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

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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, 50kHz phase data was input into the numerator while 20kHz–500kHz 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—50kHz 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 50/200kHz 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.

Keywords

electrical impedance myography; subcutaneous fat; Duchenne muscular dystrophy; optimization; functional measures

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INTRODUCTION

Electrical impedance myography (EIM) is an easily applied technology for the noninvasive 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 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 10X 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, Gabriel et al., 1996b). 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.

MATERIALS AND METHODS

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).

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 to 13 years, with a baseline mean age ($\pm$ 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 kilograms. 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 6 MWT 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 wheelchair-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.

Six-minute walk test

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

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., 2013). 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.

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.

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 by maintaining the 50 kHz EIM data in the numerator and by inputting in the denominator a range of EIM phase values starting at 20 kHz and extending up to 500 kHz, plotting the absolute values of the correlation coefficient (R) versus the denominator frequency values for 6MWT and SF thickness. The ratio and 50 kHz raw data were then applied to the baseline data from the healthy boys to see how much the SF thickness-EIM correlation decreased across that population of individuals and whether it reduced the coefficient of variation in the raw data. All analyses were performed using Matlab (Mathworks, Natick MA). Significance was determined at $p = 0.05$, two-tailed for all calculations.

RESULTS

Figure 1 shows data from the rat and pig tissues, demonstrating the marked difference in frequency dependence of the impedance signatures for muscle and fat. As can be seen, muscle has a peak phase between 10–100 kHz, whereas fat values are low in this range, only increasing at higher frequencies.

Figure 2 shows an example of typical multifrequency EIM phase data obtained from a healthy boy and one with DMD. Figure 3A shows the relatively strong correlation between EIM 50 kHz phase and SF thickness measured with $R = -0.67$, $P < 0.001$. Figure 3B shows the respective relationship between EIM 50 kHz phase 6-muscle average and 6MWT data in all 26 visits of the 13 boys with DMD. As can be seen there is a moderate correlation to the 6MWT with $R = 0.52$, $p = 0.0066$.

Figure 4A plots the pre-optimization Pearson correlation coefficients between EIM phase and 6MWT (black) and SF (gray) across the range of applied frequencies of current. As can be seen, the SF correlations range from about $R=0.55$ to 0.75 , increasing slightly at higher frequencies, whereas the correlations to 6MWT are about $R=0.6$ at low frequencies and trend downward with increasing frequency. Figure 4B shows the optimization procedure. The frequency referred to in the x-axis of the plot is the frequency of the denominator of the ratio (ranging from 20–500 kHz) with the numerator being held constant with 50 kHz values. As can be seen, the SF thickness correlation reach a minimum near 200 kHz (arrow), while actually providing a slightly stronger correlation to 6MWT at that same frequency. Figure 5 is organized in an analogous fashion to Figure 4, showing several individual muscles; most muscles follow this pattern, while others do not (Figure 5C–F). Figures 6A and B show the same plots as shown in Figure 3, but now using the optimized phase 50/200 ratio. As can be seen, the ratio actually strengthens the correlation with the 6MWT with respect to both R and p values to $R=0.60$, $P=0.001$ from $R=0.52$, $p=0.007$) while dramatically reducing the impact of the SF thickness to the point that it becomes non-significant ($R=-0.075$, $P=0.45$).

Finally, we compared the 50 kHz phase data to the 50/200 kHz data in the group of healthy boys. Similar to the pattern seen in subjects with DMD, the correlation to SF thickness decreased markedly from $R=-0.61$, $P<0.001$ to $R=0.16$, $P=0.061$ (Figure 7). The 6MWT values remained essentially unchanged altering from 0.44 to 0.45 ($p<0.001$ for both), in contrast to the strengthened correlation seen in DMD subjects. Of note, there was also a marked reduction in the coefficient of variation in this normal group of children in the ratio as compared to the raw data, decreasing from 0.4 to 0.15 in this group of children further supporting that much of the variation in the 50 kHz phase value in healthy children is related to SF thickness.

DISCUSSION

In this study, we have shown that by creating a two-frequency ratio of EIM phase values, we are able to substantially reduce the impact of SF thickness on the resulting impedance values while still maintaining a good correlation with functional measures. At 50 kHz, the phase has a strong correlation with the SF level ($R=-0.67$) that is even higher than that of the 6MWT. However, using the ratio brings the SF correlation down to non-significant levels ($R=-0.075$), while the correlation to the 6MWT actually increases. The 50/200 frequency ratio we have chosen to highlight here is not necessarily the best for all diseases or for all muscles, nor is it necessarily the most truly optimized of values. As can be seen in Figures 4, other ratios are also possible, giving even lower correlations to SF thickness but have the undesirable effect of slightly reducing the correlation to the 6MWT. The choice of 50/200 is thus as much for convenience as it is for accuracy for the muscle group average. In fact, single muscle data (Figure 5) shows some variability as to where the optimized values lie. For example, in quadriceps, a lower frequency choice may be more optimal than the 50/200 ratio utilized here. The reason for this variability is uncertain. But it is conceivable that one could apply individual ratios in specific muscles. Another uncertain aspect of these results is the odd oscillatory phenomenon present in the optimization graphs (Figures 4 and 5). It is

not clear why the R values should fluctuate in this seemingly orderly fashion across this frequency range.

We have chosen to focus on phase values in this analysis rather than the reactance or resistance measures, two other impedance measures, since phase has been the primary focus of much of our work (Rutkove, 2009). Preliminary studies of ratios of these other values are also promising and may be pursued in the future. In addition, our data here focus on impedance values obtained at 90 degrees relative to the long axis at the limbs—in other words the transverse impedance values. Much of our previous work has focused on the longitudinal values. This choice was made in part since we have observed in both our animal studies of mdx mice and our human data thus far, that the transverse values in DMD appear more robust (Li et al., 2013). However, we note that an analogous analysis of the 0 degree longitudinal values provides similar results in the DMD patients with the 50 kHz phase values giving $R = 0.49$ $P = 0.010$ for the 6MWT and $R = -0.69$ $P < 0.001$ for SF, respectively, and with the 50/200 ratio giving $R = 0.58$, $P = 0.0020$ for the 6 MWT and $R = -0.24$, $P = 0.015$ for SF thickness.

In this analysis, we have focused only on varying the denominator of the ratio while maintaining the 50 kHz values in the numerator. We have also evaluated the opposite approach: changing the numerator while keeping the denominator constant. This, however, provided no definite improvement over the 50 kHz data, and this additional analysis is not presented here.

Another aspect to using the phase ratio over a single 50 kHz phase value is that the ratio reduces the variation observed in values across a population of individuals. As can be seen in the final analyses presented in the results section, the ratio substantially reduced the coefficient of variation in phase values across a group of normal subjects, supporting the basic premise that a major contributor to population variation in EIM 50 kHz phase values in normal subjects is SF thickness rather than inherent differences in the muscle condition.

We have previously studied the use of a slope measure of the impedance curve as a means of collapsing the multifrequency measure into a single value (Rutkove et al., 2010). The advantage of a slope measure over a simple ratio is that it encompasses many more points and thus is less likely to be subject to random errors or noise in single frequency data. In addition, studies using these measures have shown them to be more sensitive to muscle change than the single frequency measures (Rutkove et al., 2010, Wang et al., 2011b). The major disadvantage, however, is that the slopes of the impedance curves tend to be very small and straddle zero, gradually shifting from negative to positive values with progressive disease. This characteristic makes them challenging to analyze and especially sensitive to noise. In addition, the fact that various impedance-measuring devices do not measure values at identical intervals means that the slope may be weighted toward lower frequency values where a denser set of measurements is typically obtained. Also of note, there is precedence for this approach, as others have used simple ratios to serve as multifrequency measures, although not for the purpose of reducing the impact of SF on measurements (Rigaud et al., 1994).

Perhaps the most important question is why should this technique be effective? As noted earlier, the inherent electrical material properties of fat and muscle are distinct (Gabriel et al., 1996a, Gabriel et al., 1996b), and this was confirmed in our simple experiment measuring the impedance characteristics of excised animal tissues. Fat contributes similarly to the measured data at both 50 and 200 kHz, whereas muscle is mainly contributing at 50 kHz and only minimally at 200 kHz. Thus by taking a ratio, it is possible to cancel out the effect of fat. Clearly, further study of this complex topic is required to fully understand the effects both in healthy individuals and those with underlying neuromuscular disease.

While our intent here was to reduce the impact of SF on the EIM measures, in point of fact, this technique is likely reducing the effect of all fat, including intramuscular fat. Whereas intramuscular fat could be considered a confounder in certain situations, such as when obesity is present in the setting a neuromuscular disease, fatty infiltration is a common finding in many chronic neuromuscular conditions, including DMD. Thus, measuring fat content may be an important aspect of EIM measurement in such conditions. Yet in this evaluation, it appears its impact is likely less important than that of the SF thickness since the 6MWT correlation improved with the application of the ratio measure.

It is worth considering that such effects of SF on measurements are not unique to the EIM technique. Indeed, several studies have suggested that the thickness of the SF and overlying skin impacts standard nerve conduction parameters as well, including compound motor action potential and sensory nerve action potential amplitude values (Buschbacher, 1998, Cinar et al., 2013, Fujimaki et al., 2009). As the distance between the depolarizing muscle and the recording electrodes increases due to increasing SF skin thickness or edema, the tissue acts as a low pass filter, reducing the magnitude of the measured depolarization. However, such an effect is generally smaller than those observed with EIM since mainly distal extremity muscles/nerves are evaluated in which the fat and skin thickness variability is far less than in the more proximal muscles being measured here.

One of the main criticisms of EIM as a biomarker is that it is challenging to define exactly what the technique is measuring. As discussed elsewhere (Rutkove, 2009), unlike standard needle electromyography, EIM does not assess the electrical stability of the muscle membrane nor does it provide an indirect assessment of the motor unit morphology/size or number present. Rather it provides a quantification of muscle fiber size, structure and overall composition. However, extracting the true muscle properties from the overall tissue impedance remains a challenge. From this work, it appears that the frequency dependence can be exploited to help extract a muscle-specific signal and thus improve its overall value. Our results here show that taking the ratio of two different frequencies is a highly effective way to minimize the effect of the SF on the EIM data, providing results that are more closely reflective of the underlying muscle quality, as demonstrated by functional measures. The application of this and similar frequency-optimized EIM indices to the evaluation of other neuromuscular disease should be studied in future clinical trials.

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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.

Figure 1.

Impedance phase data from excised rat muscle and pig fat obtained from freshly killed animals, demonstrating the distinctly different impedance properties of the two tissues types. The data is plotted on a logarithmic scale.

Figure 2.

Raw EIM phase data verses frequency for DMD (black) and healthy (gray). Note that the frequency data is plotted on a logarithmic scale. The 50 kHz data is identified as the standard frequency at which measurements are made.

Figure 3. Pre-Optimization

Average 6-muscle EIM Phase Data in DMD boys at 50kHz versus A. Six minute walk distance. B. Subcutaneous skin fat thickness. Since only a subset of 13 boys underwent the 6MWT as compared to 28 who underwent SF measurement, the number of points is not equivalent in these two graphs.

Figure 4.

Optimization curves for the 6-muscle average EIM phase data. The lines represent correlation coefficients of phase versus six minute walk (black), and phase versus SF thickness (gray) at: A. Correlations of raw EIM phase data from 20 to 500kHz. B. Correlations of EIM phase ratio as a function of frequency. In this analysis, the numerator of the ratio is held constant with the 50 kHz phase data for each subject, while the denominator is varied with EIM phase data ranging from 20–500 kHz for each subject. Note the marked noise in the region around 50–100 kHz in the R values for both correlations of the ratio, due to instability of relationships in this frequency range.

Figure 5.

Optimization curves for single muscle EIM phase data, analogous to the two plots shown in Figure 4. Black lines represent the correlation coefficient of phase to 6MWT and gray lines represent correlation coefficient of phase to SF thickness at: A–C. Correlations of raw EIM phase data from 20 to 500kHz. D–F. The phase ratio denominator frequencies. Note the variation in quadriceps as compared to some of the other muscles presented; the explanation for this is uncertain.

Figure 6. Post-Optimization

Average 6-muscle EIM 50kHz/200kHz phase ratio data in DMD boys. A. Six minute walk distance B. Subcutaneous skin fat thickness. Note the major reduction in correlation compared to the 50 kHz data shown in Figure 3. Again fewer points are present for 6MWT since only a subset of children underwent this testing.

Pre-optimization

Post-optimization

Figure 7. Pre- and post-optimization data on the healthy boys

A total of 24 healthy boys underwent measurements with a subset of 20 subjects having the 6MWT. The correlation to SF thickness dropped markedly while the correlation to 6MWT remained virtually unchanged.