Electrical Impedance Myography (EIM) and Quantitative Ultrasonography (QUS) Measurements of the Tongue: Biomarkers of Bulbar Dysfunction

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INTRODUCTION

Speech and swallowing abnormalities accompany many neurological disorders (1-10). These oropharyngeal impairments can ultimately limit communication, negatively impact quality of life, and shorten survival (11-15). Reliable tools that quantify underlying motor dysfunction are needed for use in clinical care and therapeutic trials (16). While valuable, commonly employed measures of bulbar dysfunction such as self-report surveys, video fluoroscopy, and needle electromyography have important drawbacks including subjectivity, risk (e.g. radiation exposure), and discomfort (17-20). As painless, non-invasive, objective techniques, electrical impedance myography (EIM) and quantitative ultrasonography (QUS) are well-suited to provide biomarker information.

In EIM, a painless, high frequency, low intensity alternating electrical current is applied at a range of frequencies to a muscle of interest (21). Resultant voltages are measured in the form of resistance (R) or reactance (X) (21). From resistance and reactance, phase, which is often used as a major outcome variable, can be calculated using the equation phase = arctan(X/R) (21). Differences in resistance, reactance, and phase values within individuals over time, or between groups of people at a single time point, reflect changes in muscle architecture that correlate with disease status (21). In general, phase decreases as illness advances in a given muscle (21).

In QUS, acoustic energy can be used to capture the properties of a muscle in an image (22). Using one of various available methods, the pictorial data can then be given a numerical value based on muscle brightness (23, 24). During a transition from healthy
to unhealthy, muscle on ultrasound becomes brighter, corresponding to increasing QUS values (22).

This document discusses the use of EIM and QUS to quantify bulbar dysfunction in neurological diseases including amyotrophic lateral sclerosis (ALS) in the forms of a funded National Institutes of Health (NIH) grant application and selected abstracts. Preliminary findings indicate that the measures are reliable and well-tolerated when applied to the tongue muscle. Further, trends in tongue data correspond with themes previously documented in limb measurements.

REFERENCES CITED


PROJECT SUMMARY / ABSTRACT

Bulbar dysfunction inevitably develops in the course of amyotrophic lateral sclerosis (ALS), and approximately 25% of patients have predominantly bulbar symptoms at the time of disease onset. In addition to characterizing the evolution in muscle architecture that could underlie associated orofacial weakness, identifying new biomarkers is critical to the development and testing of novel therapeutic agents. As painless, non-invasive, portable technologies, quantitative ultrasonography (QUS) and electrical impedance myography (EIM) could meet the need for objective measures of bulbar dysfunction. In QUS, acoustic energy is applied to a muscle of interest; the resultant raw gray-scale or backscatter pictorial data are translated into a single value that reflects the health of the imaged muscle. Similarly, in EIM, a high-frequency, low-intensity alternating electrical current is applied to individual muscles, and the resulting voltages measured. Impedance values reflect changes in muscle architecture, including fiber atrophy, inflammation, and the replacement of muscle with fat or connective tissue. Both of these user-friendly methods can provide sensitive indicators of neuromuscular disease status when applied to the limbs. Although they have also been used to evaluate orofacial muscles in healthy volunteers and patients with primary muscle disorders, they have not yet been systematically studied in the ALS community. We hypothesize that quantitative ultrasonography (QUS) and electrical impedance myography (EIM) measurements can provide consistent, clinically meaningful information about orofacial muscle health in ALS. In a longitudinal natural history study of 32 individuals...
with ALS and 32 healthy subjects, we will test this hypothesis via three aims: 1.) To assess the reliability of orofacial QUS and EIM in individuals with and without ALS; 2) To determine how the measurements of selected orofacial muscles in individuals with ALS evolve over an 18-month period; 3) To evaluate preliminarily whether orofacial QUS and EIM measurements in ALS patients correlate with results of functional tests. If QUS and EIM do provide reliable, meaningful surrogate information about ALS status as expected, they could be used to make proof-of-concept therapeutic trials more efficient. Furthermore, the innovative technologies could have broader application to the diagnosis and surveillance of other central and peripheral nervous system disorders with bulbar involvement.

SPECIFIC AIMS
A disorder of anterior horn cells, amyotrophic lateral sclerosis (ALS) is invariably fatal, with no truly effective interventions (1). Identifying new biomarkers is therefore critical to the development and testing of novel therapeutic agents (2). To that end, serum, cerebrospinal fluid, protein, and imaging parameters have been proposed as surrogates of disease state (3-5). While they represent promising advances, they do not specifically reflect changes in the bulbar system. This is important because bulbar dysfunction inevitably develops in the course of ALS, and approximately 25% of patients have predominantly bulbar symptoms at the time of disease onset (1, 6). As user-friendly bedside tools that directly evaluate the health of orofacial muscles independent of an ALS patient’s cognitive status, quantitative ultrasonography (QUS) and electrical impedance myography (EIM) could meet the need for objective measures of bulbar
dysfunction.

In QUS, acoustic energy is applied to a muscle of interest; the resultant raw gray-scale or backscatter pictorial data are translated into a single value that reflects the health of the imaged muscle. In EIM, a painless, high-frequency, low intensity alternating electrical current is applied to individual muscles of interest, and the resulting voltages measured (7). Impedance values reflect changes in muscle architecture, including atrophy, inflammation, and the replacement of muscle with fat or connective tissue. These two painless, non-invasive technologies have been shown to be sensitive biomarkers of neuromuscular diseases, including ALS, for appendicular assessment (8-10). We therefore hypothesize that quantitative ultrasonography (QUS) and electrical impedance myography (EIM) measurements can provide consistent, clinically-meaningful biomarker information about orofacial muscle health in ALS. We will test this hypothesis via three aims:

Specific Aim #1: Goal: To assess the reliability of orofacial QUS and EIM in individuals with and without ALS. Approach: Repeated measurements will be taken of selected orofacial muscles of 32 individuals with ALS aged 40-79 years and 32 age- and gender-matched healthy volunteers. Trained team member A will perform ultrasound measurements of selected orofacial muscles. Trained team member B will then perform ultrasound measurements of the same selected orofacial muscles within 30 minutes of the initial measurements. Trained team member A will then repeat all of the initial ultrasound measurements. These procedures will allow assessment of both
inter- and intra-rater reliability of QUS. The same approach will be used with EIM in the same study session. **Expected Outcome:** We will establish a high level of inter- and intra-rater reproducibility for QUS and EIM when applied to orofacial muscles of individuals with and without ALS. **Impact:** Having established a high degree of reliability for these innovative technologies across patients and raters at one time point, it will then be possible to evaluate how well these technologies measure subtle orofacial muscle alterations due to ALS progression longitudinally.

**Specific Aim #2: Goal:** To determine how QUS and EIM measurements of selected orofacial muscles in individuals with ALS evolve over an 18-month period. **Approach:** The 32 patients with ALS and 32 healthy volunteers will be evaluated at approximately 3-month intervals for up to 18 months. At each return visit, the standard orofacial muscles will be evaluated with QUS and EIM. In addition, the data collected in the first six months of the study will be used to calculate trajectories of disease status (including decline, if present). Further, detected changes over time will be compared between the ALS and control groups. **Expected Outcome:** We anticipate that QUS and EIM measurements will change in parallel with evolutions in orofacial muscle architecture. Additionally, the differences in measurement values from ALS and healthy subjects are expected to become more obvious over an 18-month timeframe. **Impact:** These results will confirm the validity and sensitivity of QUS and EIM in the evaluation of orofacial muscles in patients with ALS. This work could represent the first step towards use of the innovative technologies to provide biomarker information in clinical therapeutic trials.
Specific Aim #3: Goal: To evaluate preliminarily whether orofacial QUS and EIM measurements in ALS patients correlate with results of IOPI tongue endurance and other functional tests. Approach: At each visit, individuals with ALS will participate in several functional measures designed to interrogate bulbar integrity and more global health status. Specifically, they will answer questions outlined by the revised ALS Functional Rating and Sydney Swallow Questionnaires, perform tongue and lip strength tasks using the Iowa Performance Instrument (IOPI), and demonstrate speed of speech and swallowing. The results of QUS and EIM orofacial measurements will be compared to the findings of these multi-disciplinary parameters, with the IOPI measure of tongue endurance serving as the primary dependent variable. Expected Outcome: Any evolution in QUS and EIM measurements will closely parallel declines in the functional measures. Impact: With the successful completion of this aim, we will establish that QUS and EIM values provide meaningful measures of ALS status and progression on both cross-sectional and longitudinal bases.

RESEARCH STRATEGY

Significance: QUS and EIM could meet the need for new biomarkers for ALS

The identification of new amyotrophic lateral sclerosis (ALS) biomarkers is essential for use in clinical therapeutic trials (2, 11, 12). Results of commonly employed functional measures, including the neurological examination, pulmonary function tests, and the revised ALS functional rating scale, can fail to translate into phase III trial survival endpoints and depend on patient effort (2, 11). Conversely, “biomarkers,” as defined by members of the Food and Drug Administration (FDA), are independent of
patient motivation and comprise objective indicators of disease process and response to treatment (2, 13). By generating surrogate data on early therapeutic effects, ALS biomarkers could allow for smaller and more efficient proof-of-concept drug studies (2). QUS and EIM are ideally suited to provide ALS biomarker information because the methods are painless, non-invasive, and appropriate for use in patients with physical and cognitive limitations (5). An added benefit is that QUS and EIM could provide insight into the pathological and structural changes underlying bulbar dysfunction (11).

Bulbar dysfunction inevitably develops in the course of ALS, and approximately 25% of patients present with bulbar symptoms (1). Speech and swallowing abnormalities can limit communication and markedly impact quality of life, while predisposing to co-morbidities associated with diminished survival (6, 14-17). Despite the ubiquity and clinical importance of bulbar dysfunction in ALS, no user-friendly instruments have been developed to objectively measure it in multicenter clinical trials. This project could therefore fill a critical need by exploring the role of QUS and EIM in the quantification of orofacial muscle dysfunction in patients with ALS.

Applying QUS and EIM to orofacial muscles is the next logical step, as the techniques have already been used to successfully assess appendicular muscle status in ALS. For example, QUS has identified decreased muscle thickness and increased echo intensity in the limb muscles of ALS patients (18). EIM has sensitively detected ALS progression using appendicular measurements (9). Further, limb EIM values
correlated with standard measures of disease severity including hand-held dynamometry and ALSFRS-R limb sub-scores (19). Notably, the EIM limb measurements did not correlate with ALSFRS-R total score, suggesting that the examination of non-limb regions (e.g. craniobulbar muscles) will add valuable information (19). Indeed, even orofacial muscles are heterogeneously affected in ALS, with initial and disproportionate involvement of the tongue (20, 21). While work has begun to quantify parameters of facial muscles in healthy volunteers (22, 23), this type of investigation has not yet been systematically conducted in individuals who have ALS. The transition to orofacial measurements would be especially timely, as biomarkers of orofacial function are needed to more comprehensively and accurately gauge the effect of emerging experimental therapies aimed at slowing progression of ALS.

*Significance: Transforming ultrasound into a tool for assessment of orofacial muscles in ALS*

Quantitative ultrasonography applies acoustic energy to an anatomical region, such as a muscle, to create a black and white image with varying degrees of brightness (24). In the process of QUS, these raw gray-scale or backscatter pictorial data are translated into a single value that reflects the health of the muscle being imaged. QUS has been studied as a biomarker for assessing disease status in the limb and axial muscles of patients with ALS, congenital myopathies, and myotonic dystrophy (8, 18, 25, 26). More recently, QUS has been used as a biomarker to quantify the echo intensity and thickness of facial muscles in healthy volunteers (22, 23, 27). In addition, it has been used as a biomarker in the assessment of the tongue and submental
muscles of individuals with Duchenne Muscular Dystrophy (25). In these trials, muscle echo intensity was correlated with severity of neuromuscular disease (8, 18). While all of these studies have laid an important foundation for the use of QUS as a biomarker in neuromuscular conditions, there has been no specific effort to follow participants longitudinally as will be done here.

**Significance: Transforming EIM into a tool for assessment of orofacial muscles in ALS**

In EIM, a painless, high-frequency, low intensity alternating electrical current is applied to individual muscles of interest, and the resulting voltages measured (7) (Figures 1 a and b on the following page). Impedance values reflect changes in muscle architecture, including atrophy, inflammation, and the replacement of muscle with fat or connective tissue; they can therefore serve as valuable biomarkers of disease status (7). Indeed, EIM has been shown to be a sensitive biomarker for disuse atrophy, myopathy, and ALS progression, (9, 10, 28). Specifically, declines in EIM measurements parallel clinical worsening consistent with other functional measures of ALS status (Figure 1c, from Rutkove et al 2012 on the following page). To date, the virtually all EIM biomarker work has been performed using measurements of appendicular muscles.
Approach: Preliminary Data.

1. QUS discriminates between healthy and diseased muscle.

Consistent with pediatric work showing that QUS can differentiate non-neuromuscular from neuromuscular conditions (29), preliminary data collected from 25 healthy adult subjects were quantitatively and qualitatively different from that of a patient with ALS. For instance, the average tongue QUS values for healthy subjects was 26 (standard deviation 6.2) while the value for two ALS patients was 43. Additionally, the average masseter QUS value for 33 healthy subjects was 49.8 (standard deviation 6.98) while the QUS masseter value was 57.5 for the two ALS patients. As shown below (Figures 2a-c), the image of a healthy tongue has more clearly defined anatomical features and appears darker than the image of a tongue in a patient who has ALS.
2. EIM discriminates between healthy and diseased muscle.

When measuring masster in the longitudinal plane, the average EIM value for healthy subjects and the EIM value of a patient with motor neuron disease were 6.8 (standard deviation 2.1) and 3.24 respectively. Similarly, the average EIM value for the tongues of 16 healthy controls measured from the submandibular region was 7.97 (standard deviation 2.34) and the average value for two ALS patients was 4.42. The tracings of diseased and healthy EIM measurements clearly differ (Figures 3 b, c).

In addition, Shellikeri, Yunusova, Green, Pattee, Berry, Rutkove, and Zinman have shown how EIM tongue measurements demonstrate meaningful differences between healthy subjects and patients with ALS (40). Compared to values from the control group, EIM phase values from the ALS patients were significantly lower (p=0.007).

3. EIM of the tongue correlates with a standard measure of bulbar function.

In addition to distinguishing between healthy and diseased muscle, EIM measurements of the tongue correspond with results of standard assessments of tongue function. In a pilot study of 17 ALS patients, EIM phase values were positively correlated with Iowa Oral Performance Instrument (IOPI) tongue endurance measurements in ALS patients (r
These findings form a foundation for further studies evaluating the agreement between tongue EIM data and results of commonly employed metrics of bulbar dysfunction.

4. **Composite biomarkers can offer even greater accuracy and sensitivity to disease status.**

Both QUS and EIM can offer valuable information when employed independently. However, the quality of muscle data could be optimized by using the techniques together, since they have complementary strengths and weaknesses. For instance, ultrasound data can change as a function of transducer angle, which is not a concern in EIM (30). Conversely, the accuracy of EIM measurements can be affected by the degree of subcutaneous fat, which is much less of a factor in QUS (31). There are a number of approaches for merging QUS and EIM information, ranging from simple z-score combinations to higher-order mathematical techniques such as machine learning (e.g. support vector machines.) In fact, we have recently shown that combining QUS and EIM data sets mathematically can yield even more powerful biomarkers than either set alone (32), as shown in Figure 4.

**Approach: Methods**

**Participants.** 32 patients with probable, probable lab supported, or definite ALS (El
Escorial Criteria(33)) aged 40 to 79 years; 32 age- and gender-matched healthy volunteers will also be enrolled. Participants will be recruited so that enrollment ultimately mirrors the 2:1 frequency of ALS in men versus women. **ALS patient inclusion criteria:**

1. Established diagnosis of ALS;
2. Age 40-79 years. Note: Given the preliminary nature of this work, we will specifically aim to recruit individuals at all phases of disease (from early to end-stage) to identify a range of orofacial EIM values in ALS. **Exclusion criteria:** History or presence of a neurological disorder, or other medical condition, that substantially impacts bulbar function.  

**Site/Necessary Facilities:**

Beth Israel Deaconess Medical Center includes all necessary facilities for this study.  

**Recruitment:** Healthy volunteers will be recruited to join the study through internet postings. Individuals with ALS will be recruited from our outpatient neuromuscular clinics. We will also perform outreach to colleagues at other medical centers in the greater Boston area.  

**Visit Protocol and Measures:**

1. **History and examination** (All participants). After reviewing the purpose and procedures of the study, each subject will be asked to sign the IRB-approved informed consent form. Basic medical, social, and family history will then be obtained and recorded. Previous medical records will be reviewed and the diagnosis of ALS confirmed. Body mass index, presence or absence of a gastrostomy, most recent pulmonary function data, and results of swallowing studies will be noted. Ancillary information including any relevant serology (e.g. creatine kinase level), cerebrospinal fluid, and muscle biopsy results will be reviewed. Brief general and complete neurological examinations, including gross assessment of bulbar function and tongue atrophy, will then be performed.

2. **ALSFRS-R and functional assessments** (Patients only). The full ALSFRS-R will be
administered and bulbar subscore calculated (34). 3. **Iowa Oral Performance Instrument (IOPI) Measurements of Tongue and Lip Strength** (Patients only) (35). Orofacial strength measurements will be made by Dr. McIlduff or a trained research assistant using the Iowa Oral Performance Instrument (IOPI Medical LLC, Redmond, WA). Lip (interlabial) compression, cheek (buccodental) compression, tongue elevation strength, and tongue endurance will be assessed according to procedures outlined by Clark and Solomon (35) The IOPI measure of tongue endurance will represent the primary dependent variable. 4. **Sentence Intelligibility Test (SIT)** (Patients Only) (36). We will digitally record patients reading aloud 11 randomly generated sentences of successively longer length. The recordings will then be transcribed by an individual unfamiliar with the speakers. From this transcription information, the percentage of intelligibly produced words will be calculated. The number of words per minute will also be recorded (37). 5. **Timed Tasks** (Patients only) (38). Individuals with ALS will be asked to repeat “ticker” ten times as quickly as possible; the time it takes to perform this task will be recorded in seconds. Individuals with ALS will be asked to repeat “pepper” ten times as quickly as possible; the time it takes to perform this task will be recorded in seconds. If safe, individuals with ALS will be asked to swallow three ounces of water; the time it takes to perform this task will be recorded in seconds. 6. **Sydney Swallow Questionnaire (SSQ)** (Patients only) (39). Individuals with ALS will complete this self-report questionnaire about the ease of swallowing items of different consistencies. 7. **QUS** (All participants) (25). Static measurements will be performed on selected muscles (Table 1) using a Terason t3000 system, with quantification of gray-scale and backscattered data (25). Measurements will be performed first by Dr.
McIlduff, then by a trained research assistant, and then again by Dr. McIlduff to obtain intra- and inter-rater variability values. 8. **EIM** (All participants). Static multifrequency EIM measurements will be performed using pre-configured electrode arrays and a commercial multifrequency bioimpedance device (Imp SFB7®, Impedimed, San Diego, CA) on selected muscles (Table 1).

Again, measurements will be performed first by Dr. McIlduff, then by a trained research assistant, and then again by Dr. McIlduff to obtain intra- and inter-rater variability values. **Visit**

**Schedule:** At the conclusion of the first session, participants will be asked to return for follow-up visits at approximately 3-month intervals for up to 18 months (Table 2). To help ensure retention of subjects, study visits will be specifically coordinated for a date when patients are returning for regularly scheduled clinic visits to obviate the need for additional trips to the hospital. If necessary, home visits will be conducted as well; this is an approach we have taken in other studies (9). All procedures will be repeated at each subsequent appointment. **Reimbursement:** With a goal of optimizing participant retention, all subjects will be reimbursed for the cost of transportation ($30 cap per visit) and their time at a $20/visit rate.

| Table 1. Selected Orofacial and Appendicular Muscles to be evaluated using QUS and EIM |
|------------------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Orofacial Muscles** | **Limb Muscles** |
| Frontalis | Biceps |
| Masseter | Brachii |
| Orbicularis oculi | Tibialis |
| Orbicularis oris | anteriors |
| Sternomastoid | Temporalis |
| Tongue | |

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Specific Aim # 1: To assess the reliability of orofacial QUS and EIM in individuals with and without ALS. For the QUS and EIM measures described above, we will have obtained two sets of data from the initial visit (an intra-rater and an inter-rater set). Therefore, standard tests of inter-and intra-rater reproducibility, (including intraclass correlation coefficients, the coefficient of variation in the percent difference between measures, and Bland-Altman analysis), will be completed. In addition, we will compare the reliability of each of the technologies in individuals with and without ALS. If the coefficients of variation are greater than anticipated (e.g. approximately 15%) for QUS and EIM based on preliminary data, then potential technique modifications will be explored. The inclusion of normal subjects in this part of the analysis bolsters sample size and facilitates a comparison of repeatability between individuals with and without ALS.

Specific Aim # 2: Goal: To determine how QUS and EIM measurements of selected orofacial muscles in individuals with and without ALS evolve over an 18-month period. According to individual subject, for each QUS and EIM result, we will calculate the difference between the baseline observation at the initial visit and the value at each follow-up visit at three-month intervals. In the ALS group, we expect these values to decline over time. In the normal subject group, these values are expected to remain stable over time. Thus, the individual difference measures are expected to differentiate between the two study groups. We also will plot the individual data points against time for both normal subjects and ALS. We will then perform a random effects analysis where we assume that the rate of change over
time is linear with slopes and intercepts randomly distributed about average values. Thus, for each patient, the data over time will be fitted to a line and the slopes of those lines used in the analysis. The dependent variables are the outcome measures’ slopes and the independent variable is time. We will perform a similar analysis for the normal subject data. We will thereby characterize how the orofacial muscles of patients with ALS evolve over time and how they differ from healthy individuals. This data could potentially serve as an important baseline for the evaluation of therapeutic effects in clinical trials, the normal subject data serving as a substitute for patients being treated with an effective drug.

**Specific Aim #3: To determine preliminarily whether orofacial QUS and EIM measurements correlate with results of bulbar and systemic functional tests.** For patients, we will assess the relationships between the results of standard measures with the values generated by QUS and EIM. As with Aim 2, data will be plotted cross-sectionally to identify relationships between QUS and EIM and standard measures. Results will also be analyzed longitudinally to determine how QUS and EIM values change over time and how these compare to standard measures, using a fixed linear model. The response will be a functional measure and the primary predictor of interest a QUS or EIM measure. A positive slope for a QUS or EIM measure would be evidence of a significant association between the functional measure and the QUS or EIM measure. To account for the repeated measures, we will model subject-specific slopes and intercepts as random effects. Additional covariates will be added to the model if the p-value from the likelihood ratio test for their inclusion is < 0.05.
Candidate covariates include age and time since disease onset. To assess the degree of association between the functional measures and QUS or EIM, the t-score for the QUS or EIM predictor will be used. **Sample size estimation:** Based on other conditions (for example Duchenne muscular dystrophy) for which we have ample data, with 32 patients per group, we anticipate that there will be a 90% power to detect a 0.5 standard deviation difference in a given ultrasound value and a 0.3 standard deviation difference for EIM data point in a patient with ALS as compared to a normal subject (Aim 2). Given the limited data set, we have not attempted to perform any power analysis for Aims 1 or 3.

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{SELECTED ABSTRACTS}

1. Electrical Impedance Myography and Quantitative Ultrasonography: Tools to Quantify Tongue Health
Courtney McIlduff MD, Sung Yim BA, Tom Geisbush BA, Aleksandar Mijailovic BS, Adam Pacheck BS, and Seward Rutkove MD (Boston, MA)

BACKGROUND: Speech and swallowing abnormalities accompany many neuromuscular disorders for which there are limited treatment options. Reliable tools that quantify underlying motor dysfunction are needed for use in clinical therapeutic trials. Electrical impedance myography (EIM) and quantitative ultrasonography (QUS) are well-suited to provide biomarker information because the methods are painless, non-invasive, and user-friendly. Existing work indicates that EIM and QUS measurements of craniobulbar muscles, including the tongue, could provide valuable information about disease status.
OBJECTIVES: 1. To compare tongue EIM and QUS data from healthy controls and neuromuscular patients with bulbar symptoms; 2. To determine the reliability of tongue EIM and QUS measurements in healthy participants

METHODS: EIM and QUS tongue measurements were performed in 36 healthy individuals and five patients with neuromuscular disease of both genders aged 22-71 years. EIM 50 kHz phase values (degrees) and QUS grayscale level (GSL) were the primary endpoints used to compute descriptive statistics. Reliability was assessed by calculating the intraclass correlation coefficient (ICC) and percent variability.

RESULTS: For EIM, the average tongue values were 11.47±1.02° for healthy participants and 10.78±1.97° for patients. The intra- and inter-rater reliability ICC values obtained in the healthy subjects were 0.75 and 0.68 with 4.47% and 6.36% variability, respectively. For QUS, the average tongue values were 25.67±6.04 for healthy participants and 45.92 ±10.88 for patients. Intra- and inter-rater reliability ICC values obtained in the healthy subjects were 0.75 and 0.78 with 18.3% and 17.7% variability, respectively.

CONCLUSIONS: EIM and QUS are two techniques that provide reliable tongue measurements in a group of healthy subjects, and may be helpful in distinguishing diseased from healthy tongue muscle. Further study of both techniques is warranted to determine their role as biomarkers.
An Improved Electrical Impedance Myography (EIM) Tongue Array for Use in Clinical Trials

Courtney McIlduff MD, Sung Yim BA, Adam Pacheck BS, Tom Geisbush BA, Aleksandar Mijailovic BS, and Seward Rutkove MD

**BACKGROUND:** Electrical impedance myography (EIM) measurements of the tongue could provide valuable information about bulbar disease status in amyotrophic lateral sclerosis (ALS) clinical therapeutic trials. A prototype tongue depressor EIM array produced gag reflexes.

**OBJECTIVE:** To determine the reliability, tolerability, and normal values of tongue EIM measurements using a smaller electrode array in healthy volunteers and neuromuscular patients

**METHODS:** Tongue EIM measurements were performed in a total of 35 participants using a novel electrode array. Reliability was assessed by calculating the intraclass correlation coefficient (ICC) and percent difference in addition to performing Bland-Altman analyses. Standard descriptive statistics were also determined.

**RESULTS:** At the 50 kHz frequency, the ICCs for intra- and inter-rater reliability were 0.76 with 5.17% difference and 0.78 with 5.34% difference respectively. The mean EIM phase values were 11.61 +/- 1.00° (healthy participants) and 9.87 +/- 1.28° (patients). None of the participants experienced gag reflexes or discomfort.
**CONCLUSIONS:** The small tongue array provided good inter- and intra-rater reliability, and was well-tolerated.

3. **Electrical Impedance Myography of the Tongue at 300 kHz Provides Improved Distinction Between Healthy and Diseased Muscle**

Courtney McIlduff MD, Sung Yim BA, Adam Pacheck BS, and Seward Rutkove MD

**BACKGROUND:** Electrical impedance myography (EIM) data is routinely collected and analyzed at 50 kHz, the frequency at which healthy muscle is most reactive. Recent work has shown that 50 kHz EIM measurements of the tongue also reflect clinical status of patients with neuromuscular disease. However, direct comparisons of healthy and diseased muscle might not be most apparent at this frequency.

**OBJECTIVE:** To determine at what frequencies of current EIM may distinguish healthy from diseased tongue muscle.

**METHODS:** Tongue EIM measurements from 31 healthy participants and 5 neuromuscular patients were collected and analyzed at frequencies between 50 and 500 kHz.

**RESULTS:** The difference in mean tongue EIM phase value (in degrees) between the healthy and diseased participants was 1.74° at 50 kHz and 4.20° at 500 kHz. However,
a maximum difference of 4.70° was observed at 300 kHz, with a mean value (± standard deviation) of 18.60±1.76° for healthy subjects versus a mean of 14.03±1.69° for neuromuscular disease patients.

**CONCLUSIONS:** In this small cohort, EIM detected differences between healthy and diseased tongue muscle at frequencies ranging from 50-500 kHz, with the most pronounced separation at 300 kHz.

**DISCUSSION AND FUTURE DIRECTIONS:**

These data suggest EIM and QUS are reliable tongue measures, and provide preliminarily support for the role of the tools as surrogates for bulbar status in neuromuscular disease. The analyses included here involve neuromuscular patients who have evidence of lower motor neuron dysfunction on examination; whether EIM and QUS can detect preclinical signs of disease is yet to be determined. Additional work is also needed to learn if the techniques can discriminate upper from lower motor neuron deficits.

EIM data were analyzed at 50 kHz because most commercially-available bio-impedance devices provide only a single 50 kHz frequency of current. In addition, healthy muscle is most reactive at 50 kHz. Future work will assess data at several different frequencies since disease-associated changes in cellular structure do alter EIM tracing trajectories along the multi-frequency spectrum.
EIM and QUS provide distinct but complementary information about muscle health. Both techniques represent promising candidates to provide biomarker information since they are quantitative, painless, portable, and user-friendly. Going forward, the technologies could play important roles in the investigational and clinical evaluation of a variety of neurological disorders associated with tongue weakness and dysfunction.

This work was funded by the National Institutes of Health R01AR060850.