ABSTRACT: Aging impairs the ability of muscle to adapt to exercise or injury. The goal of this study was to determine whether age-related changes in muscle adaptability could be the result of satellite cell apoptosis. Ten days after exposure to an injury protocol, estimates of edema in the exposed tibialis anterior muscles were higher in old (30 months) than young (3 months) rats, and isometric force levels were lower in old rats. Both young and old rats displayed an increase in MyoD labeling in the exposed muscle, indicating that injury induced satellite-cell activation. However, there were more MyoD-labeled cells that coexpressed the proapoptotic factor, Bax, in old than in young rats, suggesting that decrements in muscle recovery may be associated with an increase in satellite-cell apoptosis. Based on these findings we conclude that reducing satellite-cell apoptosis in aged animals may improve muscle recovery after injury.

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PROAPOPTOTIC FACTOR BAX IS INCREASED IN SATELLITE CELLS IN THE TIBIALIS ANTERIOR MUSCLES OF OLD RATS

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With age, the ability of skeletal muscle to adapt to strenuous physical activity or regenerate after injury is impaired.[18] A number of mechanisms underlie this age-related decline in muscle regeneration including reductions in muscle innervation,[14] altered production of systemic and local factors that regulate regeneration and repair processes,[10] and reduced satellite-cell activation.[19]

Satellite-cell proliferation and differentiation play a critical role in skeletal muscle recovery after injury and adaptation in response to hypertrophic stimuli.[19] During the regeneration and repair process, chemotaxic signals from surrounding skeletal muscles and infiltrating immune cells stimulate satellite-cell activation and differentiation.[28] Satellite cells can differentiate into myoblasts and fuse with existing myofibers to repair muscle, or become quiescent and return to the pool of cells available for proliferation.[31] Pathologic processes or manipulations that interfere with satellite-cell activation and differentiation hinder the regeneration of muscle after contraction-induced injury.[7]

Normal aging hinders satellite-cell proliferation and differentiation,[7,25] and age-related changes in these repair processes appear to underlie the reduced rate of tissue and functional recovery often displayed in old animals after injury. In addition, aging is associated with an increased expression of a number of proapoptotic factors in skeletal muscle.[27] Thus, it is possible that age-related changes in satellite-cell apoptosis also might underlie changes in skeletal muscle adaptation and regeneration. Although it has been implied that myonuclear apoptosis may occur with loading and unloading in aged animals,[27] it is not clear how changes in satellite-cell apoptosis are associated with age-related decrements in functional recovery.

Abbreviations: AIF, apoptosis inducing factor; Bax, Bcl-2 associated X protein; Bcl-2, β-cell leukemia/lymphoma 2; Id2, inhibitor of differentiation factor 2; IGF-I Ea, insulin-like growth factor (liver type); MGF, mechanogrowth factor; PBS phosphate-buffered saline; PBS-Tx, PBS with Triton X-100; qRT-PCR, quantitative reverse transcriptase–polymerase chain reaction; SSCs, stretch-shortening cycles; TA, tibialis anterior

Key words: aging; apoptosis; contraction-induced injury; isometric force; satellite cells

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The goal of this study was to determine whether age-related differences in force production in injured tibialis anterior (TA) muscles of rats were associated with increases in satellite-cell or myofiber apoptosis. In addition, we examined the effects of aging and injury on the expression of genes involved in mediating apoptosis and cell survival in young and old animals to begin to understand the mechanisms that might underlie changes in satellite-cell and myofiber survival after injury. Understanding the processes that underlie the age-related declines in the ability of muscle to recover after injury may provide information for developing treatment strategies to improve recovery in the elderly.

MATERIALS AND METHODS

Animals. Young (n = 6, 3 months old, 330 ± 28 g) and old (n = 6, 30 months old, 588 ± 32 g) male Fischer 344 × Brown Norway (FBNF1) rats were obtained from the National Institute on Aging colony housed at Harlan (Indianapolis, Indiana). Rats were housed in accredited facilities, where the temperature and the light/dark cycle were controlled, and food and water were provided ad libitum. All procedures were approved by the National Institute for Occupational Safety and Health Animal Care and Use Committee and were in compliance with Public Health Service Policy.

Equipment. Rats were tested on a custom-built rodent dynamometer designed to test the dorsiflexor muscle group. The dynamometer and software used for conducting the experiment and collecting data has been described elsewhere.[4,5,12]

Functional Testing. Rats were anesthetized with 5% isoflurane gas using a small animal anesthetic system (Surgivet Anesco, Waukesha, Wisconsin). The knee was secured in flexion (at 1.57 radians) 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.[12] After being positioned in the dynamometer, the joint position of each rat 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 the angular position of the ankle. A calibrated potentiometer measured the angular position of the load cell fixture in real-time during testing. Vertical forces applied to an aluminum sleeve fitted over the dorsum of the foot were translated to a load cell transducer (Sensotec, 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, Quincy, Massachusetts) were placed subcutaneously to span the peroneal nerve. The first electrode was placed lateral to the tibial notch and the second electrode was placed ~5 mm distal and 3 mm posterior to the first electrode. Activation of the electrical stimulator resulted in muscle contraction of the dorsiflexor muscle group. Stimulator settings were optimized 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 recovery times between stimulations.[30]

SSC Exposure. After 1 week of acclimation, the left hindlimb of all rats was exposed to 150 stretch-shortening cycles (SSCs). The SSC exposure protocol consisted of 15 sets of 10 continuous stretch-shortening contractions. Each set was administered at 1-min intervals. We previously used this protocol to generate injury in the TA muscle and reductions in force in young Sprague Dawley rats.[15]

Isometric Force Tests. A single isometric force test was performed preceding and immediately following the SSC exposure to evaluate changes in force production. The duration of the contraction was 300 ms at an ankle angle of 90°. Isometric force was also measured 1, 2, 3, 5, and 10 days after the SSC exposure.

Muscle Processing. Immediately after the last force measurements, animals were anesthetized with pentobarbital (100 mg/kg), weighed, and euthanized by exsanguination. The left (exposed) and right (control) TA muscles were removed and weighed. The mid-belly of each TA muscle was then dissected, mounted onto cork board with OTC (VWR, West Chester, Pennsylvania), frozen in liquid nitrogen–cooled isopentane, and stored at −80°C until sectioned for histology. The proximal portion of each TA muscle was frozen in liquid nitrogen and stored at −80°C until used for PCR. Tibia lengths also were measured with calipers.

Table 1. Primer sequences for RT-PCR

Transcripts	Primer sequences
AIF-F*	5′-GGTATCCGTTCGGAGAGTGA-3′
AIF-R*	5′-CGTAGTCCTCCCCTTCAACA-3′
Bax-F	5′-TGTTTGCTGATGGCAACTTC-3′
Bax-R	5′-GATCAGCTCGGGCACTTTAG-3′
Bcl-2-F	5′-GGGATGCCTTTGTGGAACTA-3′
Bcl-2-R	5′-CTCACTTGTGGCCCAGGTAT-3′
Id2-F	5′-ATCCCCCAGAACAAGAAGGT-3′
Id2-R	5′-CTGTCCAGGTCTCTGGTGGT-3′
IGFI Ea-F	5′-AGGGCACAGGCTGGCTTTGTAC-3′
IGF-Ea-R	5′-CGATCCAAGTGGCAGCTCCTTC-3′
MGF-F	5′-TCCGCTGCAAGCCTACAAAGTC-3′
MGF-R	5′-CTTTCCTTCTCCTTTGCAGCTTCC-3′

**F denotes the sequence for the forward primer and R denotes the sequence of the reverse primer.*

Quantitative Reverse Transcriptase–Polymerase Chain Reaction (qRT-PCR). qRT-PCR was used to measure transcript levels for the proapoptotic factors, β-cell leukemia/lymphoma 2 (Bcl-2), Bcl-2 associated X protein (Bax), apoptosis-inducing factor (AIF), inhibitor of differentiation Id2, the insulin-like growth factor-1 splice variants IGF-I Ea and mechanogrowth factor (MGF), and ribosomal 18s which served as an RNA loading control. The primer sequences for all transcripts examined are presented in Table 1.

RNA was isolated and purified as previously described.[5] First-strand cDNA was synthesized from 1 μg of total RNA using Invitrogen's Reverse Transcription System (Invitrogen, Carlsbad, California). The general protocol for performing qRT-PCR has been described previously.[5] Control RNA from heart tissue was run at ×10 dilutions for each transcript to establish a standard curve of relative transcript levels. Samples that did not show a single defined melt peak in the 80°C range were not included in the dataset. Changes in the relative levels of transcript (calculated using the standard curve) were used for analyses. Changes that were 2-fold or greater and were statistically different from each other were considered significant. Smaller changes were considered to be too unreliable.

Histology and Immunohistochemistry. Frozen sections (10 μm) from the mid-belly of the TA muscles were cut on a cryostat, thaw-mounted onto SuperPlus slides (Fisher Scientific, Pittsburgh, Pennsylvania) and stored at −80°C until processing.[4] One slide from the exposed and control limb of each rat was stained with hematoxylin and eosin using Harris' procedure. Muscle morphology was assessed using stereological methods.

Dual immunohistochemistry for MyoD, a marker of activated satellite cells and myonuclei, and the proapoptotic protein Bax was performed on one set of sections (one slide) from the control and exposed TA of each animal. Serial sections from a subset of young and old rats were also labeled with MyoD and dystrophin to determine whether MyoD was labeling satellite cells, myofibers, or both structures. All antibodies were diluted in 0.01 M phosphate-buffered saline (PBS, pH 7.4) containing 4% Triton X-100 (PBS-TX). Sections were rinsed in PBS between incubations. Both the Bax and MyoD primary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, California). The Bax and MyoD antibodies were used at 1:500 and 1:100 dilutions, respectively. The dystrophin antibody was a mouse monoclonal antibody (Chemicon, Temecula, California) used at a 1:200 dilution. The fluorescently tagged secondary Cy2 and Cy3 antibodies and normal donkey serum for blocking were obtained from Jackson ImmunoResearch Laboratories (West Grove, Pennsylvania). The secondary antibodies were used at a 1:100 dilution.

The procedures used to perform dual immunohistochemistry were modified from a previously published protocol.[22] Modification included fixing frozen sections in ice-cold acetone for 5 min prior to immunolabeling and coverslipping slides with Vectashield Mounting Medium with DAPI (Vector Laboratories, Burlingame, California).

Photomicrographs were taken using a Zeiss LSM510 laser scanning confocal microscope, with HeNe, argon, and ultraviolet lasers, and integrated 2D and 3D image processing software. MyoD and MyoD/Bax immunolabeled cells were counted in two sections from the exposed and control TA muscles from each animal. A grid (1-mm square) was placed over the end of each muscle and the number of MyoD cells was counted at a wavelength of 550 nm. The number of MyoD cells expressing Bax was counted using a triple-band filter (400 nm excitation; Chroma Technologies, Rockingham, Vermont) that allowed for the simultaneous visualization of DAPI, Cy2, and Cy3. Only MyoD immunopositive cells that appeared to be in the interstitial space or adjacent to myofibers were counted. The grid was moved across the tissue so that all MyoD and MyoD/Bax immunolabeled cells in each section could be counted. Data from the two sections were averaged and analyzed as described below.

Stereology. The surface densities of normal myofibers, degenerative myofibers, the cellular interstitium, and the noncellular interstitium in tissue sec-

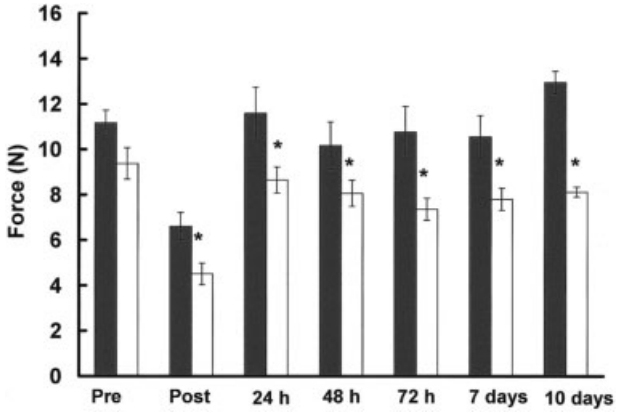

FIGURE 1. Average isometric forces (N) ± SEM in the exposed tibialis anterior (TA) of young (black) and old rats (white) prior to and after exposure to SSCs. Before the exposure, isometric forces were similar in young and old rats. However, after SSC exposure isometric forces were lower in old than young rats at all time points (*$P < 0.0001$).

tions from the mid-belly of the exposed and control TA muscles of each animal were measured using standard morphometric procedures adapted for skeletal muscle tissue.[4,5,29] A stage micrometer was used to identify the mid-point of the tissue section. Point and intercept counts using a 121-point/11-line overlay graticule (12.5 mm square with 100 divisions) at ×40 magnification were taken at five 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 1,210 points and 110 intercept lines per section. Volumes were computed as the percentage of total points over the tissue section to points over each of the defined structures. Points and intercepts over blood vessels greater than 25 μm in diameter were excluded.[4,5,29] One section from each limb of each animal was evaluated.

Data Analyses. Preexposure measures of isometric force in young and old animals were compared using a Student's *t*-test. Measures of isometric force during the recovery period (24–240 h) were analyzed using a two-way repeated measures analysis of variance (ANOVA) (age × day). To compare force during the recovery period to preexposure force values, a Dunnet's test was used.

All histological, immunohistochemical, and PCR data, with the exception of the measures of degenerative myofibers, were analyzed using two-way ANOVAs (age × limb with limb nested in subject). Tibia lengths were analyzed using a one-way ANOVA with animal as a random variable. Muscle weights from control and exposed TA muscles were compared using an ANCOVA, with tibia length as a

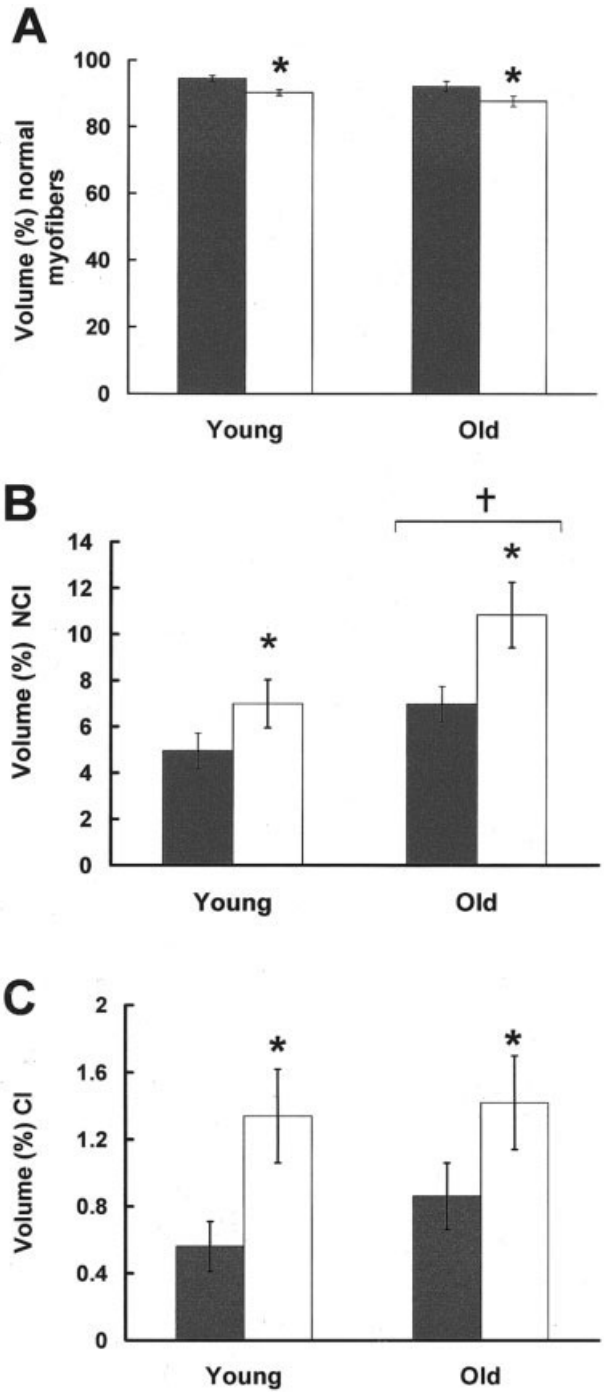

FIGURE 2. The volume (%) of normal myofibers **(A)**, noncellular interstitium (NCI; **B)** and cellular interstitium (CI; **C)** in the mid-belly of TA muscles. The volume of normal myofibers was lower in the exposed (white bars) than the control TAs (black bars) in both groups. In addition, the percent volume of the NCI and CI was greater in the exposed than control TAs in all rats, suggesting that exposure to SSCs resulted in injury to the exposed TA. The only age-related change was in the volume of the NCI, which was higher in old than young rats (*different from control TA values, $P < 0.001$; †different from young values, $P < 0.001$).

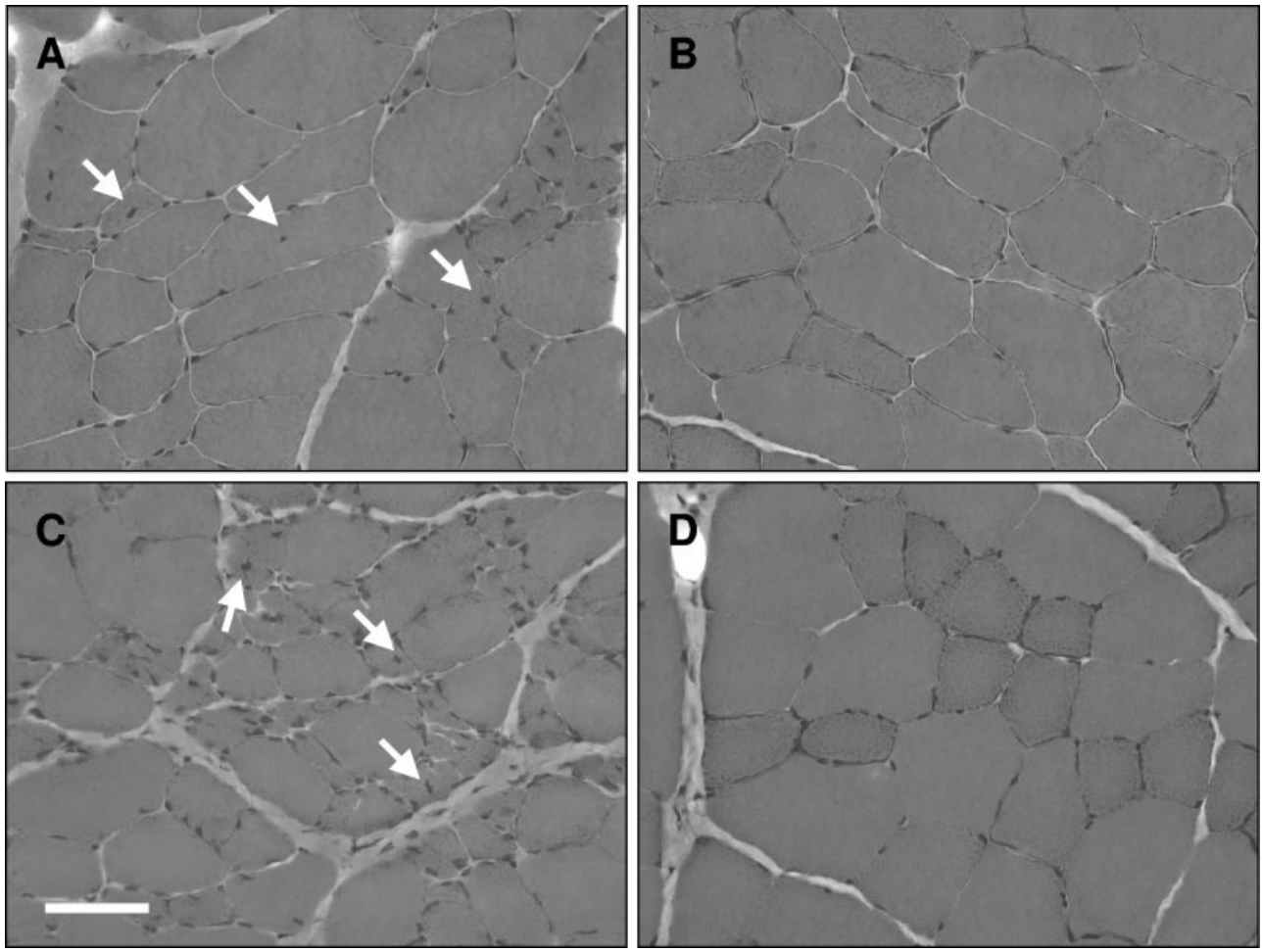

FIGURE 3. Hematoxylin and eosin–stained myofibers with central nuclei (arrows) were present in the exposed TA muscles of both young **(A)** and old **(C)** rats, indicating that exposure to SSCs 10 days earlier had resulted in myofiber injury. In contrast, no central nuclei were seen in the control TA muscles of young **(B)** or old **(D)** rats (scale bar, 50 μm).

covariate to control for age-related differences in the sizes of young and old rats. All data are presented as means ± SEM unless otherwise noted. Differences with a probability of $P < 0.05$ were considered significant. Data were analyzed using JMP v. 5.0.1 or SAS/STAT software, v. 9.1 of the SAS System for Windows (SAS Institute, Cary, North Carolina).

RESULTS

Muscle Weights. There was a significant effect of tibia length (the covariate) on TA weight [$F(1,10) = 5.07$, $P = 0.043$] in young and old rats. Analyses of tibia lengths demonstrated that the tibias of old rats were longer than tibias of young rats [46.38 ± 0.46 mm vs. 40.26 ± 0.02 mm, respectively, $t(10) = 12.17$, $P < 0.0001$]. The ANCOVA also uncovered a significant limb × age interaction [$F(1,10) = 10.45$, $P = 0.0053$]. Aging did not affect TA weights after they were adjusted for tibia length. However, in young rats there was a difference between exposed and control TA weights, with exposed TA weights being lower than control TA weights (means ± SEM adjusted for tibia length, 518 ± 52 mg vs. 676 ± 47 mg, respectively). In contrast, there were no significant differences between exposed and control TA weights in old rats (505 ± 48 mg vs. 543 ± 49 mg).

Isometric Force Tests. Mean levels of maximal isometric force for each group over time are presented in Figure 1. Although the maximal isometric force of the dorsiflexor muscles was lower in old than young rats during the preexposure test, this difference did not reach significance [$t(10) = 4.15$, $P = 0.07$]. In addition, isometric force measures collected during the recovery period (1–10 days postexposure) were not significantly different from preexposure values

in either group of animals. However, on days 1–10 after the exposure, isometric force generation in old rats was less than in young rats [main effect of age, $F(1,10) = 37.49$, $P < 0.0001$], with the average force in young animals being 11.21 ± 0.45 N and the force in old animals being 8.12 ± 0.22 N.

Histology and Stereology. The volume (%) of normal myofibers, cellular interstitium, and noncellular interstitium are presented in Figure 2A–C. Analyses of the stereological data demonstrated that the volume of normal myofibers was lower in the exposed than control TA of all animals [Fig. 2A; $F(1,10) = 11.76$, $P < 0.007$]. The reduction in normal myofiber volume was associated with a significant increase in the volume of the noncellular interstitium [Fig. 2B; $F(1,10) = 8.14$, $P < 0.02$), and cellular interstitium [Fig. 2C; $F(1,10) = 10.24$, $P < 0.01$] in the exposed TA of all rats. In addition, the volume of the noncellular interstitium, an indicator of edema, in the TA muscles of old rats was greater than the volume in young rats [$F(1,10) = 8.11$, $P < 0.02$].

Degenerative myofibers were identified in the exposed TA of one young rat and the control TA of one old rat. No degenerative fibers were identified in any other animals. However, sections from the exposed TA muscles of all rats contained myofibers with central nuclei, indicating that the exposure resulted in injury to the exposed TA muscles in both groups of rats. No central nuclei were detected in the control TA muscles of either group of rats (Fig. 3A–D). These morphological changes are indicative of SSC-induced muscle injury[4,11] and suggest that the tissue disruption caused by SSC exposure was similar in young and old rats, except that old rats were still exhibiting edema 10 days after the injury and young rats were not.

qRT-PCR. Bax expression and the ratio of Bax to Bcl-2 was increased in the exposed TA compared to the control TA in all animals (Fig. 4A,B) [Bax $F(1,10) = 49.75$, $P < 0.0001$; Bax/Bcl-2 $F(1,10) = 26$, $P = 0.0005$]. However, the expression of the AIF and Id2 and Bcl-2 were not affected by contraction exposure or age. The only transcript affected by age and contraction exposure was MGF (Fig. 4C). Exposure to contractions was associated with an increase in MGF expression in the exposed TA of both young and old animals [$F(1,10) = 12.10$, $P < 0.006$]. However, MGF expression generally was higher in TA muscles of young than old animals, regardless of limb [$F(1,10) = 5.00$, $P < 0.04$]. In contrast, IGF-I Ea expression in the exposed and control TA was not significantly affected by age or exposure to contractions.

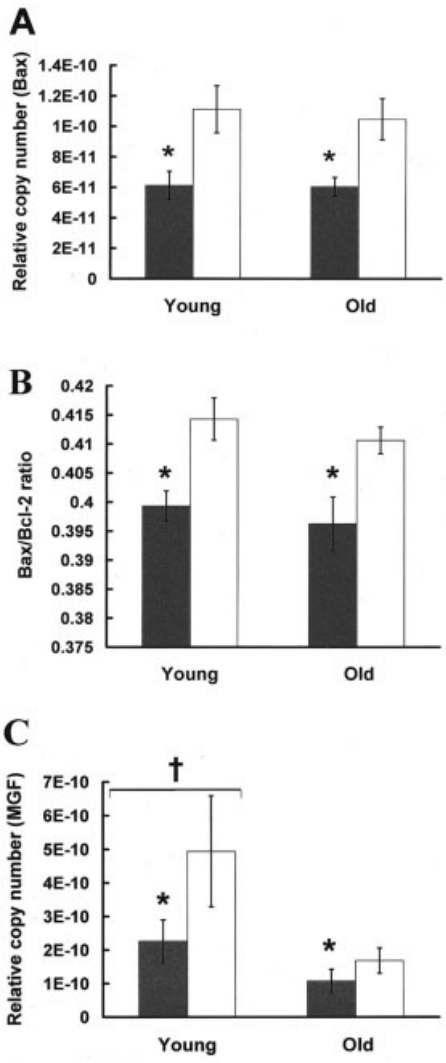

FIGURE 4. Relative transcript levels of Bax, Bcl-2, and MGF as determined by qRT-PCR in the control (black bars) and exposed TA muscles (white bars) of young and old rats. Bax expression **(A)** was higher in the exposed than control muscles of all animals. Bcl-2 transcript levels were not significantly altered in any group, but the ratio of Bax/Bcl-2 also was higher in the exposed than control TA muscles. Although MGF was higher in exposed than control TA muscles of all rats, MGF expression was generally higher in young than old animals (*different from exposed TA values, $P < 0.0005$; †different from old values, $P < 0.0006$).

Immunohistochemistry. Figure 5 shows MyoD- or MyoD/Bax-labeled satellite cells in the exposed TA of a young and old rat. The average number of MyoD- and MyoD/Bax-labeled cells are presented in the graphs in Figure 6. Exposure to contractions resulted in an increase in the number of MyoD-immunopositive cells in both young and old rats [Fig

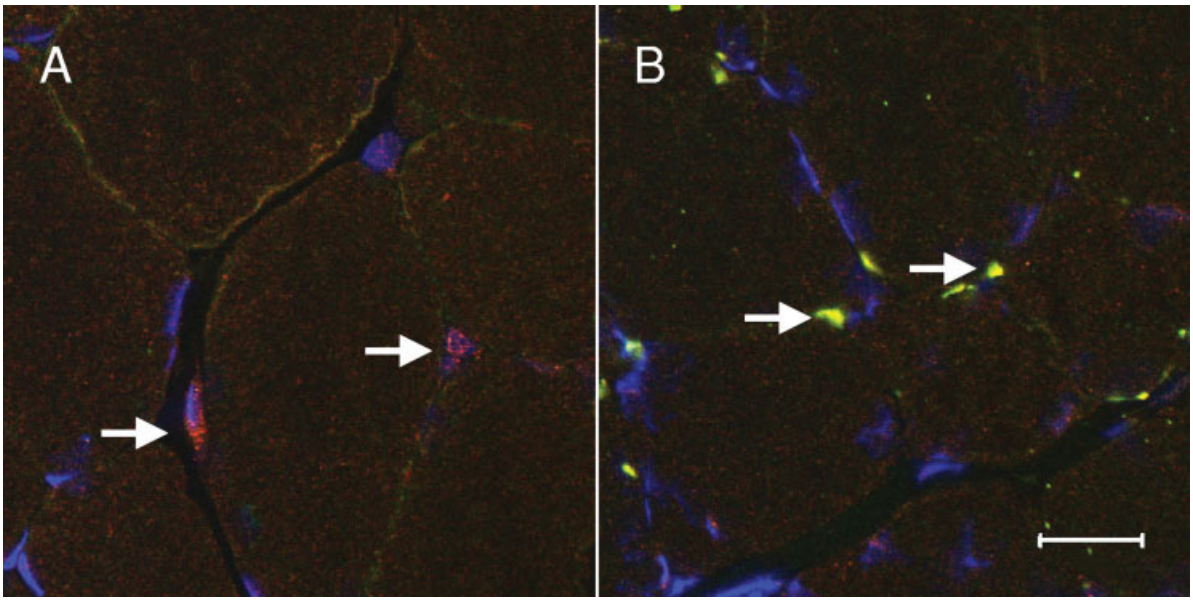

FIGURE 5. These photomicrographs show MyoD immunopositive cells (white arrows) in the exposed TA muscles of young **(A)** and old **(B)** rats. Cells immunopositive for MyoD are red, cells in which MyoD and Bax are colocalized are yellow, and DAPI stained nuclei are blue (scale bar, 20 μm).

6A; $F(1,9) = 116.65$, $P < 0.0001$]. However, there was an age × limb interaction when the number of cells coexpressing MyoD and Bax was analyzed [Fig. 6B; $F(1,9) = 4.98$, $P < 0.05$]. The analysis of this interaction revealed that the cellular response of young and old animals to SSC-induced injury was different. In old rats the number of MyoD/Bax-labeled cells was higher in the exposed than control TA [$F(1,9) = 17.74$, $P < 0.003$]. However, there was no difference in the number of MyoD/Bax-labeled cells in the exposed and control TAs of young rats. In addition, analyses comparing the number of MyoD/Bax cells in the exposed TAs of young and old rats revealed that there were more double-labeled cells in old than young animals [$F(1,9) = 20.47$, $P < 0.002$]. We also analyzed the percent of MyoD cells that coexpressed Bax and found a main effect of age [$F(1,9) = 36.48$, $P = 0.0002$], with old animals having a higher percentage of coexpressing cells than young animals (Fig. 6C). Although the number of MyoD/Bax colabeled cells was different in the exposed and control TA of old rats, the percent of cells coexpressing these factors was similar in both limbs.

To determine whether the MyoD-labeled cells were satellite cells or myonuclei, dual-immunostaining for MyoD and dystrophin was performed. Dystrophin was used to identify sacrolemma. When these slides were examined, we found that most MyoD staining was outside or adjacent to the sacrolemma (Fig. 7A–D), indicative of a sub-basal lamina localization. Very little MyoD staining was located clearly within myofibers.

DISCUSSION

The primary findings of this study were that after exposure to the SSC protocol, isometric force production in the exposed limb was lower in old than young rats. These age-related differences in force were associated with an increase in the number of MyoD-labeled cells in the exposed limb of both young and old rats. However, the number of MyoD-immunopositive cells that coexpressed Bax, a pro-apoptotic marker, was significantly higher in the old than young animals. Although exposure to mechanical stress resulted in an increase in the number of MyoD- and MyoD/Bax-expressing cells in the exposed TA muscle, the coexpression of MyoD and Bax was not limited to that limb, but was apparent in both limbs. When the percent of MyoD cells expressing Bax in each limb was calculated, there was no difference in the percentage of cells coexpressing these factors in the exposed and control TA muscles within each age group. However, the percent of colabeled cells was higher in the TA muscles of old than young rats, indicating a general effect of age on the colocalization of these factors. In addition, at 10 days after exposure, most of the cells expressing MyoD were outside of the sacrolemma, suggesting that they were satellite cells. Based on these findings,

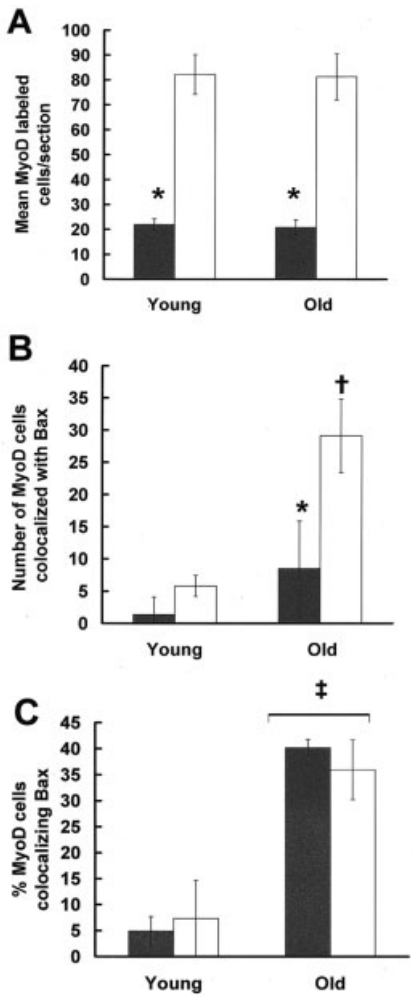

FIGURE 6. The average number of MyoD **(A)** and MyoD/Bax **(B)** immunopositive cells/section, and the percent of MyoD/Bax cells **(C)**, in control (black bars) and exposed TA muscles (white bars) of young and old rats. The number of MyoD cells was higher in the exposed than control TA of all rats **(A)**. The number of MyoD cells that coexpressed the proapoptotic factor, Bax, was higher in the exposed TA muscles of old than young rats, and in old rats the number of double-labeled cells was higher in the exposed than control TA muscles **(B)**. When the percent of double-labeled cells were analyzed, no differences were seen between the control and exposed TA muscles, but the percent of double-labeled cells was higher in old than young rats (*different than exposed TA, $P < 0.003$; †greater than the exposed TA of the young animal, $P < 0.002$; ‡greater than young animals, $P < 0.0002$).

we suggest that satellite cells or myofibers in old animals are more likely to undergo apoptosis.

Most studies examining the effects of aging on muscle maintenance and recovery have focused on reductions in satellite-cell proliferation.[18] However, we did not find a significant effect of age on the number of MyoD-immunopositive cells. There are a number of factors that could account for this. First, we used MyoD immunostaining to identify satellite cells. In adult skeletal muscle, MyoD is expressed in satellite cells and myonuclei in response to injury or exercise.[13] In this study, only MyoD-labeled cells that appeared to be in the interstitial space were counted. In addition, dual labeling with MyoD and dystrophin demonstrated that most MyoD-immunopositive cells were located outside the sacrolemma, and were probably satellite cells. However, it is possible that some myonuclei were counted and may have inflated our estimates of the number of activated satellite cells in older animals. Second, in most studies satellite-cell proliferation is determined 1–2 days after the initial injury,[9] whereas we assessed the number of MyoD-labeled cells 10 days after the initial injury. Thus, it is possible that there was an age difference in the number of activated satellite cells in the first 2 days after SSC exposure, which was no longer apparent 10 days after exposure. Finally, animals in our study also were anesthetized and exposed to five isometric force tests over the 10-day recovery period, in addition to the acute exposure to SSCs. Although previous studies have demonstrated that exposure to repetitive isometric contractions does not cause tissue necrosis, isometric contractions can cause acute inflammation and edema.[15] Thus, it is possible that the additional stimulation from the isometric force tests could have enhanced satellite-cell proliferation or myonuclei activation, thereby masking any aging effect.

Even though SSC-induced MyoD staining was similar in young and old rats, MyoD-labeled cells in the TA of old rats expressed the apoptotic factor Bax, and thus may have been more prone to undergo apoptosis than MyoD-labeled cells in young rats. Satellite-cell apoptosis is displayed under a number of conditions associated with muscle degeneration, such as denervation[21] and inclusion-body myositis.[24] In addition, dystrophin-deficient *mdx* mice, which are more prone to exercise-induced muscle injury and show deficits in recovery, also show greater levels of satellite-cell apoptosis than wild-type mice.[23] Muscle degeneration in response to age also appears to be associated with satellite-cell apoptosis. Primary satellite cells collected from old rats are more prone to undergo apoptosis in response to a stressor than satellite cells collected from young rats,[20] and loading and unloading results in an increase in satellite-cell apoptosis in aged quail.[27] In addition, nuclear apoptosis is increased in muscles of older animals.[3] Thus, our data are consistent with the results of other studies indicating that satellite-cell and myofiber apoptosis are associated with muscle degeneration and reduced muscle recovery.

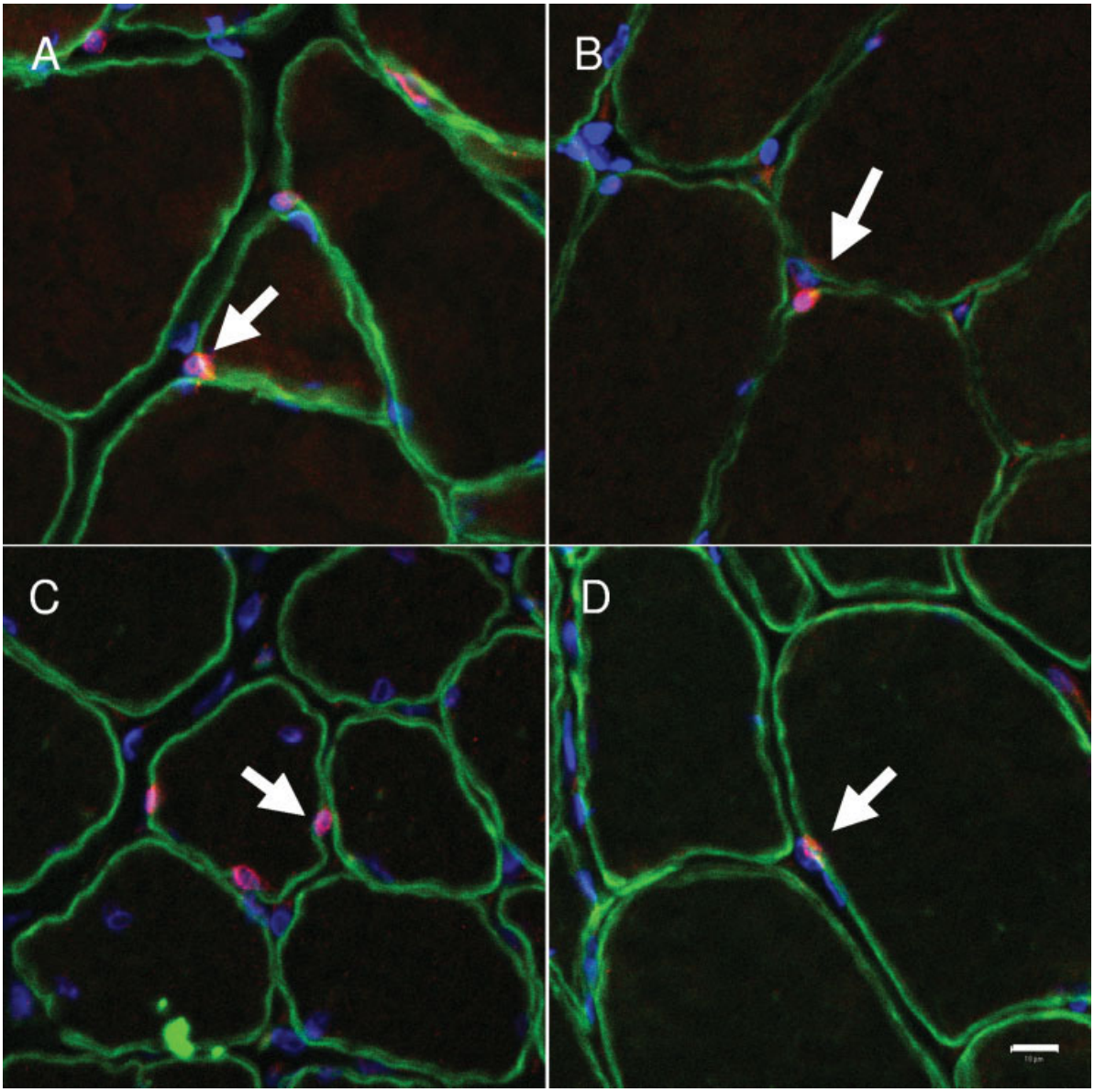

FIGURE 7. MyoD and dystrophin immunolabeling in the exposed **(A,C)** and control TA muscles **(B,D)** of young **(A,B)** and old rats **(C,D)**. The majority of MyoD-labeled cells (red) were located in the interstitial space (arrows), outside the dystrophin-labeled (green) borders of the myofibers (scale bar, 10 μm).

The number of MyoD/Bax-labeled cells was higher in the exposed than control TA muscles of old rats, but the percent of dual-labeled cells was not different between the two limbs. The increase in both MyoD- and MyoD/Bax-immunostained cells in the exposed limbs of old rats suggests that mechanical stress was capable of stimulating satellite-cell proliferation, but that satellite cells may not survive in muscle of older animals. In addition, the finding that the percent of MyoD cells immunopositive for Bax is similar in both the exposed and control limbs of old rats indicates that aging, and not mechanical exposure, is associated with an increased probability of apoptosis in skeletal muscle. Thus, it is possible that apoptosis might hinder general muscle maintenance and muscle recovery in aged individuals.

Transcript levels of the proapoptotic factor, Bax, were increased in our aged animals, but two other factors associated with apoptosis, Id2 and AIF, were unaffected. Id2 is a repressor of myogenic regulatory factors and is increased with unloading-induced apoptosis in patagialis muscle of quail[3] and in the hindlimbs of old rats.[2] However, Id2 was not increased in old animals after hindlimb denervation.[1]

Thus, changes in Id2 expression are not needed to induce apoptosis in skeletal muscle. However, previous studies also demonstrated that aging increases Id2 expression[1] and that aging, unloading-, or oxidative stress–induced apoptosis result in changes in AIF protein concentrations.[26] The absence of age-related changes in these transcripts may be due to a number of factors. For example, it is possible that measurements at the single time point chosen in this study missed changes in Id2 and AIF transcription, or that the changes in protein concentrations reported in other studies were not accompanied by significant changes in transcription. Thus, although we did not find changes in Id2 and AIF transcript levels, the increase in Bax gene and protein expression and the increased Bax/Bcl-2 ratio support our conclusion that proapoptotic pathways were activated in the exposed TA muscles and that apoptosis may be increased in old rats.

Mitochondrial-associated apoptosis has been linked to dysfunction in aged skeletal muscle.[26] However, changes in the cellular environment also have a significant effect on satellite-cell proliferation and skeletal-muscle recovery. For example, treating old rats with IGF-I increases satellite-cell proliferation and force recovery and also rescues the loss of muscle mass after an unloading–reloading injury in the hindlimb.[8] Exogenous IGF-1 administration also prevents age-related muscle atrophy in senescent mice.[6] In addition, parabiosis studies in mice have demonstrated that serum factors in young animals help stimulate satellite cell proliferation and recovery from injury.[10] Based on these findings, we hypothesize that changes in the cellular environment might also underlie the increased expression of proapoptotic factors in skeletal muscle from old rats. We found that, although SSC-induced injury resulted in an increase in MGF expression in the exposed TA of all animals, transcript levels were generally higher in TA muscles from young than old rats. Other studies have demonstrated that MGF plays a role in repair and regenerative mechanisms in skeletal muscle[17] and that age-related reductions in the expression of MGF are associated with a decline in muscle repair and sarcopenia.[16] Thus, age-related changes in MGF, or other locally and systemically produced growth factors, may result in an increased susceptibility of satellite cells to apoptosis.

In conclusion, the results of this study demonstrate that activated satellite cells are more likely to express proapoptotic factors in aged animals, and an increased susceptibility to apoptosis could in part underlie age-related decrements in recovery after injury. In addition, our data demonstrate that the increase in the expression of apoptotic factors is associated with a decrease in MGF expression. Future studies should focus on determining the precise roles that locally produced and systemic growth factors play in mediating satellite cell survival.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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