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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

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HIGHLIGHTS

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

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 ($p = 0.019$).
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.

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

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(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 (Fig. 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 (Fig. 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).

Fig. 2A shows an example of the raw longitudinal versus transverse EIM reactance data across a range of applied frequencies from 10 kHz to 1 MHz 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 Fig. 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).

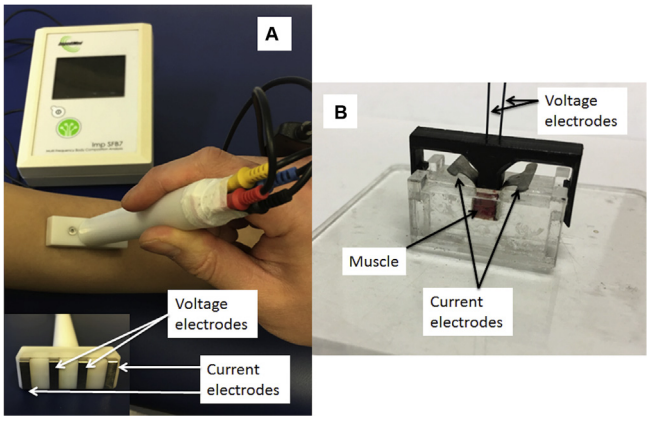

Fig. 1. Measurement techniques. (A) Custom-designed handheld array used with Imp SFB7® for collecting muscle impedance data. (B) Plastic impedance measuring cell with mouse muscle placed inside. The voltages are measured by the needle electrodes inserted into the top of the cell. The current is applied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

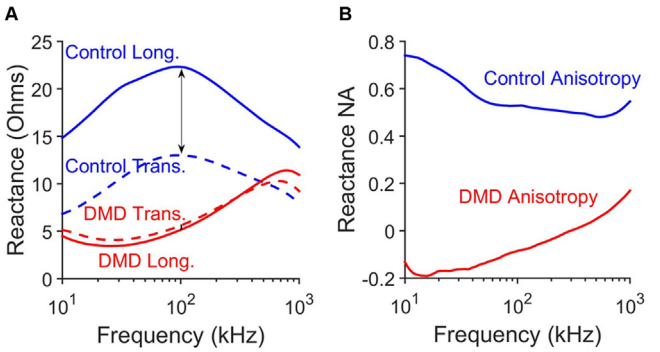

Fig. 2. Derivation of anisotropy data. Example of raw longitudinal and transverse multifrequency reactance data (A) and calculated anisotropy difference (B) in the biceps brachii muscle of a healthy 12-year-old boy and a 13-year-old boy with DMD.

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 2–14 ($n = 36$) 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 Fig. 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 boney prominences (e.g., for biceps, 2/3rd 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