INSIGHTS FROM A MULTISITE STUDY UTILIZING IMPROVED TECHNOLOGY TO ASSESS ELECTRICAL IMPEDANCE MYOGRAPHY AS AN OUTCOME MEASURE FOR DUCHENNE MUSCULAR DYSTROPHY

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INTRODUCTION

Duchenne muscular dystrophy (DMD) remains a disease of significant unmet treatment need. One major obstacle to developing disease-modifying therapies is the lack of suitable outcome measures to quantitate and evaluate muscle condition and response to therapy. For example, the 6-minute walk test (6MWT) has been one of the standard approaches utilized in DMD clinical trials. It is contested, however, that the test often shows variability between assessments and is dependent upon a boy's motivation. In addition, the 6MWT has both lower and upper age limits which constrict its utility to a relatively narrow age range of boys: It actually shows improving values up until approximately 7 years of age in many boys with DMD, and the upper age limit is set by the transition all boys make to becoming non-ambulatory — usually in their teens. Other functional outcome measures are hindered by requiring subjective evaluations by the evaluators and/or dependent on the boys completing a given task, bringing similar limitations.

A basic approach for circumventing these issues is to use quantitative therapy-response measures that neither rely on subjective assessments nor are impacted by motivation. One technology that offers substantial promise is electrical impedance myography (EIM). In EIM, a weak, high-frequency electrical current is applied across a muscle of interest and the resulting voltages measured. Changes in the voltage characteristics provide insights into the health of the muscle, the impact of disease, and the effect of therapy. Numerous studies have shown that EIM has high inter and intra-rater reliability. It is has practical advantages as well: it is quick and painless, less costly relative to other imaging options, and easily applied from infants to the elderly. In DMD, a single-site longitudinal study provided evidence that EIM was sensitive to disease progression as well as to the beneficial effects of corticosteroid therapy. However, that study was limited in that it utilized an off-the-shelf impedance-measuring system not intended for muscle assessment, and did not assess older non-ambulatory boys.

Here we report results using a dedicated EIM system in the evaluation of a group of ambulatory and non-ambulatory boys with DMD, as well as healthy boys, all between ages 5-17, followed longitudinally for 6-12 months, to assess EIM's potential value as a tool for tracking disease status with implications for its application in future DMD clinical therapeutic trials.

METHODS

General: We performed a 5-site multicenter study including Boston Children's Hospital (Basil Darras, PI) Washington University, St. Louis (Craig Zaidman, principal investigator (PI)), Colorado Children's Hospital (Michele Yang, PI), Cincinnati Children's Hospital (Brenda Wong, PI), and Myolex, Inc (Laura Freedman, PI). At all sites both healthy and DMD boys were enrolled, except for the Myolex site, which enrolled only healthy boys. Institutional review board (IRB) approval was obtained at each of the 5 sites, and parental written informed consent and verbal or written assent was obtained from each child participant.

Participants. ***DMD boys.*** 53 Boys with DMD aged 5-17 years were recruited through the neuromuscular clinic at each of the 4 academic sites. All boys were required to have genetic confirmation of disease or to have a brother with genetically confirmed DMD and a characteristic clinical picture. DMD boys were excluded if they were enrolled in a therapeutic clinical trial or had a concomitant condition that substantially impacted health. Boys were enrolled regardless of corticosteroid use and ambulatory status.

We divided the group into 2 cohorts for analysis: an ambulatory cohort of 29 DMD boys stably on steroids, and a non-ambulatory cohort of 15 DMD boys (with a mix of steroid status).

Healthy boys. 57 healthy age-similar controls were also recruited across the 5 sites and enrolled over the same time period. Healthy boys had no history of neuromuscular disease or any other disorder that would be anticipated to affect muscle health, and were recruited via IRB-approved advertisement and word of mouth.

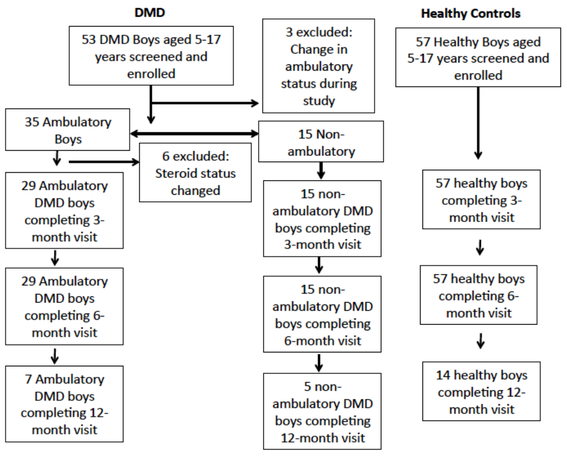
Study design. Study visits included baseline (0), 3, 6, and 12 months. At each visit, medications were reviewed, interim medical history obtained, and weight and height measured. In addition to the EIM measurements, a standard set of age- and ability-appropriate motor function tests were also performed in the DMD boys only.

EIM measurements. EIM was performed using the Myolex mView® system (Myolex (formally Skulpt), Inc, San Francisco, CA); see Figure 1. All evaluators were trained in proper use of the system prior to initiation of the study at a single investigator meeting held in Boston, MA. The system consists of a handheld EIM device with disposable electrode pads. Each electrode array contains 3 electrode sets and thus 3 sets of data are obtained virtually simultaneously, with applied electrical current frequencies between 1 kHz and 10 MHz. The handheld device is connected via a cable to a power convertor box, which itself is directly connected to a laptop. After wetting the skin overlying a muscle of interest with saline, the electrode array is applied and an EIM measurement taken (in about 4 seconds). This entire process is briefly repeated two times to ensure stability/consistency of the data before moving on to the next muscle. Seven muscles were studied unilaterally: lateral deltoid, biceps brachii, forearm flexors, forearm extensors, rectus femoris, tibialis anterior, and medial gastrocnemius. The right side was chosen for measurement unless clear left side dominance was present, in which case the left side was measured.

Figure 1. mView EIM system and boy undergoing testing

Functional measurements. In addition to EIM data, several functional measures were assessed longitudinally in the DMD boys where possible. These included: **1. 6-minute walk test (6MWT).** Boys were asked to walk as far as they could in 6 minutes and the distance recorded; the outcome was total distance walked. **2. North Star Ambulatory Assessment.** This performance battery captures a number of predominantly lower extremity functions, including timed tests. **3. Handheld Dynamometry.** This was performed unilaterally on the corresponding muscles to those assessed with EIM, using a standard handheld dynamometer.

Data analysis. Prior to a formal data analysis, raw multifrequency EIM data was assessed for artifacts or other technical factors that negatively impacted data quality using an automated algorithm developed in MATLAB (The Mathworks, Inc, Natick, MA). This algorithm was sensitive to noise across the frequency spectrum or extreme or negative values at low frequencies (under 30 kHz) consistent with poor electrode contact (the most common cause for data artifacts). After data affected by artifact was removed, longitudinal analysis was performed using a linear mixed-effects model for each muscle's EIM data with random intercept and slope terms to account for within-subject correlations and between-subject variability under the missing-at-random assumption. The main result of interest was the slope difference between healthy and DMD boys, since this would enable sample size estimation (effect sizes). Univariate correlations were also performed comparing EIM change with functional change across the boys out to 1 year. Thus, our main focus was to identify those outcomes that would have the largest effect sizes, thereby providing the greatest sensitivity to disease-related change and enabling the use of smaller patient sample sizes and/or shorter trials.



RESULTS

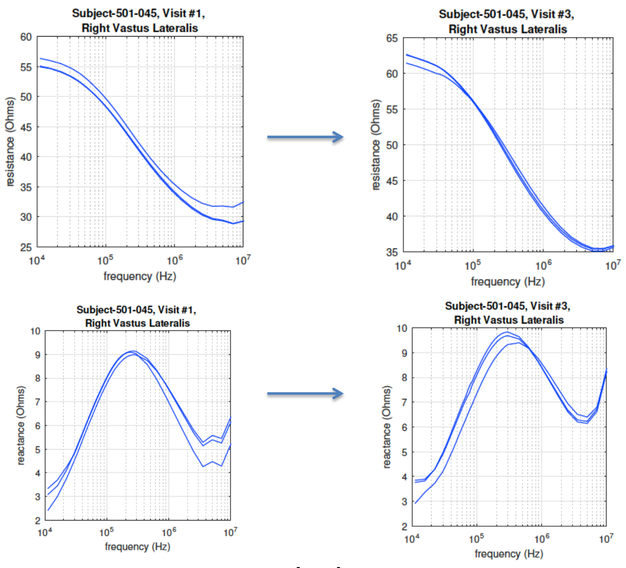


Figure 2. Example of Resistance and Reactance Change over time in a single muscle in a 12 y.o. DMD boy

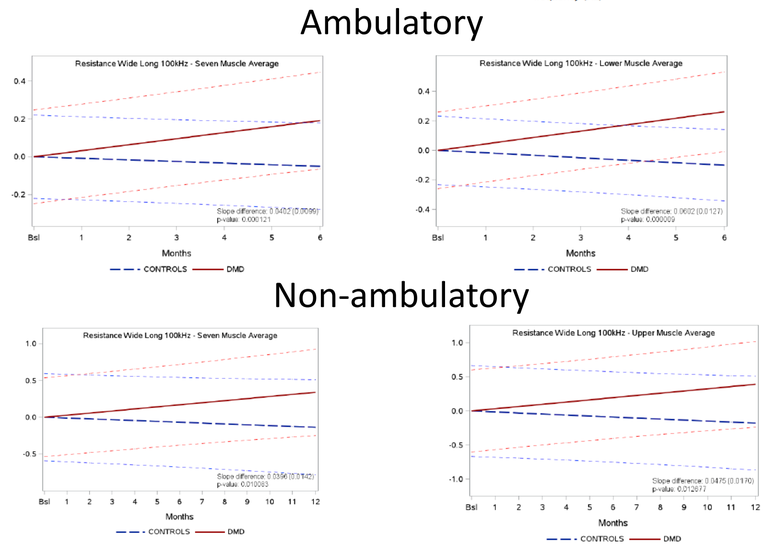


Figure 3. Examples of changes in DMD and healthy boys over time. Both ambulatory and non-ambulatory boys show similar changes.

Table 1: Effect size/sample size 5-12 y.o. ambulatory boys (12 mo)

Measure	SS@25%	SS@50%	Effect Size
Resistance Narrow Trans 100kHz - Tibialis	74	19	1.8497
AUC Reactance Narrow Long 450kHz-10mHz - Tibialis	77	20	1.815
Resistance Narrow Long 100kHz - Tibialis	78	20	1.8061
Resistance Narrow Trans 50kHz - Tibialis	80	20	1.7729
Resistance Narrow Long 100kHz - Quads	84	21	1.7306
Resistance Narrow Long 50kHz - Tibialis	85	22	1.7212
Resistance Wide Long 50kHz - Quads	85	22	1.7193
Resistance Wide Long 100kHz - Quads	88	22	1.6964
Resistance Narrow Long 50kHz - Quads	89	23	1.6823
Resistance Narrow Long 100kHz - Lower Muscle Average	97	25	1.6168

Table 2: Effect size/sample size 9-17 y.o. non-ambulatory boys (12 mo)

Measure	SS@25%	SS@50%	Effect Size
Resistance Narrow Long 100kHz - Biceps	69	18	1.919
AUC Reactance Narrow Long 50-100kHz - Wrist Flexors	79	20	1.7881
Reactance Narrow Long 50kHz - Wrist Flexors	84	21	1.7298
Resistance Narrow Long 50kHz - Biceps	90	23	1.6712
Resistance Wide Long 50kHz - Biceps	97	25	1.6103
Resistance Wide Long 100kHz - Seven Muscle Average	105	27	1.551
Resistance Wide Long 100kHz - Upper Muscle Average	105	27	1.551
Reactance Narrow Long 100kHz - Wrist Flexors	106	27	1.5418
Resistance Wide Long 100kHz - Biceps	111	28	1.51

A)

Measure	Effect size
Supine-to-stand	0.85
HHD-EF	0.56
HHD-WF	0.47
North Star	0.44
HHD-KE	0.38
HHD-AD	0.34
North Star w/o 12	0.33
6MWT	0.25
HHD-SA	0.10
HHD-WE	0.08
4-stair climb	0.01

Longitudinal Effect Size (A) and Correlations (B) for Functional Measures in the Ambulatory Cohort.

B)

	EIM metric with highest correlation	Muscle with Highest Correlation
North	RES=0.48	Lower
supine	AUC=0.46	WE
hhd ef	MAXX=0.39	7 Muscle
hhd ke	AUC=0.55	Quad
hhd sa	AUC=0.83	WE

DISCUSSION

This study further extends our original observations that EIM is sensitive to DMD progression. Unlike earlier work, here we utilized a dedicated system in a multi-site study demonstrating its application in a real-world situation. The sample size estimations included here are similar to those identified in the earlier study of EIM, and are similar to those identified for MRI.

Part of our effort in this study was to identify the optimal EIM metrics and the optimal muscles for future clinical trial use, using sensitivity to disease change over time across the widest age range as our main criteria. **The key findings of these studies include that resistance, in lower limb muscles (tibialis and quadriceps) for ambulatory boys and upper limb muscles (especially biceps) for non-ambulatory boys, is the most sensitive and reliable measure of disease progression across disease stage, will be very valuable for future studies.**

Ultimately, while promising, EIM remains a relatively new technique and its application in DMD even more recent. Only through the dedicated application and incorporation of this technology to future clinical trials can we fully understand and further refine the EIM technique to evaluate its ability to offer meaningful outcomes in DMD. To this end, we encourage academic researchers and pharmaceutical industry alike to incorporate this promising measure in DMD studies.

CONCLUSIONS

1. EIM is sensitive to DMD progression in both younger and older boys.
2. Of all the parameters studied, 50 and 100 kHz resistance appeared to be most promising, showing consistent changes in healthy and DMD boys in both age groups.
3. Alterations in the 50 and 100 kHz resistance parameter correlated with the Northstar Ambulatory Assessment in younger, ambulatory boys.
4. Given its ease of use and sensitivity to change, EIM is a promising biomarker that should be considered in future use in all clinical therapeutic DMD trials.

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