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Optimizing electrical impedance myography measurements by using a multifrequency ratio: A study in Duchenne muscular dystrophy

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HIGHLIGHTS

- By exploiting differences in the inherent electrical properties of tissues, it is possible to reduce the contribution of subcutaneous fat in electrical impedance myography data.
- A ratio of phase values obtained at two frequencies of applied electrical current provides a measure of muscle condition that is only minimally impacted by subcutaneous fat.
- This advance will improve the muscle tissue specificity of electrical impedance myography, helping to further strengthen its value as a tool for the non-invasive assessment of neuromuscular disease.

ABSTRACT

Objective: Electrical impedance myography (EIM) is an electrophysiological technique for neuromuscular evaluation that is impacted by subcutaneous fat (SF). Exploiting the differing frequency dependences of muscle and fat, we assessed a 2-frequency EIM phase ratio in Duchenne muscular dystrophy (DMD) boys.
Methods: Twenty-eight DMD boys aged 2–13 years underwent EIM and the 6-minute walk test (6MWT). For each subject, 50 kHz phase data was input into the numerator while 20–500 kHz phase values were input into the denominator. We then performed correlation analyses seeking to identify the denominator frequency that simultaneously optimized SF and 6MWT correlations. This optimized ratio was then tested in 24 healthy boys.
Results: 50 kHz phase correlated to 6MWT in DMD boys with $R = 0.52$, $p = 0.0066$, and to SF thickness with $R = -0.67$, $p < 0.001$. An optimized ratio of 50/200 kHz phase reduced the correlation of SF thickness to $R = -0.075$, $p = 0.45$ while improving the relationship to the 6MWT ($R = 0.60$, $p = 0.001$). In normal subjects, the optimization decreased SF correlation from $R = 0.61$ from $R = 0.16$ with 6MWT correlation remaining unchanged.
Conclusions: The 50/200 kHz EIM phase ratio removes the impact of SF while maintaining EIM's association with function.
Significance: The use of a phase ratio may enhance EIM's application for evaluation of neuromuscular disease.

1. Introduction

Electrical impedance myography (EIM) is an easily applied technology for the non-invasive assessment of neuromuscular disease in which a high-frequency, very low-intensity electrical current is passed through a localized area of muscle and the consequent surface voltages analyzed (Rutkove, 2009). Studies in a variety of disorders, including spinal muscular atrophy, amyotrophic lateral sclerosis, and Duchenne muscular dystrophy (DMD) have shown that EIM is sensitive to neuromuscular disease severity (Rutkove and Darras, 2013; Rutkove et al., 2010; Tarulli et al., 2009). Recently, work has shown that the EIM phase value is

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exceptionally sensitive to disease progression in amyotrophic lateral sclerosis and thus has the prospect of serving as a novel biomarker in clinical therapeutic trials (Rutkove et al., 2012a).

Despite EIM's promise, one potential limitation of the technique is the impact of subcutaneous fat (SF) on the resulting impedance phase data. Whereas electrical current preferentially travels through muscle more than through fat (since muscle is 10× more conductive than fat (Faes et al., 1999)), EIM data still can be impacted by it. This was recently demonstrated in a study in healthy individuals (Sung et al., 2013) and another utilizing computer modeling (Jafarpoor et al., 2013). In fact, it was for this reason that early approaches to performing EIM had the current emitting electrodes placed far from the voltage electrodes (on the hands or feet) to ensure that the current was flowing mainly through muscle (Rutkove et al., 2002). However, this approach had a number of disadvantages, including being clumsy to perform and highly sensitive to joint angle. It has since been abandoned in favor of our current approach in which the entire electrode array (both the two current-emitting and two voltage-measuring electrodes) is placed over a single muscle of interest (Rutkove, 2009).

Whereas the effect of SF is only modestly important for longitudinal studies of neuromuscular disease, in which alterations in muscle typically far outweigh any alterations in SF, reducing the impact of SF on the measurements would clearly be advantageous, especially since some therapies, such as corticosteroids, may alter SF thickness. One approach for achieving this utilizes the idea of performing the measurement at multiple frequencies. In many EIM studies to date, only a single frequency (50 kHz) of electrical current has been used for measurement. This frequency choice is based on the fact that skeletal muscle tends to be most reactive around 50 kHz and that most inexpensive impedance devices provide electrical current at this single frequency. More recently, however, studies have shown that utilizing multifrequency measures may be more sensitive to disease status and progression over time (Rutkove et al., 2012b). In addition, the frequency dependence of the inherent electrical material properties of fat and muscle, their conductivity and permittivity, are quite different (Gabriel et al., 1996a,b). Thus, it is possible that by utilizing multifrequency measures we will be able to help extract the impedance characteristics of muscle from those of fat.

In this study, we first confirm the reported marked differences in impedance spectra between fat and muscles by studying excised animal tissues. We then attempt to exploit this differing frequency dependence by using a 2-frequency impedance phase ratio that would optimize EIM measurements by simultaneously reducing the impact of SF while maintaining a strong correlation to function in a group of children with DMD. For this purpose, we chose to optimize the ratio to the 6MWT, the functional measure that is being used as the outcome measure in most clinical trials. By optimizing to this metric, we were hoping to develop a measure that would likely have the greatest clinical value and could potentially serve as a surrogate marker in future DMD clinical trials.

2. Materials and methods

2.1. Animal tissue and data analysis

In order to provide simple experimental proof that fat and muscle have very different impedance values, as an initial experiment, we first measured fresh rat muscle and pig subcutaneous fat that were obtained from animals that had been sacrificed for unrelated experiments. The tissue was placed in an impedance-measuring cell and impedance data collected from 3 kHz to 1 MHz. using the Imp SFB7 (Impedimed, Inc.), as previously described (Wang et al., 2011a).

2.2. Patient demographics

The clinical study was approved by the institutional review board of Boston Children's Hospital; all parents provided informed written consent and verbal assent was obtained from all children. Twenty-eight boys with DMD were studied for this analysis, ages 2–13 years, with a baseline mean age (± standard deviation) of 7.9 ± 3.3 years at the time of baseline visit. The patients had a mean body weight of 30.7 ± 19.8 kg. All 28 boys had SF thickness measured over a total of 106 visits; a subset of 13 boys had the 6-minute walk test (6MWT) performed over a total of 26 visits. The reason for the difference in the number of boys undergoing the 6MWT than measurement of SF thickness is that the main focus of this study was on the longitudinal progression of disease, and thus age-specific outcome measures were utilized. The 6MWT was performed only in children who were old enough to walk and yet not wheechair-bound whereas SF thickness measurements were performed in all.

In addition, 24 healthy subjects with a mean age 7.7 ± 3.2 years at baseline were also included to determine how the optimized ratio compared to the 50 kHz phase value. The healthy boys' body weight was 27.0 ± 9.5 kg at baseline. A subset of 20 of the 24 healthy boys had a 6MWT performed over 57 visits. All 24 subjects had SF thickness measurements over 132 visits, and SF thickness measurements of all 6 measurements for each visit were calculated.

2.3. Six-minute walk test

The standard 6MWT was performed by a physical therapist experienced in the measure (AP) using identical standard conditions (McDonald et al., 2010).

2.4. EIM testing

EIM testing was performed on six muscles on each patient's dominant side: the unilateral deltoid, biceps, wrist flexors, quadriceps (rectus femoris), tibialis anterior, and medial gastrocnemius. EIM measurements were taken both transversely and longitudinally with respect to the long axis of the limb, since most previous human work has been completed in the longitudinal direction, yet recent data in mdx mice suggest that in DMD the transverse direction may be more sensitive to disease status (Li et al., 2014). EIM was performed with the Imp SFB7 (Impedimed, Inc., Sydney Australia). The device was connected to a hand-held electrode array similar to that which was used previously (Narayanaswami et al., 2012). Three different sizes of electrode array were used, based on the size of the child, so as to maximize the region of muscle being evaluated.

2.5. Ultrasound measurements of SF thickness

Ultrasound measurements were taken with a Terason t3000 system (Teracorp, Inc., Burlington, MA) with a 10 MHz probe. The tests were performed on the same muscles as the EIM testing, in precisely the same location that the EIM array was placed. Ultrasound measurements were taken transversely to the muscle fiber direction. Using the system's software, an electronic caliper was used to measure SF thickness on each image.

2.6. Data analysis

Standard Pearson correlation analyses were performed correlating 6-muscle mean EIM 50 kHz phase values to the 6MWT and SF thickness values to establish the baseline correlation between these different measures. A range of ratios was then developed