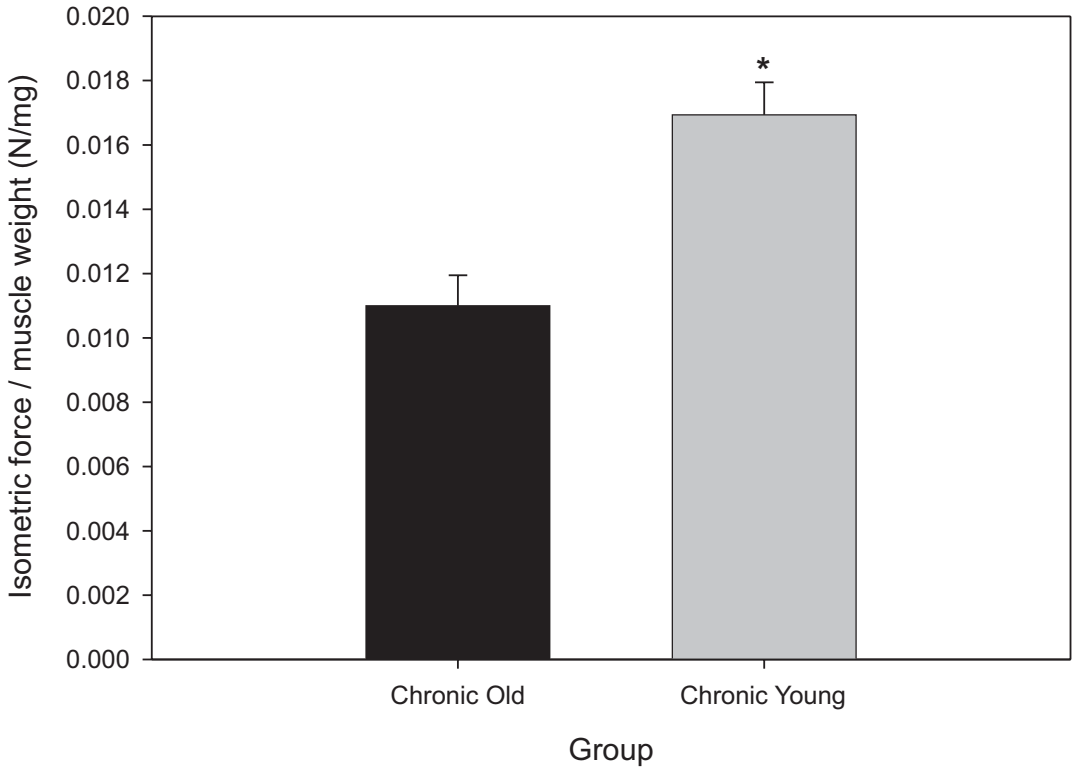
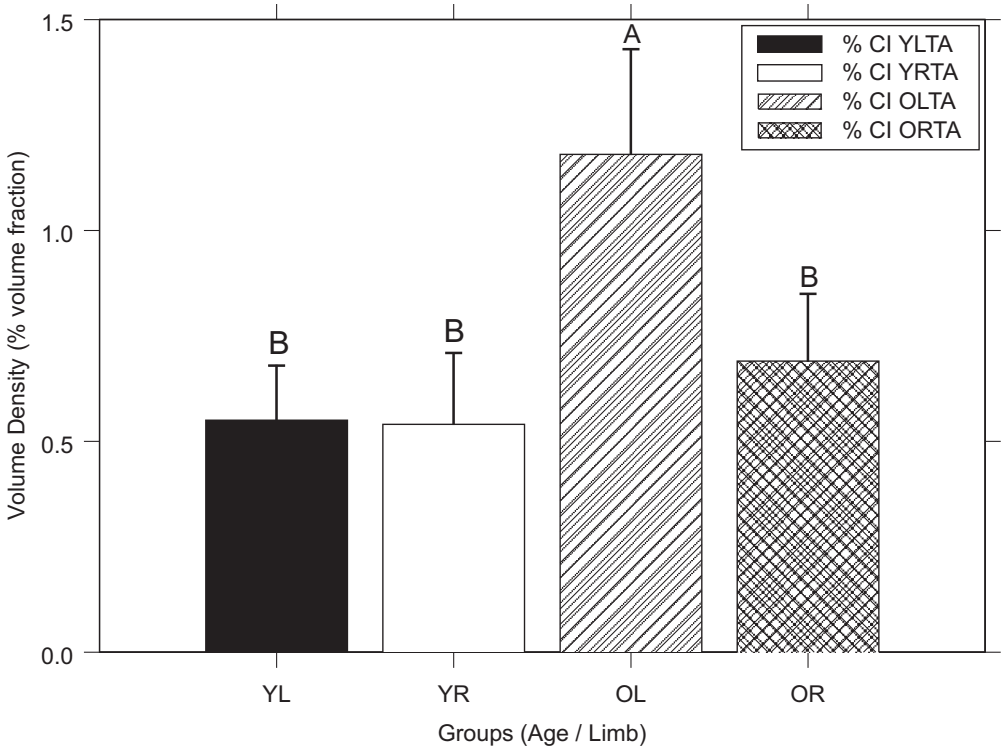



**Fig. 10.** Volume density (% volume fraction) of the cellular interstium (CI) in the young and old animals' left (treated) and right (control) tibialis anterior. The volume density of the CI was greater in the treated limb of the old animals than in all other groups. All other comparisons were not significantly different. Different letters denote significance at the 0.05 level. Data are reported as mean values ± standard error.



this, adult and old mice exhibited similar isometric and peak force deficits of the dorsiflexors after the initial exposure, and the recovery of those forces during rest periods between bouts of exposures was also similar (Brooks et al. 2001). Moreover, after 6 weeks of conditioning, the maximal isometric force was not different between age groups. In spite of this, the amount of rest between exposures can affect the ability to adapt to potentially beneficial and (or) injurious contractions, particularly in aged animals. The exposure paradigm used in the current study resulted in an average increase in force of 25% above the pre-test force in young animals (indicative of adaptation), while producing a 34% deficit in old animals (indicative of maladaptation). The peak eccentric force also increased approximately 28% in young animals, while decreasing 22.4% in the old animals. In contrast, Brooks et al. (Brooks et al. 2001) reported a significant increase in peak force by week 6 for both adult and old animals over baseline values; however, this was reported in mice. Thus, a differential effect may exist between species, along with the previously noted difference in rest between exposures.

To our knowledge, this is the first study to report that changes due to repetitive SSC resulted in a significant increase in negative and positive work in young animals over baseline values that was commensurate with increases in isometric force and peak eccentric forces. Conversely, the protocol produced significant decreases in negative and positive work in the old animals as compared with baseline values that was commensurate with losses in isometric force and peak eccentric forces. The results from the repetitive-loading model of Brooks et al. (Brooks et al. 2001) indicated that older animals require more time to adapt to injurious muscle contractions as assessed by a force decrement after exposure. Our results are consistent with this observation; because exposure to injurious contractions is repetitively administered; older animals were less able to adapt.

The morphological changes that occurred during the chronic exposure period were quite different between the 2 age groups. The exposed muscle mass in young animals was ~17% greater than the contra-lateral control muscle after 4.5 weeks of repetitive loading. This corresponds well with the magnitude of hypertrophy in young animals reported in previous work in which dorsiflexor muscles from young male mice were exposed to a 6 week conditioning program (Faulkner et al. 1992). Also, during a 10 week training protocol, the TA muscle of rats exhibited a 16%–30% increase in muscle mass compared with the contralateral limb (Wong and Booth 1990). Moreover, a 6 week conditioning program in dorsiflexor muscles of female mice resulted in protection from contraction-induced injury in both old and young animals, despite no measurable hypertrophy. Brooks et al. (Brooks et al. 2001) hypothesized that this must be due to intrinsic strengthening of the sarcomeres within the myofibrils via regeneration of stronger sarcomeres, which are more resistant to injury. Earlier work conducted by Devor and Faulkner supports this hypothesis (Devor and Faulkner 1999). In our study, performance significantly declined over the 4.5-week exposure period in the old animals, even though eccentric contractions were incorporated in the exposure paradigm.

In our study, stereological analyses detected no degenerative myofibers or indications of edema following the terminal session, even though there was a significant increase in the volume of CI, indicative of inflammation, in the exposed muscles when compared with the contralateral limb. This absence of degenerative myofibers, or necrotic fibers, is in apparent contrast to previous studies of acute exposure to eccentric contractions (Faulkner et al. 1989; Koh et al. 2003; Van Der Meulen et al. 1997; Devor and Faulkner 1999; Lieber and Friden 1993), and SSC exposure (Geronilla et al. 2003) where necrotic fibers have been reported. Moreover, this absence of degenerative myofibers in old animals exhibiting a significant decrement in functional performance is a novel finding, which needs further exploration regarding the causal mechanisms. Thus, an important factor involving the remodeling of skeletal muscle during adaptation is the modifications made to the interstitial space. This is not only accomplished through interactions with the myofiber (muscle specific factors), but also through coordinated interaction with the CI (systemic, non-muscle specific factors). Therefore, the inability of old animals to adapt may be more influenced by the interstitial space and extrinsic environment (Conboy et al. 2005).

The data in the present study clearly show that exposed muscles in young rats adapt to chronic SSC exposure by increasing muscle mass. The increase in muscle wet mass could have resulted from and be attributed to chronic edema, but based on our findings, we do not believe this is the case. Results from our muscle cross-sectional area data support the observation that there was a substantial degree of myofiber hypertrophy in the young, exposed animals as evidenced by a shift to larger fibers. In addition, stereological analysis of exposed muscle from young rats showed no increases in NCI response, (i.e., no edema). The observed increase in CI response, indicative of inflammation, may have prevented the decline in muscle wet mass that would have been expected by the decline in muscle force and power. However, the observation that old animals did not show signs of degeneration supports the hypothesis established by Conboy and colleagues that there is at least a minimal regenerative potential (Conboy et al. 2005) as indicated by the maintenance of normal myofibers, which is preserved with aging. Presumably, this is accomplished with modifications to the interstitial space via signals to muscle satellite cells (Conboy et al. 2005). Despite previous reports that indicate skeletal muscles in old animals are more susceptible to injury (Brooks and Faulkner 1996; Zerba et al. 1990) and recover more slowly (McBride et al. 1995; Brooks and Faulkner 1990) from a single exposure to injurious contractions, in support of our findings is evidence that old animals can be conditioned for protection from contraction-induced myofiber injury (Brooks et al. 2001). Muscle hypertrophy and improvements in force production occur in response to constant or chronic loading in aged animals, although the extent of muscle enlargement is attenuated relative to young animals (Alway 1995; Alway et al. 2002; Carson et al. 1995; Klitgaard et al. 1989*a*, 1989*b*; Lowe et al. 1998). However, in our current study the frequency and (or) intensity of exposures likely exceeded the ability to recover from the preceding SSC exposure in muscles from old animals, thus exceeding their safety threshold or tolerance.

In addition to age, several factors may explain the differ-

ence in performance between the 2 groups during the chronic protocol. The older animals may not have tolerated the repeated exposure to isoflurane as well as their younger counterparts that could have affected contractile performance. Also, there may have been differences in excitation–contraction coupling or muscle activation sensitivity between age groups as the chronic protocol progressed. Myofiber cross-sectional area and stereology data from the contralateral limbs of both groups were not different, which suggests that there was not a different systemic response. The lack of myofiber degeneration in the exposed limbs of the old group suggests that the loss of function is not due to fiber necrosis. Further studies are needed to delineate the contributions of repeated anesthesia and multiple exposures of SSCs. Reducing the number of exposures per week may allow the older animals to adapt to the repetitive exposures. Titrating the number of exposures per week and number of repetitions per exposure to further explore the safety threshold of aged skeletal muscle is needed.

Our findings indicate that the frequency of exposures may have profound implications on the ability to adapt to repetitive SSCs. Identifying the safety threshold of skeletal muscle under repeated loading conditions and how it is affected by age, physical training, and lifestyle (e.g., diet, supplementation), is of major importance when designing preventative strategies in vocational and recreation arenas, as well as for understanding the etiology of repetitive loading injuries. In the present study, we show concurrent adaptation in young animals and maladaptation in old animals in the absence of myofiber degeneration. This strongly suggests that there is a level of exposure where the ability to adapt to mechanical exposure is severely compromised by age (Degens and Alway 2003). Whereas muscle strength may be maintained and, in some instances, enhanced in senescence via resistive loading, the frequency of loading may play a key role in the ability of skeletal muscle to adapt in elderly populations. Although identifying the mechanisms responsible for the maladaptation to repetitive loading in aged muscles is beyond the scope of this study, further investigation is warranted.

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