Electrical impedance myography in spinal muscular atrophy: a longitudinal study

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ABSTRACT

Introduction: New approaches for assessing disease progression in spinal muscular atrophy (SMA) are needed. In this study, we evaluate whether electrical impedance myography (EIM) can detect disease progression in SMA compared to a group of healthy children of similar age.

Methods: Twenty-eight children with SMA and 20 normals underwent repeated EIM testing in four muscles at regular intervals for up to 3 years. An average rate of change of EIM was calculated for each subject and normalized to subcutaneous fat thickness and muscle girth.

Results: Multiple EIM parameters showed change in normal subjects over a mean of 16.7 months; however, no change was found in SMA patients over this period.

Discussion: These results indicate that EIM can detect non-mass-dependent muscle maturation in healthy children. In contrast, the muscle of children with SMA as measured by EIM is virtually static, showing evidence of neither growth nor active deterioration.

Keywords: SMA, impedance, clinical trials, pediatric, muscle
INTRODUCTION

A major challenge to clinical trials in spinal muscular atrophy (SMA) is the very slowly progressive nature of the disease. Although motor neurons are lost early in the disease course, later in the disease, functional decline is very slow. For example, in one study, 50% of children diagnosed with SMA Type 3 and whose onset was between 2 and 6 years of age lost their ability to walk at a median age of 44 years. Others have also found similar evidence of very slow loss of muscle strength and function, while others have described essentially a static course over a 12-month period.

This extremely slow rate of progression poses a major challenge to clinical trials in SMA since, from the standpoint of feasibility, most trials need to be completed within 1-2 years. Thus, if there is hope of identifying a treatment for this disease, a very sensitive biomarker that can identify decline over this period of time is required. One technique that may offer promise is electrical impedance myography (EIM), a method based on non-invasive application of a low intensity, high-frequency electrical current and measurement of the resulting surface electrical patterns. EIM data have shown substantial alterations in a variety of neuromuscular disease states. In amyotrophic lateral sclerosis (ALS), EIM has specifically shown promise as a sensitive measure of disease progression. EIM is especially well-suited for use in children, since it is non-invasive, painless, and is easily tolerated, as confirmed by an initial cross-sectional study in children with SMA.

In this investigation, we extend our initial observations in SMA into a longitudinal study to determine whether EIM can detect pathologic alterations in the impedance data over a several-year period of time in children and teenagers. In addition to studying a group of children with
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SMA, we also study a group of healthy children to determine whether EIM is effective at sensing muscle growth and maturation.

MATERIALS AND METHODS

Standard Protocol Approvals, Registrations, and Patient Consents: All procedures were approved by the Institutional review board at Children’s Hospital Boston, and signed consent was obtained from patients over 18 years or from parents with verbal or written assent obtained from individuals under 18 years of age.

Normal subjects: Normal subjects were recruited by advertisement, by word-of-mouth through family members and through the department of Neurology at Children’s Hospital Boston. Normal subjects were required to be under 30 years of age, have no history of neuromuscular or other neurological disease, and to be in otherwise good health.

SMA patients: SMA patients were identified through the Neurology Clinic at Children’s Hospital Boston, through the Pediatric Neuromuscular Clinical Research (PNCR) network, and by advertisement. Patients were required to have a positive genetic test for SMA or have a sibling with a known positive SMN gene mutation and the appropriate clinical phenotype. SMA patients were differentiated into Type 1, Type 2 and Type 3 based on the standard clinical criterion of their maximum level of motor function achieved at any point, including whether the child was able to walk (Type 3), only sit (Type 2) or achieved neither milestone (Type 1). Type 4 (adult onset) patients were not enrolled.
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**Strength measurements:** Patients underwent limited dynamometry using a handheld device (Manual Muscle Test System Model 01163, Lafayette Instrument, Lafayette, IN) of the four muscles/muscle groups to be studied: biceps (via elbow flexion), wrist extensors (via wrist extension), quadriceps (via knee extension), and tibialis anterior (via foot dorsiflexion) on one side. Subjects were asked to sustain a maximum contraction for 5 seconds, and each measurement was performed twice; the higher value was recorded each time. If the two values differed by more than 15%, a third measurement was taken, and the highest value utilized. Evaluators were trained through the PNCR Network (which had established this approach for strength testing) and had previously established their reliability. Subjects too young to follow directions (two of the SMA Type 2 children) were excluded from this testing in the results that follow; their remaining data, however, are included in the analyses.

**EIM electrode placement and measurement:** For each muscle, a series of four strip electrodes (part number 019-766400, Viasys Healthcare/Nicolet Biomedical, Madison, WI, cut to one quarter length) were used (Figure 1). The current-emitting (outer) electrodes were placed over the same four muscles studied with dynamometry (unilateral biceps, wrist extensors, quadriceps, and tibialis anterior muscles) 5 cm on either side of the approximate center of each muscle along the long axis of the limb. The voltage-measuring (inner) electrodes were placed 2 cm within them. In order to ensure good electrical contact and the absence of electrode movement during measurement, a standard medical adhesive tape was placed over the array to affix the electrodes firmly to the skin.

Multifrequency EIM measurements were performed with the Imp SFB7 ® (Impedimed, Inc, San Diego, CA). This device provides a frequency spectrum of electrical current from 2 kHz...
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to 1 MHz; however, for the purposes of our analyses, the current frequency range was limited to 10 kHz to 500 kHz. Each multifrequency measurement was repeated 2-3 times and averaged.

The data were downloaded, and the reactance (X), resistance (R) and phase \[\theta = \arctan(X/R)\] values were plotted against frequency.

In addition to the 50 kHz phase (50 kHz \(\theta\)), the standard single-frequency parameter we have used for many earlier studies, we included 3 collapsed parameters, as previously described in our cross-sectional study.\(^{13}\) These included: 1. the reactance-slope (X-slope), which is obtained by performing a linear regression of the reactance values from 100-500 kHz and calculating its slope; 2. The log-resistance slope (log-R-slope), which is calculated by taking the resistance data from 10-500 kHz, performing a log transformation of both the measured values and the applied current frequencies and taking the slope of a linear regression that fit those points; and 3. the phase-slope (\(\theta\)-slope), by performing the same procedure as for the reactance-slope except using phase values.

**Ultrasound measurements:** To assess the skin-fat thickness, ultrasound measurements were also made using a Terason Model 2000 Imaging System (Teratech Corporation, Burlington, MA) with a 5 MHz probe. The probe was placed approximately at the midway point of the electrode array. An image was saved after the subject was asked to briefly contract and relax the muscle of interest to help distinguish the subcutaneous fat layer from the underlying muscle. The thickness was then measured with electronic calipers.

**Data analysis:** Raw EIM data were visually reviewed prior to any statistical analysis, and any data sets that were distorted by artifacts were excluded. Typical impedance artifacts included
low-frequency noise or negative values at any frequency. Both of these artifacts are due to electrode-skin contact impedance problems and are easily recognized.

For the formal analysis, we were primarily interested in determining whether the EIM and strength parameter trajectories for the SMA patients over the course of this study were different from the trajectories of the normal subjects and whether both were different from zero.

The EIM and strength parameters are partially dependent on the specific muscle studied and therefore cannot be averaged in a meaningful way. In order to circumvent this problem but still utilize a single value per muscle per patient, a z-score transformation can be performed. A z-transformation in this case allowed us to normalize between muscle types, thus avoiding a potential confounding variable in the analysis. This essentially places every muscle, both from normal subjects and from patients, on the same scale. The muscle-specific mean and standard deviation needed for this transformation were taken from the normal subjects at the time of their initial visit. For both groups, the trajectories of individual muscle z-scores were followed over time, and the average of these trajectories was taken to provide the overall rate of change in EIM and strength over time for that patient.

To test our hypothesis, we fit a linear mixed model with the EIM parameter as the response. The fixed effect predictors were a binary disease status variable (control or SMA) and days after the initial visit as a continuous measure. Both subcutaneous fat thickness and limb girth, each a potential confounder, were also included in the model. Subject specific random intercepts were modeled as random effects. There was a small degree of model building especially in determining the random effect structure. Terms were dropped from the model based on likelihood ratio tests. In particular, we considered modeling subject-specific slopes as random effects. Evidence of different trajectories for SMA subjects versus normal controls was
taken from the regression coefficients, in particular, the regression coefficient for the time-
disease/normal status interaction. In our parameterization, this coefficient represents the
difference in slopes between the SMA subjects and the healthy controls.

RESULTS

Subject demographics: Subject demographic data are summarized in Table 1. As can be seen,
we achieved a very close match in terms of ages, with the 20 normal subjects ranging from 0.8 to
22.6 years (mean age 9.8 years) and 28 SMA patients ranging from 1.2 to 20.8 years (mean 9.6
years). However, we were not as successful in matching gender between the groups; the SMA
group was 64% female/36% male, and control group was 45% female/55% male.

Subject retention: Of the SMA patients, 10 had only two visits (they had one six-month follow
up), 8 had four visits (they were followed for 1.5 years), and 10 had five visits (followed for 2
years). Of the normal subjects, 5 had only two visits (one six-month follow-up), 8 had three
visits (1 year follow-up), 5 had five visits (followed for 2 years); 1 subject had 4 visits and
another had 6 visits. Overall, the two groups were followed for a similar period of time: normal
subjects for a mean of 17.2 months and SMA patients for a mean of 16.7 months.

EIM, ultrasound, and strength testing tolerability: All subjects who participated experienced
no related adverse event from the procedures and easily tolerated all testing.

EIM and strength over time: The analyses are summarized in Table 2 and Figure 2. As can be
seen, the 50 kHz single frequency EIM data failed to reveal a significant trend in either the
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normal subjects or the patients over the approximately 18-month period of time. However, the 3 multifrequency parameters did show a significant or near-significant change in the normal subjects over time as compared to zero. No change, however, was observed in these same parameters for the SMA patients, as their trajectories on average were not different from zero (even when accounting for age at the time of enrollment). This was even the case when the data from the three Type 1 patients was removed. When compared to the SMA patients, the normal subject trajectory data also showed a significant difference for all three measures. We note that for the most part, controlling for the subcutaneous fat thickness and girth had little impact on the ultimate EIM results; in fact, in nearly all cases, performing the same analyses excluding these values from the regression equation would not have altered the overall statistical results in a substantive way. A post-hoc analysis of the data from patients aged 10-15 years only (ie those likely growing most quickly during puberty) did not alter the significance of the results.

Similarly, a post-hoc analysis of individual muscles over time showed no significant differences between the groups.

The z-score strength data followed the EIM data closely, as the normal subjects showed an overall increase in strength over time whereas the SMA patients had a non-significant decrease in strength (Table 2). Among normal subjects, EIM phase-slope trajectories had a significant correlation with changes in strength over time (p = 0.0017); the reactance-slope trajectory had a borderline significant correlation with strength change (p = 0.054), and the log-resistance slope correlation was non-significant (p = 0.49).

DISCUSSION
The longitudinal EIM data we obtained indicate that there is a major difference in muscle maturation in normal children vs. those with SMA. Specifically, in normal children, the 3 multi-frequency parameters all showed a significant alteration in a direction consistent with improving or maturing muscle, whereas those of subjects with SMA showed no alteration over time (i.e. the results were not significantly different than zero). Even in that subset of SMA children followed the longest (2-2.5 years) there was no significant alteration in the impedance data. These findings paralleled those observed in muscle strength over the same time period.

The alterations in EIM observed in the healthy children are not just due to increasing muscle bulk. Indeed, when we included limb girth as a covariate, this only increased the significance of the observed change over time, for example improving the p value for the reactance-slope from 0.027 to 0.0012. To some extent, the impact of muscle size on EIM data has remained an open question. It is reassuring to see that the alterations in the EIM data in normal children are not simply an effect of increasing muscle mass, but rather they are likely an indication of alterations in the structure and composition of the muscle itself.

However, in the children with SMA, our EIM data suggest that over a mean period of 17 months there is no significant change in muscle structure/composition. This implies an absence of maturation or significant decline (or possibly that both maturation and decline are balanced). Of course, it is possible that there are subtle changes in muscle architecture that are not being identified by EIM or strength testing over this time period. As noted above, previous studies have shown evidence of slow functional decline over many years or decades. Thus, it is certainly possible that if this study had a very large number of subjects (about 600) or had been extended for a considerably longer period of time, a small but significant change in the SMA patients may have been identified. Such a change, however, could still simply reflect the effects of
longstanding disuse, with consequent muscle fibrosis and fatty infiltration\textsuperscript{14} rather than actual ongoing motor neuron loss. Indeed, in one recent study, no substantial decline in motor unit number on MUNE testing was observed over time.\textsuperscript{6}

In order to identify new treatments to slow progression based on the concept of forestalling motor neuron loss, a meaningful degree of decline over an acceptable period of time needs to be identified. If after 18 months, no such change can be identified, the practical possibility of performing a study of a therapy focused on slowing disease progression becomes dubious. In contrast, however, there is good evidence that motor neurons are lost early in the disease course.\textsuperscript{1} Thus, for a therapeutic trial to be successful in this disease, a focus on early treatment may be absolutely essential. This study thus further strengthens the argument for performing newborn screening for SMA so that affected individuals can be more rapidly enrolled in clinical therapeutic trials at a time when therapy has a chance of making a difference. Second, it also argues for the need to seek alternative therapies that are not based on purely on forestalling motor neuron loss. For example, approaches that provide for actual replacement of motor neurons and/or myocytes or improve neuronal or muscle function by increasing the production of full-length SMN protein may be needed. One recent example of such a potential therapy is the use of antisense oligonucleotides to increase SMN protein in motor neurons to a level that could restore neuronal function.\textsuperscript{15}

There are several limitations to this study worth highlighting. First, the number of subjects enrolled, although substantial, was not high and thus negative statistical analyses are more likely a result of Type II error. This small number of patients essentially also made any effort to complete a meaningful subgroup analysis on SMA Type 2 or Type 3 patients impossible. Moreover, the broad age range of patients studied and the relatively short time in
which they were followed limits the power of this study and our ability to determine whether age could have impacted the rate of progression of the disease. Thus, additional larger studies following more homogeneous groups of SMA patients over longer periods of time are needed. Second, the use of z-scores and our multiple regression models made certain assumptions (e.g., linearity across age of certain measures for example) which may not be true. Ideally, such a model would not even be necessary, but given the relatively small group of subjects and multiple confounders, this approach helps pull out a potential effect that might otherwise have been lost in the noise. Third, the impedance system being used, the SFB7 from Impedimed, Inc, is a multifrequency device not intended for localized impedance measurements; accordingly, it is not calibrated to the relatively small impedances of the limbs and thus could reduce reliability of the data to some extent. Finally, there was an imbalance in gender between the groups. Fortunately, however, gender was not found to be a significant predictor when all the other variables were included, a decision based on the significance of the likelihood ratio test.

One major question that this study leaves unanswered is the mechanism underlying the observed EIM data. Studies in healthy rodent models have similarly identified a strong effect of animal maturation on the EIM data. In addition, in experimental neurogenic disease (e.g., sciatic crush and ALS) the severity of the EIM abnormalities mirror the severity of neurophysiologic, behavioral and pathological data. The frequency-dependent changes in the EIM signature may be due, in part, to atrophy (or hypertrophy) of the myocytes. In EIM, muscle can be modeled as a complex, three-dimensional network of resistors and capacitors; such networks display unique frequency-dependent characteristics, the properties of which will change if alterations are made to the component circuit elements. Thus, changes in myocyte size and number would be expected to lead to analogous changes in the impedance spectra obtained.
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in humans. In addition, in more advanced chronic disease, myocytes are replaced with connective tissue and fat, and these changes in muscle composition also will distort the normal spectra. Why these multifrequency measures should be better than single frequency remains uncertain; however, single frequency measures are likely to be more affected by electrode placement and positioning than the multifrequency-derived parameters. For example, simply placing the voltage electrodes further apart would result in a change in the measured phase but in little alteration in the collapsed multifrequency parameters.

In summary, the longitudinal EIM and strength data obtained here confirms other work which suggests that over a mean period of 1.5 years, children with SMA failed to show evidence of significant disease progression.\textsuperscript{6,18} The results in healthy subjects also support that EIM is sensitive to muscle status and that this status is not simply a reflection of muscle size, but rather assesses more intrinsic qualities of the muscle. Given that this is the first longitudinal study of EIM in SMA, further studies applying EIM to SMA disease assessment, possibly in more restricted age groups and over longer periods of time, are warranted, especially considering its ease of use in both non-ambulatory subjects and very young children. In addition, technological improvements to EIM are currently in development that will likely help further streamline its clinical application.\textsuperscript{19}
ABBREVIATIONS

ALS – amyotrophic lateral sclerosis
EIM – electrical impedance myography
PNCR – Pediatric Neuromuscular Clinical Research
R – resistance
SMA – spinal muscular atrophy
X – reactance
θ – phase
REFERENCES


## Table 1. Demographic data

<table>
<thead>
<tr>
<th>Type</th>
<th>Male</th>
<th>Female</th>
<th>Mean Age (years) at enrollment</th>
<th>Age Range (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11</td>
<td>9</td>
<td>9.8</td>
<td>0.8-22.6</td>
</tr>
<tr>
<td>SMA 1</td>
<td>2</td>
<td>1</td>
<td>8.7</td>
<td>4.2-17.0</td>
</tr>
<tr>
<td>SMA 2</td>
<td>6</td>
<td>9</td>
<td>8.0</td>
<td>1.2-20.8</td>
</tr>
<tr>
<td>SMA 3</td>
<td>2</td>
<td>8</td>
<td>12.0</td>
<td>9.1-16.4</td>
</tr>
<tr>
<td>Total SMA</td>
<td>10</td>
<td>18</td>
<td>9.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Table 2. Summary of trajectories (changes over time) for each of the collapsed parameters*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>50 kHz</th>
<th>X-slope</th>
<th>slope</th>
<th>θ-slope</th>
<th>Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects mean trajectory (units/month)</td>
<td>0.0181</td>
<td>-0.113</td>
<td>-0.115</td>
<td>-0.18</td>
<td>0.635</td>
</tr>
<tr>
<td>Normal subjects’ trajectories different from zero?</td>
<td>p = 0.74</td>
<td>p = 0.051</td>
<td>p = 0.047</td>
<td>p = 0.0049</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>SMA subjects mean trajectory (units/month)</td>
<td>-0.03</td>
<td>0.0409</td>
<td>0.0615</td>
<td>0.0219</td>
<td>-0.1078</td>
</tr>
<tr>
<td>SMA subjects’ trajectories different from zero?</td>
<td>p = 0.65</td>
<td>p = 0.98</td>
<td>p = 0.19</td>
<td>p = 0.97</td>
<td>p = 0.28</td>
</tr>
<tr>
<td>Difference between normal/SMA trajectories (units/month)</td>
<td>0.048</td>
<td>0.154</td>
<td>0.177</td>
<td>0.20</td>
<td>0.74</td>
</tr>
<tr>
<td>Normal subjects trajectories different than SMA subject trajectories?</td>
<td>p = 0.61</td>
<td>p = 0.0012</td>
<td>p = 0.018</td>
<td>p = 0.018</td>
<td>p = &lt;0.001</td>
</tr>
</tbody>
</table>

*All values are presented as z-score-trajectories of each individual collapsed parameter over time.
FIGURE LEGENDS:

**Figure 1:** Example of EIM being performed on wrist extensors using the Impedimed SFB7 device. The outer electrodes supply the electrical current at different frequencies; the inner electrodes measure the resulting voltages.

**Figure 2.** Plots of average trajectories for the 4 major EIM parameters as z-scores (+/−standard error of the mean) normalized to the first visit, as assessed across 20 normal subjects and 28 SMA patients. In the normal subjects, all three multi-frequency parameters show significant changes over time whereas in SMA patients, the average trajectory is not significantly different than zero. The actual length of time individual subjects were followed varied, but is set at the mean value of 500 days. In addition, for purposes of presentation and for consistency with the 50 kHz phase data, the signs of the data for the 3 multifrequency parameters have been reversed.
Figure 1: Example of EIM being performed on wrist extensors using the Impedimed SFB7 device. The outer electrodes supply the electrical current at different frequencies; the inner electrodes measure the resulting voltages.

38x50mm (300 x 300 DPI)
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85x70mm (300 x 300 DPI)