Published in final edited form as:

Muscle Nerve. 2017 December ; 56(6): E85–E94. doi:10.1002/mus.25559.

Non-invasive assessment of muscle injury in healthy and dystrophic animals with electrical impedance myography

Benjamin Sanchez, PhD^{1,*}, Shama R. Iyer, PhD², Jia Li, PhD¹, Kush Kapur, PhD³, Su Xu, PhD⁴, Seward Rutkove, MD¹, and Richard M. Lovering, PhD, PT^{2,*}

¹Department of Neurology, Harvard Medical School, Boston, MA 02115, USA

³Boston Children's Hospital, Boston, MA 02115, USA

²Department of Orthopaedics, University of Maryland School of Medicine, Baltimore, MD 21201, USA

⁴Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Abstract

Introduction—Dystrophic muscle is particularly susceptible to eccentric contraction-induced injury. We tested the hypothesis that electrical impedance myography (EIM) can detect injury induced by maximal-force lengthening contractions.

Methods—We induced injury in the quadriceps of wild type (WT) and dystrophic (*mdx*) mice with eccentric contractions using an established model.

Results—*mdx* quadriceps had significantly larger losses in peak twitch and tetany compared to losses in WT quadriceps. Injured muscle showed a significant increase in EIM characteristic frequency in both WT ($177 \pm 7.7\%$) and *mdx* ($167 \pm 7.8\%$) quadriceps. EIM also revealed decreased extracellular resistance for both WT and *mdx* quadriceps following injury.

Discussion—Our results show overall agreement between muscle function and EIM measurements of injured muscle, indicating that EIM is a viable tool to assess injury in dystrophic muscle.

Keywords

in vivo injury; electrical impedance myography (EIM); Duchenne muscular dystrophy (DMD); edema

*Correspondence to: Benjamin Sanchez, Department of Neurology, Harvard Medical School, Boston, MA 02115 USA, Phone: 617-667-3057, bsanchez@bidmc.harvard.edu. Richard M. Lovering, University of Maryland, School of Medicine, Department of Orthopaedics, AHB, Rm 540, 100 Penn St., Baltimore, MD 21201 USA, phone: 410-409-1077, rlovering@som.umaryland.edu.

Disclosure/conflict of interest

Dr. Sanchez is named as an inventor on a patent application in the field of electrical impedance. Dr. Sanchez receives consulting income from Maxim Integrated, Inc. Dr. Rutkove has equity in, and serves a consultant and scientific advisor to, Skulpt, Inc. a company that designs impedance devices for clinical and research use; he is also a member of the company's Board of Directors. The company also has an option to license patented impedance technology of which Dr. Rutkove is named as an inventor. This study, however, did not employ any relevant company or patented technology.

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked myopathy characterized clinically by severe, progressive, and irreversible muscle wasting, and loss of muscular function.^{1–3} This disease is due to the lack of dystrophin, a large membrane-associated protein expressed in striated muscle and localized to the inner surface of the sarcolemma.^{4, 5} Dystrophin is part of the dystrophin-associated glycoprotein complex, which connects the internal cytoskeleton of the muscle fiber to the extracellular matrix. The *mdx* mouse, the most common animal model for DMD, also lacks dystrophin and shares similar muscle pathology to that found in DMD patients.

A number of potential medical treatments have been the focus of recent clinical trials in DMD (clinicaltrials.gov NCT02255552, NCT02257489), but only a small handful of studies have examined the potential benefit of exercise in boys with this disorder.^{6–11} Unfortunately, these exercise studies are limited by inconsistency in study parameters, such as intensity, frequency, duration, and mode of exercise, which makes comparisons of outcomes difficult.^{12–14} Further complicating matters, there are no easily applied biomarkers or outcome markers in this population to assess the effects of therapy outside of the 6-minute walk test.¹⁵ Thus, a convenient and easily applied tool that is sensitive to disease status, the effects of therapy, and superimposed injury would be very useful when developing and studying the impact of exercise programs in DMD boys.

Muscle electrical impedance, also known as electrical impedance myography (EIM), has been introduced as a novel neuromuscular assessment method.¹⁶ Unlike conventional electrophysiological measures of muscle and nerve,¹⁷ EIM does not assess the active electrical properties of tissues; instead, it evaluates the underlying structural and passive electrical properties of muscle.^{18–21} EIM works on the premise that these properties are altered in disease,¹⁶ and earlier work has demonstrated differences in the electrical material properties of healthy muscle compared to diseased muscles. To date, EIM has proven a valuable tool for detecting a variety of neuromuscular diseases both in humans^{22–29} and in animal models.^{30–33} EIM has also been shown to be sensitive to the effects of therapy in spinal muscular atrophy and recovery from disuse.³⁴ Whether contraction-induced injury confers additional alterations on electrical impedance characteristics of muscle is not known. If EIM can detect such superimposed injury in DMD muscle, it would suggest that impedance-based methods could serve as convenient, easily-applied biomarkers to assess both potential improvement and injury during exercise therapies.

In this study, we tested the hypothesis that EIM can detect the interstitial edema and inflammatory response to skeletal muscle injury induced by maximal-force lengthening contractions and subsequent recovery in wild type (WT) and dystrophic (*mdx*) mice.

Materials and Methods

Animals

All protocols were approved by the University of Maryland Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guidelines for the Care

and Use of Laboratory Animals. We measured muscle function in 10 wild type (WT, male, 2 month old, body weight 27.4 ± 2.7 g, C57BL/10ScSnJ, Jackson Laboratory, Bar Harbor, ME) and in 10 *mdx* mice (male, 2 month old, body weight 27.8 ± 2.1 g, C57BL/10ScSn-*DMD*^{mdx/J}, Jackson Laboratory, Bar Harbor, ME). Experimentation was performed with the animal anesthetized under 2% inhaled isoflurane anesthesia, which has no known effect on muscle electrical current or contractile activity³⁵ or the propagation of an externally applied electrical current. Body and muscle temperature were maintained by a heating lamp. The opposite quadriceps served as a control.

In vivo injury protocol

Quadriceps *in vivo* injury induced by maximal lengthening contractions was performed as described using an established model.^{36, 37} With the animal placed in a supine position, the thigh and pelvis were stabilized, and the ankle was secured onto a lever arm (Fig. 1A). The axis of the knee was aligned with the axis of the stepper motor (model T8904, NMB Technologies, Chatsworth, CA), and a torque sensor was (QWFK-8M, Sensotec, Columbus, OH) used to measure torque in Newton-millimeters (Nmm). The femoral nerve was stimulated via subcutaneous needle electrodes (J05 Needle Electrode Needles, 36BTP, Jari Electrode Supply, Gilroy, CA). Proper electrode position was determined by a series of isometric twitches and by observing isolated knee extension in the anesthetized animal. A custom program based on commercial software (LabView version 8.5, National Instruments, Austin, TX) was used to synchronize contractile activation and the onset of forced knee flexion. Injury resulted from 15 forced lengthening contractions superimposed onto maximal quadriceps contractions through a 40° – 100° arc of motion spaced approximately 1 minute apart. This range of motion is similar to that used in human studies.³⁸ Maximal isometric torque was measured before lengthening contractions and 5 minutes after the last lengthening contraction, and were used to calculate force deficits. *In vivo* functional measures were assessed in the same animals over time before injury (*n*=10), and 0 h (WT *n*=10, *mdx* *n*=9), 24 h (WT *n*=3, *mdx* *n*=2) and 48 h (WT *n*=4, *mdx* *n*=4) after injury.

Measurements of impedance

We measured left and right longitudinal quadriceps impedance between 10 kHz and 1 MHz (EIM1103, Skulpt Inc., San Francisco, California, USA) using a surface electrode array described elsewhere (Fig. 1B).³⁰ Data were collected after the fur was clipped and all remaining fur was removed using a depilatory agent. The skin was cleaned with 0.9% saline solution to ensure good electrode contact. Quadriceps impedance was measured in the same animals over time before injury (WT *n*=10, *mdx* *n*=10) and 0 h (WT *n*=10, *mdx* *n*=10), 24 h (WT *n*=10, *mdx* *n*=10), and 48 h (WT *n*=6, *mdx* *n*=6) after injury.

Torque measurements

All torque values reported were normalized to the resting torque immediately prior to stimulation, i.e. peak torque attained during stimulation minus resting torque immediately prior to stimulation. A twitch was elicited by a single supramaximal pulse (200 μ s duration) adjusted after several twitches, separated by 1 minute. Tetanic contractions were elicited by 1 ms duration pulses in 300 ms train duration with frequency between 85–100 Hz, which was optimized for each animal. The greatest peak torque attained in the twitch and tetanic

experiment was taken as peak twitch torque and peak tetanic torque. The contraction time was calculated as the time from onset of torque to peak force. Half relaxation time was calculated as the elapsed time until the peak twitch torque decreased to its half value. Peak rates of torque contraction (+dT) and decline (-dT) were determined from the maximum and minimum values of the first derivative of the twitch torque, respectively. Times to +dT and -dT were calculated as the time from onset of contraction to the time of peak rate contraction and decline.

Impedance data analysis

Raw phase angle values were analyzed at 50 kHz for the injured muscle group. The averaged multi-frequency impedance of the quadriceps muscle was modeled using the impedance model proposed by Kenneth S Cole.³⁹ The model was adjusted using a weighted nonlinear least square algorithm, and their standard errors were calculated⁴⁰ using Matlab (Mathworks, Natick, MA). As the measurement frequency is changed, the Cole plot describes a circular arc of impedance.

The Cole model summarizes multi-frequency impedance dependence into a reduced set of parameters which have a direct interpretation in terms of the geometrical and (passive) electrical properties of skeletal muscle fibers⁴¹. The muscle fibers were assumed to be essentially a cylinder of radius a composed by the surface membrane represented as a parallel combination of the membrane resistance r_m and capacitance c_m and the bulk resistive properties of the intracellular r_i and extracellular media r_e . The apex of the impedance arc (i.e. peak reactance) corresponds to the characteristic frequency at which the current is evenly split between the intracellular and extracellular compartments. The greatest resistance value in the abscissa of the Cole plot is the resistance measured by the low frequencies and is by definition, r_e . The lower resistance value in the abscissa of the Cole plot is the resistance measured at high frequencies calculated as the parallel combination between r_e and r_i . The change of c_m can be calculated from the characteristic frequency assuming constant both a and r_i after injury. The membrane resistance is $r_m \gg c_m$ and therefore negligible. Unless otherwise noted, values are reported as specific.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) studies were performed on a Bruker Biospec 7.0 Tesla 30-cm horizontal bore scanner using Paravision 5.1 software (Bruker Biospin MRI GmbH, Germany). A Bruker 4-element ^1H surface coil array was used as the receiver, and a Bruker 72 mm linear-volume coil was used as the transmitter. One mouse of each genotype (WT $n=1$, mdx $n=1$) was anesthetized in an animal induction chamber with a gas mixture of O_2 (1 L/min) and isoflurane (3%). The animal was then placed supine on a custom-made body holder bed, and the radio frequency coil was positioned and fixed with surgical tape in the region of interest on the animal leg. After the animal was moved into the center of the magnet, the isoflurane level was maintained at 1.0 to 1.5% for the remainder of the experiment. An MR-compatible small-animal monitoring and gating system (SA Instruments, Inc., New York, NY) was used to monitor animal respiration rate and body temperature. Mouse body temperature was maintained at 36 – 37° C using a warm water circulator. Three-slice (axial, mid-sagittal, and coronal) scout rapid acquisition with fast low

angle shot MR imaging was used to localize the leg. High resolution T₂-weighted MRI images in the cross-sectional view between the hip joint and the knee were acquired using rapid acquisition with relaxation enhancement (RARE) sequence with TR/TE (repetition time/echo time) = 5000/32 ms, RARE factor = 8, field of view = 30 × 30 mm², matrix size = 250 × 250, slice thickness = 0.5 mm without a gap, averages = 16, number of slices = 32.

Statistics

The Mann-Whitney test with Bonferroni correction was used to identify differences in twitch and tetanic torque parameters between genotypes before injury and 0 h, 24 h, and 48 h after injury. Dependent impedance variables were defined as the paired difference of injured and non-injured phenotypes over time points and were analyzed using linear mixed-effects regression models with random intercept term to account for correlations within measurements over multiple time-points. The time in the model was coded using 3 indicator variables for 0 h, 24 h, and 48 h post-injury with pre-injury as the reference. Similarly, the response trajectories of only injured phenotypes were analyzed using a linear mixed-effects model. A Bonferroni-Sidak approach was used to adjust for the entire set of multiple comparisons based on the models for the trajectories of paired difference (injured vs non-injured) and only injured phenotypes within each impedance variable. All statistical tests were 2-tailed, and the significance was set at $P < 0.05$. Statistical calculations were conducted using Prism (GraphPad Software, La Jolla, CA, USA) and SAS version 9.4 (Cary, NC). All data are presented as mean ± standard error of the mean (S.E.M.) unless otherwise noted.

Results

mdx quadriceps muscles are more susceptible to injury than healthy muscle

Since proximal muscles are affected earlier and to a greater extent in DMD^{42, 43}, and a similar pattern has been documented in *mdx* mice,⁴⁴ we performed muscle contractility and EIM on the anterior thigh muscles. *mdx* mice are known to generate at least as much absolute force as WT mice (Table 1, peak tetany), but less “specific force” (amount of force per unit of muscle physiological cross-sectional area) as we and others have shown previously.^{45, 46} Muscles in *mdx* mice show severe susceptibility to injury,^{45, 47} as demonstrated in various protocols, including a high number of repetitions through a low strain (e.g., 150 reps through a 40° arc of motion), or fewer repetitions using a larger strain.^{48, 49} We used the latter (15 repetitions through 60° arc of motion) for the *in vivo* quadriceps eccentric injury protocol. As expected, *mdx* mice sustained a significantly greater drop in isometric strength ($68.4 \pm 4.5\%$ loss in maximal tetanic torque) after injury compared to WT ($22.1 \pm 5.0\%$ loss in maximal tetanic torque), $P < 0.0001$. We also include analysis of twitch torque (Table 1), which paralleled the findings of tetanic torque, with a significantly larger drop in *mdx* mice ($66.6 \pm 5.5\%$) than in WT ($37.3 \pm 6.5\%$), $P < 0.01$ (Fig. 2C and D). Although maximal isometric torque is the standard metric for measuring contractile strength, if changes in maximal twitch are valid, this provides another possible means to assess dystrophic muscles, without subjecting them to the large forces developed during maximal isometric testing.

Single and multi-frequency muscle impedance values change after injury

Single-frequency changes at 50 kHz in phase angle normalized to pre-injury values show a decrease in WT and *mdx* mice (Fig. 3A and B) of $12.0 \pm 6.1\%$ and $14.4 \pm 4.7\%$ 0 h after injury ($P < 0.0001$); and $35.2 \pm 9.3\%$ and $46.0 \pm 3.0\%$ at 24 h after injury ($P < 0.0001$), respectively. Both WT and *mdx* mice had an increase in phase angle values after 48 h of recovery, and WT mice recovered faster to pre-injury values ($18.3 \pm 5.1\%$, $P < 0.001$) than *mdx* mice ($30.0 \pm 5.7\%$, $P < 0.001$). These changes are indicative of gross structural and morphological alterations occurring in muscle after similar large-strain injury protocols we have used previously.^{46, 50–53} We analyzed multiple frequencies and model the data using an equivalent circuit to disentangle the source(s) that cause such changes in phase angle, (Fig. 3C and D). During the first 24 h, WT and *mdx* arcs of impedance shrink and shift to lower resistance (*R*) and reactance (*X*) values. Forty-eight hours post-injury WT and *mdx* impedance recover and shift back toward *R* and *X* pre-injury values.

Characteristic frequency increases after muscle injury

Control data from the non-injured right quadriceps muscle are shown in Figure 4, solid lines. The characteristic frequency in WT muscle at 0 h and 24 h after injury increases by $137.0 \pm 8.3\%$ and $182.2 \pm 15.0\%$, $P < 0.05$ and $P < 0.0001$, respectively (Fig. 4B). In *mdx* mice, however, there is a significant ($P < 0.0001$) change only at 24 h, showing a $170.0 \pm 9.1\%$ increase (Fig. 4C). In WT and *mdx* mice, at 48 h post-injury, the increase in quadriceps characteristic frequency ($137.1 \pm 9.2\%$ and $138.4 \pm 12.3\%$) is not significantly different to the non-injured values, indicating recovery of muscle physiology to pre-injury values. The factors that determine the characteristic frequency will be discussed in detail.

Decrease in extracellular resistance after injury indicates interstitial edema

Specific extracellular (Fig. 4D and E) and intracellular (Fig. 4F and G) resistances from the quadriceps muscles of WT and *mdx* mice were measured pre-injury and 0 h, 24 h, and 48 h post-injury. Muscle injury results in interstitial edema,^{54–57} which is confirmed by the loss in extracellular resistance, the latter being measured by the low-frequency current flowing through the extracellular compartment (Fig. 4A, blue sine-wave). At 0 h, 24 h, and 48 h, the loss in extracellular resistance in WT mice is $28.0 \pm 4.3\%$ ($P < 0.001$), $45.7 \pm 5.6\%$ ($P < 0.0001$), and $27.0 \pm 8.3\%$ ($P < 0.01$), respectively. In *mdx* mice, however, the loss of extracellular resistance is only significant after 24 h ($43.8 \pm 3.7\%$, $P < 0.001$). Lack of substantial post-injury changes in intracellular resistance as measured by high-frequency current (Fig. 4A, red sine-wave) indicates minimal cell swelling in WT or *mdx* mice after injury (Fig. 4F and G).

Changes in membrane capacitance suggest sarcolemmal damage after injury

There was significant interaction between changes in injured and non-injured myofiber membrane permeability as measured by the membrane capacitance (Fig. 4H and I). Normalized to pre-injury values, the membrane capacitance at 0 h, 24 h, and 48 h in WT muscle is $75.5 \pm 4.7\%$ ($P < 0.01$), $59.6 \pm 6.5\%$ ($P < 0.0001$), and $74.5 \pm 4.7\%$ ($P < 0.05$), respectively. In *mdx* mice, however, the membrane capacitance is only significant after 24 h ($61.9 \pm 5.3\%$, $P < 0.001$) and 48 h ($76.0 \pm 8.6\%$, $P < 0.05$).

Non-invasive imaging confirms edema after injury

In vivo magnetic resonance imaging (MRI) was performed at the level of the thighs. The representative images from a WT and an *mdx* mouse show transverse (axial) sections of T₂-weighted MRI (Fig. 5). There is clearly increased signal intensity (edema) in the quadriceps on the injured side (red arrows) compared to the uninjured control side. This increase in edema over time [within 1 hour after injury (0 h) to 24 h] was present after injury in both WT and *mdx* mice, paralleling the findings of a decrease in extracellular resistance after injury.

Discussion

Skeletal muscle damage is often accompanied by a variety of clinical symptoms and signs, including localized pain, fatigue, loss of strength, and muscle tenderness. Similarly, alterations induced by damage can be readily identified with imaging and biopsy. The purpose of this work was to determine whether *in vivo* surface-based EIM measurements could also reveal changes in murine skeletal muscle after contraction-induced injury. We compared values pre- and post-injury in the quadriceps muscles from both WT and *mdx* mice. Our findings indicate that the expected finding of loss of torque after injury was accompanied by major alterations in EIM values after injury. Although the changes in EIM did not exactly parallel the changes in torque (see 0 h), these results show that it is possible to obtain meaningful information on the condition of muscle after *in vivo* injury using the easily applied surface EIM technique.

Standard functional experiments showed a loss in maximal isometric torque after injury, as expected from other studies.^{37, 45, 58, 59} Comparison of contractile twitch in WT and *mdx* mice has previously been studied,⁶⁰ but here we show a change in twitch after eccentric injury including multiple time points. Maximal tetanic force or torque is the most common measure to assess muscle strength. Here both WT and *mdx* mice experience a loss in maximal tetanic torque after injury (~22% and 68%, respectively) as well as a reduction in twitch torque (~37% and 65%, respectively). In terms of magnitude, it is unclear why there was a corresponding reduction in twitch only for *mdx* mice. This could reflect change in excitation-contraction coupling (EC coupling),^{61, 62} or be related to changes in muscle stiffness.⁶³ In any case, since maximal isometric testing generates large forces, such testing is imprudent in patients with muscular dystrophy. If the twitch response provides the same information as maximal isometric force with regard to muscle injury, it could offer a means to assess dystrophic muscles without subjecting them to the greater forces that occur with maximal isometric testing.

Single EIM phase frequency measured at 50 kHz can detect gross alterations in muscle; however, the changes in this single value are non-specific, as they do not reflect specific muscle characteristics *per se*. Modeling multi-frequency EIM data allows us to go a major step further and infer the specific muscle properties that were affected after injury.^{41, 64} For skeletal muscle tissue, the characteristic frequency can increase due to a reduction in the intracellular resistance, membrane capacitance, average myofiber radii, or cell volume concentration.⁶⁵ The lack of substantial changes in intracellular resistance rule out cellular swelling, thus indicating that the single-frequency changes in phase were the result of a

combined effect between interstitial edema and disruption of cell structure integrity, as detected by a reduction in extracellular resistance and membrane capacitance values. These changes in muscle extracellular resistance due to edema are similar to those found in patients with fluid removal during peritoneal dialysis.⁶⁶ In fact, cell swelling would have led to a decrease in the characteristic frequency, with an increase in myofiber radii and intracellular resistance.⁴¹ In addition, the accumulation of small inflammatory cells due to injury could have also contributed to the decrease of overall cell volume fraction measured with EIM.

The pathogenic mechanisms of dystrophin loss can explain the lack of significant differences in *mdx* model parameters immediately after injury (0 h) as compared to baseline. In *mdx* muscle, the chronic inflammatory response and persistent repair processes lead to continuous muscle regeneration.^{67–69} However, we detected changes in *mdx* model parameters after 24 h, once the eccentric injury-induced process dominated over the underlying response of disease. This would imply that if the tool were used to evaluate DMD patients who sustained stretch injury to muscle, they would need to be followed over a period and not simply immediately after injury.

Thus far, muscle strains are revealed best by T₂-weighted MRI images, which optimize contrast between injured muscles with edema (increased signal intensity) and normal uninjured muscles. The signal intensity in MRI increases substantially, and gradually reaches the peak after injury as early as 7 hours⁷⁰ or as late as 24 h⁷¹. In this study, we used *in vivo* MRI to evaluate changes in edema. The imaging signal intensity (white in image) continued to increase after injury (from 0 h to 24 h). This further increase has been noted in other studies and has been termed a “secondary injury”; it is due to the onset of inflammation.^{72–74} The onset and duration of inflammation can vary from muscle to muscle, especially depending on the injury, and the exact timing of a secondary injury can be missed if imaging or histology is not performed hourly. The inflammation observed with MRI is seen in both WT and *mdx* quadriceps after injury and supports the decrease in extracellular resistance found with EIM at 24 h.

There is an important broad translational aspect to this murine-based work. Specifically, these data suggest that EIM technology can be utilized to evaluate noninvasively the development of injury after exercise protocols in boys with DMD as well as to monitor longer term muscle condition. This is critical, since exercise and physical activity continue to be considered potentially important interventions but with undetermined value.^{3, 13} The ability to obtain valuable insights noninvasively into how muscle condition is impacted by these therapies may be important on both the individual and collective basis. It can help clarify the beneficial effects of exercise over the long term while also identifying potential short-term injurious effects. Importantly, these interventions may play roles in conjunction with potential therapies. For example, it may become valuable to non-invasively assess the impact of exercise interventions in boys treated with antisense oligonucleotides in order to ensure they lead to improvement in muscle condition rather than inadvertent worsening. Given the fact there is delayed EIM response after injury in dystrophic muscle, patients should return to the clinic for follow-up visits. In the future, however, it may become possible for them to perform daily measurements at home using a personal EIM device, and visits could be scheduled accordingly.

Previous work has shown that EIM can detect differences in WT versus *mdx* muscle. Our study shows that skeletal muscle injury results in significant alterations in modeled EIM parameters in WT and *mdx* mice. Despite the focus on dystrophic muscle in these experiments, the results suggest that EIM can be used as a biomarker to noninvasively detect alterations in skeletal muscle associated with injury in otherwise healthy muscle, further widening its future clinical applications.

Acknowledgments

Funding

This work was supported by grants from the National Institutes of Health, including training grant T32 AR-007592 (SRI), and research grants R01-AR059179 and R21-AR067872-01 (RML); R01NS055099 (SR)

Reference List

1. McDonald CM, Abresch RT, Carter GT, Fowler WM Jr, Johnson ER, Kilmer DD, et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. Am J Phys Med Rehabil. 1995; 74:S70–S92. [PubMed: 7576424]
2. Brooke MH, Fenichel GM, Griggs RC, Mendell JR, Moxley R, Florence J, et al. Duchenne muscular dystrophy: patterns of clinical progression and effects of supportive therapy. Neurology. 1989; 39:475–481. [PubMed: 2927672]
3. Lovering RM, Porter NC, Bloch RJ. The muscular dystrophies: from genes to therapies. Phys Ther. 2005; 85:1372–1388. [PubMed: 16305275]
4. Tinsley JM, Blake DJ, Zuellig RA, Davies KE. Increasing complexity of the dystrophin-associated protein complex. Proc Natl Acad Sci U S A. 1994; 91:8307–8313. [PubMed: 8078878]
5. Blake DJ, Tinsley JM, Davies KE. Utrophin: a structural and functional comparison to dystrophin. Brain Pathol. 1996; 6:37–47. [PubMed: 8866746]
6. Eagle M. Report on the muscular dystrophy campaign workshop: exercise in neuromuscular diseases Newcastle, January 2002. Neuromuscul Disord. 2002; 12:975–983. [PubMed: 12467755]
7. Jansen M, de GI, van AN, Geurts AC. Physical training in boys with Duchenne Muscular Dystrophy: the protocol of the No Use is Disuse study. BMC Pediatr. 2010; 10:55. [PubMed: 20691042]
8. Dunn JF, Tracey I, Radda GK. Exercise metabolism in Duchenne muscular dystrophy: a biochemical and [31P]-nuclear magnetic resonance study of mdx mice. Proc Biol Sci. 1993; 251:201–206. [PubMed: 8097327]
9. Scott OM, Hyde SA, Goddard C, Jones R, Dubowitz V. Effect of exercise in Duchenne muscular dystrophy. Physiotherapy. 1981; 67:174–176. [PubMed: 7029578]
10. de Lateur BJ, Giacon RM. Effect on maximal strength of submaximal exercise in Duchenne muscular dystrophy. Am J Phys Med. 1979; 58:26–36. [PubMed: 434131]
11. Sockolov R, Irwin B, Dressendorfer RH, Bernauer EM. Exercise performance in 6-to-11-year-old boys with Duchenne muscular dystrophy. Arch Phys Med Rehabil. 1977; 58:195–201. [PubMed: 851390]
12. Lovering RM, Brooks SV. Eccentric exercise in aging and diseased skeletal muscle: good or bad? J Appl Physiol (1985). 2014; 116:1439–1445. [PubMed: 23471953]
13. Markert CD, Case LE, Carter GT, Furlong PA, Grange RW. Exercise and Duchenne muscular dystrophy: where we have been and where we need to go. Muscle Nerve. 2012; 45:746–751. [PubMed: 22499105]
14. Grange RW, Call JA. Recommendations to define exercise prescription for Duchenne muscular dystrophy. Exerc Sport Sci Rev. 2007; 35:12–17. [PubMed: 1721188]
15. McDonald CM, Henricson EK, Han JJ, Abresch RT, Nicorici A, Elfring GL, et al. The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. Muscle Nerve. 2010; 41:500–510. [PubMed: 19941337]

16. Rutkove SB. Electrical impedance myography: Background, current state, and future directions. *Muscle Nerve*. 2009; 40:936–946. [PubMed: 19768754]
17. Daube JR, Rubin DI. Needle electromyography. *Muscle Nerve*. 2009; 39:244–270. [PubMed: 19145648]
18. Epstein BR, Foster KR. Anisotropy in the dielectric properties of skeletal muscle. *Med Biol Eng Comput*. 1983; 21:51–55. [PubMed: 6865513]
19. Cole, KS. *Membranes, Ions, and Impulses: A Chapter of Classical Biophysics*. University of California Press; 1972.
20. Eisenberg, RS. *Impedance Measurement of the Electrical Structure of Skeletal Muscle*. Wiley Publishers; 2011.
21. Sanchez B, Li J, Bragos R, Rutkove SB. Differentiation of the intracellular structure of slow-versus fast-twitch muscle fibers through evaluation of the dielectric properties of tissue. *Phys Med Biol*. 2014; 59:2369–2380. [PubMed: 24743385]
22. Zaidman CM, Wang LL, Connolly AM, Florence J, Wong BL, Parsons JA, et al. Electrical impedance myography in Duchenne muscular dystrophy and healthy controls: A multicenter study of reliability and validity. *Muscle Nerve*. 2015; 52:592–597. [PubMed: 25702806]
23. Shklyar I, Pasternak A, Kapur K, Darras BT, Rutkove SB. Composite biomarkers for assessing Duchenne muscular dystrophy: an initial assessment. *Pediatr Neurol*. 2015; 52:202–205. [PubMed: 25447928]
24. Schwartz S, Geisbush TR, Mijailovic A, Pasternak A, Darras BT, Rutkove SB. Optimizing electrical impedance myography measurements by using a multifrequency ratio: a study in Duchenne muscular dystrophy. *Clin Neurophysiol*. 2015; 126:202–208. [PubMed: 24929900]
25. Rutkove SB, Geisbush TR, Mijailovic A, Shklyar I, Pasternak A, Visyak N, et al. Cross-sectional evaluation of electrical impedance myography and quantitative ultrasound for the assessment of Duchenne muscular dystrophy in a clinical trial setting. *Pediatr Neurol*. 2014; 51:88–92. [PubMed: 24814059]
26. Rutkove SB, Darras BT. Electrical impedance myography for the assessment of children with muscular dystrophy: a preliminary study. *J Phys Conf Ser*. 2013:434.
27. Rutkove SB, Caress JB, Cartwright MS, Burns TM, Warder J, David WS, et al. Electrical impedance myography as a biomarker to assess ALS progression. *Amyotroph Lateral Scler*. 2012; 13:439–445. [PubMed: 22670883]
28. Rutkove SB, Shefner JM, Gregas M, Butler H, Caracciolo J, Lin C, et al. Characterizing spinal muscular atrophy with electrical impedance myography. *Muscle Nerve*. 2010; 42:915–921. [PubMed: 21104866]
29. Esper GJ, Shiffman CA, Aaron R, Lee KS, Rutkove SB. Assessing neuromuscular disease with multifrequency electrical impedance myography. *Muscle Nerve*. 2006; 34:595–602. [PubMed: 16881067]
30. Li J, Yim S, Pacheck A, Sanchez B, Rutkove SB. Electrical Impedance Myography to Detect the Effects of Electrical Muscle Stimulation in Wild Type and Mdx Mice. *PLoS One*. 2016; 11:e0151415. [PubMed: 26986564]
31. Wu JS, Li J, Greenman RL, Bennett D, Geisbush T, Rutkove SB. Assessment of aged mdx mice by electrical impedance myography and magnetic resonance imaging. *Muscle Nerve*. 2015; 52:598–604. [PubMed: 25597760]
32. Li J, Geisbush TR, Rosen GD, Lachey J, Mulivor A, Rutkove SB. Electrical impedance myography for the in vivo and ex vivo assessment of muscular dystrophy (mdx) mouse muscle. *Muscle Nerve*. 2014; 49:829–835. [PubMed: 24752469]
33. Nie R, Sunmonu NA, Chin AB, Lee KS, Rutkove SB. Electrical impedance myography: transitioning from human to animal studies. *Clin Neurophysiol*. 2006; 117:1844–1849. [PubMed: 16807097]
34. Arnold W, McGovern VL, Sanchez B, Li J, Corlett KM, Kolb SJ, et al. The neuromuscular impact of symptomatic SMN restoration in a mouse model of spinal muscular atrophy. *Neurobiol Dis*. 2016; 87:116–123. [PubMed: 26733414]

35. Kunst G, Graf BM, Schreiner R, Martin E, Fink RH. Differential effects of sevoflurane, isoflurane, and halothane on Ca²⁺ release from the sarcoplasmic reticulum of skeletal muscle. *Anesthesiology*. 1999; 91:179–186. [PubMed: 10422943]
36. Pratt SJ, Lovering RM. A stepwise procedure to test contractility and susceptibility to injury for the rodent quadriceps muscle. <http://dx.doi.org/10.14440/jbm.2014.34.2014>
37. Pratt SJ, Shah SB, Ward CW, Inacio MP, Stains JP, Lovering RM. Effects of in vivo injury on the neuromuscular junction in healthy and dystrophic muscles. *J Physiol*. 2013; 591:559–570. [PubMed: 23109110]
38. Paulsen G, Crameri R, Benestad HB, Fjeld JG, Morkrid L, Hallen J, et al. Time course of leukocyte accumulation in human muscle after eccentric exercise. *Med Sci Sports Exerc*. 2010; 42:75–85. [PubMed: 20010127]
39. Cole KS. Permeability and impermeability of cell membranes for ions. *Cold Spring Harbor Symp Quant Biol*. 1940; 8:110–122.
40. Sanchez B, Li J, Yim S, Pacheck A, Widrick JJ, Rutkove SB. Evaluation of Electrical Impedance as a Biomarker of Myostatin Inhibition in Wild Type and Muscular Dystrophy Mice. *PLoS One*. 2015; 10:e0140521. [PubMed: 26485280]
41. Valdiosera R, Clausen C, Eisenberg RS. Circuit models of the passive electrical properties of frog skeletal muscle fibers. *J Gen Physiol*. 1974; 63:432–459. [PubMed: 4820090]
42. Cros D, Harnden P, Pellissier JF, Serratrice G. Muscle hypertrophy in Duchenne muscular dystrophy. A pathological and morphometric study. *J Neurol*. 1989; 236:43–47. [PubMed: 2915226]
43. Mathur S, Lott DJ, Senesac C, Germain SA, Vohra RS, Sweeney HL, et al. Age-related differences in lower-limb muscle cross-sectional area and torque production in boys with Duchenne muscular dystrophy. *Arch Phys Med Rehabil*. 2010; 91:1051–1058. [PubMed: 20599043]
44. Muntoni F, Mateddu A, Marchei F, Clerk A, Serra G. Muscular weakness in the mdx mouse. *J Neurol Sci*. 1993; 120:71–77. [PubMed: 8289081]
45. DelloRusso C, Crawford RW, Chamberlain JS, Brooks SV. Tibialis anterior muscles in mdx mice are highly susceptible to contraction-induced injury. *J Muscle Res Cell Motil*. 2001; 22:467–475. [PubMed: 11964072]
46. Xu S, Pratt SJ, Spangenburg EE, Lovering RM. Early metabolic changes measured by ¹H MRS in healthy and dystrophic muscle after injury. *J Appl Physiol*. 2012
47. Head S, Williams D, Stephenson G. Increased susceptibility of EDL muscles from mdx mice to damage induced by contraction with stretch. *J Muscle Res Cell Motil*. 1994; 15:490–492. [PubMed: 7848493]
48. Rathbone CR, Wenke JC, Warren GL, Armstrong RB. Importance of satellite cells in the strength recovery after eccentric contraction-induced muscle injury. *Am J Physiol Regul Integr Comp Physiol*. 2003; 285:R1490–R1495. [PubMed: 12920057]
49. Lovering RM, Roche JA, Bloch RJ, De Deyne PG. Recovery of function in skeletal muscle following 2 different contraction-induced injuries. *Arch Phys Med Rehabil*. 2007; 88:617–625. [PubMed: 17466731]
50. Lovering RM, De Deyne PG. Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *Am J Physiol Cell Physiol*. 2004; 286:C230–C238. [PubMed: 14522817]
51. McMillan A, Shi D, Pratt SJP, Lovering RM. Diffusion tensor MRI to assess damage in healthy and dystrophic skeletal muscle after lengthening contractions. *J Biomed Biotechnol*. 2011 article ID 970726.
52. Lovering RM, McMillan AB, Gullapalli RP. Location of myofiber damage in skeletal muscle after lengthening contractions. *Muscle Nerve*. 2009; 40:589–594. [PubMed: 19760787]
53. Dipasquale DM, Bloch RJ, Lovering RM. Determinants of the repeated-bout effect after lengthening contractions. *Am J Phys Med Rehabil*. 2011; 90:816–824. [PubMed: 21862912]
54. Evans GF, Haller RG, Wyrick PS, Parkey RW, Fleckenstein JL. Submaximal delayed-onset muscle soreness: correlations between MR imaging findings and clinical measures. *Radiology*. 1998; 208:815–820. [PubMed: 9722865]

55. Tidball JG. Inflammatory cell response to acute muscle injury. *Med Sci Sports Exerc.* 1995; 27:1022–1032. [PubMed: 7564969]
56. Stauber WT, Fritz VK, Dahlmann B. Extracellular matrix changes following blunt trauma to rat skeletal muscles. *Exp Mol Pathol.* 1990; 52:69–86. [PubMed: 2307215]
57. Jarvinen TA, Jarvinen TL, Kaariainen M, Kalimo H, Jarvinen M. Muscle injuries: biology and treatment. *Am J Sports Med.* 2005; 33:745–764. [PubMed: 15851777]
58. Grange RW, Gainer TG, Marschner KM, Talmadge RJ, Stull JT. Fast-twitch skeletal muscles of dystrophic mouse pups are resistant to injury from acute mechanical stress. *Am J Physiol Cell Physiol.* 2002; 283:C1090–C1101. [PubMed: 12225973]
59. Hayes A, Williams DA. Contractile function and low-intensity exercise effects of old dystrophic (mdx) mice. *Am J Physiol.* 1998; 274:C1138–C1144. [PubMed: 9575811]
60. Quinlan JG, Johnson SR, McKee MK, Lyden SP. Twitch and tetanus in mdx mouse muscle. *Muscle Nerve.* 1992; 15:837–842. [PubMed: 1501627]
61. Hollingworth S, Zeiger U, Baylor SM. Comparison of the myoplasmic calcium transient elicited by an action potential in intact fibres of mdx and normal mice. *J Physiol.* 2008; 586:5063–5075. [PubMed: 18772198]
62. Hollingworth S, Marshall MW, Robson E. Excitation contraction coupling in normal and mdx mice. *Muscle Nerve.* 1990; 13:16–20. [PubMed: 2183043]
63. Wood LK, Kayupov E, Gumucio JP, Mendias CL, Claflin DR, Brooks SV. Intrinsic stiffness of extracellular matrix increases with age in skeletal muscles of mice. *J Appl Physiol (1985).* 2014; 117:363–369. [PubMed: 24994884]
64. Eisenberg RS. The equivalent circuit of single crab muscle fibers as determined by impedance measurements with intracellular electrodes. *J Gen Physiol.* 1967; 50:1785–1806. [PubMed: 6034768]
65. Foster KR, Schwan HP. Dielectric properties of tissues and biological materials: a critical review. *Crit Rev Biomed Eng.* 1989; 17:25–104. [PubMed: 2651001]
66. Davenport A. Does peritoneal dialysate affect body composition assessments using multi-frequency bioimpedance in peritoneal dialysis patients? *Eur J Clin Nutr.* 2013; 67:223–225. [PubMed: 23249878]
67. Porter JD, Khanna S, Kaminski HJ, Rao JS, Merriam AP, Richmonds CR, et al. A chronic inflammatory response dominates the skeletal muscle molecular signature in dystrophin-deficient mdx mice. *Hum Mol Genet.* 2002; 11:263–272. [PubMed: 11823445]
68. Chamberlain JS, Metzger J, Reyes M, Townsend D, Faulkner JA. Dystrophin-deficient mdx mice display a reduced life span and are susceptible to spontaneous rhabdomyosarcoma. *FASEB J.* 2007; 21:2195–2204. [PubMed: 17360850]
69. Pratt SJ, Xu S, Mullins RJ, Lovering RM. Temporal changes in magnetic resonance imaging in the mdx mouse. *BMC Res Notes.* 2013; 6:262. [PubMed: 23837666]
70. Mattila KT, Lukka R, Hurme T, Komu M, Alanen A, Kalimo H. Magnetic resonance imaging and magnetization transfer in experimental myonecrosis in the rat. *Magn Reson Med.* 1995; 33:185–192. [PubMed: 7707908]
71. Wishnia A, Alameddine H, Tardif de GS, Leroy-Willig A. Use of magnetic resonance imaging for noninvasive characterization and follow-up of an experimental injury to normal mouse muscles. *Neuromuscul Disord.* 2001; 11:50–55. [PubMed: 11166166]
72. Zerba E, Komorowski TE, Faulkner JA. Free radical injury to skeletal muscles of young, adult, and old mice. *Am J Physiol.* 1990; 258:C429–C435. [PubMed: 2316631]
73. Tidball JG. Interactions between muscle and the immune system during modified musculoskeletal loading. *Clin Orthop.* 2002:S100–S109. [PubMed: 12394458]
74. MacIntyre DL, Reid WD, Lyster DM, Szasz IJ, McKenzie DC. Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise. *J Appl Physiol.* 1996; 80:1006–1013. [PubMed: 8964718]

Figure 1.

Apparatuses used to measure contractility and electrical impedance myography (EIM). (A) To produce the injury, the femur was stabilized, and the ankle was attached to a motor-driven lever arm. The femoral nerve was stimulated supramaximally (causing knee extension, black arrow), while the lever arm forced the knee joint into flexion (curved arrow). To complete 1 repetition, the quadriceps was stimulated for 200 ms to induce a peak isometric contraction prior to lengthening by the lever arm. Reproduced with permission (Pratt et. al, *J Physiol*, 2013). (B) Photograph of setup for EIM measurements. Under anesthesia, the mouse was placed in a supine position, and the hindlimb was placed in a fixed position. The EIM electrode was placed onto the mid-belly of the quadriceps muscle. The cartoon inset shows the electrode array arrangement and connection to the EIM measuring device.

Figure 2.

Representative pre-injury and immediately after injury (0 h post-injury) tetanic (A) and twitch (C) torque waveforms of WT and *mdx* mice. Loss of peak tetanic (B) and twitch (D) torque immediately after injury were normalized in each WT and *mdx* animal to pre-injury (0% loss in torque). After 15 lengthening contractions, *mdx* mice had a larger loss in peak twitch and tetanic torque, $66.6 \pm 5.5\%$ and $68.4 \pm 4.5\%$, respectively, compared to WT mice, $37.3 \pm 6.5\%$ and $22.1 \pm 5.0\%$, respectively. Additional twitch parameters and time points post-injury are listed in Table 1. Data shown as mean $\pm$ S.E.M. Mann-Whitney test, 2-tailed. ** $P < 0.01$, **** $P < 0.0001$.

Figure 3.

Specific single-frequency muscle phase angle changes of WT (A) and *mdx* (D) mice measured at 50 kHz before injury, and 0 h, 24 h, and 48 h post-injury. Averaged multi-frequency impedance loci from the quadriceps muscles of WT (C) and *mdx* mice (D). During the first 24 hours, WT and *mdx* impedance loci shrink and shift to lower resistance (*R*) and reactance (*X*) values. Forty-eight hours post injury WT and *mdx* impedance loci recover and shift towards *R* and *X* values immediately after injury. Representative frequencies are shown only in the impedance locus pre-injury for illustration purposes. Data shown as mean $\pm$ S.E.M. One-way ANOVA test with repeated measures, 2-tailed. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Figure 4.

Electrical impedance before and after injury. Illustration shows the relationship between the alternating electrical current (sine-waves) and electrical circuit parameters of a cylinder-shaped muscle fiber (A). The circuit parameters are the intracellular resistance r_i ; extracellular resistance r_e ; membrane capacitance c_m ; membrane resistance r_m ; and myofiber radius a . Changes in quadriceps characteristic frequency (B, C) within 48 h after *in vivo* injury (dashed lines) indicate muscle recovery. At 48 h post-injury, the increase in characteristic frequency drops to $126.5 \pm 7.0\%$ (non-significant) and $133.8 \pm 7.7\%$ ($P < 0.05$) in WT and *mdx* mice, respectively. Specific extracellular (D, E) and intracellular (F, G) resistances and membrane capacitance (H, I) from the quadriceps muscles of WT and *mdx* mice were also measured. Lack of substantial post-injury changes in intracellular resistance and membrane capacitance, measured by high-frequency current (Fig. 4A, red sine-wave), suggest that there was no cell swelling or cell integrity disruption in WT or *mdx* after injury. Non-injured data from the right quadriceps are shown as control (solid lines). Data shown as mean $\pm$ S.E.M. Two-way ANOVA test, 2-tailed. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Figure 5. Edema after injury

Representative images show transverse sections of T₂-weighted MRI (magnetic resonance imaging) in a WT and an *mdx* mouse before and after contraction-induced injury to the thigh (F = Femur). The injured quadriceps muscle (red arrows) is easily distinguished from the non-injured quadriceps based on the increased T₂ signal (edema, white signal). Development of edema over time (0 h to 24 h) is present after injury in both WT and *mdx* mice. Note that in *mdx* muscle that, even with no injury, there are regions of hyperintensity (dotted circle) in various muscles, a characteristic finding in dystrophic muscle. The overall shape of the thigh is altered due to compression by the apparatus used to stabilize the legs.

Table 1Twitch and tetanic torque parameters from healthy WT and *mdx* mice

	PRE-INJURY				0 h				24 h				48 h			
	MEAN	STD	n		MEAN	STD	n		MEAN	STD	n		MEAN	STD	n	
Peak twitch (mN mm)	WT	2.17	0.78	10	1.36	0.73	10		2.64	0.45	3		2.40	0.43	4	
	<i>mdx</i>	2.19	0.92	10	0.76	0.47	9		2.19	0.56	2		2.61	0.82	4	
	<i>p</i>	0.950			0.065				0.600				0.829			
Contraction time (ms)	WT	19.67	1.88	10	18.08	1.81	10		16.22	3.53	3		13.44	1.64	4	
	<i>mdx</i>	19.60	2.11	10	18.24	3.61	9		22.92	2.00	2		23.88	2.25	4	
	<i>p</i>	0.941			0.899				0.099				*			
Half relaxation time (ms)	WT	14.31	2.43	10	12.54	2.72	10		14.28	1.31	3		11.66	1.16	4	
	<i>mdx</i>	14.07	3.36	10	13.75	4.74	9		21.06	3.39	2		21.44	2.59	4	
	<i>p</i>	0.858			0.500				0.045				*			
Time to peak dT (ms)	WT	8.67	1.58	10	8.50	2.37	10		6.39	0.98	3		3.69	1.68	4	
	<i>mdx</i>	8.85	1.23	10	9.41	4.58	9		4.00	1.41	2		9.71	0.82	4	
	<i>p</i>	0.775			0.589				0.106				*			
Peak +dT (mN mm/ms)	WT	0.19	0.08	10	0.13	0.07	10		0.27	0.08	3		0.27	0.07	4	
	<i>mdx</i>	0.20	0.07	10	0.08	0.05	9		0.17	0.03	2		0.20	0.07	4	
	<i>p</i>	0.703			0.131				0.200				0.189			
Time to peak -dT (ms)	WT	30.37	3.26	10	27.43	3.59	10		29.56	6.55	3		22.75	2.47	4	
	<i>mdx</i>	29.65	4.37	10	28.52	4.84	9		42.83	8.25	2		39.13	6.35	4	
	<i>p</i>	0.683			0.581				0.135				0.003			
Peak -dT (mN mm/ms)	WT	-0.10	0.04	10	-0.08	0.04	10		-0.14	4.53	3		-0.15	0.04	4	
	<i>mdx</i>	-0.10	0.03	10	-0.04	0.02	9		-0.09	0.02	2		-0.09	0.02	4	

PRE-INJURY			0 h			24 h			48 h		
	MEAN	STD	n	MEAN	STD	n	MEAN	STD	n	MEAN	STD
Peak tetany (mN mm)	0.978			0.019			0.053			0.032	
	WT	6.41	1.48	10	5.01	1.49	10	8.53	0.95	3	8.22
										2.15	4
	<i>mdx</i>	7.16	1.59	10	2.17	0.83	9	4.78	1.23	2	7.71
	<i>p</i>	0.290			**		0.014			0.706	
Peak twitch/tetany	WT	0.34	0.10	10	0.26	0.07	10	0.31	0.02	3	0.31
										0.09	4
	<i>mdx</i>	0.30	0.10	10	0.32	0.14	9	0.45	0.04	2	0.35
	<i>p</i>	0.471			0.202		0.011			0.639	

Data are shown as mean and standard deviation. Mann-Whitney test, 2-tailed, with Bonferroni correction for multiple comparisons. Statistical significance was set after correction at $P < 0.001$.

* $P < 0.001$;

** $P < 0.0001$.

The non-significant P -values reported are the actual P -values before correction. Abbreviations: +dT, rate of torque development; -dT, rate of torque decay; EIM, electrical impedance myography; WT, wild type; *mdx*, dystrophic; MRI, magnetic resonance imaging