ELECTRICAL IMPEDANCE MYOGRAPHY IN DUCHENNE MUSCULAR DYSTROPHY AND HEALTHY CONTROLS: A MULTICENTER STUDY OF RELIABILITY AND VALIDITY

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ABSTRACT: *Introduction:* Electrical impedance myography (EIM) is a non-invasive, painless, objective technique to quantify muscle pathology. *Methods:* We measured EIM in 8 arm and leg muscles in 61 boys with Duchenne muscular dystrophy (DMD) and 31 healthy boys, ages 3–12 years, at 5 centers. We determined the reliability of EIM and compared results in boys with DMD to controls and to 6-minute walk distance (6MWD), North Star Ambulatory Assessment (NSAA), timed functional tests (TFTs), and strength (hand-held dynamometry). *Results:* EIM was well tolerated and had good inter- and intrarater reliability (intraclass correlation coefficient 0.81–0.96). The averaged EIM phase value from all muscles was higher ($P < 0.001$) in controls (10.45 ± 2.29) than boys with DMD (7.31 ± 2.23), and correlated ($P \leq 0.001$) with 6MWD ($r = 0.55$), NSAA ($r = 0.66$), TFTs ($r = -0.56$), and strength ($r = 0.44$). *Conclusion:* EIM is a reliable and valid measure of disease severity in DMD. Longitudinal studies comparing EIM with other assessments over time in DMD are warranted.

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Additional Supporting Information may be found in the online version of this article.

Abbreviations: 6MWD, 6-minute walk distance; ALS, amyotrophic lateral sclerosis; DMD, Duchenne muscular dystrophy; EIM, electrical impedance myography; ICC, intraclass correlation coefficient; MRC, Medical Research Council; NSAA, North Star Ambulatory Assessment; SMA, spinal muscular atrophy

Key words: biomarker; children; Duchenne muscular dystrophy; electrical impedance myography; myopathy

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*See Appendix for list of clinical evaluator participants.

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Quantifying change in slowly progressive neuromuscular disorders, such as Duchenne muscular dystrophy (DMD), requires that assessments be highly reliable and sensitive to pathology in order to capture an effect. Functional outcomes such as the 6-minute walk distance (6MWD),[1] North Star Ambulatory Assessment (NSAA),[2] and manual muscle testing using Medical Research Council (MRC) testing[3] are useful measures of disease progression in boys with DMD in whom they can be performed; however, in non-ambulatory and severely weak older boys and young men, they are either impractical or suffer from floor effects.[4] Furthermore, despite disease progression, young children with DMD initially gain function and strength as they develop, although not at the same pace as their healthy peers.[5,6] These initial gains with development complicate longitudinal assessments of therapeutic efficacy and require that gains over time be interpreted relative to age-specific norms. Such norm-based testing can detect deviations from expected performance in boys with DMD who are <3 years of age.[6]

Electrical impedance myography (EIM) is a painless, non-invasive technique that can rapidly and quantitatively assess children and adults with suspected neuromuscular disease regardless of age or ability at the bedside.[7] In EIM, a high-frequency, low-power electrical current is passed through a local volume of body tissue, and the resulting surface voltages are measured on the skin.[7] Variations in the structure and composition of the underlying soft tissue alter the flow of electrical current and, consequently, the voltage signals measured at the skin surface. By taking the ratio of the measured voltage and applied current, tissue impedance can be calculated to reveal properties

of the underlying tissue. By further altering properties of the applied current, such as its frequency or direction, and by measuring different properties of the electrical signal, including its resistance and reactance, a variety of different parameters can be ascertained and optimized to selected disease characteristics. One regularly reported EIM parameter, phase, is the offset between the sinusoidal curves of the applied current and measured voltage. The phase angle expresses the relationship between the reactance and resistance of the underlying tissue.

EIM is altered from normal by a variety of neuromuscular pathologies in children and adults, including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and disuse atrophy.[8–10] EIM is also sensitive to alterations in dystrophic muscle in the muscular dystrophy (*mdx*) mouse model.[11] In this study, we investigated the feasibility and performance of EIM in healthy controls and boys with DMD. We determined the reliability of EIM when measured by multiple examiners from multiple sites, as would occur during a large clinical trial. We also investigated the construct validity of EIM by comparing assessments of strength and function to EIM measurements from healthy controls and boys with DMD. Finally, we demonstrated that, by selecting certain EIM parameters from the multiple results obtained, EIM can be optimized to demonstrate a particular feature, in this case correlation with 6MWD.

METHODS

The study protocol was approved by the institutional review board of each center. All subjects/guardians were willing and able to comply with the study protocol and evaluations and gave assent/written consent. All evaluators received training in the protocol and technique in a single-day session.

We enrolled 61 boys with DMD and 31 healthy male controls, aged 3–12 years. Subjects with DMD had either absent dystrophin on immunohistochemistry of muscle or a pathogenic mutation, physical symptoms consistent with DMD (proximal muscle weakness), ability to walk 10 meters unassisted, no daytime ventilator assistance, and no concurrent enrollment in a therapeutic trial. Of the 61 subjects with DMD, 52 were taking steroids. All control subjects had a normal neuromuscular evaluation.

Assessments of strength and function were performed at each site by an experienced and trained neuromuscular clinical evaluator (See Appendix). Strength and function tests were performed in both DMD and healthy boys and included handheld dynamometry of the deltoid, biceps brachii, triceps brachii, wrist flexors, quadriceps, tibialis anterior, and hamstrings; NSAA[2]; 6MWD[1]; and timed functional testing, including standing from supine, climbing 4 standard steps, and a 10-meter walk/run.[12]

EIM was performed using the EIM 1103 System (Skulpt, Inc., Boston, Massachusetts). After applying saline to the skin using a disposable, prepackaged wipe, EIM data were collected 3 times in immediate succession without moving the device. This was performed to ensure data quality and stability. The best of the 3 sets of data, as determined by automated selection of the lowest Cole–Cole plot (reactance vs. resistance) fit error,[13] was utilized in further analysis. This method automatically selects the data set with the least noise or jitter.

To measure intra- and interrater reliability, 2 raters performed repeated EIM measurements. Rater 1 and rater 2 each performed measurements separately over the unilateral deltoid, biceps brachii, triceps brachii, anterior forearm compartment, quadriceps, tibialis anterior, hamstring, and medial gastrocnemius muscles. For intrarater reliability, rater 1 then repeated the EIM measurements on a subset of muscles, excluding the triceps brachii and hamstring muscles to shorten the length of the study. Rater 1 also measured skin-fold thickness using calipers at each muscle site.

From the multifrequency data set obtained for each muscle, single-frequency, 53-kHz resistance, reactance, and phase parameters were extracted. This frequency was chosen for initial assessment, as it was the closest available frequency to 50 kHz, the standard single frequency usually assessed. Conservative outlier detection and removal was performed using standard deviation (SD)-based techniques to remove any spurious values due to faulty electrode contact or equipment malfunction.[14] This conservative approach removed only extreme values, likely resulting from technical malfunction. Subjects with more than half of all muscles judged to be outliers at 53 kHz were removed completely from analysis. In the remaining subjects, outlying values from individual muscle(s) were replaced by the second measurement on the same muscle (performed by rater 1). These replaced measures were excluded from reliability analysis.

We analyzed the EIM 53-kHz phase from individual muscles, and an average EIM score was computed for each subject from the average phase across all muscles studied. In addition to these single-frequency analyses, we also evaluated other EIM parameters, including data obtained at different frequencies, to determine whether such data correlated more strongly with an outcome of interest than the data obtained at only 53 kHz. To demonstrate this principle, we created an "optimized EIM score" from a subset of the multifrequency,

Table 1. Reliability of EIM phase at 53 kHz.

	DMD		Controls	
Muscle	Intrarater ICC	Interrater ICC	Intrarater ICC	Interrater ICC
Biceps brachii	0.94	0.94	0.94	0.92
Triceps brachii	NP	0.88	NP	0.81
Anterior forearm	0.97	0.95	0.97	0.96
Deltoid	0.93	0.92	0.96	0.92
Quadriceps femoris	0.90	0.89	0.97	0.92
Tibialis anterior	0.96	0.92	0.95	0.91
Medial gastrocnemius	0.93	0.94	0.94	0.91
Hamstrings	NP	0.86	NP	0.88
Average—upper limb muscles	0.98	0.95	0.97	0.97
Average—lower limb muscles	0.97	0.98	0.98	0.96
Average—all muscles	0.99	0.99	0.98	0.98
Optimized EIM score	0.93	0.92	0.88	0.95

NP, not performed.

multimuscle data selected to optimize the correlation with the 6MWD in all boys with DMD. The optimized EIM score was the average of the phase and reactance results selected from multifrequency current applied to the biceps brachii (118-kHz phase, 15-kHz reactance), wrist flexors (118-kHz phase, 118-kHz reactance), quadriceps (15-kHz phase, 15-kHz reactance), and gastrocnemius (118-kHz phase, 15-kHz reactance). These frequencies were selected because they yielded the strongest correlation with the 6MWD in DMD boys without reducing reliability [intraclass correlation coefficient (ICC) >0.8]. We included phase and reactance to best measure changes in tissue capacitance, which we hypothesized would alter with dystrophic pathology. Optimized EIM scores were calculated for controls and boys with DMD. We compared relationships to functional assessments between the optimized EIM score and the 53-kHz phase data in boys with DMD.

Statistical calculations were performed using MATLAB 2011b, including the Statistics Toolbox (The MathWorks, Inc., Natick, Massachusetts). All values are expressed as mean (SD), unless otherwise stated. Parametric distributions were confirmed via visual inspection of the data. Reliability and evaluation for systemic bias between raters was assessed using ICC and Bland-Altman plots. The means and SDs of the dynamometry and timed functional tests in the healthy control subjects were used to determine the *Z*-scores for each subject. *Z*-scores from each timed functional test and from dynamometry measurements for each muscle were summed, respectively, to determine a combined timed functional test and overall strength score for each subject. Rater 1 EIM results were evaluated with a 2-sample *t*-test for DMD vs. control group comparisons and with Pearson correlation coefficients (*r*-values) to define relationships with strength, function, and subject characteristics.

RESULTS

Subject Characteristics. There was no difference ($P \geq 0.4$) between controls and boys with DMD for age (controls 7.7 ± 2.6 years, DMD 7.6 ± 2.6 years), weight (controls 26.5 ± 8.7 kg, DMD 28.3 ± 10.5 kg), or skin-fold thickness averaged from all body regions (controls 11.3 ± 5.6 mm, DMD 12.1 ± 7.3 mm). Compared with healthy controls, *Z*-scores in boys with DMD showed slower combined timed functional testing (0.0 ± 2.8 vs. 16.6 ± 15.4, $P < 0.001$) and weaker overall strength (0.0 ± 11.1 vs. -18.5 ± 6.6, $P < 0.001$). In boys with DMD, age did not correlate with either the NSAA ($r = -0.15$, $P = 0.27$) or 6MWD ($r = 0.12$, $P = 0.37$).

Safety, Tolerability, and Feasibility of EIM. No adverse events were reported, and all subjects were able to complete the EIM evaluation without difficulty. The most common complaint from the boys was that the saline wipes were cold when placed on the skin. A complete (8 muscle) evaluation (by rater 1) took an average of 18 minutes. Three subjects (2 DMD, 1 healthy) had technically invalid results at 53 kHz in more than half of all muscles and were excluded. In the remaining subjects, of 1,958 individual muscle measurements, only 15 were technically invalid and were replaced by a repeated measurement. These replaced measures were excluded from reliability analysis.

Reliability of EIM. Inter- and intrarater reliability were high in all examined muscles (Table 1) and did not show a systematic bias between raters (Fig. 1). ICCs for interrater reliability ranged from 0.81 in the triceps brachii to 0.96 in the anterior forearm muscles. ICCs for intrarater reliability were ≥ 0.90 for each muscle.

EIM in Healthy Controls and Boys with DMD. In healthy controls, the average EIM phase at 53 kHz did not correlate with weight ($r = -0.27$, $P = 0.16$)

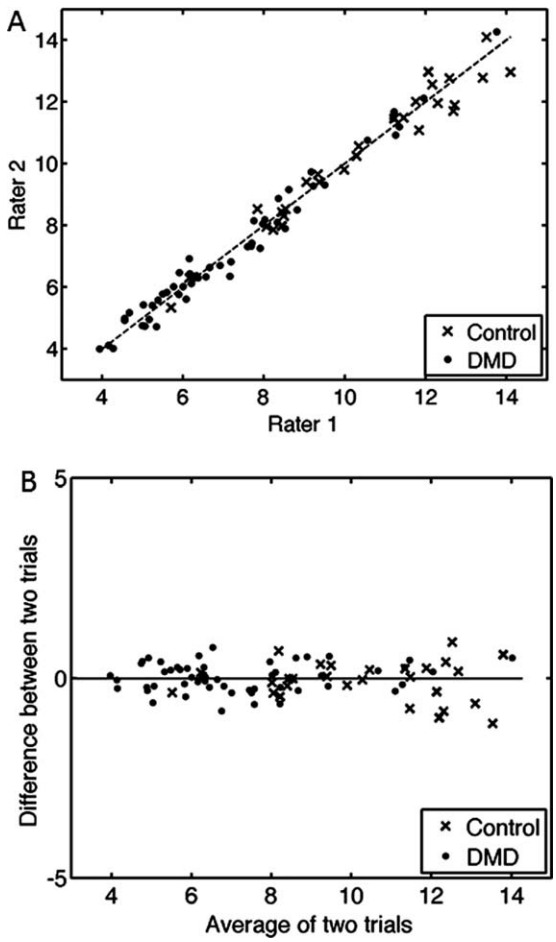

FIGURE 1. Interrater reliability of EIM. The EIM phase, averaged from all examined muscles, is similarly measured by 2 raters in both healthy and dystrophic muscle and without bias across results (ICC = 0.99). The average EIM phase at 53 kHz is higher in healthy controls (10.45 ± 2.29) than in boys with DMD (7.31 ± 2.23) ($P < 0.001$) (area under the receiver operator characteristic curve = 0.83).

or age ($r = -0.053$; $P = 0.78$), but it did correlate inversely with skin thickness overlying each muscle ($r = -0.45$ to -0.68; $P \leq 0.01$). Average EIM phase at 53 kHz in healthy controls correlated inversely with timed functional tests ($r = -0.67$; $P < 0.001$; Table 2).

In boys with DMD, EIM phase at 53 kHz was lower compared with healthy controls, on average (controls 10.45 ± 2.29, DMD 7.31 ± 2.23; $P < 0.001$), and in each muscle individually (all $P < 0.001$), except for the medial gastrocnemius ($P = 0.06$; Fig. 2). In both younger (3–7 years) and older (8–12 years) boys with DMD, average EIM phase at 53 kHz increased with better function and strength (Table 2). In all boys with DMD, the average EIM phase at 53 kHz correlated positively with 6MWD ($r = 0.55$; $P < 0.001$; Fig. 3), NSAA score ($r = 0.66$; $P < 0.001$), and muscle strength ($r = 0.44$; $P = 0.001$), and correlated negatively with the amount of time needed to complete the timed functional tests ($r = -0.56$; $P < 0.001$).

Optimization of the EIM parameters further improved correlation with function (Table 2, see also Fig. S1 in Supplementary Material, available online) while retaining high reliability (ICC = 0.97). The optimized EIM score was the average of the phase and reactance results selected from multifrequency current applied to the biceps brachii (118-kHz phase, 15-kHz reactance), wrist flexors (118-kHz phase, 118-kHz reactance), quadriceps (15-kHz phase, 15-kHz reactance), and gastrocnemius (118-kHz phase, 15-kHz reactance). Compared with the average EIM phase at 53 kHz, the optimized EIM score better differentiated boys with DMD from healthy controls (area under the receiver operator characteristic curve = 0.91, vs. 0.83 for average EIM 53-kHz phase) and showed

Table 2. EIM: Correlation (*r*) with function and strength.

Test	Age group (years)	Average EIM score		Optimized EIM score
		Controls (*r*)	DMD (*r*)	DMD (*r*)
6-minute walk distance	All	0.33	**0.55**	**0.65**
	3-7	0.14	**0.58**	**0.60**
	8-12	0.57*	**0.57**	**0.71**
North Star Ambulatory Assessment	All	0.43*	**0.66**	**0.67**
	3-7	0.28	**0.66**	**0.60**
	8-12	0.49	**0.65**	**0.76**
Timed functional tests (all combined)	All	**−0.67**	**−0.56**	**−0.59**
	3-7	**−0.68**	**−0.57**	**−0.57**
	8-12	**−0.68**	**−0.51**	**−0.61**
Standardized strength (all muscles combined)	All	0.30	**0.44**	0.19
	3-7	0.31	0.46*	−0.09
	8-12	0.37	**0.50**	0.43*

Average EIM is the phase at 53 kHz averaged from all (8) examined muscle groups. Optimized EIM is the average multiple-muscle, multifrequency phase and reactance optimized to the 6-minute walk distance. Values in bold are $P \leq 0.006$.

**$P < 0.03$.*

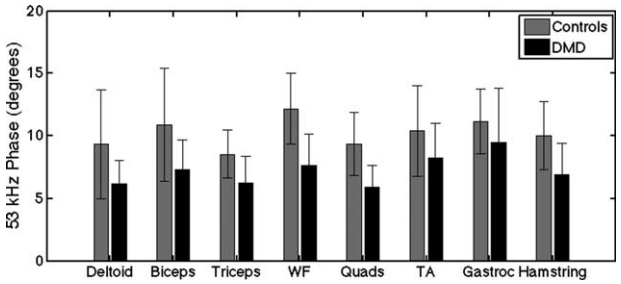

FIGURE 2. EIM in dystrophic and healthy muscle. EIM phase at 53 kHz was lower in boys with DMD than in healthy controls for all muscles ($P < 0.001$) except medial gastrocnemius ($P = 0.06$).

stronger correlations with the 6MWD in DMD, particularly in the older boys. In 8-12-year-old boys with DMD, the optimized EIM score improved the correlation with 6MWD ($r = 0.71$; $P < 0.001$) compared with the average EIM phase at 53 kHz ($r = 0.57$; $P = 0.002$). In 3–7-year-old boys with DMD, the optimized score had less impact on correlation with the 6MWD ($r = 0.60$; $P = 0.001$) compared with average EIM phase at 53 kHz ($r = 0.58$; $P = 0.001$).

DISCUSSION

EIM can be performed safely, rapidly, and reliably in healthy boys and boys with DMD and is a valid measure of disease severity. Examiners obtained reliable EIM measurements from multiple muscles of the arm and leg in both healthy boys and ambulatory boys with DMD (ages 3-12 years) after only a single half-day training session. Our protocol involved repeated acquisition of data 3 times in rapid succession from each muscle. This allowed for selection of the most reliable data set, and likely contributed to the high inter- and intrarater reliability. Measuring EIM is feasible with the EIM 1103 System (Skulpt, Inc.). Only 3 of 92 boys (3%) were excluded for technically invalid measurements, and only 15 of the remaining 1,958 individual muscle measurements (<1%) were technically invalid. Thus, from a practical point of view, EIM would be very simple to implement in a clinical trial of boys with DMD. It takes only a few minutes to measure EIM, and all children were able to complete the EIM protocol without any serious adverse events.

EIM measures are abnormal in boys with DMD and correlated with assessments of function and strength. In DMD, EIM phase is lower than in healthy controls and decreases with weaker strength and lower function. Unlike functional assessments, EIM can be performed in children and adults of any age and ability and does not depend on patient effort or willingness to cooperate with functional testing. Outcome measures like EIM that are not dependent on ability could expand eligibility in clinical trials of DMD to

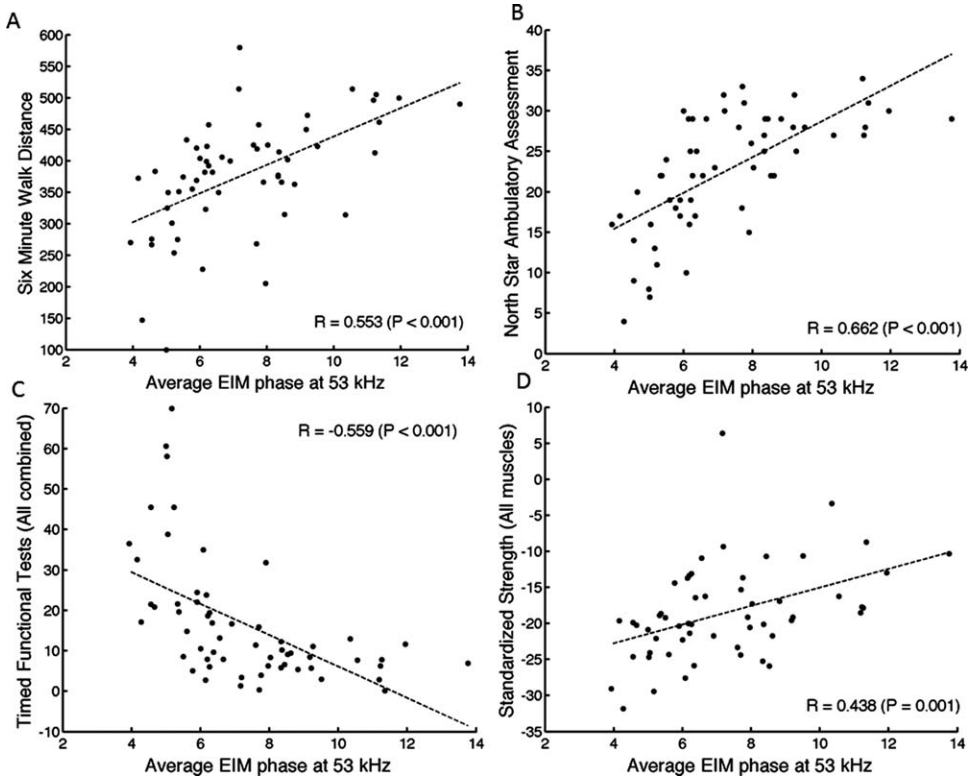

FIGURE 3. Correlations between EIM phase at 53 kHz and strength and function in boys with DMD. The average 53 kHz phase in boys with DMD is higher with longer 6MWD **(A)** (in meters), better NSAA score **(B)**, faster timed function **(C)**, and greater strength **(D)**. Timed functional **(C)** and strength **(D)** scores are normalized based on matching-age healthy controls.

include the very young or very weak. Our findings further support the use of EIM for assessment of boys with DMD. The results of this multicenter study are similar to those of an earlier single-site study of a single-frequency EIM device in DMD[15] and to studies of EIM in other neuromuscular diseases. For instance, in ALS, EIM phase is measured reliably, is reduced compared with healthy adults, and shows a lower coefficient of variation in the rate of decline over time than the ALS Functional Rating Scale and hand-held dynamometry.[8] Additional study is required to determine how EIM performs in females, younger and older males, non-ambulatory boys and men with DMD, and after treatment, as compared with other outcome measures over time.

The specific tissue characteristics that affect the EIM phase are not certain. We found that EIM phase is reduced with thicker subcutaneous fat and in dystrophic muscle, which is characterized by increased intramuscular fat. It is unlikely that the differences in EIM between DMD and controls resulted only from effects of subcutaneous fat, as skin-fold thickness was similar in the 2 groups. Reductions in cell size and the presence of edema or connective tissue in the muscle also decrease the EIM phase values.[11] Muscle fiber hypertrophy, such as occurs in the gastrocnemius of boys with DMD,[16] may explain why the EIM phase in dystrophic and healthy gastrocnemius muscles was more similar than in other muscles. Additional studies comparing EIM with muscle and subcutaneous fat characteristics measured by imaging or biopsy are required to better determine what tissue characteristics most affect EIM measurements.

Our goal is to develop a valid, sensitive measure to detect changes in muscles of boys with DMD over time and in response to therapy that can be performed in patients of all ages and abilities. In this study, we measured multiple EIM parameters (reactance, phase) at multiple frequencies. We then selected data optimized to correlate with the 6MWD in boys with DMD and compared the data with EIM phase obtained at a single frequency. Compared with EIM results obtained at a single frequency, the optimized EIM score showed a stronger correlation with the 6MWD and some other functional outcomes in boys with DMD and also increased the resolution of EIM as a binary classifier of disease. This demonstrates the utility and versatility of multifrequency EIM measurements. It is likely that the EIM parameters selected for optimization will differ based on subject characteristics and the outcome of interest, such as changes over time.

In conclusion, EIM can be measured safely and reliably in healthy boys and boys with DMD, and can be implemented practically at the bedside and in multicenter studies. In DMD patients, EIM phase is lower than in controls and varies with the degree of impairment. Additional longitudinal studies of EIM in older and younger boys with DMD are warranted and are currently underway.

APPENDIX

The DART-EIM Clinical Evaluators Consortium consists of: Betsy C. Malkus, PT, MHS, Catherine Siener, PT, MHS, and Jeanine R. Schierbecker, PT, MHS (St. Louis, MO); Lisa Stover, PT, MSPT, PCS, Paula Morehart, BSN, RN, and Lauren Miller, BA (Cincinnati, OH); Michele Yang, MD, Terri Carry, PT, and Melissa Gibbons, MS, CGC (Denver, CO); and Leslie Vogel, PT, Randal Richardson, MD, and Elise L. Townsend, PT, DPT, PhD, PCS (Seattle, WA).

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