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Loss of electrical anisotropy is an unrecognized feature of dystrophic muscle that may serve as a convenient index of disease status

Seward B. Rutkove^a, Jim S. Wu^b, Craig Zaidman^c, Kush Kapur^d, Sung Yim^a, Amy Pasternak^d, Lavanya Madabusi^d, Heather Szelag^d, Tim Harrington^d, Jia Li^a, Adam Pacheck^a, and Basil T. Darras^d

^aDepartment of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Ave, Boston, MA, 02215, USA

^bDepartment of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Ave, Boston, MA, 02215. Boston, USA

^cDepartment of Neurology, Washington University in St. Louis, 660 S Euclid Ave, St. Louis, MO 63110, USA

^dDepartment of Neurology, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA

Abstract

Objective—We sought to understand the alteration in the anisotropic, or direction dependent, character of muscle as measured by electrical impedance myography (EIM) in subjects with Duchenne muscular dystrophy (DMD) and its potential to serve as a biomarker of disease status.

Methods—Thirty-six boys with DMD and 27 healthy controls were measured with EIM, with electrical current applied both parallel and perpendicular to the major muscle fiber direction. In addition, muscle extracted from 10 *mdx* and 10 wild-type mice were measured analogously.

Results—Normalized reactance anisotropy, a direction-dependent measure of membrane charge storage capability, was significantly lower in the four muscles of DMD subjects as compared to controls ($p < 0.01$). Normalized reactance anisotropy also decreased with increasing age in DMD subjects ($r = -0.36$, $p = 0.031$), but not in healthy boys. Analogous changes were observed in *mdx* mouse gastrocnemius as compared to wild type ($p = 0.019$).

Correspondence to: Seward Brian Rutkove, MD, Professor of Neurology, Harvard Medical School, 330 Brookline Avenue, TCC-810, Boston, MA 02215, USA, Tel.: +1-617-667-8130, srutkove@bidmc.harvard.edu.

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Competing interests

Dr. Rutkove has equity in, and serves as 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.

Conclusion—These results support that loss of electrical anisotropy is a previously unrecognized feature of dystrophic muscle.

Significance—Anisotropic alterations may offer novel indices to assist in neuromuscular disease diagnosis and to serve as easy-to-obtain biomarkers in clinical therapeutic trials.

Keywords

Duchenne muscular dystrophy; anisotropy; impedance; biomarker

1. INTRODUCTION

Anisotropy refers to any directionally dependent property of tissue. A common use of the term is in diffusion tensor magnetic resonance imaging (MRI), in which the direction-dependent diffusion of water is defined as the tissue's fractional anisotropy (Basser et al. 1994; Le Bihan et al. 2001). This MRI technique has been especially useful in the field of neurology, allowing for the evaluation of white matter tracts in the brain and spinal cord, in disorders ranging from stroke (Werring et al. 2000) to multiple sclerosis (Werring et al. 1999) to cerebral malformations (Lim et al. 2005). However, the concept has been applied outside the central nervous system, including to the kidney (Thoeny et al. 2005) and, most relevant to this discussion, to skeletal muscle (Lansdown et al. 2007; Damon et al. 2011). Similar to nerves, skeletal muscle is anisotropic since normal muscle fibers have a fairly uniform orientation throughout the tissue. Disruption of the organized muscle fiber architecture by certain diseases or injury will lead to changes in the anisotropic nature of the muscle (Zaraiskaya et al. 2006; McMillan et al. 2011; Cermak et al. 2012). Diffusion tensor imaging of muscle has shown reductions in fractional anisotropy in Duchenne muscular dystrophy (DMD) (McMillan et al. 2011; Ponrartana et al. 2014). This result is explained by the fact that marked pathological change is one of the outstanding characteristics of DMD muscle impacted by DMD. On histological examination, dystrophic muscle shows a variety of abnormalities including myocyte atrophy and hypertrophy, degenerating, regenerating myocytes and multinucleated myocytes, and in muscle with longer standing disease, connective tissue deposition and fat infiltration (Dubowitz et al. 2014).

However, the concept of anisotropy is not restricted to MRI. One more convenient and less costly approach than MRI for evaluating tissue anisotropy non-invasively is via the direct application and measurement of electrical current flow through tissue using electrical impedance-based methods. In the case of skeletal muscle evaluation, the technique has been termed electrical impedance myography (EIM) (Rutkove 2009). Specifically, in EIM a low-intensity multifrequency alternating electrical current is passed between two current electrodes and measured with two voltage electrodes placed over a muscle of interest (Figure 1A) (Rutkove 2009). The major outcome parameters include the resistance and the reactance, the latter offering a measure of the capacitance or charge-storage capability of the tissue. These two characteristics are dependent on both the volume and geometry of the tissue and the specific electrode topology used. A third parameter, the phase angle, can be calculated directly from these two and has also been useful in disease assessment. It is also possible to evaluate the inherent passive electrical properties of the muscle, including its relative permittivity (charge storage capability) and resistivity (inherent resistance to current

flow), by measuring excised muscle tissue in a chamber of known dimensions (Figure 1B) (Li et al. 2014). Indeed, we and others have been able to show that healthy muscle has strong electrical anisotropic characteristics with electrical current flow being several times greater in the longitudinal direction (i.e. parallel to the muscles fibers) as compared to the transverse direction (i.e. perpendicular to them) (Aaron et al. 1997; Tarulli et al. 2006). We have also previously demonstrated that patients with a variety of neuromuscular diseases have a disrupted muscle anisotropy compared to healthy individuals (Chin et al. 2008; Garmirian et al. 2009).

Figure 2A shows an example of the raw longitudinal versus transverse EIM reactance data across a range of applied frequencies from 10 kHz to 1MHz in a healthy boy and one with DMD and the resulting differences in anisotropy, normalized to the mean of the two values at each frequency. For example, as shown in Figure 2B the resulting 100 kHz normalized anisotropy (NA) is greatly reduced in the DMD boy (-0.085) as compared to that of the healthy boy (0.53).

In this study, we evaluate the nature and extent of electrical anisotropic disruption in DMD by studying a group of children with DMD and control subjects with the EIM technique. In addition, we evaluate a group of wild-type (*wt*) and muscular dystrophy (*mdx*) mice muscle *ex vivo*, revealing that the fundamental nature of the impedance alteration is at the level of the electrical material properties of the muscle itself.

2. MATERIALS AND METHODS

2.1 Participants

Boys with DMD aged 2 to 14 ($n = 36$) years were recruited into the study through the neuromuscular clinic at Boston Children's Hospital, as part of our study of EIM and quantitative ultrasound as biomarkers of disease progression in DMD (Rutkove et al. 2014). All subjects with DMD had genetic confirmation of disease or were a brother of a family member with DMD and had a characteristic clinical picture. DMD boys were excluded if they were involved in an ongoing therapeutic clinical trial or if they had a concomitant neuromuscular or other medical condition that substantially impacted health. Healthy boys ($n = 27$) had no history of neuromuscular disease or any other disorder that would affect muscle health and were recruited separately via advertisement and word-of-mouth.

2.2 EIM measurements

EIM measurements were obtained with the Imp SFB7® (Impedimed, Sydney, Australia) with the leads attached to a custom handheld array as previously described and shown Figure 1A (Rutkove et al. 2014). Measurements were performed separately on four muscles unilaterally (on the dominant side or the right side if dominance could not be determined), including deltoid, biceps, rectus femoris, and medial gastrocnemius. Prior to placement, the skin was moistened with saline. The probe was positioned over the bulk of each muscle using simple measurement paradigms based on the location of standard bony prominences (e.g., for biceps, $2/3^{\text{rd}}$ the distance between the acromion process and midpoint of the antecubital fossa). EIM measurements were performed on each muscle with the array first

placed longitudinally (to obtain muscle impedance properties with electrical current flowing along/in parallel to the muscle fibers) and then transversely (to obtain muscle impedance properties with electrical current flowing across/perpendicular to the muscle fibers).

2.3 Functional measurements

The Northstar Ambulatory assessment (Mazzone et al. 2010), a functional mobility assessment validated for DMD, was performed by a trained pediatric physical therapist on all boys with DMD who were ambulant and could follow instructions successfully.

2.4 Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at the Beth Israel Deaconess Medical Center. Ten two-month old male *mdx* animals (C57BL/10ScSn-Dmd^{mdx}/J) and 10 two-month old wild-type (*wt*, C57BL/6J) male controls were obtained from Jackson Labs (Bangor, Maine). Animals were sacrificed with CO₂, and the gastrocnemius was immediately surgically exposed and removed. The excised muscle was weighed and then cut to an approximately 5mm X 5mm X 3mm block using a razor blade. The tissue was then placed in a 5mm X 5mm base impedance-measuring cell configured with 2 broad, stainless steel electrodes on 2 sides and 2 needle electrodes positioned on top, as previously described and shown in Figure 1B (Li et al. 2013). The muscle was placed in the cell such that current flow was parallel to the fibers to obtain longitudinal data. Impedance data were obtained using the Imp SFB7®, the same device used to collect the human data. The tissue then was rotated 90 degrees and the measurements repeated to obtain transverse data.

2.5 Statistics

A total of 36 boys with DMD (mean age 7.3 ± 3.4 years, range 2.2 - 13.1 years) and 27 healthy controls (mean age 7.3 ± 3.4 years, range 2.0 - 14.6 years) were studied. Since the data was collected with the same impedance-measuring device for both animal and human studies (though obviously with different sized electrode arrays), it was handled similarly for both. The two raw impedance measures, the reactance and the resistance are obtained directly from the device. They were plotted across frequency, from 10 kHz – 1 MHz. These EIM data were reviewed for quality, and any spurious data (as indicated by erratic multifrequency and low-frequency artifact, denoting poor electrode contact) were excluded. Since the baseline impedance values are affected by disease, it is critical to normalize the measured anisotropy difference to the underlying values. Thus, we defined the normalized anisotropy (NA) as the transverse – longitudinal EIM values/(transverse + longitudinal)/2 at each frequency between 10 kHz and 1 MHz, resulting in a dimensionless value. These multifrequency anisotropy curves were then plotted for the healthy and diseased humans and for the mouse tissue; extraction of the 100 kHz data, approximately where the peak anisotropy is found, was also performed.

The electrical material properties, including the resistivity (ρ) and relative permittivity (ϵ_r) of the mouse muscle were calculated (Wang et al. 2011), via equations (1) and (2) below:

$$\rho = \frac{A}{d} \cdot \frac{R^2 + X^2}{R} \quad (1)$$

$$\varepsilon_r = \frac{1}{\varepsilon_0} \cdot \frac{d}{A} \cdot \frac{X}{\omega(R^2 + X^2)} \quad (2)$$

where d is the distance between the voltage measuring electrodes, A is the cross-sectional area of the muscle, R and X are the measured resistance and reactance, respectively, at a specific frequency, ω is the frequency in radians, and ε_0 is the permittivity of free space. Resistivity is measured in ohm-meter; relative permittivity is dimensionless. The NA for both the permittivity and resistivity derived from the mouse muscle were calculated analogously to the human data.

Standard parametric two-group statistics were applied, including t-tests and Pearson correlations, with $p < 0.05$, two-tailed confirming significance. For correlations to age and function of the boys being studied, we calculated an average NA by averaging the NAs across the 4 muscles studied.

3. RESULTS

3.1 Multifrequency alterations in anisotropy in healthy boys and those with DMD

Figure 3A shows the anisotropic spectral data (average of values for all muscles for each child), displaying the alterations in health and disease (group mean $\pm$ standard deviation) across the frequency spectrum, for the 2 major impedance indices, resistance and reactance. As can be seen, the anisotropic alterations are most apparent in the reactance data, the parameter most tightly connected to the myocyte membrane properties; possible explanations for this finding are explored in the discussion. Since the peak of the anisotropy differences in both healthy and DMD boys is approximately 100 kHz, all subsequent single-frequency analyses are shown at 100 kHz reactance values. Figure 3B shows the 100 kHz data across the 4 muscles sampled, showing significant differences between healthy boys and those with DMD for each of the muscles sampled ($p < 0.01$ for biceps and gastrocnemius, $p < 0.001$ for deltoid and quadriceps). Of note there is considerable overlap between the groups, consistent with the fact that the boys with DMD had varying disease severity given their relatively wide range of ages. However, it is important to note that the two muscle groups most severely affected clinically in the disease, quadriceps and deltoid, also show the greatest difference between healthy and DMD boys.

3.2 Relationship between anisotropy and age and function

From a pathological standpoint, DMD worsens with progressive age. Thus, age can serve as a simple proxy for disease status across a group of individuals with the disease. Figures 4A and B shows the relationship between muscle anisotropy and age for the 4-muscle 100 kHz average for both boys with DMD and healthy boys. As can be seen, the NA decreases with

age in the DMD boys ($r = -0.36$ and $p = 0.031$) and increases non-significantly in healthy boys ($r = 0.22$ and $p = 0.26$). Similarly, in the subset of boys with DMD who completed the NSAA, there is a near-significant relationship between NA and NSAA score ($r = 0.40$ and $p = 0.074$, Figure 4C).

3.3 Analogous changes are observed in the mdx mouse

Figure 5A shows the *mdx* and *wt* mouse *ex vivo* muscle multifrequency NAs. Although spectral characteristics are different than the human data (Figure 3A), the anisotropic features are generally analogous to those observed in human subjects. Figure 5B shows the derived multifrequency relative permittivity and resistivity NAs, respectively. These reveal that the permittivity, or relative charge carrying capacity of the muscle, is similar in both groups of animals, except at the lowest frequencies; however, the resistivity, or the inherent, volume-independent resistance to electrical current flow, shows a reduction in NA in the *mdx* as compared to *wt* mice. Finally, Figure 5C shows the separate longitudinal and transverse resistivity and permittivity values from the mice, analogous to the data shown in Figure 2A, demonstrating that the reduction in resistivity NA in *mdx* muscle is mainly due to a marked reduction in transverse resistivity with only a minor reduction in longitudinal resistivity compared to healthy animals. Table 1 summarizes 100 kHz reactance data for both humans and mice.

4. DISCUSSION

These results show that reduction in the electrical anisotropy of muscle tissue is an inherent characteristic of both dystrophin-deficient human and mouse muscle, findings consistent with our previous work in other conditions (Garmirian et al. 2009). Moreover, in DMD, the degree of loss of electrical anisotropy correlates to disease status. Greater reductions in anisotropy are associated with more severe disease as indicated by older age and a trend toward worse function in the boys with DMD. The mechanism underlying this change is difficult to prove since no standard histological analysis can provide insight into these tissue electrical properties. Nonetheless, the *ex vivo* measurements in *mdx* mice and calculation of raw permittivity and resistivity values support that conclusion. In other words, the demonstration that the raw resistivity is less anisotropic in diseased mouse muscle than healthy muscle supports that the alterations identified are inherent to the muscle and, indeed, to the disease itself. Specifically, they are not due to other factors such as alterations in limb shape, size, or the presence of overlying tissues (Li et al. 2014), although these still could potentially impact the data.

The loss of electrical anisotropy likely reflects the loss of the structural anisotropy of muscle tissue—in other words the normal bundled, structure is disrupted. Several pathological features of dystrophic muscle may be involved in producing this result. First, in dystrophic muscle, the muscle fibers themselves become irregular in size and considered longitudinally, many fibers may demonstrate interruptions due to segmental degeneration and branching (Coulton et al. 1988; Deconinck and Dan 2007). Second, there is the deposition of inflammatory cells that, due to their generally round or ovoid shape, are far more isotropic in nature than long thin myofibers. Although proportionally small compared to the overall size

of myofibers, these also would be expected to reduce the measured anisotropy. Third, there are major changes to the extracellular milieu and muscle interstitium. The deposition of fat, connective tissue, and the presence of edema will further reduce the inherent anisotropic properties of the tissues. These last features, which confer no clear directional dependence, may contribute most substantially.

While it is possible to offer potential explanations, a specific pathological basis is very difficult to identify since it is not possible to isolate a unique muscle pathology and determine its effect on anisotropy. For example, we cannot induce isolated fat deposition within the muscle or induce split/branched myofibers without concomitant alterations in the tissue. Thus, a specific study correlating specific pathological features in muscle with its anisotropic alterations would be virtually impossible to achieve.

There is an important difference, however, between the *mdx* mouse muscle and the human data presented here that must be highlighted. Specifically, the *mdx* mouse tends to develop very little fat deposition at any age and at 2 months, the amount of connective tissue is also likely to be only modestly elevated above normal (Coulton et al. 1988). Thus, it is very possible that the primary cellular changes—damaged myofibers, associated edema and the presence of inflammatory infiltrates—are responsible for the loss of anisotropy in the mouse. In the human, in addition to the presence of these pathological features, the presence of greater amounts of intra-muscular fat and connective tissue are also likely to play important roles.

In this analysis, we have focused on reactance values, and the decision for doing so is partly practical and partly based on theoretical concerns. The first practical reason is that, as can be seen in Figures 3A and 5A, of the standard impedance parameters assessed, the reactance shows greater anisotropy and obeys a fairly consistent change across frequencies. Whereas resistance also shows anisotropy, the alterations are more complex to characterize. A second practical reason is that the reactance curves show a peak (near 100 kHz) that can serve as a reference point to evaluate the anisotropy. The theoretical reason for using this measure is that the reactance is produced predominantly by capacitive effects of the muscle fiber sarcolemma; since membrane integrity is so severely impacted by this disease, of the impedance parameters available, this would appear the most relevant. Importantly, both the calculated resistivity and permittivity are dependent on both resistance and reactance values (see equations in the methods section). Accordingly, it is difficult to intuit how these differences play into the conclusion that the resistivity and not the permittivity is the major inherent tissue characteristic altered in dystrophic muscle.

Limitations of this study include the fact that we have not pursued a longitudinal analysis of the data over time in boys with DMD; thus, its value as a biomarker of actual disease progression and its sensitivity to treatment is uncertain. Rather, the purpose of this study was to set the stage for such future investigations. In addition, the device being used to measure the impedance parameters, the Impedimed Imp SFB7®, is not specifically designed for measuring localized areas of muscle, and is rather built for whole body or single limb measurements. Moreover, it is impossible to know whether the handheld array was consistently aligned appropriately with the fibers. Small variations in placement could

certainly contribute to variations in the data, but are unlikely to explain the differences we observed between healthy boys and those with DMD. Finally, this study focused on a very basic approach to identifying anisotropy—namely by measuring impedance values in two directions: perpendicular and parallel to the major muscle fiber direction. The measurement of fractional anisotropy in diffusion weighted MRI, in contrast, incorporates a multi-directional approach based on the incorporation of different eigenvalues derived from a diffusion ellipsoid (Zaraiskaya et al. 2006).

Importantly, these alterations, so readily identifiable in DMD, may also extend to a variety of other myopathic disorders that have similar pathology, including other muscular dystrophies, inflammatory myopathies, and degenerative diseases of muscle, including inclusion body myositis and possibly sarcopenia. Impedance-based electrical anisotropic alterations in these conditions may offer novel indices to assist in disease diagnosis and to serve as convenient biomarkers in clinical therapeutic trials.

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REFERENCES

- Aaron R, Huang M, Shiffman CA. Anisotropy of human muscle via non-invasive impedance measurements. *Phys Med Biol*. 1997; 42(7):1245–62. [PubMed: 9253037]
- Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. *Biophys J*. 1994; 66(1):259–67. [PubMed: 8130344]
- Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, et al. Diffusion tensor imaging: concepts and applications. *J Magn Reson Imaging*. 2001; 13(4):534–46. [PubMed: 11276097]
- Cermak NM, Noseworthy MD, Bourgeois JM, Tarnopolsky MA, Gibala MJ. Diffusion tensor MRI to assess skeletal muscle disruption following eccentric exercise. *Muscle Nerve*. 2012; 46(1):42–50. [PubMed: 22644795]
- Chin AB, Garmirian LP, Nie R, Rutkove SB. Optimizing measurement of the electrical anisotropy of muscle. *Muscle Nerve*. 2008; 37:560–5. [PubMed: 18404614]
- Coulton GR, Morgan JE, Partridge TA, Sloper JC. The mdx mouse skeletal muscle myopathy: I. A histological, morphometric and biochemical investigation. *Neuropathol Appl Neurobiol*. 1988; 14:53–70. [PubMed: 2967442]
- Damon BM, Buck AK, Ding Z. Diffusion-tensor MRI-based skeletal muscle fiber tracking. *Imaging Med*. 2011; 3:675–87. [PubMed: 25429308]
- Deconinck N, Dan B. Pathophysiology of Duchenne Muscular Dystrophy: Current Hypotheses. *Pediatr Neurol*. 2007; 36:1–7. [PubMed: 17162189]
- Dubowitz, V.; Sewary, CA.; Oldfors, A.; Lane, R. *Muscle Biopsy: A Practical Approach*. Saunders; China: 2014.
- Garmirian LP, Chin AB, Rutkove SB. Discriminating neurogenic from myopathic disease via measurement of muscle anisotropy. *Muscle Nerve*. 2009; 39:16–24. [PubMed: 19058193]
- Lansdown D, Ding Z, Wadington M, Hornberger JL, Damon BM. Quantitative diffusion tensor MRI-based fiber tracking of human skeletal muscle. *J Appl Physiol*. 2007; 103:673–81. [PubMed: 17446411]
- Li J, Geisbush TR, Rosen GD, Lachey J, Mulivor A, Rutkove SB. Electrical impedance myography for the in and ex vivo assessment of muscular dystrophy (mdx) mouse muscle. *Muscle Nerve*. 2014; 49:829–835. [PubMed: 24752469]

- Li J, Jafarpoor M, Boussein M, Rutkove SB. Distinguishing neuromuscular disorders based on the passive electrical material properties of muscle. *Muscle Nerve*. 2015; 51:49–55. [PubMed: 24752678]
- Lim CC, Yin H, Loh NK, Chua VG, Hui F, Barkovich AJ. Malformations of cortical development: high-resolution MR and diffusion tensor imaging of fiber tracts at 3T. *Am J Neuroradiol*. 2005; 26(1):61–4. [PubMed: 15661702]
- Mazzone E, Martinelli D, Berardinelli A, Messina S, D'Amico A, Vasco G, et al. North Star Ambulatory Assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. *Neuromuscul Disord*. 2010; 20(11):712–6. [PubMed: 20634072]
- McMillan AB, 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; 2011:970726. [PubMed: 22190860]
- Ponrartana S, Ramos-Platt L, Wren TAL, Hu HH, Perkins TG, Chia JM, et al. Effectiveness of diffusion tensor imaging in assessing disease severity in Duchenne muscular dystrophy: preliminary study. *Pediatr Radiol*. 2014; 45:582–9. [PubMed: 25246097]
- Rutkove SB. Electrical Impedance Myography: Background, Current State, and Future Directions. *Muscle Nerve*. 2009; 40:936–46. [PubMed: 19768754]
- 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*. Jul. 2014; 51(1):88–92. [PubMed: 24814059]
- Tarulli AW, Chin AB, Partida RA, Rutkove SB. Electrical impedance in bovine skeletal muscle as a model for the study of neuromuscular disease. *Physiol Meas*. 2006; 27(12):1269–79. [PubMed: 17135699]
- Thoeny HC, De Keyser F, Oyen RH, Peeters RR. Diffusion-weighted MR imaging of kidneys in healthy volunteers and patients with parenchymal diseases: initial experience. *Radiology*. 2005; 235(1):911–7. [PubMed: 15845792]
- Wang LL, Ahad M, McEwan A, Li J, Jafarpoor M, Rutkove SB. Assessment of alterations in the electrical impedance of muscle after experimental nerve injury via finite-element analysis. *IEEE Trans Biomed Eng*. 2011; 58:1585–91. [PubMed: 21224171]
- Werring DJ, Clark CA, Barker GJ, Thompson AJ, Miller DH. Diffusion tensor imaging of lesions and normal-appearing white matter in multiple sclerosis. *Neurology*. 1999; 52(8):1626–1626. [PubMed: 10331689]
- Werring DJ, Toosy AT, Clark CA, Parker GJ, Barker GJ, Miller DH, et al. Diffusion tensor imaging can detect and quantify corticospinal tract degeneration after stroke. *J Neurol Neurosurg Psychiatry*. 2000; 69(2):269–72. [PubMed: 10896709]
- Zaraiskaya T, Kumbhare D, Noseworthy MD. Diffusion tensor imaging in evaluation of human skeletal muscle injury. *J Magn Reson Imaging*. 2006; 24(2):402–8. [PubMed: 16823776]

HIGHLIGHTS

- 1.** Surface electrical impedance measurements can reveal the anisotropic character of muscle.
- 2.** The anisotropic characteristics of muscle are disrupted in Duchenne muscular dystrophy (DMD).
- 3.** Electrical anisotropy can serve as a biomarker for evaluation of muscle condition in DMD.

Figure 1. Measurement techniques. **(A)** Custom-designed handheld array used with Imp SFB7® for collecting muscle impedance data. **(B)** Plastic impedance measuring cell with mouse muscle placed inside. The voltages are measured by the needle electrodes inserted into the top of the cell. The current is applied via two flat metal plates on the sides of the array.

Figure 2.

Derivation of anisotropy data. Example of raw longitudinal and transverse multifrequency reactance data (**A**) and calculated anisotropy difference (**B**) in the biceps brachii muscle of a healthy 12-year-old boy and a 13-year-old boy with DMD.

Figure 3.

A Mean ($\pm$ SD) resistance and reactance data averaged across all muscles of the cohort of boys with DMD ($n = 36$) and healthy controls ($n = 27$). **B.** Box-Whiskers plot of 100 kHz reactance normalized anisotropy data across the 4 muscles studied with DMD ($n = 36$) and healthy controls ($n = 27$). The edges of the box are the 25th and 75th percentiles; the middle line is the median. A two-tailed t-test was applied (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Blue, healthy controls; red, DMD boys.

Figure 4.

Correlation between clinical parameters and impedance anisotropy. (A, B.) Pearson correlations between 100 kHz reactance normalized anisotropy and age in DMD boys ($n = 36$) and healthy controls ($n = 27$). As expected, there is a significant reduction in anisotropy in DMD boys as they grow, in contrast to a non-significant increase in healthy boys. (C) Correlation between 100 kHz reactance normalized anisotropy with NSAA in boys with DMD ($n=21$).

Figure 5.

A. Mean ($\pm$ SEM) *ex vivo* anisotropy data in *mdx* (n=10) and *wt* mice (n=10), showing analogous alterations identified in human subjects, shown in Figure 3A. **B.** Mean ($\pm$ SEM) relative permittivity and resistivity anisotropy data in *mdx* (n=10) and *wt* mice (n=10), demonstrating that the effect appears to be mainly due to a relative reduction in the resistivity anisotropy and not to the effects of permittivity at the frequencies of interest. **C.** Mean ($\pm$ SEM) relative permittivity and resistivity anisotropy data in *mdx* (n=10) and *wt* mice (n=10) are broken down into their component longitudinal and transverse values, revealing the etiology of the reduction in anisotropy is mainly due to a reduction in transverse resistivity. Blue, *wt* mice; red, *mdx* mice.

Table 1

Summary of 100 kHz reactance data in humans ($\pm$ SD) and mice ($\pm$ SEM).

Reactance NA	Healthy boys	DMD boys	p value
Deltoid($\pm$ SD)	0.31 $\pm$ 0.089	0.17 $\pm$ 0.13	1.70e-05
Biceps($\pm$ SD)	0.34 $\pm$ 0.076	0.25 $\pm$ 0.15	0.0071
Quadriceps($\pm$ SD)	0.25 $\pm$ 0.063	0.14 $\pm$ 0.12	5.70e-05
Gastrocnemius($\pm$ SD)	0.34 $\pm$ 0.11	0.23 $\pm$ 0.13	0.0013
Average($\pm$ SD)	0.30 $\pm$ 0.048	0.23 $\pm$ 0.088	2.57e-04

	<i>wt</i> mice	<i>mdx</i> mice	p value
Gastrocnemius ($\pm$ SEM)	1.11 $\pm$ 0.073	0.57 $\pm$ 0.053	0.019