Past injurious exercise attenuates activation of primary calcium-dependent injury pathways in skeletal muscle during subsequent exercise

Ryo Takagi1,2, Riki Ogasawara3, Junya Takegaki2, Yuki Tamura1, Arata Tsutaki1, Koichi Nakazato1 & Naokata Ishii2

1 Graduate School of Health and Sport Science, Nippon Sport Science University, Tokyo, Japan
2 Department of Life Sciences, The University of Tokyo, Tokyo, Japan
3 Department of Life Science and Applied Chemistry, Nagoya Institute of Technology, Aichi, Japan

Keywords
Calpain, contraction, JNK, mechanical stress, repeated bout effect.

Abstract
Past contraction-induced skeletal muscle injury reduces the degree of subsequent injury; this phenomenon is called the “repeated bout effect (RBE).” This study addresses the mechanisms underlying the RBE, focusing on primary calcium-dependent injury pathways. Wistar rats were subdivided into single injury (SI) and repeated injury (RI) groups. At age 10 weeks, the right gastrocnemius muscle in each rat in the RI group was subjected to strenuous eccentric contractions (ECs). Subsequently, mild ECs were imposed on the same muscle of each rat at 14 weeks of age in both groups. One day after the exercise, the RI group showed a lower strength deficit than did the SI group, and neither group manifested any increase in membrane permeability. The concentration of protein carbonyls and activation of total calpain increased after ECs given at the age of 14 weeks. Nonetheless, these increases were lower in the RI group than in the SI group. Furthermore, calcium-dependent autolysis of calpain-1 and calpain-3 in the RI group was diminished as compared with that in the SI group. Although peak ankle joint torque and total force generation during ECs at the age of 14 weeks were similar between the two groups, phosphorylation of JNK (Thr183/Tyr185), an indicator of mechanical stress applied to a muscle, was lower in the RI group than in the SI group. These findings suggest that activation of the primary calcium-dependent injury pathways is attenuated by past injurious exercise, and mechanical stress applied to muscle fibers during ECs may decrease in the RBE.

Introduction
Excessive exercise causes skeletal muscle injury, especially after eccentric contractions (ECs) rather than concentric or isometric contractions (Howatson and van Someren 2008; Nosaka 2011). The injury induces a prolonged muscle strength deficit; furthermore, muscle fibers can undergo necrosis in case of severe injury (Kano et al. 2008). As a skeletal muscle adaptation to the injury, injured skeletal muscle reduces the severity of the symptoms of subsequent muscle injury, a phenomenon called the “repeated bout effect” (RBE) (Nosaka et al. 2001; Stupka et al. 2001; Vissing et al. 2008). McHugh et al. (1999) have suggested that complex adaptations in neurons, connective tissue, and muscle fibers are involved in the RBE. Furthermore, Hyldahl et al. (2017) have reported that neural adaptations, alterations to muscle mechanical properties, structural remodeling of the extracellular matrix (ECM), and biochemical signaling are involved in the potential mechanism of the RBE; however, the details are unknown.
In pathways of contraction-induced injury, extracellular calcium influx through stretch-activated channels (Yeung et al. 2005; Zhang et al. 2012) elevates intracellular calcium levels (Sonobe et al. 2008). The upregulated calcium activates calpain (Khordich and Ikura 2002; Murphy et al. 2007) and phospholipase (Ryan et al. 2000), and may enhance reactive oxygen species (ROS) formation (Grijalba et al. 1999; Ott et al. 2002). Studies suggest that calpain activation and ROS are related to the strength deficit in contraction-induced muscle injury (Badalamente and Stracher 2000; Moopanar and Allen 2005). Additionally, calpain and phospholipase activation events and lipid peroxidation by ROS increase membrane permeability (Duncan and Jackson 1987; Mason et al. 1997; Zhang et al. 2008), and the secondary extracellular calcium influx can induce muscle fiber necrosis in severe cases (Owensby et al. 1978).

Recently, we developed an animal model of the RBE, which is suitable for clarifying adaptations in connective tissue and muscle fibers (excluding neurons) due to controlled electrical stimulation and showed that past injurious exercise causes collagen deposition and fiber type conversion (Takagi et al. 2016), which are associated with a reduction in muscle tensile strength completely recovers 4 weeks after the injury (Takagi et al. 2016). Animals in both groups were euthanized by a combination of anesthesia and cervical spine fracture prior to or 15 min, 6 h (n = 7, respectively), or 1 days (n = 6) after ECs at the age of 14 weeks after measurement of ankle joint torque as described elsewhere (Takagi et al. 2016). Samples of the right medial gastrocnemius muscle belly were divided for histological and biochemical analyses, rapidly frozen in liquid nitrogen, and stored at –80°C.

The experimental procedure

The rats were randomly assigned to one of two groups: single injury (SI) or repeated injury (RI). The right gastrocnemius muscle of the RI group was subjected to strenuous ECs at the age of 10 weeks as reported previously (Takagi et al. 2016). Then, the rats in both groups were subjected to mild ECs (described below) at 14 weeks of age. The 4 weeks interval between two successive bouts of ECs in the RI group was employed because isometric strength completely recovers 4 weeks after the injury (Takagi et al. 2016). Animals in both groups were anesthetized with isoﬂurane and firmly fixed on a custom-made isokinetic dynamometer platform in the prone position. The right gastrocnemius muscle was electrically stimulated with electrodes attached to the muscle belly and the Achilles’s tendon percutaneously. Stimulation voltage was set to achieve maximal twitch torque, and tetanic stimulation was given for 0.3 and 2.0 sec with a train of 4 msec rectangular pulses at 10 msec intervals in the models of necrotizing and non-necrotizing injuries, respectively. At the onset of the electric stimulation, the ankle joint was isokinetically dorsiflexed to cause an EC. The speed and range of the forced lengthening were 180 and 30° per second from 60 to 125° and 60 to 120° of the ankle joint angle in the models of necrotizing and non-necrotizing injuries, respectively. The experimental procedure

Model of EC-induced necrotizing or non-necrotizing muscle injury

The models of EC-induced necrotizing and non-necrotizing injuries were based on muscle injuries at the age of 10 and 14 weeks, respectively. The rats were anesthetized with isoflurane and firmly fixed on a custom-made isokinetic dynamometer platform in the prone position. The right gastrocnemius muscle was electrically stimulated with electrodes attached to the muscle belly and the Achilles’s tendon percutaneously. Stimulation voltage was set to achieve maximal twitch torque, and tetanic stimulation was given for 0.3 and 2.0 sec with a train of 4 msec rectangular pulses at 10 msec intervals in the models of necrotizing and non-necrotizing injuries, respectively. At the onset of the electric stimulation, the ankle joint was isokinetically dorsiflexed to cause an EC. The speed and range of the forced lengthening were 180 and 30° per second from 60 to 125° and 60 to 120° of the ankle joint angle in the models of necrotizing and non-necrotizing injuries, respectively. The experimental procedure

Methods

Animals

Fifty-four 10-week-old male Wistar rats (CLEA Japan, Tokyo, Japan) were maintained in a 12:12 h light–dark cycle and allowed ad libitum access to food and water throughout the experiments. This study’s protocol was approved by the ethical committee of the Nippon Sport Science University (approval No. 014-A03).
relief medication because interventions that may affect regeneration after injury should be excluded.

Measurement of ankle joint torque
Isometric tetanic torque was measured as described previously (Song et al. 2004). Stimulus intensity was adjusted to produce the maximal isometric twitch force. Isometric planter-flexion torque of the right ankle joint was measured with a dynamometer at a joint angle of 90°.

Histological analysis
The Evans Blue dye (EBD) was used to detect partial necrosis of the muscle fibers (Matsuda et al. 1995; Barbier et al. 2004). EBD can enter only muscle fibers with a damaged membrane (Barbier et al. 2004; Lovering et al. 2007). Immediately after ECs at the age of 14 weeks, EBD (Sigma, St. Louis, MO, USA) in sterile phosphate-buffered saline (PBS) was intraperitoneally injected into the rats at a volume of 1% of the body mass (1 mg of EBD in 0.1 mL of PBS per 10 g of body mass).

Biochemical analysis
Western blotting was performed as reported elsewhere (Ogasawara et al. 2014; Takagi et al. 2016) with slight modifications to measure calpain-1, calpain-3, and total and phospho-JNK (Thr183/Tyr185) levels. For the detection of calpain-1 and calpain-3, another homogenizing buffer was applied according to another report (Kanzaki et al. 2014). Muscle samples for other detection assays were homogenized in RIPA buffer (Thermo Scientific, Waltham, USA) containing a protease inhibitor (Roche Applied Science, Upper Bavaria, Germany) and a phosphatase inhibitor (Thermo Scientific). The homogenates were centrifuged at 20,000 g for 15 min at 4°C. Twenty micrograms of protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a membrane. The membranes were blocked with 5% powdered milk or bovine serum albumin in Tris-buffered saline containing 0.1% Tween 20 for 1 h at room temperature and incubated overnight at 4°C with a primary antibody. Primary antibodies against calpain-1 (cat. #C0555; Sigma), calpain-3 (cat. #NCL-CALP-12A2; Novocastra Laboratories, Newcastle upon Tyne, UK), total c-Jun N-terminal kinase (JNK) (cat. #9252; Cell Signaling Technology, Danvers, MA, USA), or phospho-JNK (Thr183/Tyr185, cat. #9251; Cell Signaling Technology) were employed. The membranes were next incubated for 1 h at room temperature with an appropriate secondary antibody. Chemiluminescent reagents (Thermo Scientific) served for signal detection. Images were captured with Ez-capture (ATTO, Tokyo, Japan), and the signals were quantified in the CS analyzer software (ATTO). After image capture, membranes were stained with Coomassie Brilliant Blue to normalize the signal intensities to the amount of each protein loaded, in accordance with another study (Welinder and Ekblad 2011).

Protein carbonyl content was measured with a detection kit (cat. #ROIK03; SHIMA Laboratories, Tokyo, Japan). Samples were prepared as described above. The membrane after the transfer was reacted with 2,4-dinitrophenylhydrazine, and the protein-bound 2,4-dinitrophenylhydrazone was detected with an antidinitrophenyl antibody.

Total calpain activity was measured by means of the Calpain Activity Assay kit (cat. #ab65308; Abcam plc, Cambridge, UK). Frozen muscle samples were subjected to this analysis.

Statistics
Data are expressed as means ± standard deviation (SD). Differences between the two groups were determined by the Welch t tests (Figs. 1A, 4A and D). The differences in the proportion of EBD-positive fibers were determined by one-way analysis of variance, and the other differences were examined by a two-way analysis of variance followed by Bonferroni’s post hoc test. Data with P < 0.05 were considered statistically significant.

Results
The strength deficit and EBD-positive fibers after ECs at the age of 14 weeks
Isometric strengths measured 1 days after ECs at the age of 14 weeks decreased to 68.2 ± 2.3% and 87.0 ± 4.0% in groups SI and RI, respectively, as compared to strength measurements prior to the ECs (Fig. 1A). The deficit was significantly lower in the RI group than in the SI group. Figure 1B shows representative photomicrographs acquired 1 days after the ECs. Both groups SI (0.13 ± 0.04%) and RI (0.13 ± 0.02%) showed the same percentage of EBD-positive fibers relative to total number of muscle fibers as in the intact muscle (0.09 ± 0.04%). These findings suggest that past injurious exercise reduces the strength deficit during the subsequent exercise that does not substantially increase membrane permeability.

Protein carbonyls
The levels of protein carbonyls served as a marker of oxidative stress (Dalle-Donne et al. 2003). The differences
in protein carbonyl content between time points Post 0 or Post 6 and Pre were determined in each group. The SI group at Post 6 showed a higher value than that at Post 0 and the corresponding values in the RI group (Fig. 2). This finding suggested that past injurious exercise reduces protein carbonyl content during the subsequent exercise.

**Total calpain activity**

No interactions were associated with the difference in total calpain activity at Post 0 and Post 6 from each Pre; however, the RI group showed a lower average value than the SI group did, and Post 6 showed a lower average than Post 0 did (Fig. 3). This finding suggested that past injurious exercise attenuates total calpain activation during the subsequent exercise.

**Mechanical parameters with muscle contractions**

No significant differences were observed between the two groups in peak ankle joint torque (295.3 ± 38.8 mN·m in the SI group and 285.8 ± 32.1 mN·m in the RI group) and total force generation (7.27 ± 0.42 N·m·sec in the SI group and 7.31 ± 0.74 N·m·sec in the RI group) during ECs at the age of 14 weeks. These findings suggest that the mechanical stress imposed on the injured muscle was similar to that in an intact muscle.

**Phosphorylation of JNK (Thr^{183}/Tyr^{185})**

Both groups showed higher levels of phospho-JNK (Thr^{183}/Tyr^{185}) at Post 0 than at Pre and Post 6, but the SI group showed a higher value than the RI group did at Post 0 (Fig. 5). There was no difference in total JNK content between the groups prior to ECs at the age of 14 weeks (data not shown).

**Discussion**

In this study, we investigated the influence of past injurious exercise on activation of primary calcium-dependent injury pathways during subsequent exercise. Past injurious exercise attenuated calpain activation and protein

---

**Calpain-1 and -3 autolysis**

Calpain undergoes autolysis in a calcium-dependent manner (Murphy et al. 2006). The RI group showed higher total calpain-1 content than the SI group did prior to ECs at the age of 14 weeks (Fig. 4A). Autolyzed-calpain-1 content can be ranked in the following order: Pre < Post 0 < Post 6 < Post 0 in the SI group, and Pre and Post 6 < Post 0 in the RI group (Fig. 4B). Additionally, the RI group showed higher autolyzed-calpain-1 levels than the SI group did prior to ECs at the age of 14 weeks. As for the increase relative to each level before ECs, the RI group showed a lower average than the SI group did (Fig. 4C). The RI group showed higher total calpain-3 content than the SI group prior to ECs at the age of 14 weeks (Fig. 4D). As for autolyzed-calpain-3, the SI group showed a higher value at Post 0 than at Pre or Post 6 or RI group at Post 0, whereas the RI group showed no changes with time and higher values at Pre and Post 6 than the SI group did (Fig. 4E). As for the increase from each level before ECs, the SI group showed a higher value at Post 0 than the RI group did (Fig. 4F). These findings suggested that past injurious exercise reduced calpain-1 and -3 autolysis during subsequent exercise despite the increase in total calpain-1 and -3 levels.
carbonyl levels in the pathways. In conjunction with this mechanism, mechanical stress applied to muscle fibers may decrease, and consequently primary elevation in intracellular calcium levels may be attenuated.

Excessive exercise activates calcium-dependent injury pathways, results in a prolonged muscle strength deficit, and may induce necrosis of muscle fibers in severe cases (Kano et al. 2008). As for the injury at the age of 10 weeks in the RI group, we have previously shown that about 10% of muscle fibers undergo necrosis (Takagi et al. 2018). Additionally, isometric strength completely recovers, and collagen deposition and partial fiber type conversion are detectable prior to the exercise at the age of 14 weeks (Takagi et al. 2016) under the same conditions as in this study. Here, past injurious exercise reduced the strength deficit and neither group showed a remarkable increase in membrane permeability after the exercise at the age of 14 weeks. Damaged membrane induces subsequent extracellular calcium influx; therefore, the injury model at the age of 14 weeks can detect the changes in activation of primary calcium-dependent injury pathways.

Among the calcium-dependent injury pathways, calpain activation and ROS are involved in the strength deficit (Badalamente and Stracher 2000; Moopanar and Allen 2005). Calpain-3 is thought to have a role in the damage to excitation-contraction coupling (Verburg et al. 2005). In the RI group, total calpain activation and protein carbonyl content were increased by ECs at the age of 14 weeks and were lower than those in the SI group. These findings suggest that past injurious exercise attenuates calpain activation and ROS-induced injury during the subsequent exercise, resulting in a reduction in the strength deficit.

Calpain autolysis, which is thought to be necessary for calpain activation (Diaz et al. 2004) proceeds in a calcium-dependent manner (Murphy et al. 2006). Because it is difficult to measure in vivo intracellular calcium levels of the gastrocnemius muscle, calpain autolysis was investigated to estimate these levels. In this study, the increase in calpain-1 and calpain-3 autolysis after ECs at the age of 14 weeks was lower in the RI group than in the SI group despite the increase in total calpain-1 and -3 contents. These findings suggest that past injurious exercise attenuates calpain activation and ROS-induced injury during the subsequent exercise, resulting in a reduction in calpain activation and of ROS-induced injury.

Regarding the increased levels of autolysis of calpain-1 and -3 at rest in the RI group, increased calpain-3 autolysis is observed even on day 28 of muscle regeneration after nerve injury (Wu et al. 2015). In addition, calpain-3 is thought to participate in sarcomere remodeling during muscle regeneration (Hauerslev et al. 2012). Activation of

![Figure 2](image_url)

**Figure 2.** Differences in protein carbonyl content at Post 0 and Post 6 from the measurement before ECs in each group. Representative bands of protein carbonyl in each group are shown on the left. Values are presented as means ± standard deviations. * As compared to Post 0. The line between the two bars means a significant difference between groups SI and RI at the same time point (P < 0.05). SI, single-injury group; RI, repeated injury group; Post 0, at 15 min after ECs; and Post 6, at 6 h after ECs.

![Figure 3](image_url)

**Figure 3.** The difference in total calpain activity at Post 0 and Post 6 from the measurement before ECs in each group. Values are presented as means ± standard deviations. SI, single-injury group; RI, repeated injury group; Post 0, at 15 min after ECs; and Post 6, at 6 h after ECs.
these calcium-dependent pathways may be required for the later regeneration process although this notion is not yet proven.

We hypothesized that past injurious exercise attenuates activation of primary calcium-dependent injury pathways after the subsequent exercise, especially owing to the reduction in mechanical stress on muscle fibers because of collagen deposition. Intracellular calcium levels are elevated via stretch-activated channel-induced extracellular calcium influx (Yeung et al. 2005; Sonobe et al. 2008; Zhang et al. 2012). In this study, the mechanical stress imposed on the entire muscle during the exercise at the age of 14 weeks was similar between the injured and intact muscles. Nevertheless, we have reported that an injured muscle under the same conditions as this study shows greater passive resistive torque than does an intact muscle (Takagi et al. 2016). Thus, it is possible that the mechanical stress imposed on the injured muscle is lower than that on the intact muscle at the fiber level. JNKs can be activated by various factors such as growth factors (Hibi et al. 1993), cytokines (Westwick et al. 1994), and stressors (Cano et al. 1994). Among them, JNKs phosphorylation is particularly responsive to mechanical stress during muscle contractions (Martineau and Gardiner 2001; Russ and Lovering 2006). Because it is difficult to measure the stress magnitude in vivo, JNK phosphorylation (Thr183/Tyr185) was evaluated to estimate the stress. The RI group showed lower phospho-JNK (Thr 183/Tyr185) levels than the SI group did immediately after ECs. Therefore, past injurious exercise may attenuate mechanical stress applied to muscle fibers during ECs, thereby contributing to attenuation of the elevation of intracellular calcium levels.

**Figure 4.** Total calpain-1 and -3 content (A and D): a time course showing the changes in the levels of autolyzed-calpain-1 and -3 (B and E), and the increase in autolyzed-calpain-1 and -3 amounts at Post 0 and Post 6 relative to the measurement before ECs in each experimental group (C and F). Representative blots in each group appear at the top. Values are presented as means ± standard deviations. * As compared to Pre, † as compared to Post 0. The line between the two bars means a significant difference between groups SI and RI at the same time point (P < 0.05). SI, single-injury group; RI, repeated injury group; Post 0, at 15 min after ECs; and Post 6, at 6 h after ECs.
In conclusion, past injurious exercise attenuated activation of primary calcium-dependent injury pathways during subsequent exercise. Partly because of this mechanism, mechanical stress applied to muscle fibers may decrease, and consequently primary elevation in intracellular calcium levels may be attenuated. Nevertheless, further research is needed on the mechanism underlying the attenuation of activation of calcium-dependent injury pathways.

**Acknowledgments**

We thank M. Wada (Hiroshima University.) and K. Kanzaki (Kawasaki University of Medical Welfare.) for help with the experiments.

**Conflict of Interest**

The authors declare no conflicts of interest and that the results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of this study do not constitute endorsement by the American Physiological Society.

**References**

Badalamente, M. A., and A. Stracher. 2000. Delay of muscle degeneration and necrosis in mdx mice by calpain inhibition. Muscle Nerve 23:106-111.

Barbier, J., M. R. Popoff, and J. Molgó. 2004. Degeneration and regeneration of murine skeletal neuromuscular junctions after intramuscular injection with a sublethal dose of Clostridium sordellii lethal toxin. Infect. Immun. 72:3120-3128.

Boppart, M. D., D. J. Burkin, and S. J. Kaufman. 2006. Alpha7beta1-integrin regulates mechanotransduction and prevents skeletal muscle injury. Am. J. Physiol. Cell Physiol. 290:1660–1665.

Cano, E., C. A. Hazzalin, and L. C. Mahadevan. 1994. Anisomycin-activated protein kinases p45 and p55 but not mitogen-activated protein kinases ERK-1 and -2 are implicated in the induction of c-fos and c-jun. Mol. Cell. Biol. 14:7352–7362.

Dalle-Donne, I., R. Rossi, D. Giustarini, A. Milzani, and R. Colombo. 2003. Protein carbonyl groups as biomarkers of oxidative stress. Clin. Chim. Acta 329:23–38.

Diaz, B. G., T. Moldoveanu, M. J. Kuiper, R. L. Campbell, and P. L. Davies. 2004. Insertion sequence 1 of muscle-specific calpain, p94, acts as an internal propeptide. J. Biol. Chem. 279:27656–27666.

Duncan, C. J., and M. J. Jackson. 1987. Different mechanisms mediate structural changes and intracellular enzyme efflux following damage to skeletal muscle. J. Cell Sci. 87(Pt 1):183–188.

Gissel, H., and T. Clausen. 1999. Excitation-induced Ca2+ uptake in rat skeletal muscle. Am. J. Physiol. 276(2 Pt 2): R331–R339.

Grijalba, M. T., A. E. Vercesi, and S. Schreier. 1999. Ca2+-induced increased lipid packing and domain formation in submitochondrial particles. A possible early step in the mechanism of Ca2+-stimulated generation of reactive oxygen species by the respiratory chain. Biochemistry 38:13279–13287.

Hauerslev, S., M. L. Sveen, M. Dunø, C. Angelini, J. Vissing, and T. O. Krag. 2012. Calpain 3 is important for muscle regeneration: evidence from patients with limb girdle muscular dystrophies. BMC Musculoskelet. Disord. 13:43.

Hibi, M., A. Lin, T. Smeal, A. Minden, and M. Karin. 1993. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes Dev. 7:2135–2148.

Howatson, G., and K. A. van Someren. 2008. The prevention and treatment of exercise-induced muscle damage. Sports Med. 38:483–503.

Hyldahl, R. D., B. Nelson, L. Xin, T. Welling, L. Groscost, M. J. Hubal, et al. 2015. Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. FASEB J. 29:2894–2904.

Hyldahl, R. D., T. C. Chen, and K. Nosaka. 2017. Mechanisms and mediators of the skeletal muscle repeated bout effect. Exerc. Sport Sci. Rev. 45:24–33.

Kano, Y., K. Masuda, H. Furukawa, M. Sudo, K. Mito, and K. Sakamoto. 2008. Histological skeletal muscle damage and surface EMG relationships following eccentric contractions. J. Physiol. Sci. 58:349–355.

**Figure 5.** Time course of the changes in phospho-JNK (Thr183/Tyr185). Representative blots in each group appear on the top. Values are presented as means ± standard deviations. *versus Pre, †versus Post 0. The line between the two bars means significant difference between SI and RI at the same time point (P < 0.05). SI, single-injury group; RI, repeated injuries group; Post 0, at 15 min after ECs; and Post 6, at 6 h after ECs.
Kanzaki, K., M. Kuratani, S. Matsunaga, N. Yanaka, and M. Wada. 2014. Three calpain isoforms are autolyzed in rat fast-twitch muscle after eccentric contractions. J. Muscle Res. Cell Motil. 35:179–189.

Khorchid, A., and M. Ikura. 2002. How calpain is activated by calcium. Nat. Struct. Biol. 9:239–241.

Lieber, R. L., and J. Fridén. 1988. Selective damage of fast glycolytic muscle fibers with eccentric contraction of the rabbit tibialis anterior. Acta Physiol. Scand. 133:587–588.

Lieber, R. L., T. M. Woodburn, and J. Fridén. 1991. Muscle damage induced by eccentric contractions of 25% strain. J. Appl. Physiol. 70:2498–2507.

Lovering, R. M., J. A. Roche, R. J. Bloch, and P. G. De Deyne. 2007. Recovery of function in skeletal muscle following 2 different contraction-induced injuries. Arch. Phys. Med. Rehabil. 88:617–625.

Martineau, L. C., and P. F. Gardiner. 2001. Insight into skeletal muscle mechanotransduction: MAPK activation is quantitatively related to tension. J. Appl. Physiol. 91:693–702.

Mason, R. P., M. F. Walter, and P. E. Mason. 1997. Effect of oxidative stress on membrane structure: small-angle X-ray diffraction analysis. Free Radic. Biol. Med. 23:419–425.

Matsuda, R., A. Nishikawa, and H. Tanaka. 1995. Visualization of dystrophic muscle fibers in mdx mouse by vital staining with Evans blue: evidence of apoptosis in dystrophin-deficient muscle. J. Biochem. 118:959–964.

McHugh, M. P., D. A. Connolly, R. G. Eston, and G. W. Gleim. 1999. Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. Sports Med. 27:157–170.

Moopanar, T. R., and D. G. Allen. 2005. Reactive oxygen species reduce myofibrillar Ca\(^{2+}\) sensitivity in fatiguing mouse skeletal muscle at 37°C. J. Physiol. 564:189–199.

Murphy, R. M., R. J. Snow, and G. D. Lamb. 2006. u-Calpain and calpain-3 are not autolyzed with exhaustive exercise in humans. Am. J. Physiol. Cell Physiol. 290:116–122.

Murphy, R. M., C. A. Goodman, M. J. McKenna, J. Bennie, M. Leikis, and G. D. Lamb. 2007. Calpain-3 is autolyzed and hence activated in human skeletal muscle 24 h following a single bout of eccentric exercise. J. Appl. Physiol. 103:926–931.

Nosaka, K. 2011. Exercise-induced muscle damage and delayed onset muscle soreness. Pp. 179–192 in M. Cardinale, R. Newton, K. Nosaka, eds. Strength and conditioning: biological principles and practical applications. Wiley-Blackwell, Chichester.

Nosaka, K., K. Sakamoto, M. Newton, and P. Sacco. 2001. The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. Eur. J. Appl. Physiol. 85:34–40.

Ogasawara, R., K. Sato, K. Matsutani, K. Nakazato, and S. Fujita. 2014. The order of concurrent endurance and resistance exercise modifies mTOR signaling and protein synthesis in rat skeletal muscle. Am. J. Physiol. Endocrinol. Metab. 306:1155–1162.

Ott, M., J. D. Robertson, V. Gogvadze, B. Zhivotovsky, and S. Orrenius. 2002. Cytochrome c release from mitochondria proceeds by a two-step process. Proc. Natl Acad. Sci. USA 99:1259–1263.

Owney, C. L., J. B. Bjarnason, and A. T. Tu. 1978. Hemorrhagic toxins from rattlesnake (Crotalus atrox) venom. Pathogenesis of hemorrhage induced by three purified toxins. Am. J. Pathol. 93:201–218.

Roca-Cusachs, P., A. del Rio, E. Puklin-Faucher, N. C. Gauthier, N. Biais, and M. P. Sheetz. 2013. Integrin-dependent force transmission to the extracellular matrix by alpha-actinin triggers adhesion maturation. Proc. Natl Acad. Sci. USA 110:1361–1370.

Russ, D. W., and R. M. Lovering. 2006. Influence of activation frequency on cellular signaling pathways during fatiguing contractions in rat skeletal muscle. Exp. Physiol. 91:957–966.

Ryan, M. J., K. W. Gross, and G. Hajduczok. 2000. Calcium-dependent activation of phospholipase C by mechanical distension in renin-expressing As4.1 cells. Am. J. Physiol. Endocrinol. Metab. 279:823–829.

Song, H., K. Nakazato, and H. Nakajima. 2004. Effect of increased excursion of the ankle on the severity of acute eccentric contraction-induced strain injury in the gastrocnemius: an in vivo rat study. Am. J. Sport Med. 32:1263–1269.

Sonobe, T., T. Inagaki, D. C. Poole, and Y. Kano. 2008. Intracellular calcium accumulation following eccentric contractions in rat skeletal muscle in vivo: role of stretch-activated channels. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294:1329–1337.

Stupka, N., M. A. Tarnopolsky, N. J. Yardley, and S. M. Phillips. 2001. Cellular adaptation to repeated eccentric exercise-induced muscle damage. J. Appl. Physiol. 91:1669–1678.

Takagi, R., R. Ogasawara, A. Tsutaki, K. Nakazato, and N. Ishii. 2016. Regional adaptation of collagen in skeletal muscle to repeated bouts of strenuous eccentric exercise. Pflugers Arch. 468:1565–1572.

Takagi, R., R. Ogasawara, I. Takegaki, A. Tsutaki, K. Nakazato, and N. Ishii. 2018. Influence of past injurious exercise on fiber type-specific acute anabolic response to resistance exercise in skeletal muscle. J. Appl. Physiol. 124:16–22.

Verburg, E., R. M. Murphy, D. G. Stephenson, and G. D. Lamb. 2005. Disruption of excitation-contraction coupling and titin by endogenous Ca\(^{2+}\)-activated proteases in toad muscle fibres. J. Physiol. 564:775–790.

Vissing, K., K. Overgaard, A. Nedergaard, A. Fredsted, and P. Schjerling. 2008. Effects of concentric and repeated eccentric exercise on muscle damage and calpain-calpastatin gene expression in human skeletal muscle. Eur. J. Appl. Physiol. 103:323–332.
Welinder, C., and L. Ekblad. 2011. Coomassie staining as loading control in Western blot analysis. J. Proteome Res. 10:1416–1419.

Westwick, J. K., C. Weitzel, A. Minden, M. Karin, and D. A. Bremmer. 1994. Tumor necrosis factor alpha stimulates AP-1 activity through prolonged activation of the c-Jun kinase. J. Biol. Chem. 269:26396–26401.

Wu, R., Y. Yan, J. Yao, Y. Liu, J. Zhao, and M. Liu. 2015. Calpain 3 expression pattern during gastrocnemius muscle atrophy and regeneration following sciatic nerve injury in rats. Int. J. Mol. Sci. 16:26927–26935.

Yeung, E. W., N. P. Whitehead, T. M. Suchyna, P. A. Gottlieb, F. Sachs, and D. G. Allen. 2005. Effects of stretch-activated channel blockers on [Ca\textsuperscript{2+}]\textsubscript{i} and muscle damage in the mdx mouse. J. Physiol. 562(Pt 2):367–380.

Zhang, B. T., S. S. Yeung, D. G. Allen, L. Qin, and E. W. Yeung. 2008. Role of the calcium-calpain pathway in cytoskeletal damage after eccentric contractions. J. Appl. Physiol. 105:352–357.

Zhang, B. T., N. P. Whitehead, O. L. Gervasio, T. F. Reardon, M. Vale, D. Fatkin, et al. 2012. Pathways of Ca\textsuperscript{2+} entry and cytoskeletal damage following eccentric contractions in mouse skeletal muscle. J. Appl. Physiol. 112:2077–2086.