Neutrophil accumulation following passive stretches contributes to adaptations that reduce contraction-induced skeletal muscle injury in mice

Nicole C. Lockhart and Susan V. Brooks
Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan

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Skeletal muscles can be injured by their own contractions. Contraction-induced injury occurs during activities that involve stretching activated muscles (lengthening contractions) (10, 23) and is initiated by the mechanical disruption of force-generating (27, 44) or force-transmitting structures (7). The initial mechanical injury triggers a delayed secondary injury, which includes an inflammatory response and the degeneration of injured muscle fibers or portions of fibers (15, 23). The inflammatory response involves an increase in the injured muscle in the numbers of neutrophils as well as macrophages (33). The neutrophils contribute to the secondary injury as evidenced by the reduction in both functional and histological indicators of damage in the absence of neutrophil accumulation following lengthening contractions (35). Neutrophils may cause the secondary injury by releasing proteases and free radicals (25) that produce oxidative damage to membranes and muscle proteins (29, 35).

Prior exercise training reduces injury following subsequent lengthening contractions (16, 22, 37), but the adaptations responsible for protection from muscle injury are not well understood (24). Exposing a muscle to damaging lengthening contractions significantly decreases the amount of morphological damage, the force deficit, and the numbers of neutrophils and macrophages in the muscle resulting from a second bout of lengthening contractions (16, 22, 33). In addition, exposure to either isometric contractions or stretches without activation (passive stretches), while causing no observable damage to muscle fibers, reduces contraction-induced injury following subsequent lengthening contractions (16, 17). Despite the absence of overt injury following isometric contractions or passive stretches, these conditioning protocols do result in the accumulation of neutrophils in the muscle (33). The role of neutrophils in skeletal muscle in the absence of injury is unknown, but the possibility that neutrophils provide a mechanism for inducing protective adaptations has been suggested (33).

The purpose of the present study was to investigate the relationship between the increase in muscle neutrophil concentrations following passive stretches and the protective adaptations that result. Our working hypothesis was that neutrophil infiltration following passive stretch conditioning is necessary for the protection from contraction-induced injury provided by the passive stretches. To address this question, we treated mice with an antibody (RB6-8C5) to a neutrophil surface protein to deplete the level of circulating neutrophils before administration of passive stretches, thereby decreasing the accumulation of neutrophils in the muscle in response to the conditioning protocol. Fourteen days later, levels of circulating neutrophils were no longer depressed and muscles were exposed to an injurious protocol of lengthening contractions. Our specific hypothesis was that for muscles in mice treated with RB6-8C5 before passive stretch conditioning, the magnitude of injury following a subsequent protocol of lengthening contractions 14 days later would be of similar severity to that for nonconditioned muscles of untreated mice. Based on the importance of neutrophil infiltration following lengthening contractions as a contributor to contraction-induced injury (35), we also treated a separate group of mice with RB6-8C5 before exposure to lengthening contractions and confirmed a dramatic reduction in muscle neutrophils and in the magnitude of contraction-induced injury using this model of neutrophil depletion.

Address for reprint requests and other correspondence: S. V. Brooks, Dept. of Molecular and Integrative Physiology, The Univ. of Michigan, 2029 Biomedical Science Research Bldg., 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200 (e-mail: svbrooks@umich.edu).

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MATERIALS AND METHODS

All experiments were performed on 3- to 4-mo-old specific pathogen free (SPF) male C57BL/6 mice (Charles River Laboratories, Wilmington, MA), and all procedures were approved by the University of Michigan Committee for the Use and Care of Animals. Before experimentation, mice were housed in an SPF barrier facility in the Unit for Laboratory Animal Medicine at the University of Michigan. Between experimental procedures, mice were housed in a separate SPF return room. During all experimental procedures, mice were anesthetized with an initial intraperitoneal injection of 2% avertin (tribromoethanol; 400 mg/kg) followed by supplemental doses of avertin as needed to maintain a depth of anesthesia sufficient to prevent response to tactile stimuli throughout the duration of an experiment.

Overall experimental design. The overall experimental design is diagrammed in Fig. 1. The limb studied was randomized with the contralateral limb typically left undisturbed to serve as an uninjured control, although experimental procedures were in some cases performed on both limbs. Extensor digitorum longus (EDL) muscles were administered a conditioning protocol of passive stretches in situ followed 14 days later by exposure to a well-characterized injurious protocol of 75 lengthening contractions (Fig. 1A.1) that results in a decrease in isometric force of ~50% and morphological damage to ~20% of the fibers in a cross section after 3 days (16, 18). The magnitude of injury 3 days following exposure of passive stretch-conditioned muscles to the 75 lengthening contractions was compared with the injury evaluated 3 days following 75 lengthening contractions in nonconditioned muscles not previously exposed to passive stretches (Fig. 1A.2) as well as for muscles of mice from which circulating neutrophils were depleted before the administration of the passive stretches (Fig. 1B.1).

Depletion of neutrophils was achieved in the mice by administration of an antibody to a neutrophil surface protein, RB6-8C5 (Pharmingen, San Jose, CA), and neutropenia was verified by measuring circulating levels of neutrophils. Before any experimental intervention, blood samples were taken from the tail vein of anesthetized mice to conduct initial white cell counts. Mice were then administered a 200-µg dose of RB6-8C5 antibody by intraperitoneal injection (Fig. 1B). This dose of antibody was shown previously to cause neutropenia in mice, defined as a circulating neutrophil concentration of <50 cells/µl (8, 40). Twenty-four hours later, mice were again anesthetized, blood was taken from the tail vein, and mice were administered a second dose of 100 µg of antibody (Fig. 1B). Total white cell numbers were determined from the blood samples, and differential cell counts on Wright-stained blood smears were performed to assess numbers of neutrophils. Compared with the levels of neutrophils measured before injection of the antibody (897 ± 130 cells/µl), 24 h following the initial dose of RB6-8C5, the level of circulating neutrophils was depleted by 96%.

The period of 14 days between passive stretches and lengthening contractions was chosen for two reasons. First, our laboratory has previously demonstrated a reduction in lengthening contraction-induced injury for passive stretch-conditioned compared with nonconditioned muscles at this time point (16, 18), and second, to allow sufficient time for mice treated with RB6-8C5 to recover control levels of circulating neutrophils before exposure to lengthening contractions. Previous reports indicated that recovery from treatment with RB6-8C5 was complete within 5 days (8, 40). Blood samples collected from our conditioned mice confirmed that circulating neutrophil levels had recovered to 1,535 ± 510 cells/µl by the time the RB6-8C5-treated mice in the present study were administered the protocol of lengthening contractions.

Finally, an additional group of mice was treated with RB6-8C5 on the day before and the day of exposure to the injury-inducing protocol of lengthening contractions with no previous conditioning (Fig. 1B.2) to confirm that depleting the level of circulating neutrophils by this method inhibited neutrophil accumulation in the muscle and reduced the severity of lengthening contraction-induced injury (35).

In situ muscle preparation. Under anesthesia, the distal tendon of the right and/or left EDL muscle was exposed. Mice were placed on a platform warmed with a 37°C circulating water bath. The hindlimb was secured by pinning the knee with a blunt screw and tightly taping the foot to the platform. The intact tendon was then tied with 4.0 braided silk suture to the lever arm of a servomotor (Aurora Scientific, Richmond Hill, ON, Canada) that controlled the length of the muscle and measured the force generated. A computer controlled the servomotor and collected and stored force data. The small area of exposed tendon was kept moist by frequent administration of isotonic sterile saline. The EDL muscle was activated using an isolated stimulator (Grass Instruments, West Warwick, RI) and fine-needle electrodes placed transcutaneously adjacent and parallel to the peroneal nerve. A pulse duration of 0.2 ms was used for all contractions. Twitch contractions were initiated by stimulation at 6 V with stimulus amplitude increased in 1-V increments until a maximum force value was obtained, typically at ~10 V. The length of the muscle was then adjusted for maximum twitch tension. This optimal muscle length (L₀) was measured using well-defined anatomic landmarks as previously determined (2). Tetanic contractions were generated during trains of pulses starting at a frequency of 150 Hz and in 50-Hz increments during successive trains until the force level plateaued (P₀) typically at 250 Hz. One-minute rest periods were allowed between isometric contractions. This technique minimized the number of activations required to determine P₀.

The conditioning protocol consisted of 75 stretches without activation (passive stretches), 1 stretch each 4 s, for a total duration of 5 min. Stretches were initiated at L₀ and were of 20% strain relative to L₀ at a velocity of 1.0 L₀/s. Optimal muscle fiber length (Lₒ) was determined by multiplying L₀ by the previously determined Lₒ/L₀, ratio of 0.44 (23). Ten minutes after completion of the 75 passive stretches, Pₒ was remeasured. The small incision at the ankle was closed with 7.0 sterile monofilament nylon suture and bathed with povidone-iodine.
iodine solution, and the mice were monitored until they recovered from anesthesia.

Fourteen days following administration of the passive stretch-conditioning protocol, mice were again anesthetized, and previously conditioned EDL muscles were administered 75 lengthening contractions in situ (16). Nonconditioned EDL muscles not previously exposed to passive stretches were also administered the lengthening contraction protocol. The lengthening contraction protocol was identical to the protocol of passive stretches, except that lengthening was initiated 100 ms after the onset of 150-Hz stimulation to allow the development of near-maximum isometric force before the stretch. Ten minutes after completion of the lengthening contractions, Po was remeasured. The incision was closed as described above, and the mice were allowed to recover. Three days after administration of the lengthening contractions, mice were anesthetized a final time, and contractile properties of muscles in both legs were evaluated in situ. Force deficit was defined as the difference between the Po measured just before lengthening contractions and the Po measured 3 days after lengthening contractions expressed as a percentage of the initial Po. Histology and immunohistochemistry. After the final in situ evaluation of contractile properties, EDL muscles were removed, coated in tissue freezing medium, and frozen in isopentane cooled by dry ice. Mice were killed immediately by administration of an overdose of the anesthetic. Ten-micrometer cryosections through the midbelly of the muscles were stained with hematoxylin and eosin. As described in detail previously (16, 35), injured fibers were identified as those showing clear evidence of degeneration or regeneration, including fibers with pale or variable staining, clear signs of cytoplasmic degeneration, clear infiltration by inflammatory cells, a considerably swollen appearance, or the presence of nonperipheral nuclei without other evidence of degeneration. In a manner identical to that used by our laboratory and others in previous studies of contraction-induced injury (16–18, 35, 45), an observer blinded to the identity of the sample determined in one entire cross section from each muscle the number of fibers that demonstrated overt evidence of injury. Fiber counts were tallied with the aid of a microscope imaging system (Bioquant, Nashville, TN), and the number of overtly injured fibers was reported as a percentage of the total number of fibers in the cross section.

For each muscle, additional 10-μm sections were analyzed for the presence of neutrophils by immunohistochemistry (33). The sections for immunohistochemistry were taken from a region of the muscle near the region from which injured fibers were quantified. Sections were fixed in acetone and then quenched in hydrogen peroxide. Slides were incubated in a 1:100 dilution of RB6-8C5 antibody (Pharmingen, San Jose, CA) in phosphate-buffered saline for 2 h at room temperature. The RB6-8C5 antibody recognizes the Ly6G protein expressed on granulocytes. Slides were then incubated for 30 min in biotinylated mouse adsorbed anti-rat IgG (1:200; Vector Laboratories, Burlingame, CA), followed by a 30 min incubation in horseradish peroxidase (1:1,000; Vector Laboratories, Burlingame, CA). Slides were developed with 3-amino-9-ethylcarbazole (Vector Laboratories). As previously described (33), the number of neutrophils was counted in two entire cross sections of each muscle by an observer blinded to the identity of the sample. The area of each section was calculated using the image analysis system (Bioquant), and neutrophil concentrations are expressed per cubic millimeter (cross-sectional area × 10-μm cryosection thickness) of muscle.

Data analysis. Data are reported as means ± SE. Differences between experimental groups for force deficit, percentage of injured fibers in a muscle cross section, and concentration of Ly6G-positive cells in a muscle were determined using two-factor (nonconditioned/conditioned; untreated/treated with RB6-8C5 antibody) analysis of variance. Under circumstances when the F-statistic was significant, individual differences were assessed by a Tukey multiple comparison procedure. Despite using a two-factor statistical analysis, comparisons of conditioned and nonconditioned muscles of RB6-8C5-treated mice are not relevant physiologically, because the injury response was measured without and with neutropenia, respectively. Thus, to discourage the natural temptation to contrast the data from these two groups, data from nonconditioned muscles of RB6-8C5-treated mice are displayed separately. Statistical significance was set at P < 0.05.

RESULTS

Neither mean body mass (27.5 ± 1.8 g) nor Po (369 ± 13 mN) was different for the mice treated with RB6-8C5 antibody compared with the values for untreated mice of 27.5 ± 0.4 g and 385 ± 11 mN, respectively. All of these values were similar to those reported elsewhere for EDL muscles of male mice of this age (2, 16). Also highly consistent with published reports was the response in the present study of the control muscles to the protocol of 75 lengthening contractions (16, 18). Three days following the lengthening contractions, the force deficit for nonconditioned muscles of untreated mice was 50.7 ± 3.4% (Fig. 2) and 20.5 ± 3.3% of the fibers in a cross section showed morphological evidence of injury (Fig. 3). When passive stretches were administered 14 days before a bout of lengthening contractions in untreated mice, the magnitude of the resultant injury was reduced by approximately one-half. The force deficit 3 days following the lengthening contractions for passive stretch-conditioned muscles was only 22.0 ± 3.9% (Fig. 2), and the number of injured was reduced to 8.4 ± 1.2% (Fig. 3). Moreover, the concentration of neutrophils in muscles of untreated mice following exposure to lengthening contractions was reduced more than two-thirds by prior conditioning with passive stretches (Fig. 4).

In contrast to the substantial reduction in the severity of injury conferred by passive stretch conditioning in untreated control mice, when mice were treated with RB6-8C5 to deplete...
neutrophils before administration of passive stretches, the protective effects of the passive stretch conditioning were essentially eliminated. The force deficit 3 days following lengthening contractions for passive stretch-conditioned muscles of RB6-8C5-treated mice was 45.0 ± 4.8%, a value that was not different from the value for nonconditioned muscles of untreated mice and twofold greater than the value for conditioned muscles of untreated mice (Fig. 2). Similarly, conditioning resulted in no reduction in the number of injured fibers for muscles of mice that were treated with RB6-8C5. The percentage of injured fibers in passive stretch-conditioned muscles of RB6-8C5-treated mice 3 days after lengthening contractions, 17.4 ± 3.2%, was not different from the value for nonconditioned muscles of untreated mice and approximately twofold greater than the value given above for conditioned muscles of untreated mice (Fig. 3). For muscles of RB6-8C5-treated mice, the number of neutrophils present in the muscle 3 days following the protocol of lengthening contractions showed a pattern similar to that seen for force deficit and injured fibers (Fig. 4). Neutrophil levels in muscles of mice that had been treated with RB6-8C5 before the passive stretches were intermediate between the values for nonconditioned and conditioned muscles of untreated control mice, although not significantly different from either. Collectively, these data suggest that no protective effect of passive stretch conditioning was observed under circumstances when neutrophils were depleted during the passive stretches.

For the experiments described in the previous paragraph, neutrophils were depleted specifically during the time of the conditioning, whereas circulating neutrophil levels were not depressed at the time of the exposure to lengthening contractions. This is a critical point, because a recent report from Pizza et al. (35) demonstrated that the infiltration of a muscle by neutrophils following lengthening contractions contributed substantially to the development of injury in the muscle. Similarly, we now show that for nonconditioned muscles, depletion of circulating neutrophils with RB6-8C5 just before exposure to the lengthening contractions reduced the magnitude of injury by approximately one-half compared with muscles of untreated control mice (Fig. 5). Muscles of mice treated with RB6-8C5 on the day before and the day of exposure to...
lengthening contractions had force deficits at 3 days of only 27.2 ± 5.3%, and 10.3 ± 2.8% of the fibers in cross sections of muscles from these mice displayed evidence of injury. The reduction in force deficit and morphological injury was associated with the elimination of neutrophil accumulation in muscles of mice treated with RB6-8C5. The small number of Ly6G+ cells observed in cross sections of muscles from RB6-8C5-treated mice following lengthening contractions was not different from values our laboratory and others have previously reported for uninjured control muscles (33, 35). Thus treatment with RB6-8C5 antibody had the expected effect of dramatically reducing both neutrophil accumulation and the severity of injury induced by lengthening contractions.

**DISCUSSION**

The primary finding of the present study was that the protection from lengthening contraction-induced injury typically afforded to skeletal muscle by prior conditioning with passive stretches (16, 17) was not observed under circumstances when the accumulation of neutrophils in the muscle in response to the passive stretches was inhibited. Inhibition of neutrophil accumulation was achieved through the depletion of neutrophils from the circulation before administration of the passive stretches. Strong support for the effectiveness of this experimental approach was provided by our laboratory’s observation that muscle neutrophil concentrations were not increased following lengthening contractions in mice depleted of neutrophils, whereas in control mice lengthening contractions resulted in a considerable increase in muscle neutrophil concentration (present study; 33, 35). Moreover, the lack of neutrophils in the muscles of mice that were neutropenic during the development of the lengthening contraction-induced injury response had the expected effect (35) of dramatically reducing the severity of injury. In contrast, under circumstances when mice were neutropenic during administration of the conditioning protocol of passive stretches, but not during the lengthening contractions, force deficits and numbers of injured fibers were not different from those observed for nonconditioned muscles of untreated control mice. These findings provide support for our hypothesis that neutrophil infiltration following conditioning with passive stretches is associated with the induction of adaptations that provide protection from the injury produced by a subsequent bout of lengthening contractions.

The mechanism by which neutrophils act to initiate protective adaptations is not known. Rather than contributing to protection, neutrophils are more typically associated with the promotion of muscle damage. Following in vivo muscle injury, neutrophils are thought to phagocytize damaged tissue (19, 42) and release proteases to degrade cellular debris (42). The damaging effects on muscle cells appear to be mediated, at least in part, by a superoxide-dependent mechanism because the presence of antioxidants in cocultures of muscle cells and neutrophils prevents lysis of muscle cell membranes (25, 29). In addition, inhibition of neutrophil oxidant production in vivo decreases muscle membrane lysis following both stretch and reloading injuries (28, 29). While the generation of reactive oxygen species (ROS) by neutrophils clearly contributes to muscle damage following both stretch-induced (28) and reloading (28, 29) injuries, important roles for ROS as signaling molecules that contribute to normal cell function have also been recognized (3, 11, 20, 41). Based on a growing appreciation of the influence of redox-sensitive signaling pathways on normal cellular processes, including skeletal muscle adaptation (14, 31), ROS participation in signaling adaptive responses that provide protection from contraction-induced injury is not an unreasonable hypothesis. ROS released from infiltrating neutrophils may lead to a shift in redox status of the muscle, preparing the muscle for increases in oxidants following subsequent lengthening contractions, but this possibility remains untested. Nondamaging isometric exercise leads to increases in superoxide dismutase activity and heat shock protein content (21), and passive stretches may invoke similar responses. Finally, in addition to oxidants, neutrophils release proteases, growth factors, cytokines, and chemokines (4, 36) that may function either directly or indirectly as signals for the induction of protective mechanisms.

The apparent paradox between the known detrimental effects of neutrophils on muscle (35) and the potential suggested by the present study for neutrophils to invoke protective adaptations raises questions about possible differences in the properties of the neutrophils within the muscle following damaging vs. nondamaging exercise. The activity of neutrophils is highly dependent on their microenvironment (42), and neutrophils within an injured muscle may be exposed to factors not present following passive stretches or nondamaging isometric contractions. Such factors might be necessary for full activation of neutrophil function, whereas the neutrophils in the muscle following nondamaging exercise remain incompletely activated. While this hypothesis has not been tested in vivo, a dependence of the level of neutrophil activation on the stimulus administered has been demonstrated in studies of myotubes in vitro (43). Differences in neutrophil activity or function may well explain disassociations reported between the accumulation of neutrophils following protocols of passive stretches or isometric contractions and the absence of any force deficit or morphological evidence of muscle fiber injury (33). Discrepancies between neutrophil numbers and other measures of injury were also observed in the present study in the finding that muscles of neutropenic mice exposed to lengthening contractions showed no accumulation of neutrophils, yet they did display force deficits and morphological evidence of injury. Thus overt muscle fiber injury is not the sole determinant for the accumulation of neutrophils in skeletal muscle after exercise nor is the accumulation of neutrophils the sole determinant of the severity of the injury response. An additional consideration is our lack of understanding of the specific factors released by the muscle during contractions and/or stretches responsible for attracting neutrophils (42), raising the possibility that different neutrophil populations may be recruited either in response to different exercise protocols or by conditioned and nonconditioned muscles in response to similar types of exercise. Such specificity of response to different types of exercise has been shown for lymphocyte populations (32, 34). Further study is needed to identify, quantify, and characterize critical neutrophil chemoattractants released by skeletal muscles and to explore the properties of the neutrophils resident in muscles following passive stretches and lengthening contractions.

Whereas the RB6-8C5 antibody has been widely used to deplete neutrophils in vivo for studies of numerous tissue types
(5, 8, 40), RB6-8C5 had not been used previously in studies of contraction-induced skeletal muscle injury. Our observation that nonconditioned muscles of mice treated with RB6-8C5 antibody just before lengthening contractions demonstrated decreased contraction-induced injury was similar to previous findings of Pizza and colleagues using mice deficient in the β2-integrin CD18 (35). The study by Pizza et al. (35) showed that CD18 was required for neutrophil infiltration into skeletal muscle after lengthening contractions, and, when neutrophil infiltration was prevented, the magnitude of injury following lengthening contractions was reduced. The consistency of our findings with those of Pizza and colleagues supports the use of RB6-8C5 in studies of skeletal muscle. RB6-8C5 was chosen for this study because it allowed temporally selective neutrophil depletion, such that neutrophils could be depleted before the administration of passive stretches and replenished before administration of lengthening contractions. RB6-8C5 recognizes the Ly6G and Ly6C proteins expressed on granulocytes and transiently on monocytes during developmental stages and is thus not specific to neutrophils. RB6-C85 has been shown to deplete Ly6C+ monocytes (9) and to bind a subpopulation of T cells (12), although reports that RB6-8C5 recognizes Ly6C on lymphocytes has been refuted (26), and no reduction was observed in the present study in circulating lymphocyte concentrations following RB6-8C5 administration. Circulating concentrations of eosinophils, basophils, or monocytes were also not affected by RB6-C5 administration (data not shown), but these cell types represent much smaller proportions of the leukocytes than neutrophils and depletion may be more difficult to detect. We conclude that the effects of RB6-8C5 observed in the present study were due primarily to its effects on neutrophil levels.

A 14-day time period between passive stretch conditioning and exposure to lengthening contractions was used in the present study, but our laboratory has demonstrated a reduction in lengthening contraction-induced injury when passive stretches were administered as little as 1 h before lengthening contractions (18). Despite similar levels of protection observed with conditioning at any time between 1 h and 14 days before lengthening contractions (18), the adaptations that lead to the protection are not likely to be the same for these widely varying time points. Based on the length of time required for an increase in muscle neutrophils to occur (33), any mechanism of protection initiated by neutrophils would require hours or perhaps days to be in place, and we cannot rule out the possibility that our findings are specific to the time point used in the present study. Furthermore, while passive stretches are considered a noninjurious form of exercise due to the lack of any overt evidence of degeneration or regeneration (16), the force deficit, the number of fibers that show morphologic signs of injury, and the accumulation of inflammatory cells each appear to provide overlapping, but not identical, information about the response of muscle to exercise (present study; 17, 33). Thus we also cannot rule out the possibility that the protocol of passive stretches used in the present and previous studies (16, 17, 33) caused some damage that was not detected. Moreover, whether neutrophil accumulation in skeletal muscle after injury-inducing lengthening contractions is necessary for the protection from injury apparent during a second bout of lengthening contractions, the so-called repeated-bout effect, is not known. The protection from contraction-induced injury provided by a prior bout of lengthening contractions is substantially greater than that provided by passive stretches (16), indicating that the injury itself represents a strong stimulus for protection. The mechanism of the protection provided by previous injury may well override any protective adaptations induced by neutrophils as observed in the present study in the context of a lack of overt injury. Multiple mechanisms of protection in response to exercise training likely come into play, with the importance of specific mechanisms varying with the timing and mode of training.

In summary, our laboratory has previously shown that exposure of muscles of mice to passive stretches results in the accumulation of neutrophils (33) and reduces the magnitude of injury induced by a subsequent bout of lengthening contractions (16). The present study extends our laboratory’s previous findings by demonstrating that the protection provided by our group’s passive stretch protocols is associated, at least in part, with the neutrophil accumulation. Despite a widespread perception among athletes, coaches, and trainers that stretching before exercise provides protection from many types of sports-related injuries (38), numerous review articles have concluded that the experimental evidence remains inconclusive regarding the effectiveness of stretching for reducing either the number or severity of contraction-induced muscle injuries (6, 13, 39). Furthermore, whether plantar flexions of the ankle in mice, using static stretches that more accurately mimic the stretching typically performed by human beings before exercise, would afford protection from subsequent lengthening contraction-induced injury is not known. Despite the more severe damage resulting from lengthening contractions of muscles activated with maximal electrical stimulation (2, 7, 10, 23) compared with that following voluntary exercise in both animals (1, 30) and humans (7, 15, 27), the expectation is that the findings from the present study are applicable to the circumstances of exercise-induced muscle injury in human beings. A better understanding of the mechanisms underlying passive stretch conditioning and the role of neutrophils in the process of contraction-induced injury itself as well as in the protective adaptations would significantly improve our ability to design safe, effective conditioning programs, especially for populations susceptible to contraction-induced injury.

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Present address of N. Lockhart: National Institutes of Health, National Cancer Institute, Office of Biorepositories and Biospecimen Research, Bethesda, MD 20892-2580.

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