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ELECTRICAL IMPEDANCE MYOGRAPHY FOR THE *IN VIVO* AND *EX VIVO* ASSESSMENT OF MUSCULAR DYSTROPHY (*mdx*) MOUSE MUSCLE

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Abstract

Introduction—Sensitive, non-invasive techniques are needed that can provide biomarkers of disease status and the effects of therapy in muscular dystrophy.

Methods—We evaluated electrical impedance myography (EIM) to serve in this role by studying 2-month-old and 18-month-old *mdx* and wild-type (WT) animals (10 animals in each of 4 groups).

Results—Marked differences were observed in EIM values between *mdx* and WT animals; the differences were more pronounced between the older age groups (e.g., reactance of $92.6 \pm 4.3 \Omega$ for *mdx* animals vs. $130 \pm 4.1 \Omega$ for WT animals, $P < 0.001$). In addition, *in vivo* EIM parameters correlated significantly with the extent of connective tissue deposition in the *mdx* animals.

Conclusions—EIM has the potential to serve as a valuable non-invasive method for evaluating muscular dystrophy. It can be a useful biomarker to assist with therapeutic testing in both preclinical and clinical studies.

Keywords

biomarker; connective tissue; Duchenne muscular dystrophy; electrical impedance; *mdx* mouse

Duchenne muscular dystrophy (DMD) remains one of the most common and devastating neuromuscular diseases of childhood, affecting approximately 1 in 3500 male births.¹ Over the past decade, the only therapy, corticosteroids, has had a modest effect.² However, in the past several years, a variety of promising approaches to treatment have been developed, including drugs to improve muscle size and strength,³ stem cells,⁴ antisense oligonucleotides,⁵ and antifibrotic agents.⁶ A major limitation to the testing and approval process is that the outcome measures or biomarkers used to assess drug efficacy are inadequate. Functional measures, such as the 6-minute walk test, are limited due to variation and insensitivity to changes, and they even show relative improvements in values during

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certain periods of childhood despite worsening disease status.⁷ Other methods, such as magnetic resonance imaging, although sensitive to change, are costly and inconvenient. Moreover, no good tools are available for assessing upper extremity muscle health, which is especially critical in older, non-ambulatory boys.⁸ Thus, convenient, accurate biomarkers of drug efficacy are needed to help speed drug testing and ultimately improve office-based care of children who receive these therapies.

One methodology that could serve in this context is electrical impedance myography (EIM), because it provides a convenient indirect measure of muscle composition and structure.⁹ In EIM, a weak, painless, high-frequency electrical current is passed via surface electrodes through an area of muscle, and the consequent surface voltage patterns are analyzed. EIM has shown promise as a marker of disease status and progression in several neuromuscular conditions, including amyotrophic lateral sclerosis (ALS),¹⁰ spinal muscular atrophy,¹¹ disuse atrophy,¹² and myositis.¹³

Given these findings in related neuromuscular disorders, we hypothesized that EIM would also be sensitive to the muscle compositional and structural alterations characteristic of DMD. To evaluate this possibility preliminarily, we studied 2 cohorts of muscular dystrophy (*mdx*) mice, 1 young and 1 old, along with age-matched, wild-type animals to determine how the impedance parameters vary and how they relate to muscle composition and structure.

METHODS

Animals

All procedures were approved by the animal care and use committees at both Beth Israel Deaconess Medical Center and Acceleron Pharma. Male *mdx* animals (C57BL/10ScSn-Dmd^{*mdx*}/J) and wild-type (WT, C57BL/6J) male controls were obtained from Jackson Laboratories (Bangor, Maine). Animals were allowed to acclimate for 72 hours prior to testing and were fed a standard diet *ad libitum*. Two age groups (old/young each for WT and *mdx*) were studied. One group was age 2 months, and the second was age 18 months. There were 10 animals in each of the 4 groups.

In Vivo EIM Measurements

All EIM measurements were performed with the animals placed under 1% isoflurane anesthesia delivered through a nose cone with a heating pad underneath the limb to maintain consistent temperature, as described previously.¹⁴ The fur on the leg was first clipped and then completely removed with depilatory agent to help ensure good electrode contact. The leg was then taped to the measuring surface at an approximately 45° angle to the body, away from the head. A pinpoint tattoo was placed close to the center of the gastrocnemius muscle to ensure consistent electrode placement.

A fixed rigid 4-electrode array was applied over the gastrocnemius muscle, as described previously (Fig. 1A).¹⁴ EIM measurements were performed with a bioimpedance spectroscopy device (Imp SFB7; Impedimed, San Diego, California), which obtains impedance data by applying a high-frequency, low-intensity alternating electrical current

with a frequency range of 3 kHz to 1 MHz. From the measured voltages, the real and imaginary components of the impedance (resistance and reactance) are calculated.⁹ The impedance data are derived mainly from the tissue immediately under the electrode array (namely the gastrocnemius).¹⁵ In this study we analyzed only the single frequency 50 kHz data, because EIM values at this frequency have been shown to be sensitive to disease status in other disorders. Data were obtained both longitudinal and transverse to the major fiber direction.

Muscle Processing and *Ex Vivo* Electrical Impedance Measurements

Animals were euthanized with CO₂, and the gastrocnemius was immediately exposed and removed. Excised muscle was weighed and then cut to an approximately 5 mm × 5 mm × 3 mm block using a razor blade.

For *ex vivo* impedance measurements, the block of muscle tissue was placed in a 5 mm × 5 mm base impedance-measuring cell (Fig. 1B), configured with 2 broad, stainless-steel electrodes on 2 sides and 2 needle electrodes positioned on top. Impedance data were obtained using the aforementioned Imp SFB7 (Fig. 1B).¹⁶ Reactance (X) and resistance (R) data were collected in both the transverse and longitudinal directions. Measurements were repeated once on each specimen to confirm stability of the data.

Muscle Histology

After the EIM data were collected, the muscle was snap frozen in isopentane cooled in liquid nitrogen and stored at -80°C. The tissue was then cut into 10- μ m slices and stained with hematoxylin and eosin. Cell measurements were made using a Zeiss Axiophot microscope with a motorized stage interfaced with a Dell Optiflex 380 computer running Stereo Investigator software (MBF Biosciences, Inc., Williston, Vermont). This software provides a non-biased quantification of fiber sizes. A total of approximately 30 cells were evaluated from each animal. To further reduce the potential for bias, the evaluator (T.G.) was blinded as to the type of section being assessed.

Hydroxyproline Measurements

Hydroxyproline content of the gastrocnemius muscle, a measure of connective tissue content, was measured using a commercially available assay (Kit 6017; Chondrex, Inc., Redmond, Washington) based on the use of Ehrlich reagent.¹⁷ Specifically, muscle tissue was minced into fine pieces and hydrolyzed in a 6 M HCl solution overnight at 120°C. After hydrolysis, sample solutions were transferred to microcentrifuge tubes and spun at 10,000 rpm for 3 minutes. The supernatant of the solution was then pipetted into microplate wells and treated with Ehrlich reagent and read at 530 nm via spectrophotometry using a multimode microplate reader (Synergy MX; Biotek). Additional microplate wells were filled with known dilutions of hydroxyproline and used to create a calibration curve. Hydroxyproline content in samples was then determined using regression analysis.

Data Analysis

All results are summarized as mean $\pm$ standard error. Two-way analysis of variance was used to assess for differences of age (young and old) on 2 genotypes (WT and *mdx* mice). When

significant interactions were observed, an unpaired *t*-test was used to compare 2 groups for each main effect (either age or genotype). Pearson correlation analyses were performed to establish the relationship between impedance values and muscle fiber morphometry and hydroxyproline content. For all statistical tests, significance was determined at $P < 0.05$ (2-tailed). *F*-statistics and degrees of freedom are presented in the figure legends along with detailed results of the 2-way analysis of variance (ANOVA).

RESULTS

Standard Measures

Figure 2a and b shows the muscle fiber size and hydroxyproline content for all 4 groups of animals (2-month-old *mdx* and WT, and 18-month-old *mdx* and WT). Table 1 provides the actual data. As can be seen, there was a marked difference in muscle fiber size and hydroxyproline content between the WT and *mdx* animals, consistent with previous work¹⁸; the differences between young and old *mdx* animals were significant in the case of hydroxyproline but not for muscle fiber size; in both cases, the interaction terms were also highly significant, indicating a direct relationship between genotype and age for both measures.

In Vivo EIM Measurements

Figure 3a–f and Table 2 show the longitudinal and transverse *in vivo* EIM resistance, reactance, and phase differences between the 4 groups of animals. Table 3 provides the detailed *in vivo* transverse and longitudinal EIM data. As can be seen, there were significant differences between *mdx* and WT animals at 2 months and 18 months for both longitudinal and transverse data; however, the difference became much more pronounced at 18 months. Interaction terms were non-significant, except in the case of transverse resistance, suggesting a direct relationship between age and genotype.

Ex Vivo EIM Measurements

Figure 4a–f presents the *ex vivo* EIM data. *Ex vivo* data showed alterations similar to those observed for the *in vivo* data, although the reactance differences appeared most significant overall.

Correlations with Muscle Fiber Size and Hydroxyproline

Figures 5a and b and 6a and b show correlations between *in vivo* longitudinal impedance values and muscle fiber size and hydroxyproline, respectively. Given that muscle fiber size was virtually identical in both groups of *mdx* animals, data from all 4 groups of animals were used in the correlations between myocyte size and EIM data, providing a moderate correlation of 0.40 and 0.47 for resistance and reactance, respectively ($P < 0.05$ for both). However, only the *mdx* animals (young and old) were used to assess the association between hydroxyproline and the EIM data. As can be seen, there was a strong relationship between hydroxyproline content and the EIM values for each individual animal, with $r = 0.78$ and $r = 0.65$ for resistance and reactance, respectively ($P < 0.001$ for both).

DISCUSSION

There are 2 major findings from this study. First, we have shown that EIM is sensitive to disease status in the *mdx* animals, both in *in vivo* and *ex vivo*, with older animals exhibiting a greater departure from normal compared with younger animals. In WT animals, by comparison, there was little difference between old and young animals. Second, these results provide strong evidence that EIM values are associated with the characteristic features of *mdx* muscle, including the severity of muscle fiber deterioration and the elevated levels of connective tissue. Perhaps most importantly, this work suggests that improvements in muscle composition induced by an effective therapy could be detected by EIM. Any drug that has the potential to reverse or forestall the decline in function would be likely have to an impact on 1 or both characteristic pathological features of the disorder, muscle fiber atrophy and elevated connective tissue content.

The alterations in resistance, reactance, and phase with disease progression are somewhat different than those we have observed in ALS. Indeed, a major change observed in ALS, both in animal and disease models, is the prominent elevation in resistance values, along with reduced reactance values, leading to a major reduction in phase.^{10,14} In *mdx* animals, like ALS, reactance is also lower and appears to decline with advancing disease; this effect is likely due to reductions in muscle fiber size and increased fiber density, resulting in substantial changes in the overall capacitive nature of the network. However, resistance is lower than in WT animals both at 2 and 18 months. Because reactance decreases more than resistance, the ratio of the 2, as calculated in the phase, is also reduced, but not to the extent observed in ALS. The reason for the reduction in resistance in these animals, as compared with an increase in ALS animals, is likely secondary to the presence of increased connective tissue in *mdx* muscle. Connective tissue has higher conductivity than muscle fibers¹⁹ and, given its endomysial location, could lead to lower resistance in both the longitudinal and transverse directions. In contrast, in ALS, decreased muscle fiber size is the major driving factor (there is minimal additional connective tissue deposition), and thus increased resistance is observed.

Despite these findings, there remains no explanation why resistance values are higher in older compared with younger *mdx* animals. Moreover, it is not clear why there is also a positive correlation between hydroxyproline content and EIM resistance values (Fig. 6A). One possible explanation for this may be that resistance is also strongly dependent on another factor not being assessed in this study, namely intramuscular fat content. Intramuscular fat is known to increase in DMD, and its measurement with magnetic resonance imaging has been suggested as a potential useful biomarker of disease status.²¹ Because fat has a very low conductivity compared to that of connective tissue or muscle, its accumulation would be anticipated to outweigh the subtler opposing effects of increased connective tissue. Unfortunately, we did not include a fat assay in this analysis, so the importance of this one component of tissue composition in our findings remains uncertain. Given the complexity of the effects involved, a circuit model that attempts to incorporate alterations in *mdx* as compared with WT animals, such as those presented by Shiffman and Rutkove^{22,23} in human subjects, would likely be worthwhile to pursue; however, it was beyond the scope of this initial basic analysis.

There are several additional limitations to this study that deserve specific mention. First, we only evaluated 2 cohorts of animals of very different ages, aiming to find the most basic of differences between the groups. For EIM to serve as a valuable surrogate for any human or animal therapy trial, identifying subtle longitudinal changes over time in the same group of subjects will be critical. Evaluating this potential is planned as part of our future research efforts. Second, we have only focused on a single set of EIM parameters, the 50-kHZ reactance, resistance, and phase. It is possible that other aspects of the data may provide additional value, including evaluation of the multifrequency data, as we have shown in ALS and other work.^{14,24} Given the complexity of the analyses presented here, we chose to put aside analyzing that large additional data set for the time being. However, a preliminary analysis of the multifrequency data shows promising trends (data not shown). Third, we did not perform a detailed analysis of the electrical properties of excised muscle. Although we demonstrated differences in raw EIM values between groups, we were also able to calculate the tissue's actual conductivity (its ability to conduct an applied electrical current) and its relative permittivity (ability to develop an electric field). These so-called "material properties" can also provide a direct measure of tissue health; however, analysis of these data and their frequency dependence will be pursued separately.

In conclusion, these results show that there are major differences between EIM values in healthy and *mdx* animals, and the differences between both sets of animals increase with age. Importantly, the findings also indicate that EIM values correlate with relevant histological and compositional features of *mdx* muscle, including the amount of connective tissue and muscle fiber size. For these reasons, it appears that EIM parameters have the potential to serve as useful biomarkers of muscular dystrophy disease status. Continued study and application of this relatively simple, non-invasive technique is warranted in both animal models and children with DMD for assessing disease status and response to therapy.

Acknowledgments

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Abbreviations

ALS	amyotrophic lateral sclerosis
ANOVA	analysis of variance
DMD	Duchenne muscular dystrophy
EIM	electrical impedance myography
WT	wild-type

References

1. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol*. 2010; 9:77–93. [PubMed: 19945913]

2. Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. *Cochrane Database Syst Rev*. 2008;CD003725. [PubMed: 18254031]
3. Amthor H, Hoogaars WM. Interference with myostatin/ActRIIB signaling as a therapeutic strategy for Duchenne muscular dystrophy. *Curr Gene Ther*. 2012; 12:245–259. [PubMed: 22554312]
4. Grounds MD, Davies KE. The allure of stem cell therapy for muscular dystrophy. *Neuromuscul Disord*. 2007; 17:206–208. [PubMed: 17306535]
5. Aartsma-Rus A, van Ommen GJ. Progress in therapeutic antisense applications for neuromuscular disorders. *Eur J Hum Genet*. 2010; 18:146–153. [PubMed: 19809477]
6. Zhou L, Lu H. Targeting fibrosis in Duchenne muscular dystrophy. *J Neuropathol Exp Neurol*. 2010; 69:771–776. [PubMed: 20613637]
7. Mazzone E, Vasco G, Sormani MP, Torrente Y, Berardinelli A, Messina S, et al. Functional changes in Duchenne muscular dystrophy: a 12-month longitudinal cohort study. *Neurology*. 2011; 77:250–256. [PubMed: 21734183]
8. Mazzone ES, Vasco G, Palermo C, Bianco F, Galluccio C, Ricotti V, et al. A critical review of functional assessment tools for upper limbs in Duchenne muscular dystrophy. *Dev Med Child Neurol*. 2012; 54:879–885. [PubMed: 22713125]
9. Rutkove SB. Electrical impedance myography: background, current state, and future directions. *Muscle Nerve*. 2009; 40:936–946. [PubMed: 19768754]
10. 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 Lat Scler*. 2012; 13:439–445.
11. Rutkove SB, Gregas MC, Darras BT. Electrical impedance myography in spinal muscular atrophy: a longitudinal study. *Muscle Nerve*. 2012; 45:642–647. [PubMed: 22499089]
12. Tarulli AW, Duggal N, Esper GJ, Garmirian LP, Fogerson PM, Lin CH, et al. Electrical impedance myography in the assessment of disuse atrophy. *Arch Phys Med Rehabil*. 2009; 90:1806–1810. [PubMed: 19801075]
13. Tarulli A, Esper G, Lee K, Aaron R, Shiffman C, Rutkove S. Electrical impedance myography in the bedside assessment of inflammatory myopathy. *Neurology*. 2005; 65:451–452. [PubMed: 16087913]
14. Li J, Staats WL, Spieker A, Sung M, Rutkove SB. A technique for performing electrical impedance myography in the mouse hind limb: data in normal and ALS SOD1 G93A animals. *PLoS One*. 2012; 7:e45004. [PubMed: 23028733]
15. Jafarpoor M, Li J, White JK, Rutkove SB. optimizing electrode configuration for electrical impedance measurements of muscle via the finite element method. *IEEE Trans Biomed Eng*. 2013; 60:1446–1452. [PubMed: 23314763]
16. Ahad MA, Fogerson PM, Rosen GD, Narayanswami P, Rutkove SB. Electrical characteristics of rat skeletal muscle in immaturity, adulthood, and after sciatic nerve injury and their relation to muscle fiber size. *Physiol Meas*. 2009; 30:1415–1427. [PubMed: 19887721]
17. Blumenkrantz N, Asboe-Hansen G. A quick and specific assay for hydroxyproline. *Anal Biochem*. 1973; 55:288–291. [PubMed: 4356652]
18. Graham KM, Singh R, Millman G, Malnassy G, Gatti F, Bruemmer K, et al. Excessive collagen accumulation in dystrophic (mdx) respiratory musculature is independent of enhanced activation of the NF-kappaB pathway. *J Neurol Sci*. 2010; 294:43–50. [PubMed: 20471037]
19. Gabriel C, Gabriel S, Corthout E. The dielectric properties of biological tissues: I. Literature survey. *Phys Med Biol*. 1996; 41:2231–2249. [PubMed: 8938024]
20. Gabriel S, Lau RW, Gabriel C. The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz. *Phys Med Biol*. 1996; 41:2251–2269. [PubMed: 8938025]
21. Wren TA, Bluml S, Tseng-Ong L, Gilsanz V. Three-point technique of fat quantification of muscle tissue as a marker of disease progression in Duchenne muscular dystrophy: preliminary study. *AJR Am J Roentgenol*. 2008; 190:W8–12. [PubMed: 18094282]
22. Shiffman CA, Rutkove SB. Circuit modeling of the electrical impedance: II. Normal subjects and system reproducibility. *Physiol Meas*. 2013; 34:223–235. [PubMed: 23354000]
23. Shiffman CA, Rutkove SB. Circuit modeling of the electrical impedance: I. Neuromuscular disease. *Physiol Meas*. 2013; 34:203–221. [PubMed: 23353926]

24. Wang LL, Spieker AJ, Li J, Rutkove SB. Electrical impedance myography for monitoring motor neuron loss in the SOD1 G93A amyotrophic lateral sclerosis rat. *Clin Neurophysiol.* 2011; 122:2505–2511. [PubMed: 21612980]

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FIGURE 1.

(A) Electrical impedance myography measurements performed on the mouse hindlimb using a prefabricated electrode array. **(B)** Muscle tissue in an impedance-measuring cell. The 2 current-emitting electrodes are on the sides (the 2 small gray tabs beneath the black bar), and the 2 voltage measuring electrodes are on top (the two needle extending from the top of black bar).

FIGURE 2.

Myocyte size (a) and hydroxyproline (b) values in the 4 groups of animals. Dark bars: *mdx* mice; light bars: WT mice. Two-way ANOVA results: (a) a significant interaction between genotype and age (2WA g × a) [$F(1, 36) = 11.7, P = 0.0016$]; (b) a significant interaction between genotype and age [$F(1, 36) = 12.6, P = 0.0011$]. ** $P < 0.01$.

FIGURE 3.

In vivo EIM data (resistance, reactance, and phase) obtained in all 4 groups of animals in both the longitudinal and transverse directions. Dark bars: *mdx* mice; light bars: WT mice. Two-way ANOVA results: (a) a significant effect of genotype [$F(1, 36) = 37.7, P = 4.52 \times 10^{-7}$] and a significant effect of age [$F(1, 36) = 43.3, P = 1.18 \times 10^{-7}$], but no interaction of genotype and age [$F(1, 36) = 0.36, P = 0.55$]; (b) a significant effect of genotype [$F(1, 36) = 48.8, P = 3.46 \times 10^{-8}$] and a significant effect of age [$F(1, 36) = 25.0, P = 1.48 \times 10^{-5}$], but no interaction of genotype and age [$F(1, 36) = 0.25, P = 0.62$]; (c) a significant effect of genotype [$F(1, 36) = 17.1, P = 2.10 \times 10^{-4}$], but no effect of age [$F(1, 36) = 0.25, P = 0.62$] or interaction of genotype and age [$F(1, 36) = 0.92, P = 0.34$]; (d) a significant interaction between genotype and age [$F(1, 36) = 5.49, P = 0.025$]; (e) a significant effect of genotype [$F(1, 36) = 94.6, P = 1.30 \times 10^{-11}$] and a significant effect of age [$F(1, 36) = 6.57, P = 0.015$], but no interaction of genotype and age [$F(1, 36) = 0.0043, P = 0.95$]; and (f) a significant effect of genotype [$F(1, 36) = 31.7, P = 2.18 \times 10^{-6}$], but no effect of age [$F(1, 36) = 3.58, P = 0.067$] or interaction between genotype and age [$F(1, 36) = 3.72, P = 0.062$]. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

FIGURE 4.

Ex vivo EIM data (resistance, reactance, and phase) obtained in all 4 groups of animals in both the longitudinal and transverse directions. Dark bars: *mdx* mice; light bars: WT mice. Two-way ANOVA results: (a) a significant effect of genotype [$F(1, 36) = 34.5, P = 1.03 \times 10^{-6}$], but no effect of age [$F(1, 36) = 1.24, P = 0.27$] or interaction of genotype and age [$F(1, 36) = 1.99, P = 0.17$]; (b) a significant effect of genotype [$F(1, 36) = 10.3, P = 0.0028$], but no effect of age [$F(1, 36) = 0.005, P = 0.94$] or interaction of genotype and age [$F(1, 36) = 2.34, P = 0.14$]; (c) a significant interaction of genotype and age [$F(1, 36) = 7.32, P = 0.01$]; (d) a significant effect of genotype [$F(1, 36) = 63.0, P = 2.01 \times 10^{-9}$], but no effect of age [$F(1, 36) = 3.16, P = 0.08$] or interaction of genotype and age [$F(1, 36) = 0.0047, P = 0.95$]; (e) a significant effect of genotype [$F(1, 36) = 59.7, P = 3.75 \times 10^{-9}$], but no effect of age [$F(1, 36) = 0.12, P = 0.73$] or interaction of genotype and age [$F(1, 36) = 2.13, P = 0.15$]; and (f) a significant interaction between genotype and age [$F(1, 36) = 8.40, P = 0.0064$]. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

FIGURE 5.

Correlations between myocyte size and EIM resistance and reactance across all 4 groups of animals: ($\blacktriangle$) 2-month-old *mdx* mice; ($\triangle$) 2-month-old WT mice; ($\bullet$) 18-month-old *mdx* mice; and ($\circ$) 18-month-old WT mice.

FIGURE 6.
Correlations between hydroxyproline and EIM resistance and reactance across *mdx* animals only. ($\blacktriangle$) 2-month-old *mdx* mice; ($\bullet$) 18-month-old *mdx* mice.

Table 1

Data summary: standard measures.

	Young		Old	
	<i>mdx</i>	WT	<i>mdx</i>	WT
Muscle fiber size (μm^2)	1526 $\pm$ 80	1697 $\pm$ 88	1305 $\pm$ 115	2215 $\pm$ 140
Hydroxyproline ($\mu\text{g}/\text{mg}$)	2.06 $\pm$ 0.25	2.01 $\pm$ 0.30	3.80 $\pm$ 0.32	1.89 $\pm$ 0.15

Table 2

In vivo EIM data.

		Young		Old	
		<i>mdx</i>	WT	<i>mdx</i>	WT
Longitudinal configuration	Resistance (Ω)	185 $\pm$ 4.19	236 $\pm$ 5.49	240 $\pm$ 11.0	282 $\pm$ 7.90
	Reactance (Ω)	58.5 $\pm$ 2.71	81.7 $\pm$ 2.74	74.6 $\pm$ 3.63	101 $\pm$ 4.81
	Phase ($^{\circ}$)	17.5 $\pm$ 0.55	19.0 $\pm$ 0.29	17.3 $\pm$ 0.25	19.8 $\pm$ 0.71
Transverse configuration	Resistance (Ω)	245 $\pm$ 7.28	327 $\pm$ 8.04	307 $\pm$ 10.0	352 $\pm$ 5.59
	Reactance (Ω)	82.4 $\pm$ 3.93	120 $\pm$ 3.10	92.6 $\pm$ 4.27	130 $\pm$ 4.14
	Phase ($^{\circ}$)	18.5 $\pm$ 0.50	20.3 $\pm$ 0.33	16.7 $\pm$ 0.48	20.3 $\pm$ 0.53

Table 3

Ex vivo EIM data.

		Young		Old	
		<i>mdx</i>	WT	<i>mdx</i>	WT
Longitudinal configuration	Resistance (Ω)	330 $\pm$ 14.5	476 $\pm$ 26.5	336 $\pm$ 14.9	426 $\pm$ 22.2
	Reactance (Ω)	97.1 $\pm$ 11.1	120 $\pm$ 12.7	77.1 $\pm$ 9.38	142 $\pm$ 19.4
	Phase ($^{\circ}$)	16.1 $\pm$ 1.49	14.0 $\pm$ 1.02	12.8 $\pm$ 1.17	18.2 $\pm$ 1.80
Transverse configuration	Resistance (Ω)	536 $\pm$ 30.2	864 $\pm$ 20.6	611 $\pm$ 42.8	934 $\pm$ 59.6
	Reactance (Ω)	289 $\pm$ 21.7	549 $\pm$ 30.3	242 $\pm$ 26.9	624 $\pm$ 69.2
	Phase ($^{\circ}$)	28.2 $\pm$ 1.13	32.2 $\pm$ 1.03	21.2 $\pm$ 1.28	32.8 $\pm$ 1.73