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Inter-session reliability of electrical impedance myography in children in a clinical trial setting

Tom R. Geisbush, BA¹, Nicole Visyak, BA², Lavanya Madabusi, BA², Seward B. Rutkove, MD¹, and Basil T. Darras, MD²

¹Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

²Department of Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

Abstract

Objective—High reliability is a prerequisite for any test to be useful as a biomarker in a clinical trial. Here we assessed the reproducibility of electrical impedance myography (EIM) in children by comparing data obtained by different evaluators on separate days.

Methods—Healthy boys and boys with Duchenne muscular dystrophy (DMD) aged 2-14 years underwent EIM of multiple muscles performed by two evaluators on two visits separated by 3-7 days. Single and multifrequency data were analyzed. Reliability was assessed via calculation of the percent relative standard deviation (% RSD), Bland-Altman analysis, and the intraclass correlation coefficient (ICC).

Results—For both individual muscle data and data averaged across muscles, intra-evaluator measurements showed high repeatability for both 50 kHz phase and 50/200 kHz phase ratio values, with ICCs generally above 0.90 and % RSD below 10%. Inter-evaluator results showed very similar ICC and % RSD values as those obtained by the same evaluator.

Conclusions—Both the 50 kHz phase and 50/200kHz phase ratio are reliable measures both across time and evaluators and in both health and disease.

Significance—These results support the concept that EIM can serve as a reliable measure in clinical therapeutic trials in a pediatric population.

Please address correspondence to: Seward B. Rutkove, MD, Professor of Neurology Harvard Medical School, 330 Brookline Avenue, TCC-810, Boston, MA 02215, USA srutkove@bidmc.harvard.edu.

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Conflict of Interest

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.

None of the other authors have any potential conflicts of interest to be disclosed.

Keywords

Electrical impedance myography; reliability measures; Duchenne muscular dystrophy

INTRODUCTION

Better measures that can serve as reliable biomarkers of disease severity and drug effect in neuromuscular disease clinical trials are needed. For example, recent and ongoing clinical therapeutic trials in Duchenne muscular dystrophy (DMD) have relied on the 6-minute walk test or muscle biopsy as the major outcome measures of drug efficacy (Finkel et al. 2013; McDonald et al. 2013). However, such measures are limited for many reasons. The 6 minute walk test can be utilized in only a subset of boys - namely those who are ambulatory above the age of 5 years, requires considerable training for investigators to perform well, and has sufficient variability to have negatively impacted the results of at least two recent clinical trials (Hoffman and Connor 2013). Although muscle biopsy for dystrophin staining has also been used especially in early-stage trials, it is impractical in larger trials and its relationship to functional improvement remains uncertain.

Electrical impedance myography (EIM) is a measure that is showing promise for the quantification of neuromuscular disease severity (Rutkove 2009). In EIM, a weak, electrical current at multiple high frequencies (generally over 10 kHz) is applied to a localized area of tissue and the consequent surface voltages are measured. From these voltages, the complex impedance can be calculated, which includes the reactance (X), the resistance (R) from which the major outcome variable phase (θ) is derived via the trigonometric relationship:

$$\theta = \tan^{-1} \left(\frac{X}{R} \right).$$

To date, EIM parameters have proven to be very sensitive to disease status in a variety of disorders including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, and DMD (Tarulli et al. 2009; Rutkove et al. 2010; Rutkove et al. 2014). The alterations in EIM values are due to a variety of factors, including changes in muscle fiber size, organization, and the development of increasing connective tissue and intramuscular fat (Ahad et al. 2009; Li et al. 2014a). Recently, work has also shown that EIM is very sensitive to disease status in the muscular dystrophy (mdx) mouse, showing substantial differences compared to wild type animals at even young age (Li et al. 2014a). Importantly, EIM is very different from whole body bio-impedance techniques, which are mainly geared to the assessment of body composition, and thus provide no information on individual muscles or body regions. Also, unlike whole body bioimpedance analysis, EIM is relatively insensitive to hydration status (Li et al. 2014b) and can provide anisotropic (directional dependent) data on muscle condition (Garmirian et al. 2009).

Given the potential value of EIM for assessing neuromuscular disease, we recently initiated a longitudinal study assessing EIM changes over time in children with DMD and their relationship to standard functional measures. Indeed, early cross-sectional analysis of that baseline data has shown important correlations between function, age, and EIM data (Rutkove et al. 2014). However, before evaluating the value of these measures in assessing

disease progression, it is critical that we establish the reliability of the measures in children. In this study, we describe a detailed analysis of the reliability of the technique in both healthy boys and those with DMD by comparing data obtained several days apart by the same and different evaluators.

METHODS

Boston Children's Hospital Institutional Review Board approved the protocol, and parents and children provided written consent and verbal assent, respectively. Both healthy boys and those with DMD aged 2 years to 14 years were recruited into the study through the neuromuscular clinic at Boston Children's Hospital with the plan to follow them for up to 2 years. Subjects were excluded if they had a pacemaker or other implanted electrical device. All subjects with DMD had genetic confirmation of disease or had a consistent history and were a brother of a family member with a genetically confirmed diagnosis. DMD subjects were excluded if they were involved in an ongoing clinical trial (outside of a natural history study) or if they had a concomitant neuromuscular or another medical condition that substantially impacted health. Healthy subjects had no history of neuromuscular disease or other disorder that would substantially impact muscle health and were recruited via advertisement and word-of-mouth. For all subjects, the analysis presented here comes from data obtained at the baseline visit as well as at a second visit designed specifically to assess the technique's reproducibility, three to seven days after the initial visit.

EIM Measurements

EIM measurements were obtained with the Imp SFB7 (Impedimed, Inc, Sydney Australia), using a custom hand-held array previously described (Narayanaswami et al. 2012). Three different probe sizes were used depending on the child's size (small: 4×1.5 cm, medium: 5×2 cm, large: 7×2.5 cm). Unilateral measurements were performed on 6 muscles/muscle groups on the dominant side: deltoid, biceps brachii, forearm flexors compartment, quadriceps (rectus femoris), tibialis anterior, and medial gastrocnemius. Placement of the probe was established by measuring the distance between anatomical landmarks that coincide with the connections of tendon to bone for each muscle. EIM measurements were performed both longitudinally and transversely to the major muscle fiber direction.

Importantly, all 6 muscles were measured by the same evaluator on the two separate visits to obtain intra-rater reliability; however, in the interest of time, only 2 muscles, biceps brachii and quadriceps, were evaluated by the second evaluator. Thus, only 2-muscle comparisons were available for inter-rater reliability.

Evaluators

The evaluators, all of whom were research assistants with no previous training in radiological or electrophysiological testing, were taught how to perform the EIM measurements by one of the senior authors. They were shown where to place the probes and what data was technically sound based on the appearance of the multi-frequency resistance and reactance curves on the device's screen (e.g., absence of low frequency artifacts due to poor electrode contact or negative values). No ongoing oversight was provided outside of

this basic training, so as to be consistent with what would occur typically in a clinical trial in which this test was being utilized.

EIM procedure

For each measurement, children were placed in a sitting position with their arms and legs resting at a 90° angle at the elbow and knee. The skin was moistened with saline and the probe placed over the muscle of interest. Repeated measurements were made for all muscles at both the initial visit and at a second visit 3 - 7 days later by the same evaluator. For the biceps brachii and quadriceps only, a second evaluator made measurements as well on both days. An example of the testing being performed on a boy with DMD is shown in Figure 1.

Data Analysis

For both longitudinal and transverse measurements, the 50 kHz phase and recently developed 50/200 phase ratio (Schwartz et al. 2014) were averaged across all six muscles measured. This averaging of data across muscles is a means of compiling data with the goal of creating a robust composite score with high clinical significance. In addition, separate upper and lower extremity averages and a 2-muscle average (of just biceps brachii and quadriceps) were also included. Finally, data from each of the individual muscles studied was analyzed. Statistical procedures were performed using R statistical software and MATLAB (Mathworks, Natick, MA). For each measurement, the intra-class correlation coefficient (ICC) for the degree of absolute agreement among measurements and the percent relative standard deviation (% RSD) between the measurements were calculated (McGraw and Wong 1996). Unlike the simpler relative error (ie percent variability), the %RSD corrects for differences in mean values for different measures. In other words, it allows for the 50 kHz phase and 50/200 kHz repeatability values to be compared directly to one another. In addition, the propagation of uncertainty was calculated to account for the variability of the % RSD and is reported as the %RSD $\pm$ the propagation of uncertainty (Oberkamp et al. 2002). Finally, Bland-Altman plots were also created to provide additional visual assessment of repeatability. If a single muscle data point was missing for a given subject, the 6- or 2-muscle average to which that data point contributed for that subject was removed from the analysis.

RESULTS

Subjects

A total of 22 healthy boys and 14 boys with DMD and underwent repeated measurements 3 - 7 days after the first measurement. The age ranges for the DMD and healthy groups were 2.2 - 13.2 and 2.1 - 12.4 years, respectively. The mean age $\pm$ the standard deviations were 7.7 ± 3.0 for the DMD group and 7.1 ± 3.2 for the healthy group.

Intra-evaluator reliability of 6-muscle average values

The detailed data is presented in Table 1, and the ICC and Bland-Altman analyses presented in Figures 2 and 3, respectively. As anticipated, due to a small dynamic range of values for the 50/200 kHz data, ICC values were somewhat lower for that measure as compared to the 50 kHz data (e.g., Transverse 6-Muscle Phase 50/200 kHz ICC = 0.88, Transverse 6-Muscle

Phase 50 kHz ICC = 0.96). In contrast to the interpretation of the ICC values, the % RSD values of the 50/200 kHz data were consistently 2 to 3% lower than their 50 kHz counterparts (e.g., Transverse 6-Muscle Phase 50/200 kHz % RSD = 3.1 ± 2.5 , Transverse 6-Muscle Phase 50 kHz % RSD = 5.9 ± 3.4). Also of note, both ICC and % RSD values of longitudinal measurements performed slightly better than the transverse measurements (e.g., Longitudinal 6-Muscle Phase 50 kHz % RSD = 0.98 and 3.8 ± 3.0 , Transverse, 6-Muscle Phase 50 kHz ICC and % RSD = 0.96 and 5.9 ± 3.4 , respectively).

Intra- and inter-evaluator reliability of 2-muscle average values

Table 2 and Figure 4 include phase data both between intra- and inter-evaluator measurements of a 2-muscle average of the transverse measurements, made up of biceps brachii and quadriceps data. (Reactance and resistance data followed similarly, and are omitted in the interest of brevity.) Despite the use of only 2-muscles to create the averaged data, intra-evaluator variability remained almost identical to the 6-muscle averages shown earlier (e.g., Transverse 6-Muscle 50 kHz ICC and % RSD = 0.96 and 5.9 ± 3.4 , Intra-evaluator Transverse 2-Muscle 50 kHz ICC and % RSD = 0.95 and 7.8 ± 5.5). Moreover, the ICC and % RSD values also exhibited virtually no difference in variability whether being performed by the same or different evaluators (e.g., Intra-evaluator Transverse 2-Muscle 50 kHz ICC and % RSD = 0.95 and 7.8 ± 5.5 , Inter-evaluator Transverse 2-Muscle 50 kHz ICC and % RSD = 0.92 and 7.7 ± 5.5). Consistent with our analysis of the 6-muscle averaged data, the longitudinal measures also appeared to be more consistent than the transverse values.

Single muscle reliability

Whereas our main focus in this work is on average muscle data, it is still helpful to understand the reliability of single muscle data sets. Thus we also analyzed inter-rater reliability of quadriceps and biceps data and intra-rater of all the individual muscles, as presented in Table 3. Again, these data exhibited similar trends in their reliability to the averaged data measures. Intra-evaluator data appeared similar to inter-evaluator data (e.g., Intra-evaluator Longitudinal Biceps 50 kHz ICC and % RSD = 0.95 and 6.1 ± 6.4 , Inter-evaluator Longitudinal Biceps 50 kHz ICC and % RSD = 0.95 and 6.8 ± 5.7). The reliability of longitudinal data versus transverse data was more pronounced (e.g., Intra-evaluator Longitudinal Biceps 50 kHz ICC and % RSD = 0.95 and 6.1 ± 6.4 , Intra-evaluator Transverse Biceps 50 kHz ICC and % RSD = 0.87 and 10.9 ± 9.5). At the individual muscle level, many of the trends observed in the averaged measurement data were further confirmed including a decrease in ICC values from the 50 kHz data to the 50/200 kHz data while the 50/200 kHz data results in better (lower) % RSD values.

DMD vs. Healthy Reliability Assessment

In addition to comparing values across both patient and healthy controls together, we also separated out the groups to evaluate how the data compared between the two groups (Table 4, again showing phase data only). As can be seen, the intra- and inter- evaluator reliability was fairly similar across both groups. Again, longitudinal and 50/200 kHz ratio data showed somewhat better repeatability (as assessed by % RSD) than the transverse and 50 kHz data, respectively.

DISCUSSION

These results confirm that EIM demonstrates excellent reliability across visits separated by several days and performed by different evaluators, with errors, as measured by % RSD, well below 10% in most cases and even below 5% in some instances. The levels of repeatability are similar or surpass those for typical functional measures used in DMD clinical trials, such as the 6 minute walk test (McDonald et al. 2010; McDonald et al. 2013). One somewhat surprising aspect of the analysis performed here, is the minimal difference in variability between inter- and intra-evaluator measures. We had anticipated that measures between different evaluators might show greater variation than those from a single evaluator, but this was not the case. The absence of such an effect is crucial for the accurate completion of clinical trials since changes or unexpected unavailability of personnel to perform testing occurs commonly.

In addition, EIM measurements obtained longitudinally with respect to muscle fiber direction exhibit greater reliability than those taken transversely. The explanation for this difference is unclear, but it may be due to the fact that small variations in the relative angle of placement at 90 degrees (transversely) impact the measures more than similar variations at 0 degrees. It is also possible that it is simply more difficult to align the probe transversely with the muscle than it is longitudinally. The reason that we sought to compare both of these directions is that our early data both in humans (Rutkove et al. 2014) and in animals (Li et al. 2014a) suggests that the transverse direction may be slightly more sensitive to DMD abnormalities. Thus, the fact that longitudinal data may be more consistent implies that we will need to continue to perform measurements in both directions going forward since it remains unclear as to which position will be ultimately most valuable.

In this study we chose to do a “real world” analysis of repeatability by doing measurements separated by several days. This is in contrast to the typical, less-desirable approach of most reliability studies in which all data is obtained on one day; this is not uncommon and our group has also taken this liberty on occasion (Narayanaswami et al. 2012; Zaidman et al. 2014). Advantages of evaluating inter-session reliability (ie several days apart) is that not only do we assess intrinsic variation to the testing itself (i.e. inconsistent placement of electrode array, variation in position of the subject), but other physiological factors may also come into play, including alterations in temperature, hydration status, and previous activity, all of which have the potential for impacting the impedance data to some extent. Thus, the high reliability observed here helps confirm that the measures are stable both from an experimental (i.e. data acquisition) standpoint as well as a physiological one.

In these analyses, we also chose to focus on 6- and 2- muscle averages rather than individual muscle data. While previous studies have suggested high reliability of individual muscle data in other disorders using EIM (Rutkove et al. 2006, 2012) averaging groups of muscles in a generalized disease will ultimately yield more stable data sets and is more clinically meaningful than measuring just a single muscle in a generalized disease such as DMD. Nonetheless, single muscle repeatability is also very high, with variability almost matching that of the 6- and 2- muscle average data.

There is no single best measure of reliability and thus we have chosen to report several. While many studies use the ICC and some also Bland-Altman analyses, we also employed a calculation of the % RSD. The problem with interpretation of ICCs is that they are very much subject to data distribution width. Data with a very narrow distribution will tend to have low (i.e., poor) ICC values simply because the data is not very dispersed—thus misleadingly suggesting that the test has low reliability. In contrast, studies such as this one, which incorporates both healthy and disease data simultaneously, often will have especially high ICC values. However, this improvement of ICC values can also be an artifact of combining two well-separated groups. But, the advantage of the ICC is that it provides a quick visual assessment of repeatability. Like the ICC, the Bland-Altman analysis also provides a clear visualization of repeatability. The limitation of the Bland-Altman analysis is the absence of a simple unit-independent numerical metric that captures the repeatability. A third favored metric is simply the relative error, or percent variability, calculated as $100 \times \frac{\text{absolute value} ((\text{Measurement 2} - \text{Measurement 1}) / (\text{Mean of Measurements 1 and 2}))}{\text{absolute value} ((\text{Measurement 2} - \text{Measurement 1}) / (\text{Mean of Measurements 1 and 2}))}$. Unfortunately, the resulting percentage can be misleading if two different types of data are being compared. (One simple example is to compare the relative error using identical temperatures, with one measured in Fahrenheit and one measured in Celsius.) Thus here, we chose a variation of the relative error, the % RSD, since this value accounts for different units and allows the 50 kHz data and 50/200 kHz ratio data to be fairly compared with one another.

The focus of this study was on repeatability of the technique and not on more basic mechanistic questions as to why EIM detects differences in healthy boys versus those with DMD. Nonetheless, it is worth pointing out that EIM's ability to detect disease change in DMD likely relates to reduced muscle fiber diameter and presence of increased connective tissue and fat within the muscle tissue (Li et al. 2014). This is in contrast to ALS, another disease where EIM has been applied. In ALS, the changes likely relate to marked reductions in cell size that accompanies that disease.

This study has several limitations worth highlighting. First, we used 3 different probe sizes depending on the age/size of the patient and we have not analyzed the effect of probe size on the reproducibility, since individual numbers would be very small. Another limitation of the current analysis is that inter-evaluator measurements were limited to only 2 muscles rather than 6. Finally, our group of DMD patients is relatively small ($N = 13$). Unfortunately, it was very challenging for many of these families of children enrolled in the study to return after just a few days since they were traveling from substantial distances. Furthermore, we have chosen to forego analysis of the muscle anisotropy variability (transverse measurement minus the longitudinal measurement for a muscle) in this analysis. It may be beneficial in the future to investigate the variability of anisotropic variation as it is theorized that the increase in fat and connective tissue during muscle deterioration should reduce the relative anisotropy.

In summary, our study shows that EIM gives reliable data both across study visits and between individual evaluators. Such information helps to establish that EIM has the potential to track disease progression during clinical trials with good sensitivity. Completion

of our longitudinal natural history data collection and its analysis will ultimately clarify EIM's value as a biomarker in clinical therapeutic trials in DMD.

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Highlights

- Electrical impedance myography (EIM) is highly reliable in children even when measurements are made days apart and by different evaluators.
- EIM reliability is high whether evaluating single muscles or multiple muscles in combination.
- The reliability of EIM in healthy children and those with Duchenne muscular dystrophy is similar.

Figure 1.
EIM being performed on the biceps of a young boy with Duchenne muscular dystrophy. The array is placed longitudinally along the muscle. The connectors/wires at the end of the tube attach to the impedance-measuring device (not shown).

Figure 2.

Intra-class correlation plots of 6-muscle average transverse measurements in DMD (circle) and healthy (triangle) subjects taken at baseline and 3 – 7 days later by the same evaluator. The top row contains plots of 50 kHz data (closed icons). The bottom row contains 50/200 kHz ratio data (open icons).

Figure 3.

Bland-Altman plots of the 6-muscle average transverse measurements in DMD (circle) and healthy (triangle) subjects taken at baseline and 3 – 7 days later by the same evaluator. The top row contains plots of 50 kHz data (closed icons). The bottom row contains 50/200 kHz ratio data (open icons).

Figure 4.

ICC and Bland-Altman plots of the 2-muscle average transverse measurements in DMD (circle) and healthy (triangle) subjects taken at baseline by the same (intra-) evaluator and between two different (inter-) evaluators. The left columns contain plots of 50 kHz data (closed icons). The right columns contain 50/200 kHz ratio data (open icons).

Table 1a

Intra-evaluator reliability data of 50 kHz 6-muscle averages and upper and lower extremity averages.

	50 kHz						
	Phase		Reactance		Resistance		
	ICC	% RSD	ICC	% RSD	ICC	% RSD	N
Trans. 6-Muscle	0.96	5.9 ± 3.4	0.93	6.2 ± 4.7	0.98	3.7 ± 2.6	28
Long. 6-Muscle	0.98	3.8 ± 3.0	0.96	5.3 ± 3.3	0.98	3.4 ± 1.8	31
Trans. Upper Extremity	0.96	6.8 ± 4.5	0.93	8.0 ± 5.5	0.96	4.6 ± 2.8	31
Long. Upper Extremity	0.98	4.3 ± 3.4	0.96	5.4 ± 3.4	0.98	3.2 ± 2.5	29
Trans. Lower Extremity	0.94	6.6 ± 4.9	0.89	7.0 ± 5.6	0.95	5.3 ± 3.5	32
Long. Lower Extremity	0.96	5.8 ± 4.4	0.86	8.2 ± 5.7	0.96	5.0 ± 3.9	33

Table 1b

Intra-evaluator reliability data of 50/200 kHz ratio 6-muscle averages and upper and lower extremity averages.

	50/200 kHz						
	Phase		Reactance		Resistance		N
	ICC	% RSD	ICC	% RSD	ICC	% RSD	
Trans. 6-Muscle	0.88	3.1 ± 2.5	0.92	3.6 ± 2.7	0.97	0.9 ± 1.0	28
Long. 6-Muscle	0.93	2.5 ± 1.9	0.96	2.8 ± 2.0	0.99	0.6 ± 0.5	31
Trans. Upper Extremity	0.80	3.8 ± 3.8	0.89	4.4 ± 3.8	0.98	1.1 ± 0.9	31
Long. Upper Extremity	0.90	3.1 ± 2.2	0.94	3.3 ± 2.3	0.98	0.8 ± 0.7	29
Trans. Lower Extremity	0.89	3.1 ± 2.7	0.91	3.9 ± 2.6	0.94	1.2 ± 1.1	32
Long. Lower Extremity	0.88	3.2 ± 2.8	0.92	3.5 ± 2.9	0.97	0.8 ± 0.7	33

Table 2

Intra- and inter-evaluator reliability data of 2-muscle averages.

50 kHz Phase						
	Intra-Evaluator			Inter-Evaluator		
	N	ICC	% RSD	N	ICC	% RSD
Trans. 2-Muscle	32	0.95	7.8 ± 5.5	31	0.92	7.7 ± 5.5
Long. 2-Muscle	33	0.97	4.8 ± 4.1	32	0.96	5.5 ± 4.0

50/200 kHz Phase						
	Intra-Evaluator			Inter-Evaluator		
	N	ICC	% RSD	N	ICC	% RSD
Trans. 2-Muscle	32	0.96	3.5 ± 2.5	31	0.95	3.0 ± 2.5
Long. 2-Muscle	33	0.97	3.1 ± 2.6	32	0.96	3.1 ± 2.4

Table 3a

Single muscle comparisons for 50 kHz data.

	50 kHz						N
	Phase		Reactance		Resistance		
	ICC	% RSD	ICC	% RSD	ICC	%RSD	
Intra-evaluator							
Long. Biceps	0.95	6.1 ± 6.4	0.95	5.9 ± 4.9	0.98	4.1 ± 3.4	33
Long. Quadriceps	0.95	5.7 ± 4.7	0.82	8.8 ± 8.8	0.94	5.3 ± 4.7	34
Long. Forearms Flexors	0.95	7.3 ± 5.4	0.94	6.1 ± 5.5	0.95	5.1 ± 3.8	33
Long. Tibialis Anterior	0.93	7.6 ± 6.2	0.73	9.5 ± 10.1	0.95	6.0 ± 6.0	34
Long. Deltoid	0.95	6.9 ± 6.1	0.90	7.9 ± 7.5	0.96	5.0 ± 4.9	34
Long. Medial Gastroc	0.93	9.0 ± 6.0	0.79	10.8 ± 7.9	0.94	6.6 ± 6.0	34
Trans. Biceps	0.87	10.9 ± 9.1	0.85	11.3 ± 7.5	0.92	6.9 ± 4.0	32
Trans. Quadriceps	0.95	6.0 ± 6.6	0.87	8.4 ± 7.9	0.90	6.3 ± 5.0	34
Trans. Forearms Flexors	0.96	6.2 ± 6.2	0.91	8.2 ± 7.6	0.94	4.9 ± 3.8	32
Trans. Tibialis Anterior	0.85	8.5 ± 11.5	0.73	9.8 ± 11.2	0.97	4.3 ± 3.1	33
Trans. Deltoid	0.94	7.5 ± 7.5	0.91	8.6 ± 6.4	0.93	5.6 ± 5.1	32
Trans. Medial Gastroc	0.90	9.2 ± 7.1	0.82	9.9 ± 8.0	0.90	7.8 ± 6.4	33
Inter-evaluator							
Long. Biceps	0.95	6.8 ± 5.7	0.94	5.9 ± 4.8	0.95	6.0 ± 5.3	33
Long. Quadriceps	0.93	6.8 ± 6.6	0.90	10.2 ± 9.5	0.93	6.4 ± 4.8	33
Trans. Biceps	0.90	9.8 ± 8.3	0.84	9.1 ± 6.8	0.84	8.4 ± 6.8	32
Trans. Quadriceps	0.94	7.7 ± 5.7	0.83	10.2 ± 8.0	0.89	7.5 ± 5.0	33

Table 3b

Single muscle comparisons for 50/200 ratio data.

	50/200 kHz						N
	Phase		Reactance		Resistance		
	ICC	% RSD	ICC	% RSD	ICC	% RSD	
Intra-evaluator							
Long. Biceps	0.85	4.2 ± 3.3	0.91	4.3 ± 3.5	0.95	1.4 ± 1.3	33
Long. Quadriceps	0.85	3.9 ± 3.3	0.89	4.1 ± 3.4	0.97	0.6 ± 0.4	34
Long. Forearms Flexors	0.72	4.1 ± 4.3	0.85	4.7 ± 4.3	0.96	1.3 ± 1.1	33
Long. Tibialis Anterior	0.81	3.9 ± 3.2	0.88	4.2 ± 3.3	0.94	1.3 ± 1.2	34
Long. Deltoid	0.79	4.5 ± 4.5	0.86	5.0 ± 4.9	0.96	1.0 ± 1.0	34
Long. Medial Gastroc	0.78	6.1 ± 4.9	0.86	6.4 ± 5.0	0.95	1.4 ± 1.1	34
Trans. Biceps	0.75	5.5 ± 4.6	0.78	6.9 ± 5.3	0.93	1.9 ± 1.6	32
Trans. Quadriceps	0.87	3.4 ± 3.6	0.90	3.7 ± 3.9	0.95	0.9 ± 0.7	34
Trans. Forearms Flexors	0.43	5.4 ± 5.6	0.75	5.1 ± 5.7	0.97	1.4 ± 1.2	32
Trans. Tibialis Anterior	0.68	6.4 ± 4.7	0.71	6.8 ± 5.6	0.92	1.5 ± 1.9	33
Trans. Deltoid	0.81	5.0 ± 4.5	0.87	5.6 ± 4.7	0.95	1.3 ± 1.2	32
Trans. Medial Gastroc	0.91	4.3 ± 2.8	0.90	4.7 ± 4.0	0.90	2.1 ± 1.5	33
Inter-evaluator							
Long. Biceps	0.85	4.5 ± 3.0	0.91	4.8 ± 3.5	0.95	1.6 ± 1.2	33
Long. Quadriceps	0.85	3.7 ± 3.5	0.89	3.7 ± 3.5	0.97	0.8 ± 0.9	33
Trans. Biceps	0.85	9.6 ± 4.4	0.78	9.0 ± 4.5	0.93	1.6 ± 1.4	32
Trans. Quadriceps	0.85	5.0 ± 3.8	0.90	5.0 ± 4.0	0.95	1.5 ± 0.9	33

Table 4a

50 kHz phase data separated for DMD and healthy boys.

A. 50 kHz	Healthy	Healthy	Healthy	DMD	DMD	DMD
Intra-evaluator	N	% RSD	ICC	N	% RSD	ICC
Long. 6-Muscle	19	3.9 ± 2.9	0.95	12	3.4 ± 2.6	0.99
Long. 2-Muscle	21	4.5 ± 3.7	0.92	12	6.2 ± 5.4	0.94
Trans. 6-Muscle	17	6.0 ± 3.0	0.90	11	5.3 ± 4.4	0.97
Trans. 2-Muscle	20	6.9 ± 5.0	0.79	12	10.7 ± 6.1	0.90
Inter-evaluator						
Long. 2-Muscle	20	5.1 ± 3.8	0.91	12	7.1 ± 3.8	0.95
Trans. 2-Muscle	19	6.9 ± 4.8	0.82	12	10.3 ± 7.2	0.89

Table 4b

50/200 kHz phase ratio data separated for DMD and healthy boys.

B. 50/200 Ratio	Healthy	Healthy	Healthy	DMD	DMD	DMD
Intra-evaluator	N	% RSD	ICC	N	% RSD	ICC
Long. 6-Muscle	19	2.2 ± 1.8	0.92	12	3.0 ± 1.8	0.91
Long. 2-Muscle	21	2.9 ± 1.9	0.90	12	3.6 ± 3.6	0.81
Trans. 6-Muscle	17	2.4 ± 1.7	0.93	11	4.2 ± 3.3	0.81
Trans. 2-Muscle	20	3.0 ± 1.7	0.93	12	4.5 ± 3.4	0.78
Inter-evaluator						
Long. 2-Muscle	20	2.9 ± 2.1	0.89	12	3.5 ± 3.0	0.85
Trans. 2-Muscle	19	2.7 ± 2.2	0.93	12	3.6 ± 3.1	0.87