Effects of Streptomycin Administration on Increases in Skeletal Muscle Fiber Permeability and Size Following Eccentric Muscle Contractions

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ABSTRACT

The purpose of this study was to investigate the preventive effect of streptomycin (Str) administration on changes in membrane permeability and the histomorphological characteristics of damaged muscle fibers following eccentric contraction (ECC). Eighteen 7-week-old male Fischer 344 rats were randomly assigned to three groups: control (Cont), ECC, and ECC with Str (ECC + Str). The tibialis anterior (TA) muscles in both ECC groups were stimulated electrically and exhibited ECC. Evans blue dye (EBD), a marker of muscle fiber damage associated with increased membrane permeability, was injected 24 hr before TA muscle sampling. The number of EBD-positive fibers, muscle fiber cross-sectional area (CSA), and roundness were determined via histomorphological analysis. The ECC intervention resulted in an increased fraction of EBD-positive fibers, a larger CSA, and decreased roundness. The fraction of EBD-positive fibers was 79% lower in the ECC + Str group than in the ECC group. However, there was no difference in the CSA and roundness of the EBD-positive fibers between the two ECC groups. These results suggest that Str administration can reduce the number of myofibers that increase membrane permeability following ECC, but does not ameliorate the extent of fiber swelling in extant EBD-positive fibers. Anat Rec, 301:1096–1102, 2018. © 2018 Wiley Periodicals, Inc.

Key words: eccentric contraction; muscle damage; streptomycin

Eccentric muscle contraction (ECC), which is induced by muscle stretching during electrical stimulation-induced muscle contractions and downhill running (Takekura et al., 2001), exerts greater muscle force than concentric or isometric contractions (Barash et al., 2002; Peters et al., 2003; Mori et al., 2014). However, after repetitive ECC, muscles are more easily damaged. Stretch-induced muscle injury includes both structural disorganization and changes to the ionic regulation of muscle fibers (Yeung and Allen, 2004). The structural and functional changes that occur after repetitive ECC are characterized by sarcomere and Z-line disorganization, abnormal t-tubule arrangement, increased intracellular calcium concentration and membrane permeability, and

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decreased muscle force production (McBride et al., 2000; Takekura et al., 2001; Yeung et al., 2003, 2005; Willems and Stauber, 2005). Additionally, muscle fibers with increased intracellular calcium concentration and membrane permeability appear swollen and opaque. Several pathways for calcium entry to myofibers following ECC have been reported, but the main pathways are membrane tears and stretch-activated channels (SACs) (Allen et al., 2005).

Evans blue dye (EBD, molar mass 960.8) is a useful tool in studying the existence of membrane tears implicated in tissue damage. EBD is water soluble and membrane impermeable, has a high binding affinity for serum albumin, and can be used to identify damaged muscle fibers (Hamer et al., 2002; Mori et al., 2014; Takagi et al., 2016). Reportedly, stretch-induced muscle damage and dystrophin-deficient muscles show a large number of EBD-positive fibers, suggesting increased membrane permeability (Matsuda et al., 1995; Hamer et al., 2002; Mori et al., 2014). In addition, since calcium binds avidly to albumin (Besarab et al., 1981), EBD-positive fibers also indicate higher intracellular calcium concentrations following ECC. Increased intracellular calcium can contribute to progressive muscle damage by stimulating calcium-activated neutral protease, as such m- and μ-calpain and phospholipases, which cause membrane damage and increased membrane permeability (McNeil and Khakee, 1992; Deconinck et al., 1996). In fact, the existence of membrane tears after 1 hr of downhill running indicates uptake of albumin by muscle fibers, which is evidence of membrane permeability to large-molecular-weight markers (McNeil and Khakee, 1992).

Conversely, increased resting intracellular calcium can also be caused by ionic entry through SACs. SACs are ion channels that react to mechanical stimuli, e.g., when stretched they allow Ca$^{2+}$, Na$^+$, and K$^+$ to pass through the membrane, and they occur in myofibers and cardiac ventricular myocytes (Guharay and Sachs, 1984; Sadoshima et al., 1992). Increased intracellular calcium ion entering through SACs after ECC might be responsible for initiating the protease activity that causes damage to the membrane (Yeung and Allen, 2004). In fact, increases in resting intracellular calcium were observed in mice at 48 hr after downhill running (Lynch et al., 1997) and in single fibers after 10 stretched contractions (Yeung et al., 2005). These events, which are related to the muscle damage that follows ECC, are reportedly reduced by SAC blockers such as streptomycin (Str), gadolinium (Gd$^{3+}$), and GsMTx4 (McBride et al., 2000; Belus and White, 2003; Yeung et al., 2003, 2005; Willems and Stauber, 2005). Str, which was the first aminoglycoside antibiotic, has been used clinically to treat tuberculosis, and is incidentally one of the most potent SACs blockers. In an in vivo study, it was used to reduce EBD-induced muscle damage (number of EBD-positive fibers) and damage-related force deficit (McBride et al., 2000; Willems and Stauber, 2005; Yeung et al., 2005). SAC blockers may thus have therapeutic potential for reducing contraction-induced muscle damage (Yeung and Allen, 2004). However, all that is currently known is that Str administration reduces the number and fraction of damaged muscle fibers, such as EBD-positive fibers. Whether it affects the histomorphological characteristics of extant EBD-positive fibers, such as swelling and roundness, remains to be determined.

The aim of this study was to investigate the effects of Str administration on both the fraction of EBD-positive fibers and the histomorphological characteristics of extant EBD-positive fibers following ECC.

**MATERIALS AND METHODS**

**Animals**

Eighteen male Fischer 344 rats (CLEA, Tokyo, Japan) were housed in a temperature-controlled room at 23°C ± 2°C, humidity at 55% ± 5%, under a 12-hr light–dark cycle, and provided with CE-2 rodent chow (CE-2, CLEA) and water ad libitum. All procedures were performed in accordance with the guidelines presented in the Field of Physiological Sciences, published by the Physiological Society of Japan. This study was approved by the Animal Committee of the National Institute of Fitness and Sports and the Animal Study Committee of Niigata University of Health and Welfare.

**Experimental Protocol**

Eighteen 7-week-old male Fischer 344 rats were randomly assigned to one of three groups of six rats each: control (Cont), eccentric contraction (ECC), or eccentric contraction with Str administration (ECC + Str). Str was prepared and diluted immediately before use. Administration began six days before the ECC intervention. The rats in the ECC + Str group received Str in their drinking water, which was administered with a feeding bottle (4 g/L) (McBride et al., 2000; Tamaki et al., 2015); the other two groups were given pure drinking water. The daily amount of water drunk was measured using a precision balance. The mean Str intake in the ECC + Str group was 644 ± 26 mg/kg/day. To perform the ECC, the tibialis anterior (TA) muscles of the rats in both ECC groups were stimulated electrically under 2% isoflurane inhalation anesthesia. The rat was placed in the supine position on the supporting platform of a custom-made apparatus that was designed to stabilize the lower leg and allow full ankle rotation. The left foot was attached to a footplate connected to a servomotor (Tower Pro SG-90, Uemomo, Tokyo, Japan), and the ankle joint was set at 90-degree angle. Paired silver surface electrodes were attached to the shaved anterior surface of the leg to stimulate the left TA muscle. Percutaneous direct muscle stimulation was applied using an electrostimulator (SEN-T203, Nihon Kohden, Tokyo, Japan) and isolator (SS-201J, Nihon Kohden, Tokyo, Japan). The muscle was stimulated with an intensity of 30 V, at a frequency of 100 Hz and a pulse width of 500 μs, to induce submaximal tetanic contraction for 2 sec. The footplate was moved in synchrony with the electrical stimulator; movement was initiated 500 msec after the onset of electrical stimulation using a time-delay device (Raspberry Pi 2 Model B, Raspberry Pi Foundation, Cambridge, UK). The angular velocity of the ankle joint was set at 200 degree/second. The ECC session comprised 80 contractions at 6-sec intervals.
Muscle Sampling

In order to identify the muscle fibers responsible for increasing membrane permeability, rats were injected intraperitoneally with a solution of 1% EBD (E2129, Sigma, St. Louis, MO) at a volume of 1% of body mass (BM) (1 mg EBD/0.1 mL phosphate-buffered saline (PBS)/10 g BM) 24 hr before TA muscle sampling (Lovering and De Deyne, 2004). The rats in all three groups were anesthetized with sodium pentobarbital (50 mg/kg body weight) two days after the ECC intervention, and their TA muscles were collected and weighed. Samples for histological analyses were mounted on pieces of cork with OCT compound, and frozen in isopentane cooled by liquid nitrogen. They were stored at −80°C until use.

Staining and Histomorphometry

Frozen serial 10-μm transverse and longitudinal sections were cut from the middle portion of the TA muscle at −20°C using a cryostat (CM3050, Leica, Wetzlar, Germany) and mounted on silanized slides for glyoxal-sil (2-hydroxyanil) (GBHA) staining, immunohistochemical staining, and EBD signal detection analysis. GBHA have been reported as a free calcium indicator in a previous study (Del Bigio, 2000). Transverse sections from all groups were stained with 5% GBHA in 75% ethanol containing 3.4% NaOH for 10 min at room temperature and were rinsed with 95% and then 75% ethanol for 5 min each. Finally, the sections were dehydrated and coverslipped with mounting medium. This procedure served to visualize calcium localization as a red calcium-GBHA complex (Takano et al., 1988) under a light/fluorescent microscope (BX60, Olympus, Tokyo, Japan). For TA muscle tissue immunohistochemistry, sections were fixed in 2% paraformaldehyde for 15 min as described previously (Nakagawa et al., 2016; Tamaki et al., 2017). Sections were blocked with 10% normal goat serum (NGS) and 1% Triton X-100 in PBS containing 0.3% Triton X-100 for 1 hr, followed by two washes of 5 min each in PBS, and subsequent incubation for 16–20 hr at 4°C with a primary antibody against dystrophin (1:500 dilution; Abcam, Tokyo, Japan), laminin (1:200 dilution; Abcam), and desmin (1:200 dilution; Abcam, Tokyo, Japan) in 5% NGS in PBS containing 5% NGS and 0.1% Triton X-100 for 1 hr at room temperature, and finally mounted with Vectashield mounting medium.

Immunofluorescence and EBD signals of the transverse and longitudinal sections were detected using a light/fluorescence microscope (BX60, Olympus) with the Olympus filter set (U-MWIB2, U-MWIG3) and a CCD camera (DP73, Olympus). Digital images at a ×200 magnification were used to determine the number of EBD-positive fibers, muscle fiber cross-sectional area (CSA), and roundness (R) of each TA muscle, following previous studies but with slight modification (Nakagawa et al., 2017; Tamaki et al., 2017). The total number of EBD-positive and -negative fibers in six 1,055 × 1,404 μm fields at the center of the cross-section of the TA muscle was counted manually to determine the fraction of EBD-positive fibers. The CSA of at least 100 fibers from each muscle was measured using Image-pro Premier software (Media Cybernetics, Rockville, MD). The roundness was calculated as $R = P/(4 \times \pi \times CSA)$, where $P$ is the perimeter of the muscle fibers.

Statistical Analysis

All data are expressed as mean ± standard deviation. One-way analysis of variance followed by a post hoc Tukey–Kramer test was used to assess the significance of differences among the groups. Differences in the number of EBD-positive fibers were analyzed by student t-test. P-values less than 0.05 were considered significant.

RESULTS

There were no significant differences between the groups in terms of the body and TA muscle weights of the rats before the experiment (Table 1). EBD-positive fibers were observed in the ECC and the ECC + Str groups, but were not detected in the Cont group (Fig. 1A–C). There were significantly fewer (79%; $P < 0.05$) EBD-positive fibers in the TA muscles of the ECC + Str group than in those of the ECC group (Table 1). Immunohistochemistry revealed laminin-positive and dystrophin-negative results for the EBD-positive fibers (Fig. 1D–G), but dystrophin-negative and desmin-negative results for the EBD-positive fibers (Fig. 1H–K). Additionally, EBD-positive areas were observed only in some portions of the longitudinal muscle fiber sections (Fig. 2A,B). We observed a positive reaction for GBHA...
staining, in accordance with the location of the EBD-positive muscle fibers (Fig. 3). The CSA of the EBD-negative fibers was not significantly different between the groups, but the CSA of the EBD-positive fibers in the ECC and ECC+Str groups was 2.2–2.4 times ($P < 0.01$) that of the EBD-negative fibers in the same group (Table 1). The roundness of the EBD-negative fibers was 1.31, 1.35, and 1.38 in the Cont, ECC, and ECC+Str groups, respectively, which was not significantly different between groups (Table 1). However, that of the EBD-positive fibers in the ECC and ECC+Str groups (1.09 and 1.17, respectively) was significantly
lower \( P < 0.01 \) than the roundness of the EBD-negative fibers in the same group. Notably, however, there were no significant differences in the CSA or roundness of the EBD-positive fibers between the ECC and ECC + Str groups.

**DISCUSSION**

These findings show that ECC intervention increased the number of EBD-positive muscle fibers, which was associated with increased muscle fiber CSA and decreased roundness. Str administration reduced the number of EBD-positive muscle fibers induced by ECC, but did not affect the fiber size or roundness of extant EBD-positive fibers.

Str administration prior to ECC intervention reduced the number of damaged muscle fibers induced by ECC by 79\%. Str has been reported to block SACs, inhibit cation-permeable SACs in skeletal muscle fibers, and reduce ECC-induced muscle injury and damage-related force deficit (Sokabe et al., 1993; McBride et al., 2000; Belus and White, 2003; Allen et al., 2005; Willems and Stauber, 2005; Yeung et al., 2005). The protective effect of Str on skeletal muscle damage has been reported to be dose-dependent (Yeung et al., 2005). Additionally, other possible effects of Str on muscle include a tendency toward a lower maximal isometric force and force–time integrals evoked by electrical stimulation, as shown in Str-treated rats (Willems and Stauber, 2005; Tamaki et al., 2015); however, these results were not statistically significant. Desmin, titin, and dystrophin have been shown to be disrupted after ECC, but this effect was substantially reduced by Str administration (Zhang et al., 2012). Moreover, Str reduced the in vivo ECC-induced intracellular calcium ion increase in rat skeletal muscle by 69\% (Sonobe et al., 2008). This may be because elevations in intracellular calcium ions, which occur following stretch-induced injury, are a consequence of calcium ion influx through SACs (Yeung and Allen, 2004). In the present study, therefore, such calcium entry might be responsible for \( \sim 80\% \) of the number of EBD-positive fibers induced by ECC, since SAC blockers prevent calcium entry and reduce muscle damage following ECC (Yeung and Allen, 2004). However, Str administration did not prevent morphological changes in the extant EBD-positive fibers following ECC. This suggests that calcium also enters the myofibers via other pathways, such as membrane tears.

EBD forms a complex with serum albumin due to a high binding affinity, and albumin binds avidly to calcium (Besarab et al., 1981). EBD-positive fibers presumably therefore also indicate higher intracellular calcium concentrations following ECC. In fact, the EBD-positive fibers in our study were also positive for GBHA, which suggests higher calcium concentrations in these fibers. Reportedly, intracellular calcium ions may undergo prolonged elevations, increasing 2- to 3-fold above resting levels for up to 48 hr following a bout of ECC (Lynch et al., 1997). This excessive increase in intracellular calcium ions induces muscle injury (Yeung et al., 2005) and promotes the production of calcium-dependent proteases, such as calpain and phospholipases, which cause membrane damage and increased membrane permeability (McNeil and Khakee, 1992; Deconinck et al., 1996; Zhang et al., 2008). In addition, some studies have found that the EBD-positive fibers formed following ECC were accompanied by discontinuous dystrophin immunostaining (Lehti et al., 2007; Nakagawa et al., 2016). Our immunohistochemistry results also revealed dystrophin-negative areas around the EBD-positive fibers. Dystrophin protein, which plays a structural role in linking the sarcolemma to the underlying cytoskeleton, appears to protect the sarcolemma against stress imposed during muscle contraction or stretching. In fact, absence of dystrophin reduces muscle stiffness, increases sarcolemmal deformability, and compromises the mechanical stability of costameres and their connections with nearby myofibrils (Garcia-Pelagio et al., 2011). Additionally, Aquaporin-4 (AQP4) expression disappears in dystrophin-deficient muscles (Crosbie et al., 2002). AQP4 is present in the plasma membranes of...
specific fast-type myofibers, a selective water channel mediating water transport across cell membranes in skeletal muscles, and binds to α1-syntrophin, which is a component of the dystrophin-associated protein complex in skeletal muscles (Adams et al., 2001; Neely et al., 2001; Ishido and Nakamura, 2016). Reportedly, aquaporin-4 (AQP4) and transient receptor potential vanilloid 4, a nonselective cation channel activated by mechanical and osmotic stress, synergistically modulate cell volume (Benfenati et al., 2011; Jo et al., 2015; Mola et al., 2016). Thus, it is possible that increased intracellular calcium and/or dystrophin-disrupted myofibers following ECC lead to increased cell volume in the form of, for example, swollen fibers.

Fast-twitch fibers with myosin heavy chain (MHC) IIX and IIB appear to be more susceptible to ECC-induced damage than those containing MHC I (Lieber and Friden, 1988; Lieber et al., 1991; Mori et al., 2014; Takagi et al., 2016). Furthermore, in both the present study and a previous study that used identical ECC conditions (angular velocity of 200 degree/sec; Mori et al., 2014), EBD-positive fibers were observed throughout cross-sections of fast-type dominant TA muscle, but only some portions of the longitudinal sections were EBD-positive. In a previous study, some pattern irregularities in the otherwise well-preserved regular transverse myofibrillar bands were observed following eccentric contractions (Yu et al., 2004). In longitudinal muscle sections, sarcomere disturbances were observed in extensor digitorum longus (EDL) muscle, regions with loss of titin immunoreactivity were found next to normal regions (Friden and Lieber, 1998), and disruption of desmin was observed (Yu et al., 2003). Additionally, damaged regions have been observed adjacent to normal regions (Orfanos et al., 2016). Combined, this evidence suggests that post-ECC changes in muscle fiber morphology related to membrane permeability might not occur in the whole muscle fiber, but only in longitudinal portions of it.

In the present study, Str administration had no effect on the extent of fiber swelling following ECC. Nonetheless, our histomorphometric analysis highlights the impact of Str administration in terms of reducing the number of damaged muscle fibers induced by high-intensity exercise that includes lengthening muscle contraction. The relationships between SACs expression, dystrophin, and AQP4 following ECC are of great interest for future work, which may further our understanding of the underlying mechanisms of exercise-induced muscle fiber damage, including membrane permeability and swelling kinetics. To our knowledge, this is the first histomorphological study that quantitatively assesses the effects of Str administration on post-ECC changes in muscle fiber morphology related to membrane permeability. We have demonstrated that ECC intervention increases the fraction of EBD-positive muscle fibers, which exhibit increased CSA (2.2- to 2.4-fold), decreased roundness (15%-19%), positive GBHA staining, and absent and/or discontinuous dystrophin and desmin immunostaining. Str administration reduced the number of EBD-positive muscle fibers induced by ECC, but had no effect on fiber size and roundness in extant EBD-positive fibers. These findings suggest that Str, a SACs blocker, can mediate eccentric contraction-induced fiber damage in the rat TA muscle by reducing the number of muscle fibers that increase membrane permeability, but does not ameliorate the extent of swelling in extant EBD-positive fibers following ECC.

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LITERATURE CITED

Adams ME, Mueller HA, Froehner SC. 2001. In vivo requirement of the alpha-syntrophin PDZ domain for the sarcolemmal localization of nNOS and aquaporin-4. J Cell Biol 155:113–122.

Allen DG, Whitehead NP, Yeung EW. 2005. Mechanisms of stretch-induced muscle damage in normal and dystrophic muscle: role of ionic changes. J Physiol 567:723–735.

Barash IA, Peters D, Friden J, Lutz GJ, Lieber RL. 2002. Desmin cytoskeletal modifications after a bout of eccentric exercise in the extensor digitorum longus. Am J Physiol Regul Integr Comp Physiol 283:R958–R963.

Belus A, White E. 2003. Streptomycin and intracellular calcium modulate the response of single guinea-pig ventricular myocytes to axial stretch. J Physiol 546:501–509.

Benfenati V, Caprini M, Dovizio M, Mylonakou MN, Ferroni S, Ottersen OP, Amiry-Moghaddam M. 2011. An aquaporin-4/tran- sient receptor potential vanilloid 4 (AQP4/TRPV4) complex is essential for cell-volume control in astrocytes. Proc Natl Acad Sci USA 108:2563–2568.

Besarab A, DeGuzman A, Swanson JW. 1981. Effect of albumin and free calcium concentrations on calcium binding in vitro. J Clin Pathol 34:1361–1367.

Crosbie RH, Dovico SA, Flanagan JD, Chamberlain JS, Ownby CL, Campbell KP. 2002. Characterization of aquaporin-4 in muscle and muscular dystrophy. FASEB J 16:943–949.

Deconinck N, Ragot T, Marechal G, Perriucaudet M, Gillis JM. 1996. Functional protection of dystrophic mouse (mdx) muscles after adenovirus-mediated transfer of a dystrophin minigene. Proc Natl Acad Sci USA 93:3570–3574.

Del Bigio MR. 2000. Calcium-mediated proteolytic damage in white matter of hydrocephalic rats? J Neuropath Exp Neurol 59:946–954.

Friden J, Lieber RL. 1998. Segmental muscle fiber lesions after repetitive eccentric contractions. Cell Tissue Res 293:165–171.

Garcia-Pelagio KP, Bloch RJ, Ortega A, Gonzalez-Serratos H. 2011. Mechanisms of stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. J Physiol 352:685–701.

Hamre PW, McGechie JM, Davies MJ, Grounds MD. 2002. Evans Blue Dye as an in vivo marker of myofibre damage: optimising parameters for detecting initial myofibre membrane permeability. J Anat 200:69–79.

Ishido M, Nakamura T. 2016. Aquaporin-4 Protein Is Stably Main- tained in the Hypertrophied Muscles by Functional Overload. Acta Histochem Cytochem 49:89–95.

Jo AO, Ryskamp DA, Phuong TT, Verkman AS, Yarishkin O, Blue Dye as an in vivo marker of myofibre damage: optimising parameters for detecting initial myofibre membrane permeability. J Anat 200:69–79.

Lehti TM, Kalliokoski R, Komulainen J. 2011. Repeated bout effect on the extent of swelling in extant EBD-positive fibers following ECC.

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LITERATURE CITED

Adams ME, Mueller HA, Froehner SC. 2001. In vivo requirement of the alpha-syntrophin PDZ domain for the sarcolemmal localization of nNOS and aquaporin-4. J Cell Biol 155:113–122.

Allen DG, Whitehead NP, Yeung EW. 2005. Mechanisms of stretch-induced muscle damage in normal and dystrophic muscle: role of ionic changes. J Physiol 567:723–735.

Barash IA, Peters D, Friden J, Lutz GJ, Lieber RL. 2002. Desmin cytoskeletal modifications after a bout of eccentric exercise in the extensor digitorum longus. Am J Physiol Regul Integr Comp Physiol 283:R958–R963.

Belus A, White E. 2003. Streptomycin and intracellular calcium modulate the response of single guinea-pig ventricular myocytes to axial stretch. J Physiol 546:501–509.

Benfenati V, Caprini M, Dovizio M, Mylonakou MN, Ferroni S, Ottersen OP, Amiry-Moghaddam M. 2011. An aquaporin-4/tran- sient receptor potential vanilloid 4 (AQP4/TRPV4) complex is essential for cell-volume control in astrocytes. Proc Natl Acad Sci USA 108:2563–2568.

Besarab A, DeGuzman A, Swanson JW. 1981. Effect of albumin and free calcium concentrations on calcium binding in vitro. J Clin Pathol 34:1361–1367.

Crosbie RH, Dovico SA, Flanagan JD, Chamberlain JS, Ownby CL, Campbell KP. 2002. Characterization of aquaporin-4 in muscle and muscular dystrophy. FASEB J 16:943–949.

Deconinck N, Ragot T, Marechal G, Perriucaudet M, Gillis JM. 1996. Functional protection of dystrophic mouse (mdx) muscles after adenovirus-mediated transfer of a dystrophin minigene. Proc Natl Acad Sci USA 93:3570–3574.

Del Bigio MR. 2000. Calcium-mediated proteolytic damage in white matter of hydrocephalic rats? J Neuropath Exp Neurol 59:946–954.

Friden J, Lieber RL. 1998. Segmental muscle fiber lesions after repetitive eccentric contractions. Cell Tissue Res 293:165–171.

Garcia-Pelagio KP, Bloch RJ, Ortega A, Gonzalez-Serratos H. 2011. Mechanisms of stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. J Physiol 352:685–701.

Hamre PW, McGechie JM, Davies MJ, Grounds MD. 2002. Evans Blue Dye as an in vivo marker of myofibre damage: optimising parameters for detecting initial myofibre membrane permeability. J Anat 200:69–79.

Ishido M, Nakamura T. 2016. Aquaporin-4 Protein Is Stably Main- tained in the Hypertrophied Muscles by Functional Overload. Acta Histochem Cytochem 49:89–95.

Jo AO, Ryskamp DA, Phuong TT, Verkman AS, Yarishkin O, Blue Dye as an in vivo marker of myofibre damage: optimising parameters for detecting initial myofibre membrane permeability. J Anat 200:69–79.

Lehti TM, Kalliokoski R, Komulainen J. 2007. Repeated bout effect on the extent of swelling in extant EBD-positive fibers following ECC.
Lynch GS, Fary CJ, Williams DA. 1997. Quantitative measurement of resting skeletal muscle [Ca2+]i following acute and long-term downhill running exercise in mice. Cell Calcium 22:373–383.

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

McBride TA, Stockert BW, Gorin FA, Carlsen RC. 2000. Stretch-activated ion channels contribute to membrane depolarization after eccentric contractions. J Appl Physiol (1985) 88:91–101.

McNeil PL, Khakee R. 1992. Disruptions of muscle fiber plasma membranes. Role in exercise-induced damage. Am J Pathol 140:1097–1109.

Mola MG, Sparaneo A, Gargano CD, Spray DC, Svelto M, Frigeri A, Scemes E, Nicchia GP. 2016. The speed of swelling kinetics modulates cell volume regulation and calcium signaling in astrocytes: a different point of view on the role of aquaporins. Glia 64:139–154.

Mori T, Agata N, Itoh Y, Miyazato-Inoue M, Sokabe M, Taguchi T, Kawakami K. 2014. Stretch-speed-dependent myofiber damage and functional deficits in rat skeletal muscle induced by lengthening contraction. Physiol Rep 2:25413330.

Nakagawa K, Tamaki H, Hayao K, Yotani K, Ogita F, Yamamoto N, Onishi H. 2017. Electrical stimulation of denervated rat skeletal muscle retards capillary and muscle loss in early stages of disuse atrophy. Biomed Res Int 2017:5695217.

Nakagawa K, Tamaki H, Hayao K, Yotani K, Ogita F, Yamamoto N, Onishi H, Kasuga N. 2016. Effects of forced eccentric contractions on histomorphometric characteristics in rat skeletal muscle. J Phys Fitess Sports Med 5:426.

Neeley JD, Amiry-Moghaddam M, Ottersen OP, Frohner SC, Agra P, Adams ME. 2001. Syntrophin-dependent expression and localization of Aquaporin-4 water channel protein. Proc Natl Acad Sci USA 98:14109–14113.

Orfanoz Z, Godderz MP, Soroka E, Godderz T, Rumyantseva A, van der Ven PF, Hawke TJ, Furst DO. 2016. Breaking sarcomeres by in vitro exercise. Sci Rep 6:19614.

Peters D, Barash IA, Burdi M, Yuan PS, Mathew L, Frieden J, Lieber RL. 2003. Asynchronous functional, cellular and transcriptional changes after a bout of eccentric exercise in the rat. J Physiol 553:947–957.

Sadshima J, Takahashi T, Jahn L, Iizumo S. 1992. Roles of mechano-sensitive ion channels, cytoskeleton, and contractile activity in stretch-induced immediate-early gene expression and hypertrophy of cardiac myocytes. Proc Natl Acad Sci USA 89:9905–9909.

Sokabe M, Hasagewana N, Yamamori K. 1993. Blockers and activators for stretch-activated ion channels of chick skeletal muscle. Ann NY Acad Sci 707:417–420.

Sonobe T, Inagaki T, Poole DC, Kano Y. 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:R1329–R1337.

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

Takano Y, Matsuo S, Wakisaka S, Ichikawa H, Nishikawa S, Akai M. 1988. A histochemical demonstration of calcium in the maturation stage enamel organ of rat incisors. Arch Histol Cytol 51:241–248.

Yeung EW, Head SI, Allen DG. 2003. Gadolinium reduces short-term stretch-induced muscle damage in isolated mdx mouse muscle fibres. J Physiol 552:449–468.

Yu JG, Carlsson L, Thornell LE. 2004. Effects of stretch-activated channel blockers on [Ca2+]i and muscle damage in the mdx mouse. J Physiol 562:367–380.

Yu TG, Carlsson L, Thornell LE. 2004. Evidence for myofibril remodeling as opposed to myofibrillar damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study. Histocherm Cell Biol 121:219–227.

Zhang BT, Yeung SS, Allen DG, Qin L, Yeung EW. 2008. Role of the calcium-calpain pathway in cytoskeletal damage after eccentric contractions. J Appl Physiol (1985) 105:352–357.