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# Enhancement of Skeletal Muscle in Aged Rats Following High-Intensity Stretch-Shortening Contraction Training

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## Abstract

Exercise is the most accessible, efficacious, and multifactorial intervention to improve health and treat chronic disease. High-intensity resistance exercise, in particular, also maximizes skeletal muscle size and strength—outcomes crucial at advanced age. However, such training is capable of inducing muscle maladaptation when misapplied at old age. Therefore, characterization of parameters (*e.g.*, mode and frequency) that foster adaptation is an active research area. To address this issue, we utilized a rodent model that allowed training at maximal intensity in terms of muscle activation and tested the hypothesis that muscles of old rats adapt to stretch-shortening contraction (SSC) training, provided the training frequency is sufficiently low. At termination of training, normalized muscle mass (*i.e.*, muscle mass divided by tibia length) and muscle quality (isometric force divided by normalized muscle mass) were determined. For young rats, normalized muscle mass increased by ~20% regardless of training frequency. No difference was observed for muscle quality values after 2 days versus 3 days per week training ($0.65 \pm 0.09$ N/mg/mm vs. $0.59 \pm 0.05$ N/mg/mm, respectively). For old rats following 3 days per week training, normalized muscle mass was unaltered and muscle quality was 30% lower than young levels. Following 2 days per week training at old age, normalized muscle mass increased by 17% and muscle quality was restored to young levels. To investigate this enhanced response, oxidative stress was assessed by lipid peroxidation quantification. For young rats, lipid peroxidation levels were unaltered by training. With aging, baseline levels of lipid peroxidation increased by 1.5-fold. For old rats, only 2 days per week training decreased lipid peroxidation to levels indistinguishable from young values. These results imply that, appropriately scheduled high-intensity SSC training at old age is capable of restoring muscle to a younger phenotype in terms of lipid peroxidation levels and muscle quality.

**Keywords:** dorsiflexor muscles, dynamometer, Fisher 344XBrown Norway rats, repetitive exposure, oxidative stress

## Introduction

Exercise is an effective intervention for improving overall health and alleviating chronic diseases ranging from cancer to psychiatric, neurological, metabolic, cardiovascular, musculoskeletal, and pulmonary diseases.[1–5] Strenuous physical activity in the form of exercise is as efficacious as many commonly prescribed drug treatments for heart disease and diabetes and is more beneficial than drug intervention in rehabilitation from stroke.[4] Evidence for the pluripotency of exercise led the American Medical Association and the American College of Sports Medicine to launch the Exercise is Medicine initiative in 2007—a now global initiative to translate scientifically proven health benefits of physical exercise into the healthcare system.[5]

Among the various forms of exercise ranging from aerobic to anaerobic, resistance exercise, in particular, is promoted for increasing skeletal muscle size and strength for those with deficiencies in these attributes, such as the elderly.[1] However, the training parameters for intensity, mode, and frequency of resistance exercise to prescribe is an active area of research. Exposure to sessions of muscular activity that are extreme in terms of volume (*i.e.*, product of intensity and repetition number) result in maladaptation—especially at an advanced age.[6–8] Concerns about high-intensity training (≥70%–85% of one repetition maximum) at old age has led some researchers to investigate lower intensity training alternatives.[9,10]

Reports utilizing experimental rodent models have been especially informative in regard to negative outcomes of some high-intensity resistance-type training regimens because

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of the ability to precisely control intensity and mode. In several such studies, muscles of rats underwent maximal intensity training in terms of muscle activation 3 days per week for 1 month.[11–13] In these studies, the mode utilized was stretch-shortening contractions (SSCs), a common form of contraction mode utilized by all mammals during resistance-type exercise in which the muscle is activated before and during the initial stretch and subsequent shortening, as opposed to isolated eccentric, isometric, or shortening contractions. For young rats, this training induced gains of ~20% in muscle mass and performance. In contrast, for old rats, the exposure typically induced marginal muscle mass gains (<10%) and decreased performance output.[11–13] Such findings indicated that high-intensity SSC training has the potential to not only be ineffective at old age but also detrimental if inappropriately utilized.

Although resistance exercise programs performed at a range of intensities can produce substantial muscle hypertrophy and strength gains, high-intensity resistance training demonstrates the highest potential for maximizing these outcomes presumably because of the maximization of motor unit recruitment and, consequently, the number of muscle fibers activated in the training.[14] Therefore, the need to characterize training parameters (*e.g.*, mode and frequency), which are compatible with muscle adaptation, from high-intensity training at old age is of great importance.

This study was undertaken for SSC training at maximal muscle activation (*i.e.*, high-intensity training) to determine whether decreasing the frequency of training, and thereby increasing recovery time between training sessions, would be sufficient to induce an adaptive response. Specifically, the hypothesis tested was that decreasing the frequency of training from 3 to 2 days per week would improve the adaptive outcome for old rats.

For assessing adaptation/maladaptation, muscle mass (normalized to tibia length), isometric force, muscle quality (*i.e.*, isometric force divided by normalized muscle mass), dynamic peak force, negative work, and positive work were analyzed. Quantitative morphology for tissue percentage of normal muscle fibers, degenerative muscle fibers, noncellular interstitium, and cellular interstitium was assessed by a standardized stereological method. Lipid peroxidation was also quantified as an indicator of oxidative stress because of findings in previous reports indicating oxidative stress as a factor in SSC-induced adaptation/maladapaptation.[13,15]

In these studies, aging was accompanied by an increase in baseline levels of lipid peroxidation in skeletal muscle, and a reduction in these levels induced by Vitamin E and C supplementation improved the response to SSC training.[11,13] The measurement of lipid peroxidation in this study enabled the assessment of whether changes in lipid peroxidation levels accompany differential muscle performance outcomes when training frequency is altered. The findings demonstrate the efficacy of age-appropriate high-intensity SSC training—training with potential to reduce lipid peroxidation levels, offset sarcopenia, and promote healthy aging.

## Materials and Methods

### Animals

Male Fischer Brown Norway hybrid rats (F344 X BN F1) at 3 and 30 months of age were obtained from the National Institute of Aging colony and housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal quarters. All animal procedures were approved by the Animal Care and Use Committee at the National Institute for Occupational Safety and Health in Morgantown, WV.

### High-intensity SSC training

The 2 and 3 days per week high-intensity SSC training exposures were identical with the exception of frequency of exposure with the 2 days per week training administered Mondays and Thursdays, while the 3 days per week training was administered Mondays, Wednesdays, and Fridays. Both exposures were administered over 4.5 weeks. Each exposure was based on a previously described procedure in which rats were exposed to 80 SSCs.[11] Each rat was anesthetized with isoflurane gas and placed in dorsal recumbency on a heated table with the left knee secured in flexion at 90°. The left foot was secured to a fixture containing a load cell. Platinum electrodes were placed subcutaneously in the region of the common peroneal nerve for activation of the dorsiflexor muscles. Muscle stimulation was set at parameters for maximal contraction; 4-V magnitude, 0.2-ms pulse duration, and 120-Hz frequency.[16]

Both static and dynamic performances were assessed before exposure to the 80 SSC protocol. Static performance was assessed by exposing the muscles to a single maximal isometric tetanic contraction for 300 ms with the ankle at 90°. After a 2-minute rest, dynamic performance was assessed by exposure to a single SSC test consisting of an isometric contraction for 300 ms at an ankle angle of 70°, then rotating the ankle through the entire physiologic range from 70° to 140° at 500° per second, then returning to 70° at 500° per second, and last, continuing activation for an additional 300 ms. Peak force was determined by assessing the maximum force during the stretch phase. Negative and positive work referred to the work required to stretch the muscle and the work done by the muscle during the shortening phase, respectively.

The 80 SSC protocol was administered 2 minutes following the single SSC test. The protocol consisted of 8 sets of SSCs (2-minute intervals between sets) and 10 SSCs per set (2-second intervals between SSCs), with the interval durations chosen so as to be comparable to those typically suggested for resistance exercise training.[17] For each SSC, while the muscles were maximally activated, the ankle was set to 90° for 100 ms, rotated to 140° at 60°/s, returned to 90° at 60°/s, and deactivated 300 ms later. The ankle range of 90° (angle associated with maximum isometric force) to 140° maximized the force output during each training SSC, while decreasing the factor of fatigue, which would have been a greater factor, had the complete physiologic range (70° to 140°) been utilized.[18] The velocity of 60° per second was chosen to promote performance adaptation and hypertrophy rather than injury.[11] Muscle inflammation and degeneration are not overt for young and old rats in the days to weeks following the SSC exposure utilized in this study.[11,12,19]

The performance measures for sessions during the first and last week of the SSC exposures were averaged to determine initial and final values, respectively. At 24 hours after the final SSC exposure, the tibialis anterior (TA)

muscle was surgically removed, weighed, and the tibia length recorded. This time period was chosen to be beyond the acute effects of the first several hours posttraining, yet be within the time period conducive for detecting any overt muscle degeneration resulting from the last training session. The mid-belly of the TA muscle was covered with tissue freezing media (Tissue-Tek, 4583 O.C.T. Compound; Sakura Finetek) and frozen in cold isopentane (–80°C) for quantitative morphology. A portion of the remaining TA tissue was allocated for lipid peroxidation analysis. Normalized muscle mass was determined by dividing the muscle mass by tibia length. Muscle quality was quantified as maximum isometric tetanic force for the final week of training divided by normalized muscle mass.

### *Quantitative morphology*

The mid-belly of each TA muscle was cryosectioned at 12 $\mu$m thickness and then hematoxylin and eosin stained. A standardized stereological method was used for quantitative morphology.[20,21] At two regions, 1 mm to the right of the section midline and 1 mm to the left of the section midline, stereological analysis was performed at five equally spaced sites across the muscle section. At each site, points of a 121-point 11-line overlay graticule (0.04 $mm^2$ with 100 divisions) were evaluated at 40× magnification. Therefore, a total of 1210 points were analyzed per section since 10 total fields were evaluated.

Each point was identified as overlaying a normal muscle fiber, degenerative muscle fiber, cellular interstitium, or noncellular interstitium (Fig. 1). Degenerative muscle fibers were considered to have (1) loss of contact with surrounding fibers, (2) interdigitation of the sarcolemma by cellular infiltrates, and (3) internalization of cellular infiltrates.[20] If a muscle fiber did not have these characteristics, the fiber was considered normal. Cellular interstitium was counted when points overlaid nuclei in between muscle fibers. Points that overlaid interstitial regions without nuclei were counted as noncellular interstitium. The percentage of muscle tissue comprising normal muscle fibers, degenerative muscle fibers, centrally nucleated muscle fibers, cellular interstitium, or noncellular interstitium was calculated as the percentage of points that overlaid each type of tissue relative to the total number of points.

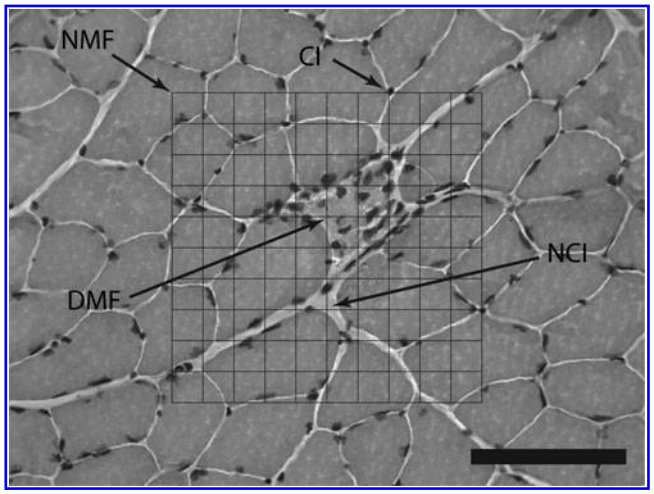


**FIG. 1.** Quantitative morphology was assessed by a standardized stereological method. Representative image for how stereological analysis was performed. At each site, points (*i.e.*, nodes where lines intersect) of a 121-point graticule overlaid a field at 40× magnification. Each point was identified as overlaying a normal muscle fiber (NMF), degenerative muscle fiber (DMF), cellular interstitium (CI), or noncellular interstitium (NCI). Examples for each of these designations are labeled (arrows). Of the total 121 points for this image, the following were the designations: 105 NMF, 6 DM, 7 NCI, and 3 CI. Scale bar = 100 $\mu$m.

### *Immunofluorescence*

Frozen TA muscle sections (cryosectioned at 12 $\mu$m thickness) were stained for 4-hydroxynonenal (4-HNE; Calbiochem; no. 393206; at 1:250) to determine lipid peroxidation distribution and $\beta$-dystroglycan (sc-33701; at 1:100; Santa Cruz Biotechnology) to outline the sarcolemma for muscle fiber size measurements. First, sections were fixed in methanol (–20°C) for 10 minutes and then acetone (–20°C) for 10 minutes. Sections were washed with phosphate-buffered saline (PBS) and then blocked with 10% goat serum at room temperature for 1 hour. Primary antibody was applied overnight at 4°C. After three washes with PBS (5 minutes each), secondary antibodies (goat anti-rabbit IgG Alexa Fluor 488 and goat anti-mouse IgG1 Alexa Fluor 594) were applied for 1 hour.

The investigator was blinded to sample identification and for each muscle section, 10 images were captured at site locations determined in the same manner as described for quantitative morphology. Images were then analyzed utilizing ImageJ (version 1.46, National Institutes of Health, USA). Each muscle fiber (208 ± 21 fibers per section) was traced to determine the minimum Feret diameter, a measure of muscle fiber size that is resistant to variations in the orientation of muscle fibers during sectioning. For analysis of 4-HNE, threshold was set (based on images of secondary-only stained sections) and percent area of signal within each muscle fiber measured.

### *Lipid peroxidation levels*

Total malondialdehyde (MDA) content was assessed using a commercially available kit (Bioxytech MDA-586; Oxis International, Inc.). TA muscle tissue was homogenized in PBS containing butylated hydroxytoluene (5 mM). The homogenate was spun at 2000 *g* at 4°C and the supernatant used for the total protein quantification and the total MDA assay as per manufacturer's instructions. Protein quantification was performed using a standard colorimetric bicinchoninic acid protein assay (Pierce). MDA levels were normalized by the amount of muscle protein for each sample.

### *Statistical analysis*

When assumptions of normality and equal variance were justified, data were analyzed using analysis of variance (ANOVA) (JMP version 11; SAS Institute, Inc.) with the animal represented as a random factor to account for repeated measures within the animal when appropriate. *Post-hoc* comparisons were performed using Fisher's least significant difference method. Regarding data for percent of tissue comprising degenerative muscle fibers, normality and equal

variance could not be assumed; so nonparametric tests (using SigmaPlot version 12.5; Systat Software, Inc.) were utilized—comparisons for groups of old versus young rats were analyzed by the Mann–Whitney Rank Sum Test and Kruskal–Wallis ANOVA on Ranks when appropriate, while comparisons for trained versus contralateral muscles within each experimental group were analyzed by Wilcoxon Signed Ranks to account for repeated measures.

Chi-square analysis (SigmaPlot version 12.5) was utilized to determine training-induced differences in the absolute frequency distributions of minimum Feret diameter. Pearson product correlations (SigmaPlot version 12.5) were performed between SSC-induced alterations in MDA levels versus muscle mass/performance outcomes. All data are expressed as means ± SD. $p < 0.05$ was considered statistically significant.

## Results

### *Muscle mass and performance*

For young rats, muscle mass and strength gains were largely independent of whether they were subjected to 2 or 3 days per week high-intensity SSC training. Specifically, such training induced increases in peak force and the capacity for negative work (Fig. 2A, B). Positive work capacity and maximum isometric force were unaltered by training regardless of frequency (Figs. 2C and 3A).

Relative to contralateral control muscles, muscle mass increases of 19% ± 4% and 20% ± 4% were observed for muscles of young rats exposed to 2 versus 3 days per week training, respectively (Fig. 3B). To determine whether muscle mass changes were reflected at the muscle fiber level, the minimum Feret diameter was measured for individual muscle fibers (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/rej). The muscle mass increases for the young rats were accompanied by shifts in muscle fiber size distribution (Fig. 4A, B).

No difference in muscle quality, a measure of maximum isometric force normalized by muscle size, was observed between the training protocols (Fig. 2C). Therefore, muscles of young rats displayed the ability to adapt in distinct performance measures following multiple SSC regimes by demonstrating comparable adaptation to both training frequencies tested.

For old rats, SSC training frequency resulted in distinct outcomes. Following 3 days per week training, the capacity for positive work diminished relative to initial values, while peak force and negative work were unaltered compared with initial values (Fig. 2A–C). Muscle mass was unchanged when considered in comparison with contralateral control muscle mass data (Fig. 3B). Muscle fiber size distribution also remained unchanged by 3 days per week training (Fig. 4C). Final muscle quality values were depressed relative to those of young rats (Fig. 3C).

Following 2 days per week training for old mice, positive work capacity was maintained, muscle mass increased by 17% ± 8% relative to contralateral control values ($p < 0.0001$), and muscle fiber size distribution shifted to larger values (Figs. 2C, 3B, and 4D). Furthermore, training 2 days per week restored muscle quality to levels indistinguishable from young levels. The implication was that at old age, decreasing frequency of high-intensity SSC training enabled muscle performance and muscle mass gains accompanied by revitalization of muscle quality.

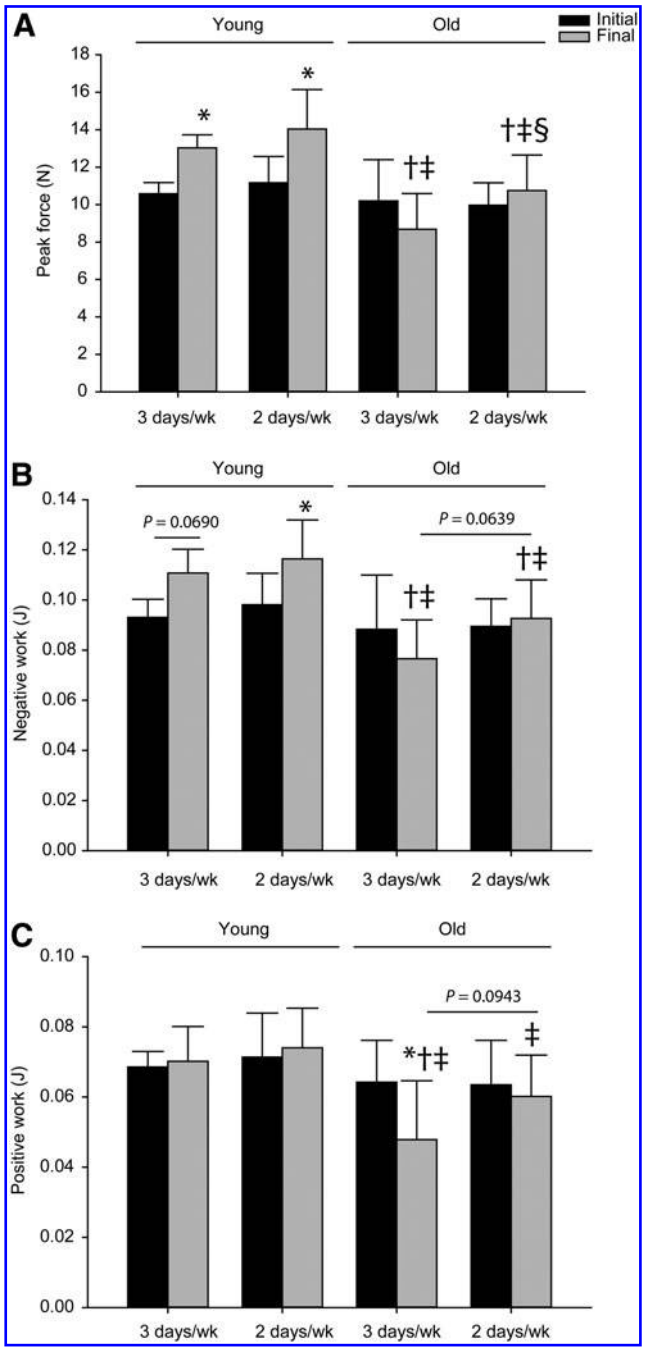


**FIG. 2.** Effect of altering training frequency on dynamic performance. Initial and final values of **(A)** peak force, **(B)** negative work (*i.e.*, work required to stretch muscle), and **(C)** positive work (*i.e.*, work done by the muscle during shortening) were assessed. For young rats (final vs. initial values), both 2 and 3 days per week training increased peak force and 2 days per week training increased negative work capacity (a trend was observed for 3 days per week training). For old rats, 3 days per week training induced a decrease in positive work capacity relative to initial values. This decrease was absent with 2 days per week training. The 2 days per week training also increased final peak force values relative to final values for 3 days per week training. Sample sizes were $N = 4$ to 8 per group. Values are mean ± SD. *Different from initial value; †Different from value for young rats exposed 3 days per week; ‡Different from value for young rats exposed 2 days per week; §Different from value for old rats exposed 3 days per week, $p < 0.05$.

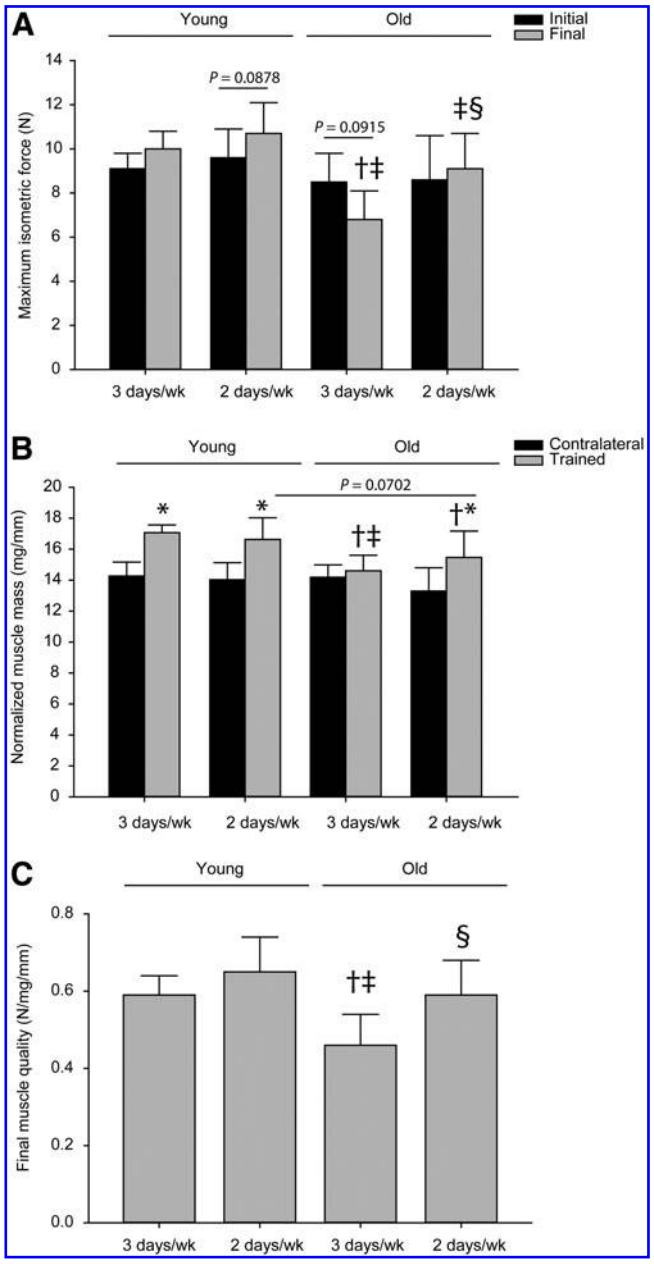


**FIG. 3.** Decreasing the frequency of training improved muscle mass and static performance for old rats. **(A)** Maximum isometric forces were assessed for the initial and final weeks of training. **(B)** Tibialis anterior muscle mass was normalized by tibia length. **(C)** Final maximum isometric forces were divided by normalized muscle mass of exposed muscles to determine muscle quality values. Sample sizes were $N=4$ to 8 per group. Values are mean ± SD. *Different from nonexposed value (*i.e.*, contralateral muscle in context of muscle mass or initial data in context of maximum isometric force and muscle quality); †Different from value for young rats exposed 3 days per week; ‡Different from value for young rats exposed 2 days per week; §Different from value for old rats exposed 3 days per week, $p<0.05$.

### *Quantitative morphology*

Analysis by quantitative morphology demonstrated an age-related decrease in tissue composed of normal muscle fibers (Table 1). Besides the difference between young and old contralateral control muscles following 2 days per week training ($p=0.039$), a main effect for age was observed by ANOVA ($p=0.01$). This decrease with age was due to an increase in the percentage of tissue other than that of normal muscle fibers, which consist of degenerative muscle fibers, cellular interstitium, and noncellular interstitium. When considering these tissue constituents separately, comparisons among individual groups did not reach significance, but trends for the main effect of age were observed by ANOVA for increases in noncellular interstitium ($p=0.057$) and cellular interstitium ($p=0.09$) with aging.

The effect of age could not be assessed by ANOVA for degenerative muscle fibers since normality and equal variance could not be assumed for that data. However, when degenerative fiber data for contralateral muscles were pooled within age groups and analyzed by Mann–Whitney Rank Sum Test, muscles of old rats comprised a greater percentage of degenerative muscle fibers, 0.4% ± 0.5%, relative to young rats, 0.2% ± 0.5% ($p=0.001$). Both the 2 and 3 days per week training had no significant effect for young and old rats (Table 1 and Fig. 5). The lack of SSC-induced histological alterations demonstrated that the factors responsible for the distinct functional outcomes following the 2 versus 3 days per week SSC training for the old rats were not apparent at the level of quantitative morphology. Rather, in agreement with multiple reports, this suggested that a more subtle process must be involved.[11,12]

### *Lipid peroxidation*

To investigate the possibility whether alterations in lipid peroxidation, an indicator of oxidative stress, correlate with the differential response in 2 days per week-exposed old rats, lipid peroxidation assays were performed. Lipid peroxidation levels rather than spatial distribution of lipid peroxidation within muscle fibers appeared to be dependent on training frequency for old rats. Spatial distribution was investigated by immunofluorescence staining for 4-HNE in transverse sections of muscles.

For all conditions (*i.e.*, nontrained, trained 2 days per week, or trained 3 days per week), lipid peroxidation was apparent both at the sarcolemma and within muscle fibers of old rats (Fig. 6). Interestingly for young rats, training either 2 or 3 days per week altered the distribution within muscles. While cytoplasmic staining of 4-HNE was present in all conditions, sarcolemma staining of HNE was modest in contralateral muscles of young rats, whereas such staining was substantial in trained muscles (Fig. 6).

These observations were consistent with quantification of muscle fiber percent area positive for 4-HNE staining; 33% ± 20% and 44% ± 39% for contralateral and exposed muscles of young rats trained 3 days per week, 49% ± 29% and 68% ± 28% for contralateral and exposed muscles of young rats trained 2 days per week, 47% ± 39% and 36% ± 22% for contralateral and exposed muscles of old rats trained 3 days per week, and 61% ± 29% and 50% ± 21% for contralateral and exposed muscles of old rats trained 2 days per week ($N=4$ to 5 per group).

While no significant differences were observed between any of these groups when considered individually, an ANOVA demonstrated a significant interaction ($p=0.02$) between age and limb (*i.e.*, right contralateral muscle vs. left trained muscle), independent of training frequency. Therefore,

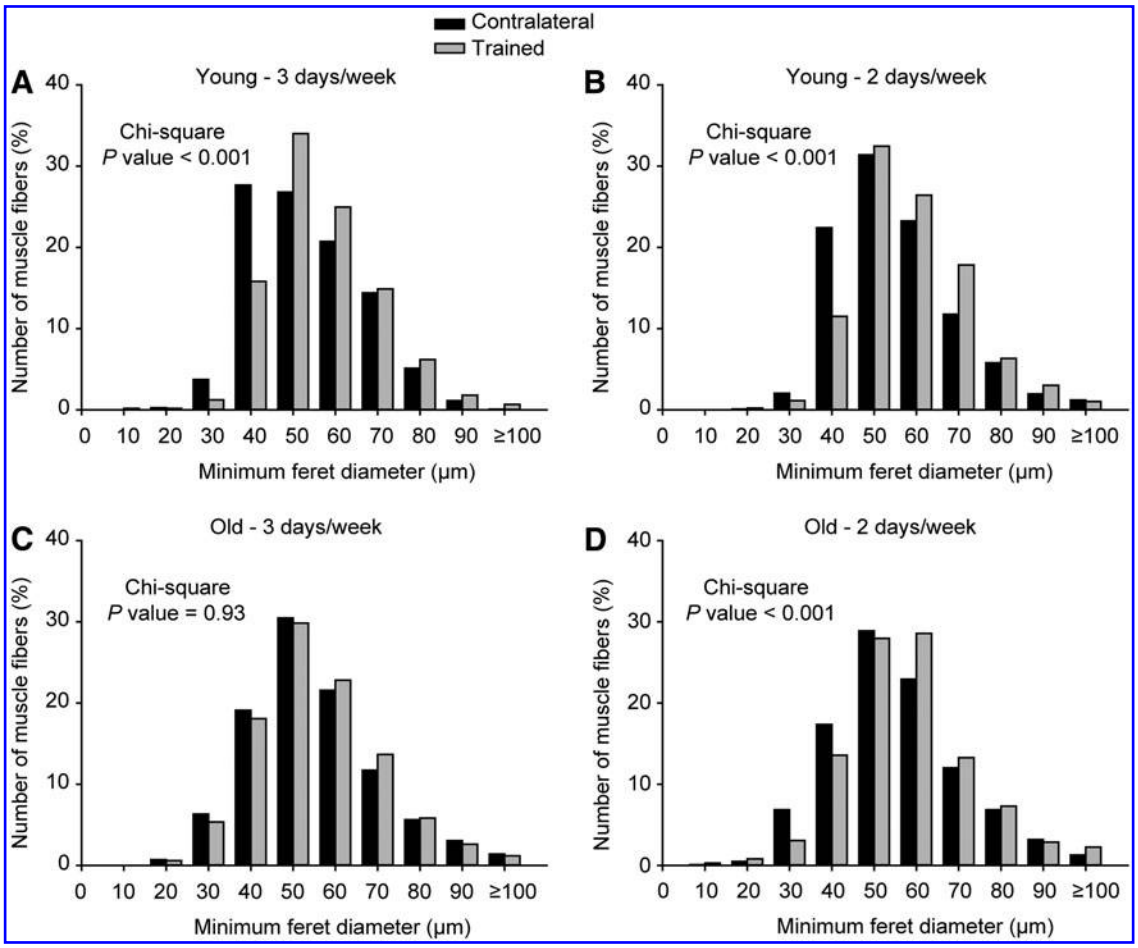


**FIG. 4.** Decreasing SSC frequency enabled training-induced shifts to larger muscle fibers for muscles of old rats. Frequency distributions of fiber minimum Feret diameters of individual muscle fibers are presented as percentage of total fibers measured for each experimental group: **(A)** young rats trained 3 days per week, **(B)** young rats trained 2 days per week, **(C)** old rats trained 3 days per week, and **(D)** old rats trained 2 days per week. Therefore, within each experimental group, the number of muscle fibers of a specific size were summed across all the muscles and then expressed as a percentage of total fibers counted for all the muscles ($N=4$ to 5 rats per group). Chi-square analysis was performed to determine alterations in distribution with training. All groups with the exception of 3 days per week trained old rats demonstrated a significant difference in distribution. SSC, stretch-shortening contraction.

TABLE 1. QUANTITATIVE MORPHOLOGY FOR STRETCH-SHORTENING CONTRACTION TRAINED AND CONTRALATERAL CONTROL MUSCLES OF YOUNG AND OLD RATS

| | *Young* | | | | *Old* | | | |
|---|---|---|---|---|---|---|---|---|
| | *3 days/week* | | *2 days/week* | | *3 days/week* | | *2 days/week* | |
| | *Contralateral* | *Trained* | *Contralateral* | *Trained* | *Contralateral* | *Trained* | *Contralateral* | *Trained* |
| Normal muscle fibers (% of tissue) | 93.4 ± 1.6 | 92.9 ± 2.6 | 95.4 ± 2.2 | 94.6 ± 2.8 | 92.7 ± 0.8 | 91.3 ± 3.1[a] | 92.4 ± 1.7[a] | 91.8 ± 1.6[a] |
| Degenerative muscle fibers (% of tissue) | 0.0 ± 0.0 | 0.6 ± 0.7 | 0.0 ± 0.0 | 0.0 ± 0.1 | 0.5 ± 0.8 | 0.1 ± 0.1 | 0.4 ± 0.3 | 0.2 ± 0.2 |
| Cellular interstitium (% of tissue) | 1.7 ± 0.6 | 1.6 ± 0.5 | 1.7 ± 0.4 | 1.3 ± 0.5 | 2.1 ± 0.3 | 2.3 ± 1.1 | 1.4 ± 0.4 | 1.9 ± 0.7 |
| Noncellular interstitium (% of tissue) | 4.9 ± 2.1 | 5.0 ± 2.6 | 2.9 ± 1.9 | 4.1 ± 2.2 | 4.7 ± 1.5 | 6.3 ± 2.2 | 5.8 ± 1.9 | 6.3 ± 1.8 |

Sample sizes were $N=4$ to 5 per group. Values are mean ± SD.
[a]Different from young 2 days/week value, $p<0.05$.

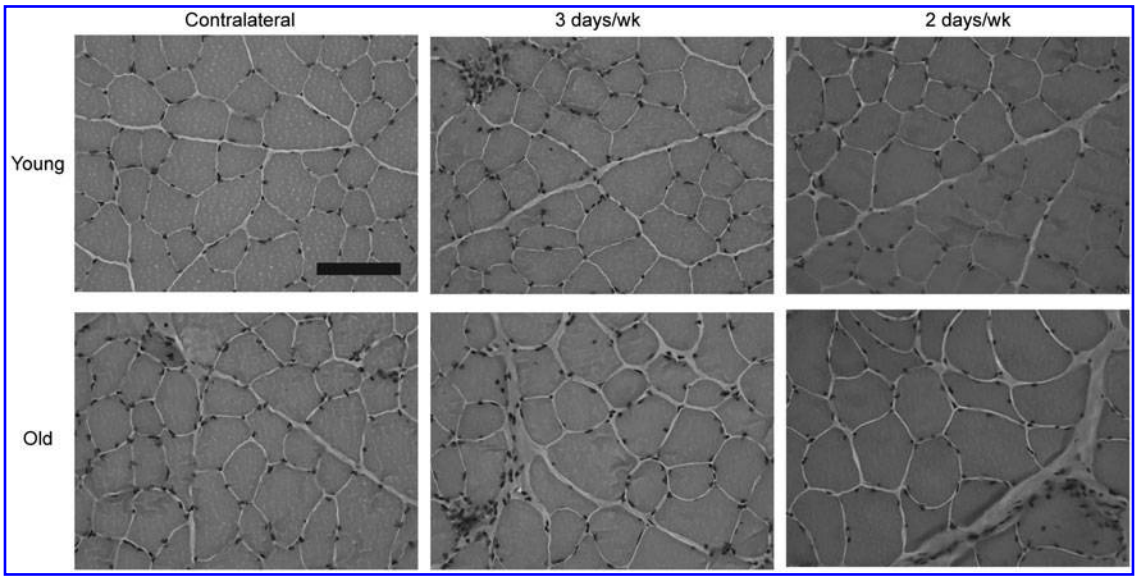


**FIG. 5.** Transverse sections of contralateral and exposed muscles following SSC training stained with hematoxylin and eosin for young and old rats. Scale bar = 100 $\mu$m.

when data for 2 and 3 days per week trained groups were pooled, the percentage of each muscle fiber positive for HNE staining in trained muscles of young rats, 56% ± 34%, was increased relative to that of contralateral muscles, 41% ± 25% ($p = 0.048$). This dispersal of lipid peroxidation was in agreement with the observation of the addition of greater sarcolemma staining of 4-HNE upon training in the young rats, indicating that a substantial level of lipid peroxidation at the sarcolemma accompanies adaptation. The percentage area of 4-HNE staining was unaltered for old rats even upon pooling of the data for 2 and 3 days per week training. This was not surprising since sarcolemma lipid peroxidation was already overtly present in the nontrained condition and remained posttraining.

To determine whether lipid peroxidation levels differ with training frequency in muscle tissue, MDA levels were quantified. For contralateral control muscles, MDA levels were 1.5-fold greater for old rats relative to those of young rats (Fig. 7). Exposure to 3 days per week of SSCs did not alter MDA levels. In contrast, 2 days per week of SSC training decreased MDA levels for muscles of old rats to levels of young rats (Fig. 7).

For old rats, Pearson product correlation analysis resulted in a negative correlation coefficient ($r = -0.719$, $p = 0.03$) between SSC-induced alterations in MDA levels versus muscle mass (*i.e.*, percentage difference in MDA levels relative to contralateral control levels versus percentage difference in muscle mass relative to contralateral control

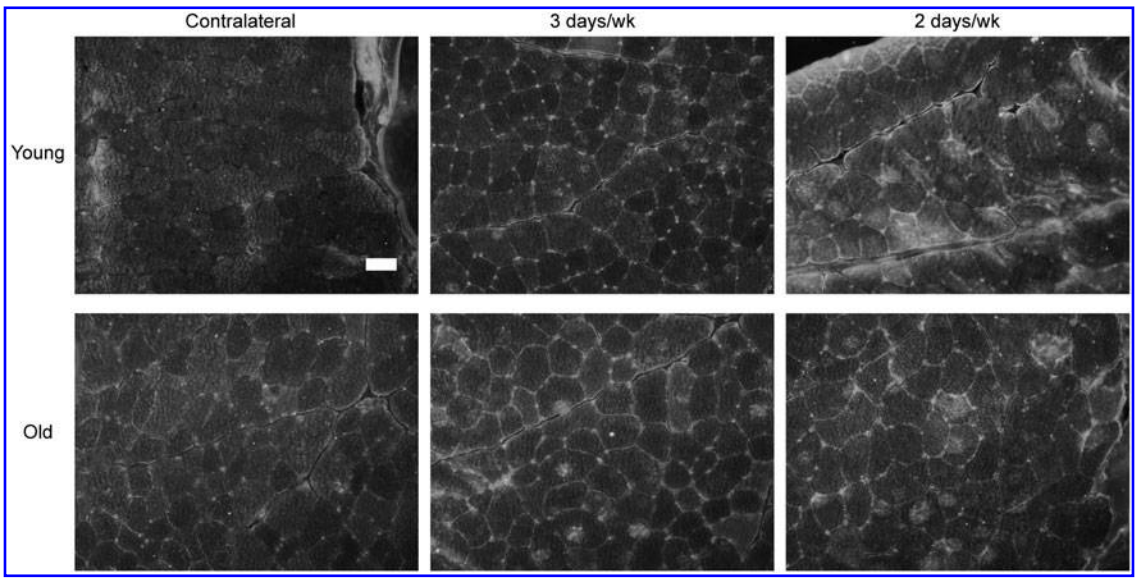


**FIG. 6.** Immunofluorescence staining for the lipid peroxidation marker 4-HNE in contralateral and trained muscles for young and old rats. Scale bar = 50 $\mu$m. 4-HNE, 4-hydroxynonenal.

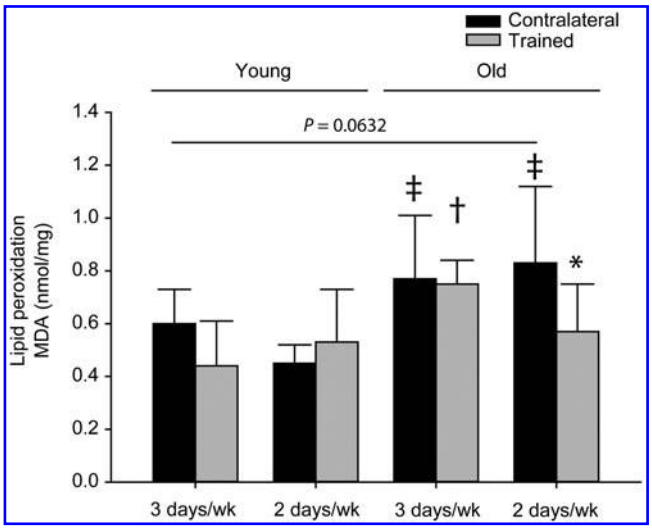


**FIG. 7.** Oxidative stress levels as determined by lipid peroxidation marker MDA was elevated in muscles of old rats and restored to young levels exclusively by SSC training 2 days per week. MDA measures were normalized to total protein content. Lipid peroxidation was elevated by aging as evident by increased levels in the contralateral muscles of old rats trained 2 and 3 days per week relative to those of the twice per week trained young rats. A trend for greater age-related lipid peroxidation was observed between contralateral control muscles of 2 days per week trained old rats and 3 days per week trained young rats. The only difference observed between trained muscles and contralateral muscles was for old rats trained 2 days per week. Sample sizes were $N=4$ to 5 per group. Values are mean ± SD. *Different from value for contralateral muscles; †Different from value for young rats exposed 3 days per week; ‡Different from value for young rats exposed 2 days per week, $p<0.05$. MDA, malondialdehyde.

values). A trend for a negative correlation was noted between SSC-induced changes in MDA levels versus positive work (*i.e.*, percentage difference in MDA levels relative to contralateral control levels versus percentage change in positive work relative to initial values; $r=-0.579$, $p=0.10$). A negative correlation trend was also observed between MDA levels of SSC-trained muscles versus final muscle quality for those muscles ($r=-0.641$, $p=0.06$). Overall, these data indicated that returning lipid peroxidation levels to young levels was inversely associated with improved skeletal muscle size, function, and quality concomitant with the 2 days per week training.

## Discussion

Sarcopenia consists of muscle mass decreases of 1%–2% per year beyond 50 years of age so that ~30% of muscle mass and strength is lost by the eighth decade of life.[22] Concomitant with this decline is an increased incidence of falls, insulin resistance, mitochondrial impairment, chronic heart failure, and bone density loss—all conditions that high-intensity resistance exercise demonstrates potential to help prevent, delay, or improve.[23–25] Unfortunately with aging, adaptation to various high-intensity resistance exercise programs becomes limited as well.[26–28] Therefore, systematic research is necessary to identify the key exercise parameters to ensure adaptation to high-intensity training at old age.

Utilizing an experimental rodent model in the present study allowed us to precisely test whether at an advanced age, muscle adaptation can occur following training at the highest intensity possible (*i.e.*, maximal muscle activation) for chronic SSC exposure. We demonstrate successful skeletal muscle enhancement following this high-intensity SSC training when frequency of training is optimized at old age.

Following 3 days per week training, positive work capacity diminished, muscle mass was unaltered, and muscle quality was low, while 2 days per week training preserved positive work capacity, induced muscle mass gains, and restored muscle quality values to values comparable to those of young rats. The restoration of final muscle quality to young levels is impressive, given the 20% decrement in nontrained muscle quality values with aging (old vs. young; $0.55\pm0.13$ N/mg/mm vs. $0.69\pm0.04$ N/mg/mm, $p=0.027$) observed for Fischer Brown Norway hybrid rats reported on in a previous study.[19] Overall, such an improvement to chronic exposure of maximal intensity contractions demonstrates that high-intensity SSC training is capable of rejuvenating skeletal muscle at an advanced age, given training frequency is modulated appropriately.

The muscles of young rats adapted to the SSC training with an ~20% increase in muscle mass and peak force capacity regardless of whether frequency of training was 2 or 3 days per week. Research regarding human subjects indicate that muscles of young individuals can also adapt similarly, in terms of muscle size and strength, to two distinct exercise modes, conventional resistance training versus plyometric training.[29] Two reports regarding human subjects suggest that a high responsiveness to exercise is present at such young ages as adolescence and the transition from adolescence to adulthood.[30,31] Compared with adults, exercise-induced growth hormone response[31] and gains in explosive power[30] were superior in young subjects. Likewise, for rats exposed to 1 month of SSCs 3 days per week, muscles of young rats (3 months old) increase isometric and dynamic force output by 20%–30%, whereas at adulthood (6 months), no such force gains are realized.[32] Overall, these robust responses to a wide range of exposures at young age are consistent with an exceptional adaptive capacity at this stage of life.

Substantial muscle fiber size gains have been realized in older men and women at such low training frequencies as 1–2 days per week.[23,33,34] Indeed for the elderly, low volume of training has been previously recommended based on the observation of compromised recovery following contractions with aging.[35,36] In a recent report focused on exercise mode, 60–70 year old males gained muscle size and strength following a regimen consisting of SSCs for 10-weeks.[37] The frequency of training was 2 to 3 days per week such that the exposure for the first week was 2 days per week, the exposure for the second and third weeks were 3 days per week, and then this exposure sequence was repeated for the remaining 7 weeks. Overall, the findings suggest that aging is accompanied by an increased sensitivity to training frequency.

For muscles of young rats, maintenance of relatively low levels of lipid peroxidation was presumably conducive for adaptation. However, a training-induced alteration in the

spatial distribution of lipid peroxidation toward an increased lipid peroxidation at the sarcolemma was observed by 4-HNE staining. This indicated that an increased dispersal of lipid peroxidation (rather than increased tissue levels) accompanies adaptation. With aging, substantial lipid peroxidation at the sarcolemma was present even in nontrained muscles. In this scenario, this may have been the result of excessive lipid peroxidation since high tissue levels of lipid peroxidation were observed. Such age-related increases in lipid peroxidation levels have been observed previously.[13,15]

The unique finding of this investigation was the lowered lipid peroxidation levels for old rats exclusively following the adaptive 2 days per week training. This implies that when baseline lipid peroxidation levels exceed a certain threshold as in the case of old rats in this study, a decreased training frequency is necessary to allow training to diminish such a heightened state of oxidative stress and permit muscle adaptation.

A negative correlation between lipid peroxidation status and SSC-induced performance is consistent with previous investigations in two studies by Ryan et al.[13,15] In these reports, 3 days per week SSC training was accompanied by decreased lipid peroxidation levels and a lack of maladaptive performance. Ryan et al. described how the lack of maladaptive performance in these two studies was atypical and attributed this to differences in individual animal cohorts obtained from the National Institute of Aging, a possibility because of the tendency for increased variation observed with increased age of organisms/subjects.[11,13,15,38,39] Nevertheless, the general finding from these studies was that low lipid peroxidation levels accompany a beneficial functional response.

Future study is required to confirm whether the lowered lipid peroxidation levels induced by 2 days per week training are indeed indicative of a role for overall oxidative stress in the outcome to high-intensity SSC training. Such a role for oxidative stress in adaptation/maladaptation is consistent with the finding that antioxidant supplementation with Vitamin E and Vitamin C increases oxidant buffering capacity and improves adaptation to SSC training for old rats.[15] Provided the lipid peroxidation data are representative of oxidative stress, this study indicates that increasing recovery time between training sessions at an advanced age decreases the redox environment to low levels, an environment important for maintaining performance during individual contractions as well as diminishing risk of other age-related oxidative stress-induced impairments.[15] Therefore, such a modulation of high-intensity resistance exercise has the potential to revitalize skeletal muscles at old age and improve quality of life at an advanced age.

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### Publications' Disclaimers

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.

### Author Disclosure Statement

No conflicting financial interests exist.

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