Experimental Gerontology 72 (2015) 138–149

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Desensitized morphological and cytokine response after stretch-shortening muscle contractions as a feature of aging in rats☆

Erik P. Rader *, Kayla N. Layner, Alyssa M. Triscuit, Michael L. Kashon, Ja K. Gu, James Ensey, Brent A. Baker

Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Morgantown, WV 26505, United States

ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form 14 September 2015
Accepted 29 September 2015
Available online 8 October 2015

Keywords:
Contraction-induced injury
Dorsiflexor muscles
Stereology
Chemokines
Muscle strain

ABSTRACT

Recovery from contraction-induced injury is impaired with aging. At a young age, the secondary response several days following contraction-induced injury consists of edema, inflammatory cell infiltration, and segmental muscle fiber degeneration to aid in the clearance of damaged tissue and repair. This morphological response has not been wholly established at advanced age. Our aim was to characterize muscle fiber morphology 3 and 10 days following stretch-shortening contractions (SSCs) varying in repetition number (i.e. 0, 30, 80, and 150) for young and old rats. For muscles of young rats, muscle fiber degeneration was overt at 3 days exclusively after 80 or 150 SSCs and returned significantly closer to control values by 10 days. For muscles of old rats, no such responses were observed. Transcriptional microarray analysis at 3 days demonstrated that muscles of young rats differentially expressed up to 2144 genes while muscles of old rats differentially expressed 47 genes. Bioinformatic analysis indicated that cellular movement was a major biological process over-represented with genes that were significantly altered by SSCs especially for young rats. Protein levels in muscle for various cytokines and chemokines, key inflammatory factors for cell movement, increased 3- to 50-fold following high-repetition SSCs for young rats with no change for old rats. This age-related differential response was insightful given that for control (i.e. 0 SSCs) conditions, protein levels of circulatory cytokines/chemokines were increased with age. The results demonstrate ongoing systemic low-grade inflammatory signaling and subsequent desensitization of the cytokine/chemokine and morphological response to contraction-induced injury with aging — features which accompany age-related impairment in muscle recovery.

Published by Elsevier Inc.

1. Introduction

Musculoskeletal disorders including contraction-induced muscle injuries are among the most common injuries precipitated during demanding work or exercise (Gallagher and Heberger, 2013). These injuries result in significant pain, weakness, and disability (Woolf et al., 2012). Contraction-induced injury is initiated by focal mechanical damage to sarcomeres and disruption of excitation–contraction coupling (Macpherson et al., 1996; Baumann et al., 2014). Studies in young rodents have established a secondary response following severe contraction-induced injury characterized by increased interstitial space indicative of edema and degeneration of damaged segments of muscle fibers (Zerba et al., 1990; Baker et al., 2007). This secondary response is critical for clearing damaged tissue for subsequent muscle fiber regeneration and recovery (Brooks and Faulkner, 1990; Mishra et al., 1995; Ploutz-Snyder et al., 2001).

The extent of the secondary response on muscle morphology is not well established at advanced age. Morphological analyses in aging studies have consisted primarily of quantifying intact and damaged fibers by histology several days after exposure to various lengthening contraction protocols (Brooks and Faulkner, 1990; McArdle et al., 2004). After 75 lengthening contractions, muscles of old animals sustain a comparable extent of overt muscle fiber damage to that of young mice despite a 1.6-fold increase in initial contraction-induced force deficit with aging (Zerba et al., 1990; Lockhart and Brooks, 2006). This finding suggests the possibility that overt segmental muscle degeneration during the secondary response of contraction-induced injury may be blunted at old age. However, whether this possibility is accurate has not been systematically studied. Furthermore, injury from stretch-shortening contractions (SSCs), contractions typical during daily living (e.g. walking and running) and occupational-related activities (e.g. reciprocal lifting and lowering tasks), have not been studied in this context (Komi, 2000).

To address whether at old age, muscles undergo a blunted morphological response to SSC-induced injury, we tested the hypothesis that compared with muscles of young rats, muscles of old rats would exhibit

☆ Publication disclaimers: "The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health."

* Corresponding author at: MS L3014, 1095 Willowdale Rd., Morgantown, WV 26505, United States.

E-mail addresses: WLZ4@cdc.gov (E.P. Rader), klayner@mix.wvu.edu (K.N. Layner), atriscui@mix.wvu.edu (A.M. Triscuit), mqk1@cdc.gov (M.L. Kashon), JGu@cdc.gov (J.K. Gu), jee6@cdc.gov (J. Ensey), bwb3@cdc.gov (B.A. Baker).

http://dx.doi.org/10.1016/j.exger.2015.09.020
0531-5565/Published by Elsevier Inc.

less pronounced SSC-induced alterations in muscle mass, degenerative muscle fiber composition, and interstitial space at 3 and 10 days after SSC protocols varying in repetition number (i.e. 0, 30, 80, 150 contractions). The repetition number range tested was based on prior research by our laboratory in which 150 SSCs induced an impaired recovery for muscles of old rats (Krajnak et al., 2006). Morphological characterization consisted of quantitative analysis of muscle fibers and interstitial space by stereology. To determine the molecular factors underlying the morphological response, transcriptional microarray analysis was undertaken and protein levels of cytokines and chemokines were evaluated. The findings are valuable for establishing the morphological and cytokine/chemokine responses which accompany viable recovery at young age and compromised recovery at old age.

2. Materials and methods

2.1. Animals

Male Fischer Brown Norway hybrid rats (F344 X BN F1) were obtained from the National Institutes of Aging colony. Young adult rats (n = 56; mean body mass ± SE, 329 ± 7 g; age, 12 weeks) and old rats (n = 54; mean body mass ± SE, 564 ± 7 g; age, 30 months) were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal quarters. After one week of acclimatization, the animals were randomly assigned to each experimental group for testing. All animal procedures were approved by the Animal Care and Use Committee at the National Institute for Occupational Safety and Health in Morgantown, WV.

2.2. SSC exposure

Rats were anesthetized with isoflurane gas and placed supine on a heated (37 °C) x–y positioning table of a custom-built rodent dynamometer system (Baker et al., 2007). The left knee was secured in flexion (90°) with a knee holder and the left foot was secured to the load cell fixture. Platinum electrodes were placed subcutaneously for activation of the common peroneal nerve. For all protocols, muscle was stimulated at 4-V magnitude, 0.2-ms pulse duration, and a 120-Hz frequency, settings optimized for maximal contraction. To assess dynamic performance, dorsiflexor muscles were exposed to a single SSC consisting of maximally activating the dorsiflexor muscles for 300 ms, then the ankle was rotated from 70° to 140° at 500° per second and returned to 70° at the same velocity (Baker et al., 2007). After cessation of ankle rotation, activation continued for an additional 300 ms. The dorsiflexor muscles were then exposed to a SSC protocol (30, 80, or 150 SSCs) or were left unexposed (0 SSCs) to serve as a control group.

The repetition range was based on previous studies in our laboratory (Krajnak et al., 2006; Baker et al., 2007; Cutlip et al., 2014). In regard to the 150 SSC protocol in particular, muscle performance was evaluated for both young and old rats (Krajnak et al., 2006). In this study, the SSC protocol induced comparable decreases in peak dynamic forces during the initial and secondary phases of injury for young rats (57 ± 2% and 81 ± 8% of pre-exposure values, respectively) and old rats (52 ± 4% and 75 ± 6% of pre-exposure values, respectively). Despite the similar force deficits initially, age-related differences were evident at later time points. Muscles of young rats functionally recovered by 7 days whereas muscles of old rats exhibited impaired recovery by generating low forces, 10.0 ± 0.7 N at 7 days (P = 0.002) and 11.8 ± 0.5 N at 10 days (P = 0.08) relative to pre-exposure peak forces of 13.4 ± 0.9 N. The finding that the 150 SSC protocol exposed age-related differences in functional recovery provided a basis to select this protocol as an upper limit. The SSC protocols with intermediate repetition numbers were selected based on previous exposure protocols tested in our laboratory with slight modification (Baker et al., 2007; Cutlip et al., 2014).

For each set of the SSC protocol, the dorsiflexor muscles were maximally activated for 100 ms with the ankle at 90°, then the ankle was rotated to 140° at a velocity of 500° per second and returned to 90° in a reciprocal fashion for 10 oscillations. Muscle activation stopped 300 ms after the final oscillation of the set. Each set was repeated at 1 min intervals until the appropriate number of SSC were achieved. At 3 min after the SSC protocol, the dorsiflexor muscles were exposed to a single SSC at the same settings as those for the single SSC before the injury protocol. SSC-induced deficits in force stabilize by 3 min thereby providing an appropriate time to measure initial functional deficits without confounding transient factors such as fatigue (Baker et al., 2006). The experiment was terminated and tissue/blood saved for analysis at 3 days, a time period marked by an intense inflammatory response in muscles of young rats exposed to 150 SSCs or 10 days, when muscles of young rats return to pre-injury force production and muscle fiber morphology (Baker et al., 2006; Krajnak et al., 2006). Upon heart puncture, whole blood was collected in a 7 mL K_2EDTA vacutainer tube for the gene expression assay and in a 7.5 mL Vacutainer Serum tube (Fisher Scientific) for protein analysis. TA muscles were surgically removed, weighed, and the tibia length recorded. Normalized muscle mass was determined by dividing muscle mass by tibia length. The mid-belly of the TA muscle was covered with tissue freezing media and frozen in cold isopentane and stored at 80 °C for histology. The remaining portions of TA muscle were allocated for gene expression assays and protein analysis.

2.3. Quantitative morphology

The mid-belly of each TA muscle was transversely cryosectioned at 10 μm thickness followed by hematoxylin and eosin staining. Histological analysis was performed by a standardized stereological method (Baker et al., 2006, 2007). On either side (by 1 mm) of the midpoint of the section, stereological analysis was systematically repeated at 5 equally spaced sites across the muscle section. At each site, points of a 121-point 11-line overlay graticule (0.04 mm^2 square with 100 divisions) were evaluated at 40× magnification. Since 121 points were evaluated in 10 fields, a total of 1210 points were analyzed per muscle section. Each point was identified as overlaying a normal muscle fiber, degenerative muscle fiber, cellular interstitium, or non-cellular interstitium. Three criteria were required for designation as a degenerative muscle fiber 1) loss of contact with surrounding fibers, 2) interdigitation of the sarcolemma by cellular infiltrates, and 3) internalization of cellular infiltrates (Baker et al., 2006). In addition, if a point overlaid a muscle fiber with at least one internal nucleus lacking contact with the sarcolemma, the muscle fiber was defined as a centrally nucleated fiber. Points which overlaid nuclei in sites between muscle fibers were counted as cellular interstitium. Points that overlaid regions between muscle fibers but did not directly overlay nuclei were counted as non-cellular interstitium. Percent of muscle tissue comprised of normal muscle fibers, degenerative muscle fibers, centrally nucleated muscle fibers, cellular interstitium, or non-cellular interstitium were computed as the percentage of points which overlaid each type of tissue relative to the total number of points.

In addition to evaluating points of the overlay graticule, the number of muscle fibers within the boundary of the graticule was counted. For a muscle fiber to be counted, the topmost point of the fiber had to be within the graticule boundary. Since degenerative fibers lacked distinct borders, only normal muscle fibers were counted. The number of fibers per unit cross-sectional area (number of fibers per mm^2) was calculated as the total number of fibers counted divided by the total area sampled over 10 regions (i.e. 0.4 mm^2). Mean muscle fiber area (μm^2) was determined by dividing the percent of tissue comprised of normal muscle fibers by the fiber number per unit area.

In addition to hematoxylin and eosin staining, other cryosections were fixed in 10% neutral buffered formalin and stained with 0.1% picrosirius red, 0.02% fast green, and Mayer's hematoxylin for 15 min,

1 min, and 1 min, respectively, with 1 min wash steps between each fixation/staining step. Similar to the methodology described above for hematoxylin/eosin stained sections, points of a 121-point 11-line overlay graticule were evaluated at various regions along the midbelly of TA muscles. Each point was classified as overlaying one of the following – cytoplasm, cellular interstitium, non-cellular interstitium stained with picrosirius red, non-cellular interstitium stained with hematoxylin, and non-cellular interstitium lacking staining. The percent of non-cellular interstitium positive for picrosirius red staining (expressed as a percentage of total interstitium sampled) was determined by dividing the point counts for picrosirius red stained non-cellular interstitium by the total point counts for non-cellular interstitium (i.e. all non-stained and stained counts).

2.4. Illumina microarray

Whole blood which was collected in a 7 mL K_2EDTA vacutainer tube via heart puncture at the termination of the experiment was immediately processed with the Leukolock RNA total isolation system kit (Ambion, Cat# AM1923). The whole blood tube was passed through a Leukolock filter to capture leukocytes then washed with 3 mL 1× PBS, followed by 3 mL of RNAlater. Samples were then stored @ −80 °C until RNA isolation could be carried out. Before RNA isolation, filters were removed from the freezer and allowed to come to room temperature before following standard kit protocols to isolate RNA. For muscle tissue that had been flash frozen and stored at −80 °C, samples were retrieved and a ~50 mg portion was cut from the frozen tissue and place into a 2 mL vial of 1 mL of 1.0 mm zirconia beads (BioSpec, Cat# 11079110zx) and 1 mL TRIzol. Samples were then placed in a Mini-BeadBeater 8 (BioSpec) for 1 min to homogenize tissue and then RNA isolation was carried out using the RNAqueous Phenol-free total RNA Isolation Kit (Ambion, Cat# AM1912) following the standard kit protocol. RNA integrity was assessed via Bioanalyzer 2100 (Agilent) with RNA Integrity Numbers (RIN) greater than or equal to 8.0 and spectrophotometry using a Nanodrop2000 (Thermo Scientific) with 260/280 ratios of 2.0–2.1.

A working solution for each sample (N = 7 to 10 per group) at a concentration of 125 ng/μl was made and 375 ng of total RNA was labeled using the Illumina TotalPrep RNA Amplification Kit (Ambion, Cat# AMIL1791). First strand cDNA synthesis was performed using a Labnet Mini-Incubator (Labnet, Cat# I-5110) for 2 h at 42 °C. Second strand cDNA synthesis was achieved using a PTC-225 DNA Engine Terad (MJ Research) for 2 h at 16 °C. An in vitro transcription reaction was run for 14 h overnight at 37 °C. Standard Illumina protocols were followed for wash steps and samples were stored at −80 °C. The cDNA (750 ng reconstituted to 5 μl final volume) was processed and loaded onto Ref12 Beadchips (Illumina, Cat# BD-27-303) for incubation at 58 °C for 20 h. The samples were scanned at the Allegheny Singer Research Institute (Pittsburgh, PA).

Metrics files from the bead scanner were checked to ensure that all samples fluoresced at comparable levels before samples were loaded into the Beadstudio (Framework version 3.0.19.0) Gene Expression module v.3.0.14. Housekeeping, hybridization control, stringency and negative control genes were checked for proper chip detection. Bead array expression data were then exported with mean fluorescent intensity across like beads, and bead variance estimates into flat files for subsequent analysis. Illumina BeadArray expression data were analyzed in Bioconductor using the 'lumi' and 'limma' packages. Bioconductor is a project for the analysis and comprehension of genomic data and operates in R, a statistical computing environment (Gentleman et al., 2004). The 'lumi' Bioconductor package was specifically developed to process Illumina microarrays and covers data input, quality control, variance stabilization, normalization and gene annotation (http://bioconductor.org/packages/2.2/bioc/vignettes/lumi/inst/doc/lumi.pdf). In short, limma fits a linear model for each gene, generates group means of expression, conducts pairwise comparisons between selected groups, and calculates P values using empirical Bayes methodology. The log fold changes of gene expression were converted to standard fold changes and raw P values were corrected for false discovery rate (FDR) using the Benjamini and Hochberg method. These lists of genes and their associated statistics were utilized as input for subsequent bioinformatic analysis.

Bioinformatic analysis was performed using IPA (Ingenuity Systems, www.ingenuity.com). Genes in which differential gene expression fulfilled the criteria of FDR P value <0.01 and fold change >1.5 were analyzed by IPA. IPA biological function categories considered for analysis consisted of the molecular and cellular function categories, the physiological system development and function categories, and the inflammatory response category. Ranking of the biological function categories was based on the summation of all the −log P values for the biological functions belonging to each category. Determination of the activation status for each biological function category, the summation of −log P values for biological functions with positive activation z-scores was subtracted from the summation of the −log P values for biological functions with negative activation z-scores. IPA Upstream

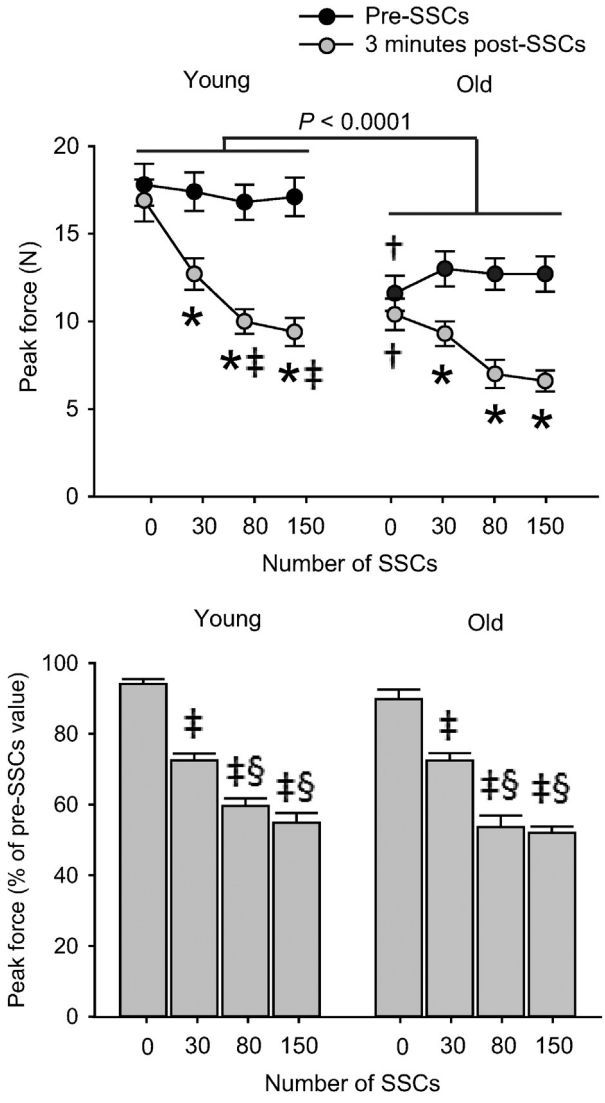


Fig. 1. Comparable initial deficits in performance for muscles of young and old rats after exposure to a SSC protocol (0, 30, 80, or 150 SSCs). Peak forces were expressed as absolute values or percentages of pre-injury protocol values. Sample sizes were N = 13 to 14 per group. Values are means ± S.E.M. Listed P value is for difference between young and old pre-SSC data. *Different from pre-injury protocol value; †different from young value; ‡different from 0 SSC value; §different from 30 SSC value, P < 0.05.

Regulator Analysis was used to predict the top 20 upstream regulators based on activation z-score.

2.5. ELISA

Blood collected via heart puncture in 7.5 ml Vacutainer Serum tubes were centrifuged at 2000 rcf for 10 min at 4 °C. Serum was drawn off and placed in labeled 2 ml cryovials and stored at −80 °C. For TA muscles, a ~75 mg piece of tissue was homogenized in 500 μl 1× PBS, aliquoted into 2 ml cryotube, and flash frozen in liquid nitrogen. SearchLight Rat Cytokine/Chemokine Arrays (Thermo Scientific Cat# 84716), a multiplex sandwich ELISA, were used to determine the protein concentration for 16 cytokines/chemokines. Samples were diluted 1:5 with Sample Diluent from the array kit and followed the kit standard protocol. Images of the 96-well plate were taken using an Alpha Innotech Fluorochem Imaging system and then analyzed by the Searchlight software to generate protein concentration against a standard curve.

2.6. Statistical analysis

Data were analyzed using ANOVA, with the animal as a random factor to account for repeated measures within animal when appropriate. Post hoc comparisons were done by Tukey's (Honest Significance Difference) analysis. All data are shown as means ± S.E.M. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparable initial performance deficits following SSC exposure with aging

Peak dynamic force was used to assess muscle performance (Fig. 1). Prior to SSC exposure, muscles of old rats generated peak forces, 12.5 ± 0.5 N, which were 73% of the forces of 17.2 ± 0.5 N for muscles of young rats ($P < 0.0001$). Exposure to SSC protocols with increasing repetition number induced a graded response in initial force deficits which were comparable for both age groups (Fig. 1). The 30 SSC protocol decreased peak force to 73 ± 2% and 72 ± 2% of pre-exposure values for muscles of young rats and old rats, respectively. The 80 SSC exposure decreased peak forces to a greater extent, i.e. to 60 ± 2% and 54 ± 3% of pre-exposure values for muscles of young and old rats, respectively. Force deficits for muscles of old rats and young rats after 150 SSC were not significantly different from deficits after 80 SSC. This indicated that post-protocol force was most sensitive to contraction number for exposures with less than 80 SSCs.

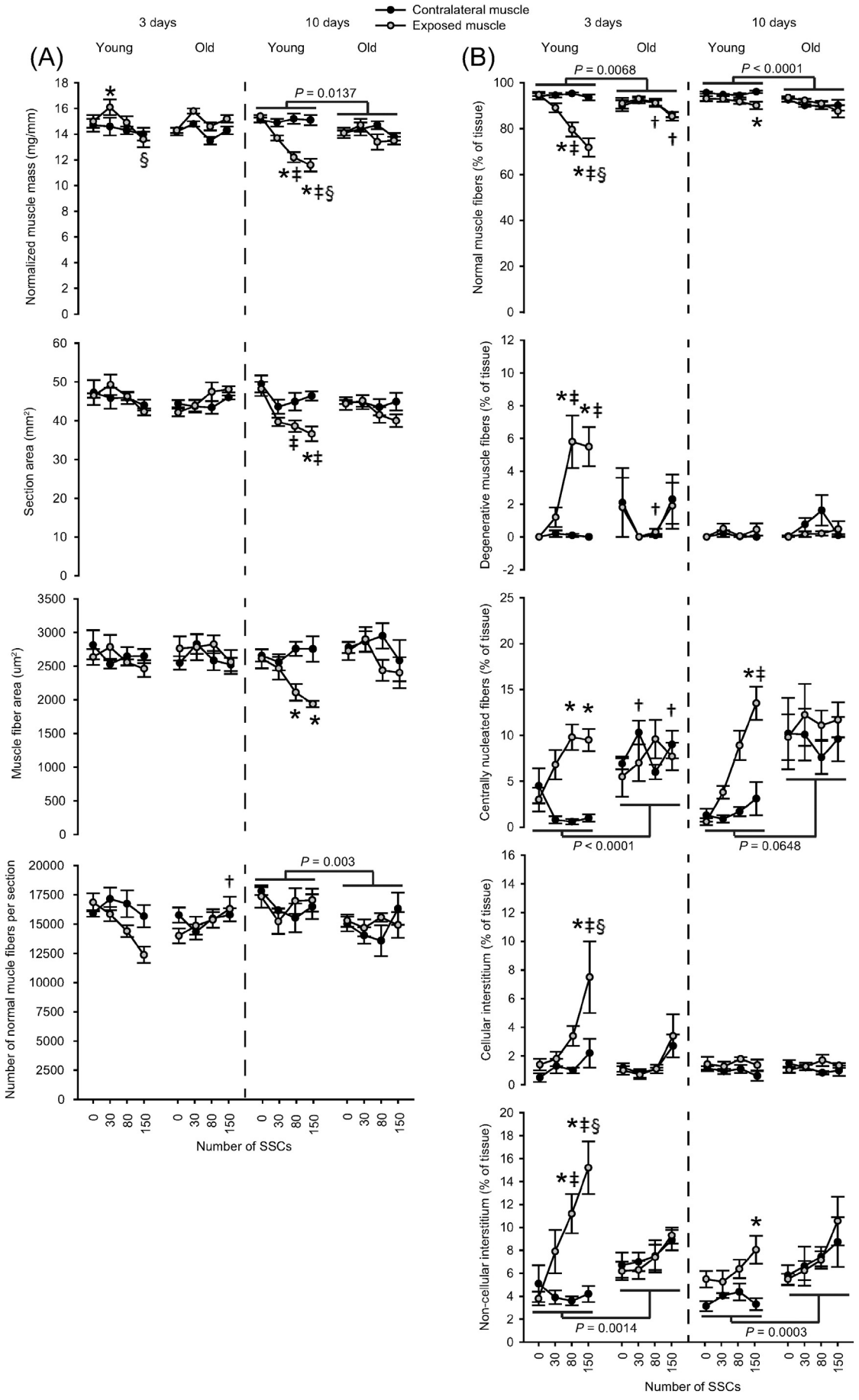
3.2. Deleterious age-related morphology at baseline and muted histological response post-SSCs

Upon removal of each muscle, muscle mass was determined and transverse muscle sections were examined. Edema post-SSCs was significant in muscles of young rats 3 days following 30 SSC exposure (Fig. 2A). At this time point, a decrease in the total number of normal muscle fibers in cross-section accompanied high repetition SSC exposure (Fig. 2A). At 10 days post-exposure, muscle masses of young rats were 81 ± 4% and 77 ± 2% of contralateral values following 80 and 150 SSCs, respectively ($P < 0.05$). Accompanying the reduced muscle mass was a comparable decrease in whole muscle section area and mean muscle fiber area. At 10 days following 150 SSCs, section area was 79 ± 3% of contralateral values and muscle fiber area was 72 ± 6% of contralateral values (Fig. 2A). Overall, these data indicated a dynamic response followed by a process of repair in muscles of young rats.

To evaluate the muscle tissue further, muscle fibers and the interstitium were characterized by quantitative morphology. In agreement with the indication of a decrease in total number of normal muscle fibers in cross-section for muscles of young rats 3 days after high repetition SSC exposure, the percentage of tissue composed of normal muscle fibers reduced to 72 ± 4% of contralateral values following 150 SSC exposure (Fig. 2B). In contrast, the percentage tissue comprised of degenerative muscle fibers, centrally nucleated fibers, cellular interstitium, and non-cellular interstitium increased 3 days after high repetition exposure for the young (Figs. 2B and 3). None of these changes were apparent in contralateral muscles. This was consistent with a robust and localized secondary phase of contraction-induced injury consisting of the following — an influx of inflammatory cells marked by increased cellular interstitium, muscle fiber degeneration of injured segments of muscle fibers, and increased interstitial space. By 10 days, the histological composition of muscles of young rats returns to that of control muscles with the exceptions of some increased non-cellular interstitium remaining and a high percentage of centrally nucleated fibers, a persistent feature of muscle fibers which recently undergo degeneration/regeneration (Fig. 3). Considering the presence of centrally nucleated fibers and the lack of major alterations in normal muscle fiber number or interstitium at 10 days, the decrease in muscle mass at that time point was consistent with a decreased muscle fiber area inherent to regenerating muscle fibers (Fig. 2A).

In contrast with the dynamic response for the muscles of young rats, muscles of old rats displayed a muted response. In the absence of exposure, Evidence of mild age-related sarcopenia was demonstrated by the finding that contralateral normalized muscle masses for old rats, 14.2 ± 0.2 mg/mm, were 94% of those for young rats ($P = 0.0137$), 15.1 ± 0.2 mg/mm (Fig. 2A). This decrease in muscle mass with age coincided with a 10% decrease in the number of normal muscle fibers while the fiber size was unchanged. Muscle sections of old rats contained 14,923 ± 319 normal fibers compared with the 16,578 ± 319 normal fibers ($P = 0.003$) for young rats (Fig. 2A). Muscle nuclei composition also changed with age. A significant percentage of tissue, 8.0 ± 0.6%, consisted of centrally nucleated muscle fibers for old rats relative to the value of 1.7 ± 0.6% for young rats ($P < 0.0001$) indicating denervation and/or muscle fiber degeneration/regeneration with aging (Figs. 2A and S1). The percentage of contralateral muscle tissue composed of normal muscle fibers was 90.3 ± 0.5% for old rats compared with 94.9 ± 0.3% ($P < 0.05$) for young rats (Fig. 2B). This age-related decrease was accompanied by a rise in the percentage of tissue consisting of non-cellular interstitium from 4.0 ± 0.3% for young rats to 7.3 ± 0.4% ($P < 0.01$) for old rats (Fig. 2B). No difference between non-exposed and SSC-exposed muscles was observed for old rats.

To evaluate the composition of the interstitium further, the non-cellular interstitium positive for picrosirius red staining (expressed as a percentage of total interstitium sampled) was evaluated for the two most extreme cases — contralateral muscles of rats exposed to 0 SSCs and exposed muscles and contralateral muscles of rats following 150 SSCs ($N = 5$ per group). For 0 SSC exposure, the percentage of picrosirius red staining within the interstitium was greater ($P = 0.04$) for muscles of old rats, 64 ± 4%, compared with that of young rats, 46 ± 6%. This suggested that the increased interstitium within non-exposed muscles with aging was concomitant with increased fibrillar collagen distribution characteristic of fibrosis. At 3 days post-150 SSC exposure, an interaction between the factors of age and SSC exposure was indicated ($P = 0.0528$). For young rats, values for exposed muscles were 67 ± 2% compared with 52 ± 6% for contralateral muscles. In contrast, contralateral and exposed muscles for old rats were not significantly different at 70 ± 4% vs 65 ± 3%, respectively. The age-sensitive response to 150 SSCs was also apparent at 10 days. Muscles of young rats attained values of 69 ± 3% with exposure compared with 48 ± 4% for contralateral muscles ($P = 0.03$). For old rats, no significant difference was observed for values of contralateral and exposed muscles, 60 ± 7% and 64 ± 4%, respectively. These results were consistent with the gene expression for various collagens (Table S1). Collagens are the most abundant proteins in mammals and make up the major


Contralateral muscle
Exposed muscle
3 days
10 days
Young
Old
(A)
(B)
Normalized muscle mass (mg/mm)
Section area (mm²)
Muscle fiber area (um²)
Number of normal mucle fibers per section
Normal muscle fibers (% of tissue)
Degenerative muscle fibers (% of tissue)
Centrally nucleated fibers (% of tissue)
Cellular interstitium (% of tissue)
Non-cellular interstitium (% of tissue)
P = 0.0137
P = 0.003
P = 0.0068
P < 0.0001
P < 0.0001
P = 0.0648
P = 0.0014
P = 0.0003
0
30
80
150
Number of SSCs

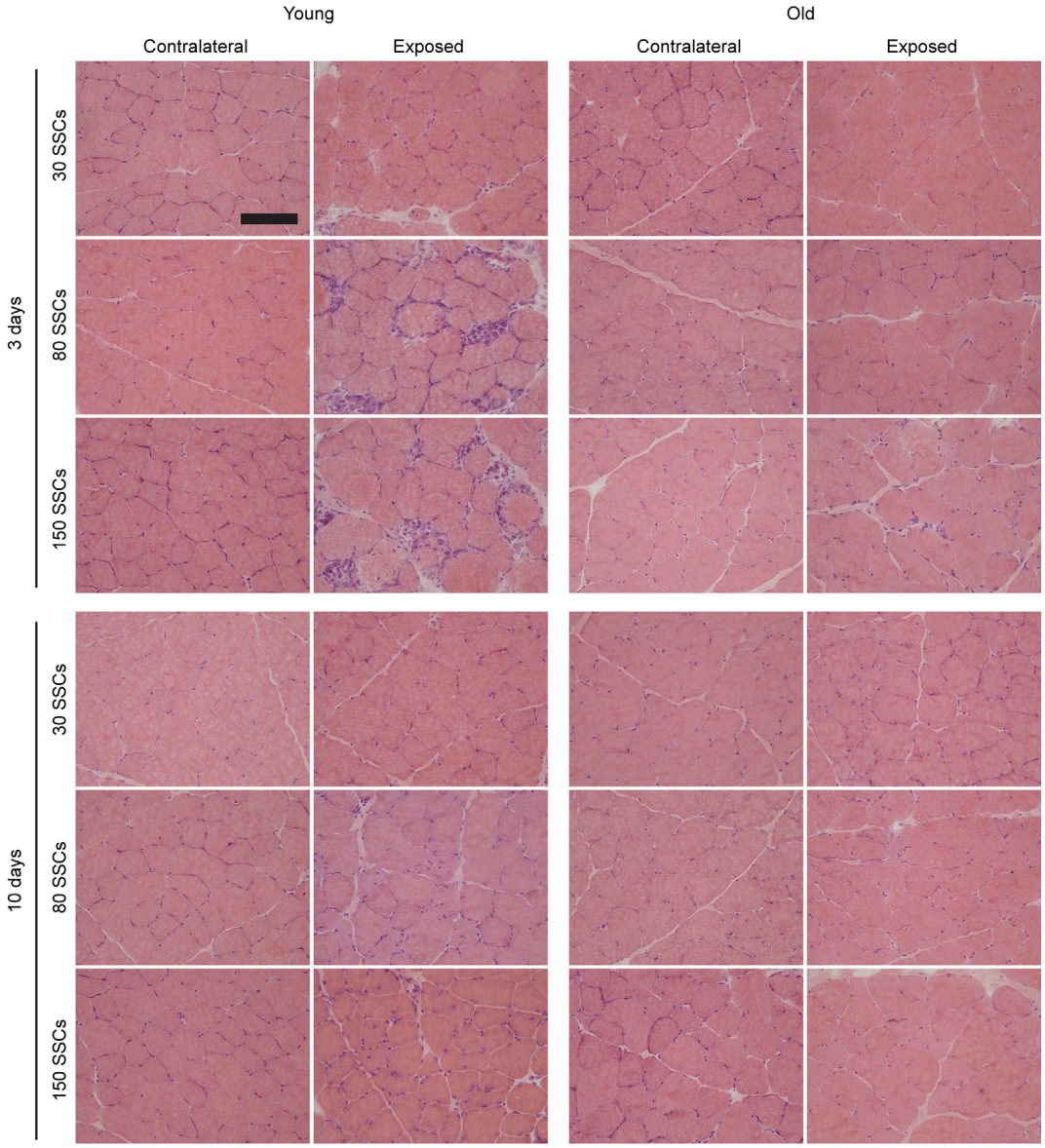


Fig. 3. Transverse sections of contralateral and exposed TA muscles following SSC exposure stained with hematoxylin and eosin for young and old rats. Scale bar = 100 μm.

protein content of the extracellular matrix with an especially important role in structural support and force transmission for skeletal muscle (Ramaswamy et al., 2011). In the interstitial matrix, fibrillar collagen types I and III predominate relative to other collagen isoforms such as the fibrillar collagen type V, microfibrillar collagen type VI, short chain collagen type VIII, and the FACIT (fibril-associated collagen with interrupted triple helices) collagen type XIV (Gelse et al., 2003). In the basement membrane, non-fibrillar type IV collagen is the major structural element (Tulla et al., 2001). For young rats of the present study, COL1A1 and COL1A2 expression (encoding for collagen type I components) increased 6.4-fold and 3.3-fold levels, respectively, 3 days post-150 SSCs and 3.2-fold and 1.5-fold, respectively, 10 days post-150 SSCs. COL3A1, COL4A1, COL5A1, COL5A2, COL6A1, COL6A3, COL8A1, and COL14A1 were also highly expressed 1.5- to 14.4-fold at those time points (encoding for components of the corresponding collagen types described above). For old rats, this response was diminished with fewer and lower (2.1- to 3.1 fold) 150 SSC-induced changes in collagen gene expression (Table S1). The implication is that muscles of old rats exhibited a muted fibrillar collagen response to SSCs after the initial setting of fibrosis at baseline.

3.3. Global gene expression profile

To investigate the molecular alterations underlying the performance and morphological changes, microarray data were analyzed. The effect of age alone was determined by evaluating non-exposed conditions (i.e. 0 SSCs). Age-related differentially expressed genes (>1.5 fold change and FDR < 0.01) were observed in muscle and, to even a greater extent, in circulatory leukocytes. A total of 122 and 190 genes were differentially expressed in muscles and leukocytes of old rats (relative to young values), respectively (Fig. S2A).

Fig. 2. Muscle mass and morphological analyses exhibited a graded response in muscles of young rats and a blunted response in muscles of old rats. For young mice, a dynamic response in (A) muscle size/cross-sectional fiber number and (B) muscle composition was observed. Sample sizes were N = 5 to 8 per group. Values are means ± S.E.M. Listed P values are for differences between young and old contralateral data with the exception of the P value for number of normal muscle fibers per section (where the P value is for the main effect of age in the ANOVA). *Different from pre-injury protocol value; †different from young value; ‡different from 0 SSC value; §different from 30 SSC value, P < 0.05.

Bioinformatic analysis by Ingenuity Pathway Analysis (IPA) identified top biological function categories for leukocytes and muscles consistent with chronic low-grade inflammatory signaling with aging. When considering ranking biological function categories by the number of differentially expressed genes or by IPA P values, besides some differences in order, the list of top categories was largely unchanged (Fig. S2B and Table S2). For leukocytes, the phagocytosis of phagocytes, recruitment of phagocytes, migration of neutrophils, and accumulation of neutrophils were among the ten most activated biological functions predicted by IPA (activation z-scores ≥2). These functions were classified under the biological function categories of cellular movement, cellular function and maintenance, hematological system development and function, cell-to-cell signaling and interaction, immune cell trafficking, and inflammatory response. The range of biological functions was more varied in muscle. The inflammatory response biological function category as a whole appeared to be indicating downregulation but significant activation z-scores were achieved for leukocyte migration and cell movement of leukocytes along with proliferation of muscle cells and angiogenesis. Despite the absence of a robust age-related difference in cytokines/chemokine gene expression (Table S3), Upstream Regulator Analysis in IPA predicted that several molecules involved in inflammatory signaling including cytokines were up-regulated in both leukocytes and muscle (Fig. S2C). The cytokines interferon gamma (IFNG), tumor necrosis factor (TNF), colony stimulating factor 1 (CSF1), and transforming growth factor beta 1(TGFB1) all were among the top 20 molecules based on activation z-scores (Fig. S2C).

The transcriptional response after SSCs was pronounced for muscles of young rats. The number of differentially expressed genes relative to 0 SSC exposure exhibited a graded response to SSC repetition number and ranged from 1194 to 2144 genes at 3 days and 141 to 505 genes at 10 days (Fig. S3). Analysis by IPA revealed that the biological function categories such as the inflammatory response, cellular movement, cell death and survival, and tissue development were among the categories with the greatest number of differentially expressed genes (Fig. S4). Cellular movement was the top ranked category according to IPA P values for all of the SSC exposures and time points with the exception of the 30 SSC exposure at 10 days (Table S4). The biological functions among the top ten activation z-scores (ranged from 4.854 to 6.221) included biological functions which were relevant to inflammation and cellular movement such as migration of phagocytes, cell movement of phagocytes, and engulfment of cells. The role of cytokine/chemokines, essential mediators of inflammatory signaling and cell movement, was implicated in the SSC-induced response because of the increased gene expression for several cytokine/chemokines and high activation z-scores upon IPA Upstream Regulator Analysis (Table S5 and Fig. S5). Of the top 20 molecules based on activation z-scores, a total of 12 cytokines were listed at 3 days and 7 cytokines were listed at 10 days (Fig. S5). No SSC-induced differentially expressed genes were observed for leukocytes extracted from blood implying the dominant exposure effect was specific to the TA muscle.

The SSC-induced transcriptional response was greatly blunted for the muscles of old rats. The number of differentially expressed genes ranged from 4 to 47 genes at 3 days and 0 to 109 genes at 10 days (Fig. S3). This decrease in transcription relative to young rats was also reflected in the IPA biological function categories (Fig. S4). For old rats, ranking of biological function categories based on IPA P values was only possible for 80 and 150 SSC protocols (Table S4). For these exposures at 3 days, despite some differences in order, the majority of the top ranked categories are in common between young and old rats. However, an important distinction is the difference in activation status of the biological function category for cell death and survival (Table S4). The indication of downregulation of this category for old rats corresponded with the absence of predicted activation for the biological functions of cell survival and cell viability, functions activated for young rats with SSC-exposure. At 10 days, the biological function category order for muscles of old rats exposed to 80 or 150 SSCs exhibited greater similarity to the young 30 SSC group than the other young groups. This implied that the response for the old rats was a muted version of that of the young rather than a completely different type of response. The age-related effect was pronounced upon Upstream Regulator Analysis. No significant activation z-scores were observed for 30 SSC exposure and at 3 days, 5 of the 12 cytokines with high z-scores for young rats did not reach significance for old rats (Fig. S5).

3.4. Age-related increase in circulatory cytokine/chemokine protein levels and desensitized SSC-induced response in muscles

To evaluate the age-related cytokine response further, a panel of cytokines and chemokines were evaluated by ELISA in muscle homogenate at the 3 day time point. Overall, the majority of the cytokines and chemokines analyzed for the young rats displayed a repetition number dependent pattern of increasing levels which plateau for the 80 and 150 SSC exposures (Figs. 4 and 5). In contrast, no such repetition number dependency was observed for the muscles of old rats. For young rats, the cytokines CSF2, IL-1β, and IL10 were increased 3- to 6-fold for the 80 SSC and 150 SSC exposures relative to the values for the 0 SSC exposure ($P < 0.05$). For IL-6, comparisons among individual groups did not reach significance but an age effect for the ANOVA was significant ($P = 0.002$). In general, chemokines displayed a more robust response compared with that of the cytokines. Chemokines CCL2, CCL3, CXCL1, and CXCL2 displayed pronounced levels at the high repetition SSC exposures with 50-fold, 30-fold, 10-fold, and 4-fold increases relative to values at 0 SSC, respectively ($P < 0.05$). In addition, a trend for a 5-fold increase was observed for CCL5 ($P = 0.0752$).

Cytokine/chemokine levels were analyzed by ELISA in serum at the 3 day time point as well to evaluate the systemic environment (Table 1). The responses of several cytokines/chemokines were dependent on age and independent of SSC exposure in the serum. Compared with levels in for young rats, values for the cytokines IFNγ and IL10 for old rats were on average 2-fold and 4-fold greater, respectively ($P < 0.05$). A 2-fold increase in the serum of old rats relative to young rats was also indicated for IL2α ($P = 0.0795$). The chemokines CCL3, CXCL1, and CXCL2 in serum of old rats were 2-fold greater on average than for those of young rats ($P < 0.05$). These data were consistent with the notion that low-level chronic inflammation develops with age and accompanies a desensitized response to contractions.

4. Discussion

Between adulthood and 80 years of age, skeletal muscle mass and strength decrease by ~30% (Lauretani et al., 2003; Lovering and Brooks, 2014). These age-related declines have a profound effect on the frailty and immobility experienced by the elderly (Hairi et al., 2010). Exercise is recommended to help offset sarcopenia and muscle weakness. However, exercise prescription is especially difficult for the elderly because of the possibility of incurring a contraction-induced injury, an injury which has the potential to exacerbate age-related muscle dysfunction (Lovering and Brooks, 2014). For severe contraction-induced injuries at adulthood, a secondary response several days following exposure is comprised of edema, inflammatory cell infiltration, and segmental muscle fiber degeneration (Lauritzen et al., 2009; Paulsen et al., 2010b; Peake et al., 2010). For mild to moderate contraction-induced injuries, such a response is largely lacking (Malm et al., 2000; Yu et al., 2013). The secondary response following severe contraction-induced injury has been well documented in rodent models especially in regards to injury induced by high-repetition lengthening contraction protocols (Zerba et al., 1990; Brooks et al., 2001; McArdle et al., 2004). Contraction-induced injury can also be induced by SSCs as well, but such research is limited (Komi, 2000). To address this, our group previously investigated a range of SSC protocols varying in repetition number and analyzed the impact on in vivo MRI imaging and muscle morphology in young rats (Baker et al., 2007; Cutlip et al.,

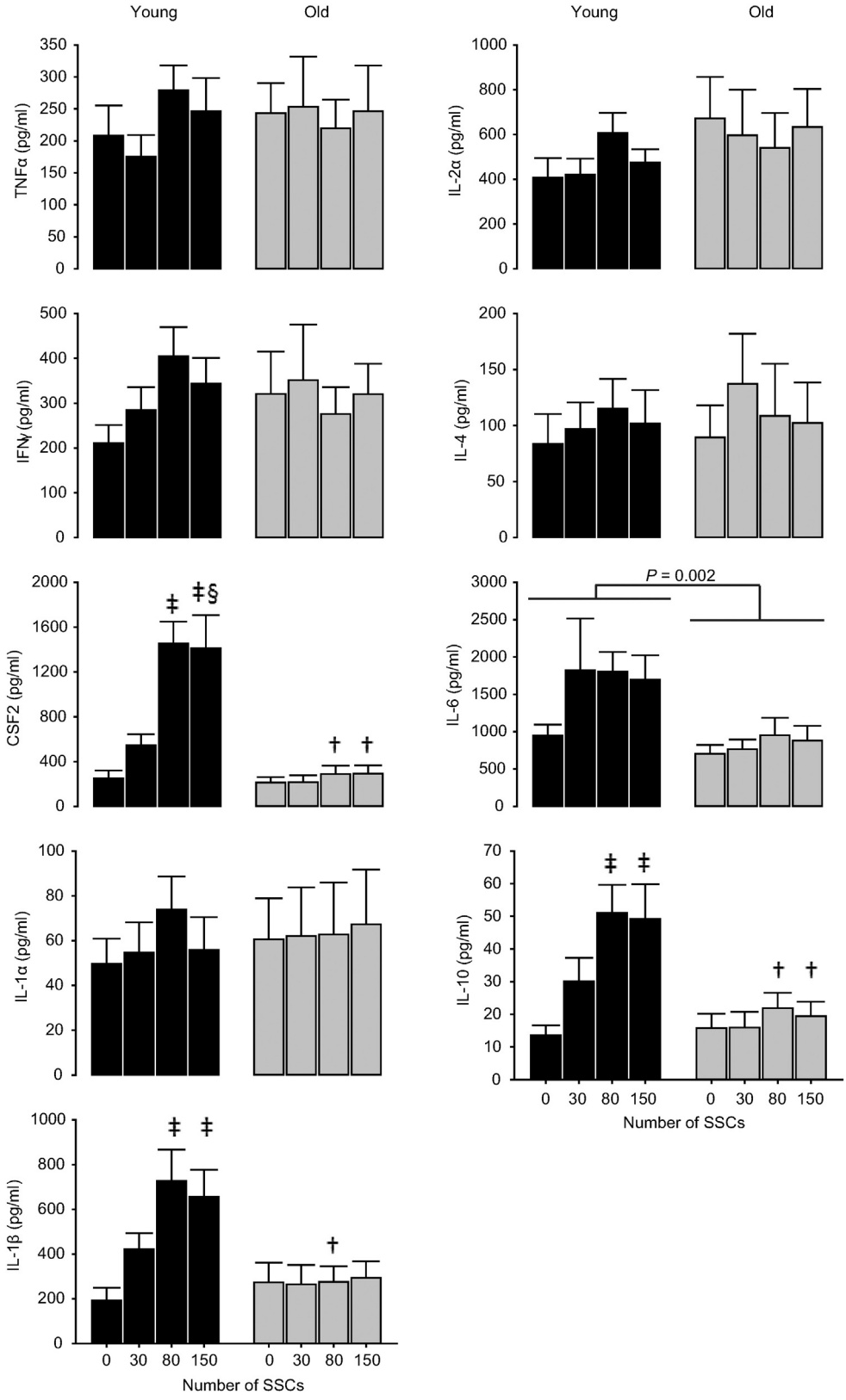


Fig. 4. Cytokine protein levels within muscle homogenates following SSC exposure. Sample sizes were N = 6 to 8 per group. Values are means ± S.E.M. Listed P value is for the main effect of age in the ANOVA. †Different from young value; ‡different from 0 SSC value; §different from 30 SSC value, $P < 0.05$.

2014). The findings identified a threshold in terms of repetition number which must be reached before a robust secondary response of edema, cellular infiltration, and segmental fiber necrosis results. Using a high-repetition SSC protocol, we then demonstrated that muscles of old rats sustain greater force deficits in the days following exposure (Krajnak et al., 2006). However, morphology was not evaluated in the immediate days following exposure for old rats and low-repetition SSC protocols were not tested. The present study advances our previous

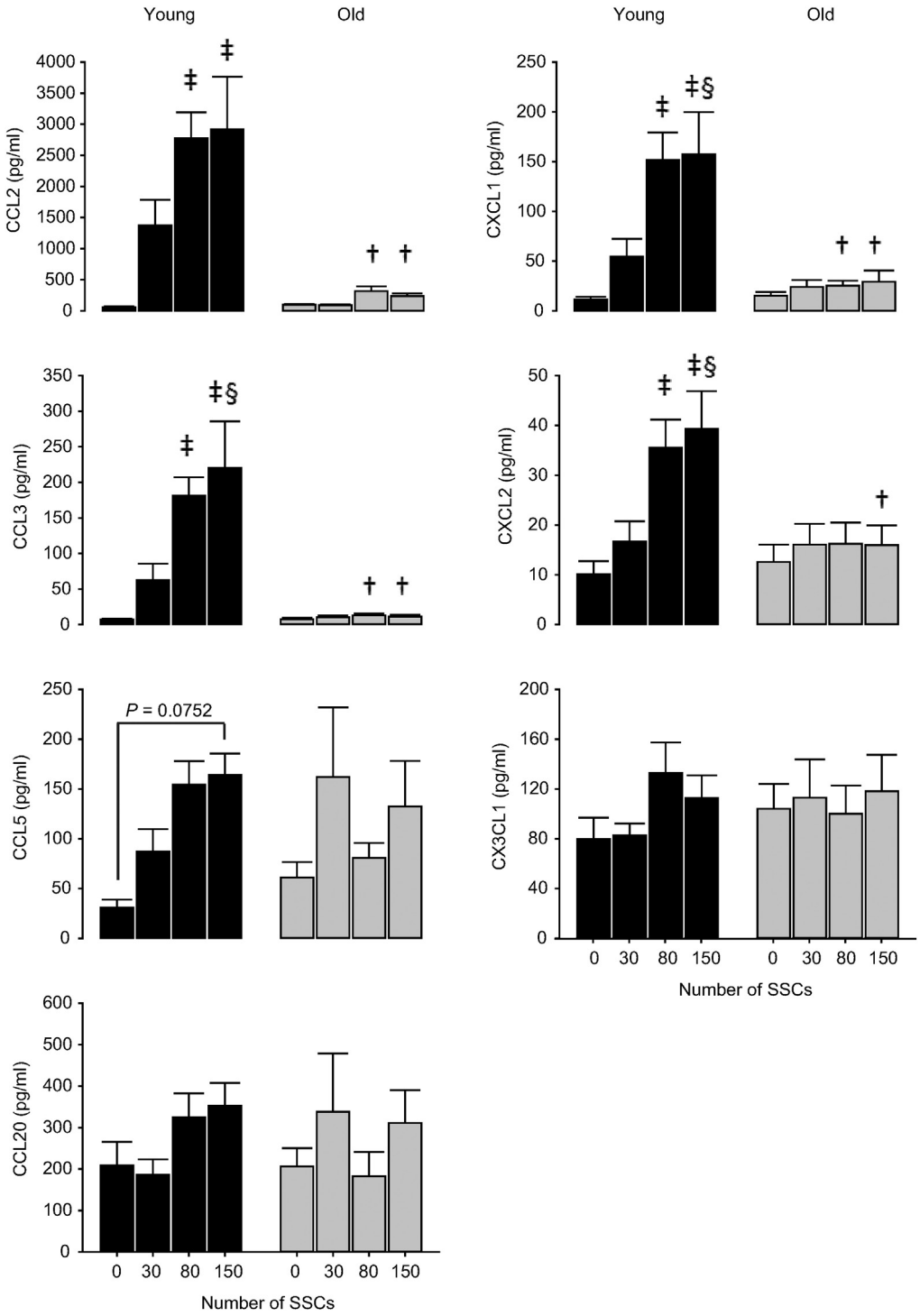


Fig. 5. Chemokine protein levels within muscle homogenates following SSC exposure. Sample sizes were N = 6 to 8 per group. Values are means ± S.E.M. †Different from young value; ‡different from 0 SSC value; §different from 30 SSC value, $P < 0.05$.

research by demonstrating that with aging, the secondary morphological response is blunted for a range of SSC protocols and is concomitant with a reduced cytokine/chemokine response. Such a diminished response may contribute to the age-related impairment in recovery following contraction-induced injury.

For adult human subjects, the magnitude of the initial force deficit following muscle contractions is predictive of whether a degenerative secondary response occurs in the subsequent days (Beaton et al., 2002; Paulsen et al., 2010a, 2012). The absence of infiltrating inflammatory cells and segmental necrosis has been observed following exercise-induced force deficits as high as ~30% or less (Beaton et al., 2002; Mikkelsen et al., 2009). More severe initial force deficits are typically followed by an overt secondary response (Paulsen et al., 2010a, 2012). The results of the present study were consistent with this trend. For muscles of young rats, the 30 SSC protocol induced an initial force deficit of 27% and no inflammatory/degenerative response. In contrast, the 80 SSC protocols induced a force deficit of 40% and an overt secondary response. No further increase in response was observed following exposure to 150 SSCs. In our prior research, 0, 30, 70, and 150 SSC protocols were evaluated for young rats (Baker et al., 2007). Unlike

Table 1
Protein levels (pg/ml) for cytokines and chemokines in serum 3 days following SSC exposure in muscle tissue.

		SSCs	Young (pg/ml)	Old (pg/ml)
Cytokines	IFNγ*	0	72.0 ± 16.3	118.8 ± 33.7
		30	44.6 ± 10.6	183.1 ± 72.6
		80	53.6 ± 24.7	170.3 ± 69.4
		150	70.6 ± 23.0	115.4 ± 30.0
	CSF2	0	106.4 ± 23.8	179.0 ± 31.2
		30	193.5 ± 73.2	154.7 ± 33.6
		80	212.6 ± 39.9	494.8 ± 307.1
		150	106.0 ± 16.6	160.0 ± 52.2
	IL1α	0	17.7 ± 9.0	26.1 ± 13.2
		30	4.4 ± 1.7	9.0 ± 1.9
		80	7.6 ± 1.8	9.2 ± 2.2
		150	12.1 ± 10.6	18.6 ± 9.6
	IL2α	0	59.8 ± 33.6	205.2 ± 87.8
		30	128.4 ± 38.8	224.2 ± 75.2
		80	141.3 ± 45.0	251.2 ± 118.3
		150	81.7 ± 58.1	156.6 ± 46.6
	IL10*	0	1.9 ± 0.1	5.7 ± 1.2
		30	2.5 ± 0.6	13.2 ± 7.0
		80	2.8 ± 0.8	9.3 ± 2.8
		150	2.0 ± 0.5	7.1 ± 3.1
Chemokines	CCL2	0	982.0 ± 143.7	1203.0 ± 164.5
		30	1114.2 ± 184.9	1037.5 ± 126.0
		80	1175.3 ± 199.7	945.8 ± 126.1
		150	1008.4 ± 148.3	1273.0 ± 100.3
	CCL3*	0	7.5 ± 0.6	12.3 ± 1.5
		30	7.5 ± 0.9	12.6 ± 3.7
		80	7.3 ± 0.9	15.4 ± 2.2
		150	7.5 ± 0.6	12.8 ± 2.4
	CCL5	0	717.1 ± 33.4	730.4 ± 43.8
		30	626.2 ± 32.4	791.1 ± 90.4
		80	775.9 ± 59.7	761.9 ± 88.1
		150	683.7 ± 16.1	743.6 ± 43.4
	CCL20	0	202.0 ± 63.4	187.5 ± 63.5
		30	176.0 ± 65.3	238.7 ± 86.4
		80	195.6 ± 67.4	204.3 ± 62.5
		150	180.9 ± 69.2	166.2 ± 55.3
	CXCL1*	0	144.8 ± 16.6	190.4 ± 21.5
		30	164.5 ± 28.1	254.9 ± 75.9
		80	153.0 ± 16.6	241.1 ± 49.4
		150	147.3 ± 15.2	309.6 ± 68.7
	CXCL2*	0	29.0 ± 2.6	35.1 ± 4.1
		30	23.9 ± 1.6	56.3 ± 20.5
		80	30.4 ± 3.6	71.3 ± 25.7
		150	25.4 ± 1.2	38.8 ± 4.5
	CX3CL1	0	54.7 ± 14.1	63.4 ± 6.1
		30	226.9 ± 197.9	74.6 ± 11.0
		80	41.0 ± 9.4	65.4 ± 9.6
		150	38.6 ± 5.5	58.8 ± 7.0

Values are means ± S.E.M. Sample sizes were N = 3 to 8 per group. Values for IL1α, IL1β, IL-4, IL-6, and TNFα are not listed because insufficient measurements passed detection.
* Main effect of age was significant for ANOVA ($P < 0.05$).

the 80 SSC-induced results of the present study, the 70 SSC-induced force deficit and muscle necrosis was distinctly less severe than that for the 150 SSC-induced response (Baker et al., 2007). This indicates that for our rodent model, the minimum number of SSCs capable of inducing severe force deficits and fiber necrosis falls within a narrow range — between 70 and 80 SSCs. Such information will be useful for future rodent studies when evaluating conditioning exposures to increase the resistance to severe contraction-induced injury.

A striking finding of the present study was the diminished secondary response for old rats in terms of edema, cellular infiltration, and overt muscle fiber degeneration. This reduction in secondary response occurred despite comparable initial force deficits between age groups. The implication is that for the SSC protocols tested, the response to the initial injury rather than magnitude of the initial injury was altered with age. Furthermore, this response appeared to be blunted rather than simply delayed as evident by the lack of SSC-induced alterations in muscle mass, muscle fiber size, and percentage of central nucleated fibers at 10 days. A diminished secondary response with aging is consistent with several studies by other groups. Following 75 lengthening contractions, old mice sustain a greater initial injury than that of young mice yet the histological muscle fiber damage is comparable with age (Zerba et al., 1990; Lockhart and Brooks, 2006). For elderly men following exercise-induced injury, macrophage infiltration in muscle is decreased relative to that of young men (Przybyla et al., 2006). Such an age-related attenuation of the inflammatory response has also been observed for various conditions including strokes (Sieber et al., 2011). The results of the present study differ from those following severe lengthening-contraction injury protocols. After exposure to high repetition lengthening contractions, muscles of old rodents exhibit the same or greater secondary response as that of young rodents (Brooks and Faulkner, 1990; McArdle et al., 2004). The protocols of these studies differed in contractions from the present study in terms of mode (lengthening contraction vs SSC) and were more severe in terms of velocity (800° per second vs 500° per second) and number (≥180 vs ≤ 150). Therefore, a robust response is possible with aging when muscle is exposed to extreme conditions. The implication is that the blunted response for old rats of the present study was not because of a complete inability to elicit a robust secondary response. Rather, the results are consistent with another alternative — an age-related insensitivity in molecular signaling for the secondary response which can be overcome only after extreme injury.

Microarray transcription analysis and protein quantification was critical for identifying the cytokine/chemokine signaling response as a major age-related deficiency in the present study. The SSC-induced increase in intramuscular levels of cytokines and chemokines in young rats was not unexpected. Mechanical loading stimulates colony stimulating factor 2 (CSF-2) release from muscle cells which promotes neutrophil chemotaxis (Peterson and Pizza, 2009). Levels of mRNA of IL-1β are increased by resistance exercise in human subjects (Jozsi et al., 2000; Przybyla et al., 2006). In addition, IL-1β has been characterized as a major factor in the activation of the acute inflammatory response at least in part by subsequently inducing gene expression of chemokines (Xu et al., 1998). The mRNA of chemokines CCL2, CCL3, CXCL1, and CXCL2, chemokines with increased levels in the present study, are also upregulated in muscle following stressors such as exercise or cardiotoxin exposure (Chen et al., 2003; Warren et al., 2004; Zhang et al., 2009; Pedersen et al., 2011). SSC-induced IL10 levels were also expected since IL10 attenuates the initial inflammatory response of muscle injury (Arnold et al., 2007). The observation of a cytokine/chemokine response in human studies regarding concentric exercise, submaximal muscle activation, and the repeated bout effect have raised the possibility that such a response is linked to the extent of energy demands during contractions rather than the magnitude of initial injury and subsequent segmental muscle degeneration (Paulsen et al., 2012). The results of the present study for young rats indicate that the link with the muscle damage may be more substantial. Despite an increased metabolic demand during the 150 SSC protocol vs the 80 SSC protocol, the cytokine/chemokine response did not change. Rather, the lack of additional cytokine/chemokine response corresponded with the plateau in initial force deficit and muscle degeneration with the additional contractions. This suggests that while some level of cytokine response may result due to the metabolic demands of mild exposures to contractions, severe exposures elicit robust cytokine responses which become insensitive to differences in energy requirements.

The lack of a cytokine/chemokine response for all of the SSC exposures in muscles of old rats was accompanied by increased baseline serum levels. A muted cytokine response to muscular activity has also been noted in human aging studies (Jozsi et al., 2000; Przybyla et al., 2006). Yet, increased pre-exercise gene expression levels of cytokines have been observed for the elderly (Przybyla et al., 2006). This observation is consistent with several age-related increases of basal measures in the present study — increased serum cytokine levels and morphological features of chronic inflammation such as increased interstitial picrosirius red staining, an indicator of fibrosis. Age-related chronic inflammation, or "inflammaging," refers to systemic low-grade inflammation present with aging in the absence of infection. This chronic inflammation has the potential to desensitize the inflammatory response to new challenges as has been indicated by studies ranging from muscular activity to lipopolysaccharide stimulation (Bruunsgaard et al., 1999; Przybyla et al., 2006; Shaw et al., 2013).

Cytokine/chemokine and inflammatory signaling desensitization possibly contributes to impaired recovery of injured muscle with aging. For instance, IL-1β and IL10, cytokines which contribute to appropriate satellite cell function, exhibited diminished SSC-induced levels with aging in the present study (Arnold et al., 2007). CCL2, also age-sensitive in the present study, has a major role in recovery as demonstrated by impaired regeneration and compromised functional recovery in CCL2 deficient mice following muscle damage (Warren et al., 2004; Martinez et al., 2010). These results call into question the use of anti-inflammatory drugs following muscle injuries of the magnitude presented here. Anti-inflammatory drugs are widely used following intense muscular activity (Brewer et al., 2014). Yet, there is growing evidence that interventions that significantly reduce the inflammatory response also compromise muscle repair (Mishra et al., 1995; Gumucio et al., 2013; Wang et al., 2014). The results of the present study and our previous research are consistent with this view in that the muscles of young rats exhibit an inflammatory response and recover rapidly whereas muscles of old mice exhibit a muted inflammatory response and lack functional recovery by 10 days (Krajnak et al., 2006). Therefore, in this situation, blocking inflammation at a young age may be detrimental to muscle repair and the use of an anti-inflammatory drug at old age may be completely unwarranted since no such inflammatory response is occurring. Overall, our findings support the research effort to determine whether inappropriate use of anti-inflammatory intervention is ineffective or even deleterious to muscle recovery at advanced age.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.exger.2015.09.020.

Conflicts of interest

No author of this manuscript has any conflict of interest regarding this work.

Acknowledgments

This study was supported by internal funds at the National Institute for Occupational Safety and Health.

References

Arnold, L., Henry, A., Poron, F., Baba-Amer, Y., van Rooijen, N., Plonquet, A., Gherardi, R.K., Chazaud, B., 2007. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J. Exp. Med. 204, 1057–1069.

Baker, B.A., Mercer, R.R., Geronilla, K.B., Kashon, M.L., Miller, G.R., Cutlip, R.G., 2006. Stereological analysis of muscle morphology following exposure to repetitive stretch-shortening cycles in a rat model. Appl. Physiol. Nutr. Metab. 31, 167–179.

Baker, B.A., Mercer, R.R., Geronilla, K.B., Kashon, M.L., Miller, G.R., Cutlip, R.G., 2007. Impact of repetition number on muscle performance and histological response. Med. Sci. Sports Exerc. 39, 1275–1281.

Baumann, C.W., Rogers, R.G., Gahlot, N., Ingalls, C.P., 2014. Eccentric contractions disrupt FKBP12 content in mouse skeletal muscle. Physiol. Rep. 2.

Beaton, L.J., Allan, D.A., Tarnopolsky, M.A., Tiidus, P.M., Phillips, S.M., 2002. Contraction-induced muscle damage is unaffected by vitamin E supplementation. Med. Sci. Sports Exerc. 34, 798–805.

Brewer, C.B., Bentley, J.P., Hallam, J.S., Woodyard, C.D., Waddell, D.E., 2014. Use of analgesics for exercise-associated pain: prevalence and predictors of use in recreationally trained college-aged students. J. Strength Cond. Res. 28, 74–81.

Brooks, S.V., Faulkner, J.A., 1990. Contraction-induced injury: recovery of skeletal muscles in young and old mice. Am. J. Physiol. 258, C436–C442.

Brooks, S.V., Opiteck, J.A., Faulkner, J.A., 2001. Conditioning of skeletal muscles in adult and old mice for protection from contraction-induced injury. J. Gerontol. A Biol. Sci. Med. Sci. 56, B163–B171.

Bruunsgaard, H., Pedersen, A.N., Schroll, M., Skinhoj, P., Pedersen, B.K., 1999. Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. Clin. Exp. Immunol. 118, 235–241.

Chen, Y.W., Hubal, M.J., Hoffman, E.P., Thompson, P.D., Clarkson, P.M., 2003. Molecular responses of human muscle to eccentric exercise. J. Appl. Physiol. (1985) 95, 2485–2494.

Cutlip, R.G., Hollander, M.S., Johnson, G.A., Johnson, B.W., Friend, S.A., Baker, B.A., 2014. Magnetic resonance imaging of graded skeletal muscle injury in live rats. Environ. Health Insights (Suppl. 1), 31–39.

Gallagher, S., Heberger, J.R., 2013. Examining the interaction of force and repetition on musculoskeletal disorder risk: a systematic literature review. Hum. Factors 55, 108–124.

Gelse, K., Poschl, E., Aigner, T., 2003. Collagens–structure, function, and biosynthesis. Adv. Drug Deliv. Rev. 55, 1531–1546.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F., Li, C., Maechler, M., Rossini, A.J., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang, J.Y., Zhang, J., 2004. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5, R80.

Gumucio, J.P., Flood, M.D., Phan, A.C., Brooks, S.V., Mendias, C.L., 2013. Targeted inhibition of TGF-beta results in an initial improvement but long-term deficit in force production after contraction-induced skeletal muscle injury. J. Appl. Physiol. (1985) 115, 539–545.

Hairi, N.N., Cumming, R.G., Naganathan, V., Handelsman, D.J., Le Couteur, D.G., Creasey, H., Waite, L.M., Seibel, M.J., Sambrook, P.N., 2010. Loss of muscle strength, mass (sarcopenia), and quality (specific force) and its relationship with functional limitation and physical disability: the Concord Health and Ageing in Men Project. J. Am. Geriatr. Soc. 58, 2055–2062.

Jozsi, A.C., Dupont-Versteegden, E.E., Taylor-Jones, J.M., Evans, W.J., Trappe, T.A., Campbell, W.W., Peterson, C.A., 2000. Aged human muscle demonstrates an altered gene expression profile consistent with an impaired response to exercise. Mech. Ageing Dev. 120, 45–56.

Komi, P.V., 2000. Stretch-shortening cycle: a powerful model to study normal and fatigued muscle. J. Biomech. 33, 1197–1206.

Krajnak, K., Waugh, S., Miller, R., Baker, B., Geronilla, K., Alway, S.E., Cutlip, R.G., 2006. Proapoptotic factor Bax is increased in satellite cells in the tibialis anterior muscles of old rats. Muscle Nerve 34, 720–730.

Lauretani, F., Russo, C.R., Bandinelli, S., Bartali, B., Cavazzini, C., Di Iorio, A., Corsi, A.M., Rantanen, T., Guralnik, J.M., Ferrucci, L., 2003. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. J. Appl. Physiol. (1985) 95, 1851–1860.

Lauritzen, F., Paulsen, G., Raastad, T., Bergersen, L.H., Owe, S.G., 2009. Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans. J. Appl. Physiol. (1985) 107, 1923–1934.

Lockhart, N.C., Brooks, S.V., 2006. Protection from contraction-induced injury provided to skeletal muscles of young and old mice by passive stretch is not due to a decrease in initial mechanical damage. J. Gerontol. A Biol. Sci. Med. Sci. 61, 527–533.

Lovering, R.M., Brooks, S.V., 2014. Eccentric exercise in aging and diseased skeletal muscle: good or bad? J. Appl. Physiol. (1985) 116, 1439–1445.

Macpherson, P.C., Schork, M.A., Faulkner, J.A., 1996. Contraction-induced injury to single fiber segments from fast and slow muscles of rats by single stretches. Am. J. Physiol. 271, C1438–C1446.

Malm, C., Nyberg, P., Engstrom, M., Sjodin, B., Lenkei, R., Ekblom, B., Lundberg, I., 2000. Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. J. Physiol. 529 (Pt 1), 243–262.

Martinez, C.O., McHale, M.J., Wells, J.T., Ochoa, O., Michalek, J.E., McManus, L.M., Shireman, P.K., 2010. Regulation of skeletal muscle regeneration by CCR2-activating chemokines is directly related to macrophage recruitment. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299, R832–R842.

McArdle, A., Dillmann, W.H., Mestril, R., Faulkner, J.A., Jackson, M.J., 2004. Overexpression of HSP70 in mouse skeletal muscle protects against muscle damage and age-related muscle dysfunction. FASEB J. 18, 355–357.

Mikkelsen, U.R., Langberg, H., Helmark, I.C., Skovgaard, D., Andersen, L.L., Kjaer, M., Mackey, A.L., 2009. Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise. J. Appl. Physiol. (1985) 107, 1600–1611.

Mishra, D.K., Friden, J., Schmitz, M.C., Lieber, R.L., 1995. Anti-inflammatory medication after muscle injury. A treatment resulting in short-term improvement but subsequent loss of muscle function. J. Bone Joint Surg. Am. 77, 1510–1519.

Paulsen, G., Crameri, R., Benestad, H.B., Fjeld, J.G., Morkrid, L., Hallen, J., Raastad, T., 2010a. Time course of leukocyte accumulation in human muscle after eccentric exercise. Med. Sci. Sports Exerc. 42, 75–85.

Paulsen, G., Egner, I.M., Drange, M., Langberg, H., Benestad, H.B., Fjeld, J.G., Hallen, J., Raastad, T., 2010b. A COX-2 inhibitor reduces muscle soreness, but does not influence

recovery and adaptation after eccentric exercise. Scand. J. Med. Sci. Sports 20, e195–e207.
Paulsen, G., Mikkelsen, U.R., Raastad, T., Peake, J.M., 2012. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? Exerc. Immunol. Rev. 18, 42–97.
Peake, J., Della Gatta, P., Cameron-Smith, D., 2010. Aging and its effects on inflammation in skeletal muscle at rest and following exercise-induced muscle injury. Am. J. Physiol. Regul. Integr. Comp. Physiol. 298, R1485–R1495.
Pedersen, L., Pilegaard, H., Hansen, J., Brandt, C., Adser, H., Hidalgo, J., Olesen, J., Pedersen, B.K., Hojman, P., 2011. Exercise-induced liver chemokine CXCL-1 expression is linked to muscle-derived interleukin-6 expression. J. Physiol. 589, 1409–1420.
Peterson, J.M., Pizza, F.X., 2009. Cytokines derived from cultured skeletal muscle cells after mechanical strain promote neutrophil chemotaxis in vitro. J. Appl. Physiol. (1985) 106, 130–137.
Ploutz-Snyder, L.L., Giamis, E.L., Formikell, M., Rosenbaum, A.E., 2001. Resistance training reduces susceptibility to eccentric exercise-induced muscle dysfunction in older women. J. Gerontol. A Biol. Sci. Med. Sci. 56, B384–B390.
Przybyla, B., Gurley, C., Harvey, J.F., Bearden, E., Kortebein, P., Evans, W.J., Sullivan, D.H., Peterson, C.A., Dennis, R.A., 2006. Aging alters macrophage properties in human skeletal muscle both at rest and in response to acute resistance exercise. Exp. Gerontol. 41, 320–327.
Ramaswamy, K.S., Palmer, M.L., van der Meulen, J.H., Renoux, A., Kostrominova, T.Y., Michele, D.E., Faulkner, J.A., 2011. Lateral transmission of force is impaired in skeletal muscles of dystrophic mice and very old rats. J. Physiol. 589, 1195–1208.
Shaw, A.C., Goldstein, D.R., Montgomery, R.R., 2013. Age-dependent dysregulation of innate immunity. Nat. Rev. Immunol. 13, 875–887.
Sieber, M.W., Claus, R.A., Witte, O.W., Frahm, C., 2011. Attenuated inflammatory response in aged mice brains following stroke. PLoS One 6, e26288.
Tulla, M., Pentikainen, O.T., Viitasalo, T., Kapyla, J., Impola, U., Nykvist, P., Nissinen, L., Johnson, M.S., Heino, J., 2001. Selective binding of collagen subtypes by integrin alpha 1I, alpha 2I, and alpha 10I domains. J. Biol. Chem. 276, 48206–48212.
Wang, H., Melton, D.W., Porter, L., Sarwar, Z.U., McManus, L.M., Shireman, P.K., 2014. Altered macrophage phenotype transition impairs skeletal muscle regeneration. Am. J. Pathol. 184, 1167–1184.
Warren, G.L., O'Farrell, L., Summan, M., Hulderman, T., Mishra, D., Luster, M.I., Kuziel, W.A., Simeonova, P.P., 2004. Role of CC chemokines in skeletal muscle functional restoration after injury. Am. J. Physiol. Cell Physiol. 286, C1031–C1036.
Woolf, A.D., Erwin, J., March, L., 2012. The need to address the burden of musculoskeletal conditions. Best Pract. Res. Clin. Rheumatol. 26, 183–224.
Xu, C., Poirier, B., Duong Van Huyen, J.P., Lucchiari, N., Michel, O., Chevalier, J., Kaveri, S., 1998. Modulation of endothelial cell function by normal polyspecific human intravenous immunoglobulins: a possible mechanism of action in vascular diseases. Am. J. Pathol. 153, 1257–1266.
Yu, J.G., Liu, J.X., Carlsson, L., Thornell, L.E., Stal, P.S., 2013. Re-evaluation of sarcolemma injury and muscle swelling in human skeletal muscles after eccentric exercise. PLoS One 8, e62056.
Zerba, E., Komorowski, T.E., Faulkner, J.A., 1990. Free radical injury to skeletal muscles of young, adult, and old mice. Am. J. Physiol. 258, C429–C435.
Zhang, L., Ran, L., Garcia, G.E., Wang, X.H., Han, S., Du, J., Mitch, W.E., 2009. Chemokine CXCL16 regulates neutrophil and macrophage infiltration into injured muscle, promoting muscle regeneration. Am. J. Pathol. 175, 2518–2527.