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Quantitative muscle ultrasound and electrical impedance myography in late onset Pompe disease: A pilot study of reliability, longitudinal change and correlation with function

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ABSTRACT

Background/objectives: Late-onset Pompe disease (LOPD) is slowly progressive, making it difficult to assess clinical change and response to interventions. In this study, quantitative muscle ultrasonography (QMUS) and electrical impedance myography (EIM) were evaluated as potential biomarkers.
Methods: 25 patients with confirmed LOPD were recruited from the Duke Pompe Clinic and evaluated with standard clinical measures, QMUS, standard EIM (sEIM) and hand-held EIM (hEIM). Patients were evaluated at baseline, 12 months and 24 months. MUS, sEIM and hEIM were compared with the clinical data. Five patients were given hEIM devices to perform measurements at home.
Results: QMUS and hEIM had good reliability as measures of muscle structure and conduction properties. Home, patient-performed hEIM measurements did not differ significantly from those performed in the clinic setting. Thirteen patients completed all follow-up measures. Most measures did not change over the study period, however, vastus lateralis echointensity increased 27%, a sign of declining muscle health. Additionally, significant correlations between QMUS, hEIM and measures of muscle strength and function were present.
Conclusions: QMUS and hEIM may provide useful outcome measures for future studies in LOPD with hEIM providing an opportunity to collect data at home. Larger, multicenter studies are needed to explore these possibilities.

1. Introduction

Late onset Pompe disease (LOPD) is an autosomal recessive disorder that results from deficiency of acid alpha-glucosidase (GAA), a lysosomal enzyme. GAA deficiency leads to accumulation of glycogen within skeletal and smooth muscle, along with other body tissues, including the liver. Hypertrophic cardiomyopathy can occur and without treatment, the infantile onset variant (IOPD) is fatal in the first 12 years of life. The presentation of LOPD has greater variation with age of presentation ranging from the first year of life to late adulthood. Characteristic symptoms include proximal limb weakness and respiratory insufficiency [2]. Recognizing and diagnosing IOPD and LOPD are critical, as enzyme replacement therapy (ERT) is available and shown to benefit both populations [19,27]. Diagnosis of LOPD has improved over the years with increased recognition by clinicians, the availability of genetic testing and the use of muscle imaging. However, there are difficulties with monitoring progression of disease.

Proving efficacy of ERT and novel therapies to augment patient response is not simple. Patients with LOPD currently undergo muscle biopsy to assess the effect of ERT and new interventions [16]. This approach is invasive and may be painful. Furthermore, the effects of LOPD on muscle can be patchy, making the results of biopsy questionable. Clinical outcome measures like the 6-min walk test are affected by numerous factors, including patient participation and disease severity, as some patients may lose the ability to ambulate [4]. Whole body MRI of muscle is an alternative outcome measure but is costly and not easy to perform in patients with advanced respiratory muscle weakness [8,11]. Non-invasive, inexpensive, point-of-care methods for assessing muscle health are needed for clinical trials and health tracking, particularly methods that can be performed at home. Two non-invasive technologies

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have excellent potential to provide sensitive, quantitative measures of muscle reserve and function – electrical impedance myography (EIM) and quantitative muscle ultrasound (QMUS).

EIM has been proposed as a potential means of tracking change in muscular disorders. [24,25,33] It is a non-invasive tool providing information about muscle integrity through the calculation of surface electrical impedance. It shows promise as being more sensitive than current clinical measures or functional rating scales, but there is little information regarding its reproducibility [13]. Recent studies have displayed its utility in Duchenne muscular dystrophy, amyotrophic lateral sclerosis and facioscapulohumeral muscular dystrophy. [14] Furthermore, new portable EIM devices provide the possibility of patients performing their own measures at home [15]..

QMUS also has potential for monitoring muscle health. Like EIM, there is a paucity of information on reliability and reproducibility in LOPD. Early studies have shown an ability to differentiate diseased from healthy muscles. [10,28] Calculation of subcutaneous fat and muscle thickness is easily performed, and muscle brightness (echointensity (EI)) correlates with fibrofatty replacement of normal muscle tissue. [1,31] Longitudinal measures of muscle thickness and EI measured through QMUS could provide another means of providing information on muscle change before it becomes clinically apparent [28]. The ability to detect pre-clinical changes in muscle could provide firm rationale for initiation of ERT in patients carrying pathogenic, late onset Pompe mutations detected on genetic screening [21]..

In this study we aim to determine the correlation between QMUS, EIM and currently accepted measures of physical function. The study will also assess the reliability of EIM measures performed in the home through use of a handheld device. As a tertiary goal, the study will examine if pre-clinical disease progression can be detected via either QMUS or EIM.

2. Methods

This study was approved by the Duke University Institutional Review Board and all patients provided written informed consent. The study was funded by Sanofi Genzyme through an Investigator Sponsored Study research grant.

Twenty-five patients were recruited through the Duke Pompe Clinic. Inclusion criteria were a genetically confirmed diagnosis of Pompe disease, plans for continued care at Duke and the ability to provide informed consent. Those unable to provide informed consent were not asked to participate. All subjects were to undergo QMUS and EIM at baseline, 12 months, and 24 months.

Five patients were asked to take home the handheld EIM device at their baseline clinic visit. Consecutive participants were asked to do so until the goal of 5 patients was reached.

2.1. Quantitative Muscle Ultrasonography (QMUS)

QMUS was performed at baseline, 12 months and 24 months in all subjects. A single Esaote MyLabSIX (Genoa, Italy) ultrasound system was used for all measures, equipped with a 6–18 MHz linear array probe. Probe frequency was held at 6 MHz with constant gain, compression and time gain compensation settings. Depth adjustments were permitted to accommodate body habitus. Ultrasound data were digitally stored in the ultrasound system and processed off-line after each visit was complete.

Imaging of the deltoid, biceps brachii, triceps brachii, forearm flexor compartment, vastus lateralis, and anterior tibialis muscles was performed over the mid-point of each muscle while it was held in a relaxed posture. Images were collected in the axial plane for each muscle, as this is the accepted standard for calculating QMUS parameters. Patients were seated upright with their feet on the ground or on a step stool for all imaging. Supine positioning was not performed as some patients were unable to safely maintain this position due to respiratory insufficiency. Still images were recorded at each site and stored for later review.

Off-line independent, analysis was performed by an examiner (LHW) blinded to the subjects' other clinical and study findings. Subcutaneous fat thickness was calculated by measuring the distance from the skin surface to the superficial fascia of the muscles using on-screen calipers. Maximal muscle thickness and echointensity (EI) was recorded for each muscle. Muscle thickness was defined as the distance from the underlying bony border to the superficial fascia of the muscle and measured with on-screen calipers. EI was measured by exporting the still images to Adobe Photoshop (Adobe Systems Incorporated, San Jose, CA) for grayscale analysis scoring. This was performed using a rectangular box placed in the superficial third of the muscle with attention to avoid bone and fascia. The size of box was not constant for this reason. Grayscale was rated 0 (pure black) to 255 (pure white) and is without units.

2.2. Electrical Impedance Myography - Standard Equipment (sEIM)

sEIM was performed at baseline and at 12 and 24 months utilizing a device (ImpediMed, Inc., Carlsbad, CA, USA) previously used in the assessment of neuromuscular disease. This device provides a painless, surface 50 kHz/200 kHz alternating current over muscle with 4 adhesive electrodes in place for recording impedance. Two measures are reported with this method – resistance and capacitance. EIM testing was performed over the deltoid, biceps brachii, forearm flexors, triceps brachii, vastus lateralis, and anterior tibialis muscles, at the sites previously described for QMUS. Measurements were taken over the muscles' axial plane, matching the probe orientation for QMUS and the handheld EIM device. Three measures were recorded for each muscle and averaged for the mean value. The subjects had EIM performed by 2 independent clinicians at each visit, creating 2 sets of measures for each muscle.

2.3. Electrical Impedance Myography - Handheld Device(hEIM)

A handheld, portable, commercially available fitness device was used for this portion of the study (AIM, Skulpt, Inc.). This smart-phone sized device uses the same methods as the previously described sEIM but provides users with both a body fat % measurement for each muscle assessed, as well as a Muscle Quality (MQ) score derived from raw EIM data. The range for MQ was 0–100 with higher scores indicating healthier muscles. Electrodes on the device are moistened with water and then placed over each muscle for approximately 5 s, while measurements are made. Muscle selection was identical to that performed with the office based, sEIM equipment. The subjects had hEIM performed by 2 independent clinicians at each visit (PJZ, SSR, LHW).

Five patients were asked to take home the hEIM device at their baseline clinic visit. Training was performed by the study coordinator (ANP) for each site prior to the subject leaving clinic with the device. All subjects demonstrated correct use prior to leaving the office visit. The subject recorded measurements of the deltoid, biceps brachii, triceps brachii, forearm flexors, vastus lateralis and anterior tibialis once weekly for 6 weeks and then return the device by mail.

2.4. Clinical measures

Patient age, sex, height and weight were recorded at the initial visit. Weight and height were recorded at subsequent visits as well, along with ERT dose and duration of ERT therapy. Clinical outcome measures are required to determine if either QMUS or EIM measures correlate with patient function. Handgrip dynamometry was utilized as a measure of upper extremity function for direct comparison with forearm QMUS and EIM measures. The 30-s chair stand and 6-min walk tests served as a functional measure of quadriceps performance in those who are able to complete the tasks and compared with vastus lateralis QMUS and EIM data. Medical Research Council (MRC) grading of strength was also performed for the deltoid, biceps brachii, triceps brachii, wrist flexors, quadriceps and anterior tibialis. All of these measures are routinely performed by physical therapy as part of the routine Pompe disease

annual clinic visits and recorded from those clinical records, as opposed to be being performed by the investigators. The physical therapists had no knowledge of any QMUS or EIM results.

The Short Form Health Survey (SF-36) and Barthel Index were administered at each clinic visit by the study coordinator (ANP) in an effort to capture the patients' functional status outside of the clinic. In the event that a patient was unable to make a follow-up visit, the study coordinator contacted the patients by phone and worked with subjects to acquire this data. The SF-36 is scored such that a higher value indicates more favorable health states on a scale of 0–100. It is a 36-item patient-reported questionnaire that covers eight health domains: physical functioning (10 items), bodily pain (2 items), role limitations due to physical health problems (4 items), role limitations due to personal or emotional problems (4 items), emotional well-being (5 items), social functioning (2 items), energy/fatigue (4 items), and general health perceptions (5 items) [7]. The Barthel Index is an ordinal scale used to measure a person's ability to perform activities of daily living. Each item on this scale has a given number of points assigned to each level or ranking. It uses ten variables to describe ADLs and mobility [9,30]. The highest score possible is 20, indicating normal function.

Study data were collected and managed using REDCap electronic data capture tools hosted at Duke University. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources. The study coordinator entered all data into RedCap and any questions were reviewed with the principal investigator (LHW).

2.5. Statistical analysis

QMUS and EIM measures were analyzed for inter-rater reliability as measured by the intraclass correlation (ICC) method. For QMUS, muscle thickness, subcutaneous fat thickness and muscle echogenicity were examined for correlation with the baseline SF-36 and Barthel Index, MRC scores, handgrip dynamometry, the 6-min walk test and the 30-s chair stand test. For EIM, the same correlations were assessed. Data were examined to determine if linear or curvilinear relationships exist, and Pearson's correlation coefficient or multiple regression were used as appropriate. The level of significance was set at <0.05.

For patients completing the 24-month study perioid, QMUS and EIM measures from the baseline, 12 month and 24 month visits were also assessed for change from baseline by the method due to Bland & Altman [5,6], as well as by stability of the measure (by ICC).

For all correlations, 0.9–1.0 were rated excellent, 0.8–0.89 very strong, 0.6–0.79 moderate and 0.4–0.59 fair. [3] Values <0.40, even if statistically significant, were considered to have no correlation.

3. Results

3.1. Patient characteristics

Twenty-five subjects with LOPD were enrolled in the study at Duke University from October 2016 through April 2018 (9 males, 16 females). Three patients completed only one visit (baseline), while two patients completed 2 of 3 visits. Twenty patients completed all three visits in some form. It should be noted that 8 patients were scheduled to complete their 24-month assessment as part of the annual Duke Pompe Clinic meeting in late March 2020, but this was cancelled due to the COVID-19 pandemic and not rescheduled. Only one of those patients lived within the geographical region and was able to visit for assessment.

The patients had a mean age of 50.1 ± 26 years and mean body mass index (BMI) of 26.8 ± 4.7. Twenty-three of 25 were receiving enzyme replacement therapy at the initial visit and all subsequently received dosing every 2 weeks. The mean dose was 22.2 ± 6.3 mg/kg. On average, patients had been receiving ERT 4.2 ± 2 years at the time of the baseline visit with a range of 0.08–14 years.

3.2. Quality of life measures

The SF-36 physical functioning, role limitations due to physical health, role limitations due to emotional health, energy/fatigue, social functioning, pain, emotional well-being, general health and health change scores did not change over the 24-month follow-up period, although there were trends toward improvement seen. The only exception to this pattern was health change, which demonstrated statistically significant improvement at the 12-month mark (43 to 54, $p = 0.049$).

The Barthel Index did not change over the length of the study (18.7 at baseline and 19.0 at 24 months, $p = 0.64$), a meaure correlating with near full independence.

3.3. QMUS measurements

3.3.1. Inter-Rater reliability

Inter-rater reliability was assessed for all QMUS data collected over the study period. Muscle thickness measures for deltoid (0.84), biceps brachii (0.84), forearm flexors (0.83), vastus lateralis (0.85) and tibialis anterior (0.95) were all in the strong/very strong range. Triceps brachii (0.68) inter-rater reliability for muscle thickness was moderate.

For subcutaneous fat thickness, deltoid (0.88), triceps brachii (0.85), vastus lateralis (0.98) and tibialis anterior (0.94) all had strong to very strong performance. Biceps brachii (0.79) and forearm flexors (0.74) subcutaneous fat thickness reliability fell into the high moderate range.

Muscle EI was expected to have the weakest performance based upon the multiple factors than can affect measurements. Correlations for deltoid (0.75), triceps brachii (0.74), forearm flexors (0.69), vastus lateralis (0.66) and tibialis anterior (0.77) were all in the high moderate correlation range. Biceps brachii (0.55) EI had only a fair agreement between examiners.

3.3.2. Baseline characteristics and change over time

Muscle thickness at baseline, 12 months and 24 months are provided in Table 1 and sample images provided in Fig. 1. Only the deltoid, biceps brachii and forearm flexors demonstrated significant changes over the study period. Deltoid and biceps brachii thickness increased between baseline and 12 months (21.0 to 24.5 mm, $p = 0.021$; and 25.7 to 31.9 mm, $p = 0.008$). This change was not significant at 24 months, although mean thickness remained increased at 23.0 mm for the deltoid and 28.8 mm for the biceps brachii. Forearm flexors had a different pattern with baseline thickness of 29.0 mm decreasing to 24.2 mm at 24 months ($p = 0.006$), although no change was evident at the 12-month mark.

Subcutaneous fat thickness did not change at 12 or 24 months in any muscle sampled (Table 1). This was expected in the absence of any significant weight changes in the study participants and provided additional reassurance regarding the reliability of QMUS measures over time.

Muscle EI was also stable in all muscles sampled, aside from the vastus lateralis (Table 1). The vastus lateralis EI was 77.1 at baseline, 84.5 at 12 months and 97.7 at 24 months. Only the 24-month measure reached significance and was increased 27% from baseline ($p = 0.04$). Increasing EI is an indicator of increased fibrofatty replacement of the muscle.

3.3.3. QMUS correlations

Deltoid, biceps brachii, triceps brachii, forearm flexor and vastus lateralis thickness, subcutaneous fat thickness and EI showed no correlations with age. For the anterior tibialis, EI had a significant correlation with age ($r = 0.46$, $p = 0.02$). Deltoid, biceps brachii, triceps brachii, forearm flexor, vastus lateralis and tibialis anterior QMUS measures had

Table 1
QMUS baseline and longitudinal measures.

Muscle	Baseline Entire Group ($n = 25$)	Baseline ($n = 13$)	12 Months (n = 13)	24 Months (n = 13)	Change at 12 Months/24 Months p
Muscle thickness					
Deltoid	21.5 ± 5 mm	**21.0 ± 5 mm**	**24.5 ± 6 mm**	23 ± 6 mm	**0.021**/0.47
Biceps brachii	28.2 ± 7 mm	**25.7 ± 7 mm**	**31.9 ± 7 mm**	28.8 ± 6 mm	**0.008**/0.32
Triceps brachii	28.1 ± 7 mm	27.3 ± 6 mm	29.5 ± 6 mm	29.9 ± 6 mm	0.47/0.45
Forearm flexors	27.9 ± 7 mm	**29.0 ± 8 mm**	31.8 ± 7 mm	**24.2 ± 6 mm**	0.5/**0.006**
Vastus lateralis	73.0 ± 17 mm	66.2 ± 16 mm	66.4 ± 16 mm	77.3 ± 25 mm	0.85/0.20
Tibialis anterior	26.2 ± 4 mm	26.7 ± 4 mm	27.6 ± 6 mm	25.0 ± 4 mm	0.62/0.19
Subcutaneous fat thickness					
Deltoid	6.3 ± 3 mm	6.2 ± 2 mm	6.3 ± 3 mm	5.8 ± 2 mm	0.14/0.20
Biceps brachii	6.9 ± 6 mm	6.6 ± 4 mm	5.6 ± 3 mm	5.2 ± 4 mm	0.76/0.37
Triceps brachii	7.7 ± 4 mm	7.9 ± 5 mm	9.3 ± 4 mm	9.1 ± 5 mm	0.10/0.17
Forearm flexors	3.7 ± 2 mm	3.5 ± 2 mm	4.3 ± 2 mm	4.7 ± 2 mm	0.25/0.09
Vastus lateralis	10.4 ± 7 mm	10.9 ± 8 mm	10.5 ± 6 mm	10.0 ± 6 mm	0.99/0.66
Tibialis anterior	4.0 ± 2 mm	4.3 ± 3 mm	5.0 ± 2 mm	3.7 ± 2 mm	0.19/0.24
Echointensity					
Deltoid	64.3 ± 20	61.2 ± 18	55.1 ± 23	59.2 ± 23	0.28/0.56
Biceps brachii	94.3 ± 20	90.5 ± 20	89.3 ± 22	88.9 ± 23	0.94/0.68
Triceps brachii	51.1 ± 18	53.3 ± 18	48.7 ± 18	48.1 ± 20	0.63/0.33
Forearm flexors	71.2 ± 18	65.5 ± 15	67.4 ± 19	78.0 ± 26	0.70/0.14
Vastus lateralis	86.4 ± 20	**77.1 ± 22**	84.5 ± 17	**97.7 ± 21**	0.45/**0.04**
Tibialis anterior	85.0 ± 17	88.4 ± 18	81.0 ± 18	91.1 ± 23	0.20/0.19

Measures listed as mean +/− standard deviation. Only 13 patients completing study analyzed for longitudinal changes. Signficant values indicated in bold type.

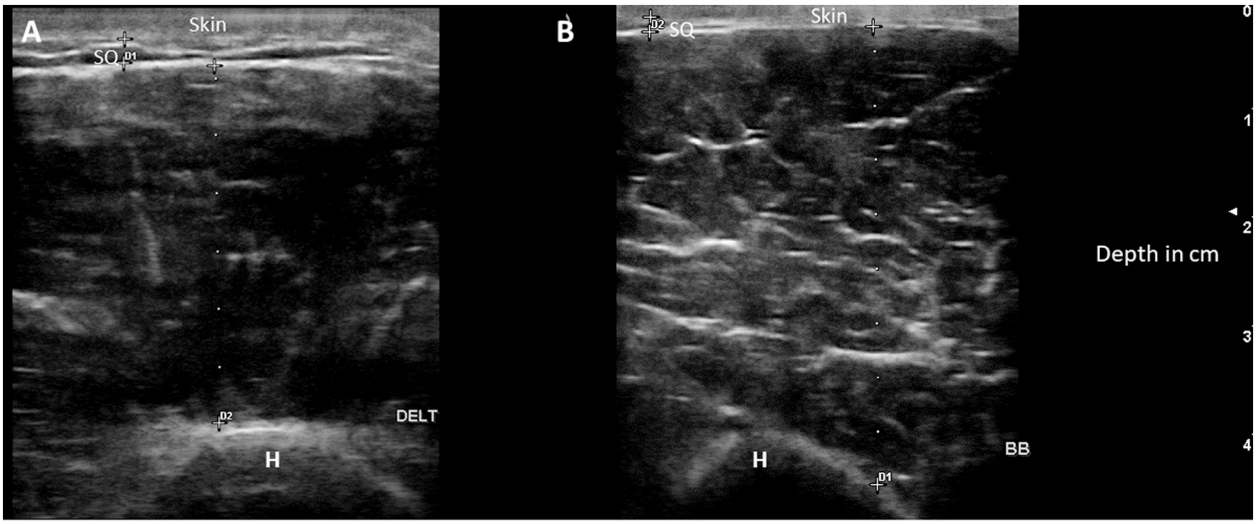

Fig. 1. Sample QMUS images of the deltoid (A) and biceps brachii (B). The calipers indicate muscle thickness. The increased echointensity (EI) of the biceps brachii relative to the deltoid is evident on visual comparison; quantitatively EI measured 24 in the deltoid and 45 in the biceps brachii. H:humerus, SQ: subcutaneous fat.

no significant correlations with duration of ERT. The forearm flexor, vastus lateralis and tibialis anterior QMUS measurements had no correlations with BMI. The biceps brachii thickness (r−0.40, $p = 0.0491$), subcutaneous fat thickness (r=0.45, $p = 0.0250$) and EI (r=0.54, $p = 0.4681$) all correlated with BMI. For the triceps brachii, there were correlations with BMI for subcutaneous fat thickness (r=0.56, $p = 0.0038$) and EI (r=0.51, $p = 0.0093$). In the deltoid, only subcutaneous fat thickness correlated with BMI (r=0.53, $p = 0.0070$).

Hand grip dynamometry measures correlated with measured muscle thickness only for the deltoid ($r = 0.71$, $p = 0.0031$) and forearm flexors ($r = 0.55$, $p = 0.0325$). For subcutaneous fat thickness, there was a negative correlation with hand grip strength for vastus lateralis ($r = -0.64$, $p = 0.0097$). In regard to EI, only deltoid was found to have a relationship with grip strength, but the negative correlation was robust ($r = -0.83$, $p = 0.0001$).

There were no significant correlations between MRC scores and QMUS muscle thickness, subcutaneous fat thickness or EI for any site sampled. This is attributed to the majority of scores rated either 5 on the MRC scale for each muscle tested. The same analysis was performed for the 6MWT, showing a moderate negative correlation only with vastus lateralis thickness ($r = -0.52, p = 0.0234$). The same was true of the 30-s chair stand test, where vastus lateralis thickness negatively correlated with performance ($r = -0.60$, $p = 0.0298$).

The relationships between SF-36 responses and QMUS measures were also examined. No relationships were present between muscle thickness and any of the any of the domains aside from a negative correlation between vastus lateralis thickness and Physical Functioning ($r = -0.42, p = 0.0347$). Subcutaneous fat thickness did not correlate with any of the domains. For EI, there were negative correlations between biceps brachii and Physical Functioning ($r = -0.46, p = 0.0223$), triceps brachii and Emotional Well-Being, Role Limitation Emotional ($r = -0.41, p = 0.0413$; $r = -0.41, p = 0.0441$) and tibialis anterior and Role

Limitation Emotional ($r = -0.42$, $p = 0.0359$). The significance of these findings is unknown, as they do not fit an expected or logical pattern.

There were correlations noted between QMUS measures and the Barthel Index. Specifically, muscle thickness of the biceps brachii ($r = 0.44$, $p = 0.0260$) and biceps brachii echointensity ($r = -0.51$, $p = 0.0086$) correlated with the index score. It makes that thicker, healthier muscle would assist in performed activities of daily living. However, no other correlations were seen in the remaining muscles tested.

3.4. Handheld EIM measurements

3.4.1. Inter-rater reliability

Deltoid (0.86), biceps brachii (0.87), forearm flexors (0.94), vastus lateralis (0.85) and tibialis anterior (0.92) muscle quality reliability were all very strong. Triceps brachii (0.77) performed slightly less well, falling into the moderate reliability range. For measured fat over muscle, deltoid (0.94), triceps brachii (0.87), forearm flexors (0.97) and vastus lateralis (0.92) all demonstrated very strong to excellent reliability between examiners. Biceps brachii (0.80) and tibialis anterior (0.79) were in the high moderate reliability range.

3.4.2. Baseline characteristics change over time

Detailed results of testing are summarized in Table 2. MQ and body fat % remained unchanged at the 12 and 24-month time periods.

3.4.3. Handheld EIM correlations

There were no correlations between age, ERT duration and MQ and body fat% over for any muscle sampled. There were negative correlations between MQ and BMI for all upper limb muscles, but not the vastus lateralis or tibialis anterior. The MQ for deltoid (-0.56 $p = 0.0039$), biceps brachii (-0.64, $p = 0.0006$), triceps brachii (-0.47, $p = 0.0186$) and forearm flexors (-0.65, $p = 0.0005$) all declined as BMI rose. Correlations were also seen between BMI and body fat% measured over the biceps brachii ($r = 0.59$, $p = 0.0020$) with trends toward this for the deltoid ($r = 0.38$, $p = 0.0608$).

As with QMUS, there were no significant correlations between MRC scores and MQ and body fat % for any site sampled. This is attributed to the majority of scores rated either 5 on the MRC scale for each muscle tested.

Hand grip dynamometry measures were found to have no correlation with MQ. However, moderate to strong negative correlations were seen between grip strength and body fat % over the measured muscle for deltoid ($r = -0.74$, $p = 0.0015$), triceps brachii ($r = -0.82$ $p = 0.0002$), and forearm flexors ($r = -0.59$, $p = 0.0217$), vastus lateralis ($r = -0.82$, $p = 0.0002$). The biceps brachii ($r = -0.50$, $p = 0.0576$) trended toward significance as well.

Unexpected results were noted when analyzing the relationship between MQ and the 6MWT. The MQ for deltoid ($r = 0.62$, $p = 0.0047$), biceps brachii ($r = 0.48$, $p = 0.0382$) and triceps brachii ($r = 0.50$, $p = 0.0282$) all had moderate positive correlations with the 6MWT, but the forearm flexors, vastus lateralis and tibialis anterior did not. Stronger, yet similar findings were observed for the 30-s chair stand test: deltoid ($r = 0.78$ $p = 0.0015$), biceps brachii ($r = 0.69$, $p = 0.0095$), triceps brachii ($r = 0.74$, $p = 0.0037$) and forearm flexors ($r = 0.58$, $p = 0.0366$). The vastus lateralis and tibialis anterior did not demonstrate correlations between MQ and the 30-s chair stand test. Body fat % measured over each muscle did not show any significant correlations with either the 6MWT or the 30-s chair stand test. However, trends toward negative correlations between body fat % and the 30-s chair stand test were seen for the deltoid ($r = -0.53$, $p = 0.06$) and possibly the biceps brachii ($r = -0.48$, $p = 0.09$).

The relationships between SF-36 responses and hand-held EIM measures were also examined. Upper limb MQ correlated positively with Physical Functioning as follows: deltoid ($r = 0.62$, $p = 0.0011$), biceps brachii ($r = 0.45$, $p = 0.0248$), triceps brachii ($r = 0.49$, $p = 0.0123$) and a trend for forearm flexors ($r = 0.49$, $p = 0.0565$). Similar results were seen for MQ and the Role Limitation Physical domain: deltoid ($r = 0.40$, $p = 0.0455$), forearm flexors ($r = 0.41$, $p = 0.448$) and a possible trend for biceps brachii ($r = 0.37$, $p = 0.0712$). There were no other correlations between the SF-36 domains and MQ aside from deltoid and Social Functioning ($r = 0.43$, $p = 0.0315$). In regard to body fat % over muscle sites and SF-36 responses, a single negative correlation was seen between the tibialis anterior and Social Functioning ($r = -0.42$, $p = 0.0392$).

For the Barthel Index, there was a correlation with forearm flexor MQ ($r = 0.434$, $p = 0.0280$) and trends toward moderate correlations for deltoid, biceps brachii and triceps brachii. As with QMUS, this may reflect the importance of upper limb function in performing the items listed on the Barthel Index.

3.4.4. Office-based EIM measurements

sEIM inter-rater reliability measures were performed at 50 kHz and 200 kHz. At 50 kHz, reliability measures were poor (<0.4) for all muscles aside from a resistance in biceps brachii (0.77) and reliability for capacitance measures over tibialis anterior (0.56). At 200 kHz, only deltoid resistance measures were reliable (0.93). Given the lack of reliability in measures and higher reliability of the hand-held EIM device, no further analysis was performed to examine for correlations. The difficulties in reliability were attributed to issues with standardization of multiple electrode placement and degree of adhesion.

3.4.5. Hand-held EIM at home

As outlined in the methods section, five patients were asked to perform EIM measures at home following their clinical visit. All 25 patients were asked before finding five willing to do so. All five patients (4 females, 1 male) completed all 36 measurements over 6 weeks unassisted. No technical difficulties were reported by the patients and none called for help. There was no statistical difference between the clinician-measured and home measured values for either MQ (59.9 vs 54.7, $p = 0.39$) or body fat percentage (24.73 vs 24.69, $p = 0.98$) overall or for

Table 2
Baseline and longitudinal hEIM measures.

Muscle	Baseline Entire group (n = 25)	Baseline (n = 13)	12 Months (n = 13)	24 Months (n = 13)	Change at 12 Months/ 24 Months p
Muscle quality					
Deltoid	39.2 ±26	40.0 ±26	39.0 ±21	39.1 ±17	0.63/0.46
Biceps brachii	41.6 ±24	43.7 ±28	40.7 ±31	41.8 ±26	0.22/0.43
Triceps brachii	38.6 ±19	41.6 ±22	33.5 ±24	42.7 ±20	0.06/0.61
Forearm flexors	44.3 ±27	44.2 ±28	49.6 ±32	38.7 ±29	0.12/0.12
Vastus lateralis	47.4 ±20	52.6 ±23	47.8 ±25	51.95 ±22	0.40/0.63
Tibialis anterior	74.25 ±21	68.0 ±25	73.3 ±18	75.9 ±21	0.63/0.15
Body Fat %					
Deltoid	33.2 ±10	32.2 ±11	33.9 ±12	32.9 ±10	0.25/0.86
Biceps brachii	28.4 ±12	30.1 ±14	29.6 ±13	27.8 ±11	0.34/0.25
Triceps brachii	35.5 ±13	34.8 ±15	37.9 ±15	37.5 ±14	0.20/0.39
Forearm flexors	21.5 ±7	21.6 ±8	20.9 ±8	21.9 ±8	0.45/0.94
Vastus lateralis	28.6 ±11	27.2 ±13	29.1 ±12	28.4 ±11	0.33/0.60
Tibialis anterior	16.9 ±9	18.0 ±9	16.0 ±6	15.7 ±6	0.71/0.51

Measures listed as mean +/− standard deviation. Only 13 patients completing study analyzed for longitudinal changes.

individual muscles. (Table 3).

4. Discussion

In the current study, QMUS and hand-held EIM were demonstrated to be promising tools for assessment of LOPD. Both had high reliability, particularly QMUS measured muscle and subcutaneous fat thickness, along with handheld EIM measured muscle quality and fat %. sEIM did not perform as well in this study, likely secondary to examiner difficulty in standardizing electrode placement. Given this finding, only QMUS and hEIM data were further examined for longitudinal change and correlations with other measures of patient function.

4.1. QMUS

There has been a paucity of other work to compare with the current study on adults with LOPD. Zaidman et al. published the earliest analysis of QMUS in LOPD patients [32]. This was a small study of 10 patients and imaging results were compared to those of 81 patients with other forms of myopathy. The authors found that most all patients with LOPD had some increased muscle EI, as measured by quantitative and qualitative approaches. A pattern of relatively normal appearing triceps brachii and abnormal elbow flexors was seen in 89% of patients with LOPD as compared to 17% of those with other myopathies. Comparing EI with a control population was not performed in the current study, but EI of the triceps brachii was lower than in other muscles and approximately half the value of that seen in biceps brachii, aligning with the Zaidman study. Increasing EI measures were correlated with reduced strength and function, but the rectus femoris was relatively insensitive as compared to elbow flexors. Much like the current study, the authors found no correlations between QMUS measures and disease duration, age, ERT duration or SF-36 scores [32]..

The current study was novel in its attempt to use QMUS to assess for longitudinal change. There were some changes in thickness over the study period with deltoid and biceps brachii thickness increasing at 12 months, while forearm flexors had reduced thickness over 24 months. Subcutaneous fat measures did not change. Muscle EI was stable, other than for vastus lateralis, where it demonstrated an increase of 27% at 24 months; lack of adequate sample size may have prevented the increased EI seen at 12 months from reaching significance. These forearm flexor and vastus lateralis findings may point to preclinical worsening over time. However, the increased in deltoid thickness argues against systemic worsening in the setting of ERT. The increased deltoid thickness may be the result of increased us of the proximal upper limbs in response to other weakness or reflect ERT-related improvement occurring in a muscle less affected by the disease process.

QMUS measured muscle thickness of the deltoid and forearm flexors also had fair to moderate correlations (0.71, 0.55 respectively) with hand grip dynamometry measures, but there was a lack of clear correlation with other tests of functioning. Of note, there were moderate negative correlations (−0.52 and − 0.60) between vastus lateralis thickness and both the 6MWT and the 30-s stand. The reasons for this isolated finding are unclear and any further discussion would be only hypothetical.

Muscle EI had a strong negative correlation (−0.81) between deltoid EI and hand grip strength, indicating that increased fibrofatty replacement of the muscles had a pronounced effect of dynamometry measures. Biceps brachii EI also demonstrated a negative correlation with the Barthel Index, indicating that elbow flexion strength is likely an important component of ADL performance in LOPD as it may reflect the importance of elbow flexion strength in performing many of the items on the index (e.g., feeding, grooming and dressing). EI of the biceps brachii and triceps brachii increased along with BMI and tibialis anterior EI had a moderate correlation with age. The latter findings are expected and would not be considered unique to LOPD.

[12] demonstrated that the posterior thigh muscles were more involved on MRI than the anterior thigh muscles [12]. In addition, the vastus intermedius was the most involved quadriceps muscle with relative sparing of the rectus femoris. Similar findings were noted in an ultrasound study by Zaidman et al. [32] In the current study, vastus lateralis was imaged and analyzed. Future studies should focus on the vastus intermedius and posterior muscles of the thigh.

4.2. hEIM

There has been little work published on EIM in Pompe disease. The EIM methods described here are based upon an older technology known as bioimpedance analysis (BIA). Muscle can conduct electricity and both BIA and EIM measure the electrical impedance (resistance and reactance) to very low levels of applied electrical current [23]. The main difference between the two is that BIA provides a whole-body assessment, while EIM provides the ability to focus on a specific muscle or muscle group. In the past, this technology has largely been used to measure and track body fat. However, in muscular diseases including LOPD, it is known that atrophic muscle is replaced by fatty tissue [18], altering impedance measures. This makes EIM an attractive investigative option for detecting muscle damage and monitoring progression of disease.

In a BIA study of 20 Pompe patients, with 11 of 20 having LOPD, the results of BIA were compared with MRI of the thigh musculature. The degree of fatty infiltration of the muscle was characterized as being either mild, moderate or severe. The authors found that in 14 of 17 patients having both tests, the results of BIA and MRI were in agreement [23]. With this information in mind, there was good rationale for using EIM as a measure of disease severity and progression in LOPD.

In the current study, hEIM showed no changes in muscle quality or body fat % over the study period. Despite an inability to detect longitudinal change, there were significant correlations with functional measures. MQ and BMI had moderate inverse correlations for upper limb muscles, demonstrating that better muscle health was present in those with lower BMI measures. There were also moderate correlations between muscle quality and both the 6MWT and the 30-s stand. Higher MQ scores correlated with longer distances walked and better scores on sit-to-stand testing. However, it should be noted that these correlations were present only for upper limb muscles. The reason for this is unclear, but perhaps reflects difficulty in assessing more severely affected muscles using EIM. Upper limb MQ also trended toward fair correlations with the Barthel Index, but only forearm flexor MQ reached significance. As previously noted, this likely reflects the importance of normal upper limb strength in performing activities of daily living.

Hand-held EIM may be an option for monitoring patient muscle health remotely, but a larger study is required. The results of the current study indicate that patients can obtain similar results to those collected

Table 3
Home handheld EIM performance.

Muscle	Office measure MQ	Home measure MQ	*p*	Office Fat%	Home Fat%	*p*
Deltoid	55.5	40.0	0.35	30.1	29.8	0.89
Biceps brachii	60.7	48.8	0.43	23.8	25.6	0.76
Triceps brachii	50.6	53.2	0.86	33.5	29.2	0.60
Forearm flexors	55.0	56.7	0.92	19.2	21.0	0.74
Vastus lateralis	60.5	60.6	0.995	26.6	25.9	0.92
Tibialis anterior	77.3	69.8	0.56	14.6	16.7	0.48
Overall Score	59.9	54.7	0.39	24.7	24.7	0.98

MQ: Muscle Quality.

in a healthcare setting. To the authors' knowledge, only one prior study has deployed a similar hand-held EIM device to patients for home measurements. The ALS AT HOME study sent an EIM device, along with other applications, to patients with amyotrophic lateral sclerosis (ALS) and healthy controls. The authors found that 88% of study subjects receiving a device were able to complete a first set of measures. Measures from the patients' biceps brachii and quadriceps did not differ from controls, but the study provided evidence that at home measures are feasible in clinical trials [26].

4.3. MRI for assessing LOPD progression

Muscle MRI is an extremely useful tool in assessing the severity of LOPD. It is known that in many muscular diseases, including Pompe disease, fat will replace muscle as the tissues atrophies. Therefore, monitoring the percentage or degree of fatty infiltration can provide a means of documenting involvement quantitatively and potentially monitoring progression of disease. It seems unlikely that QMUS and hEIM will surpass quantitative muscle MRI in this regard. However, there are some limitations to implementing MRI in the clinical setting as it is time-consuming, not inexpensive and may not be readily available to patients. Additionally, some patients with LOPD may be unable to lie supine for testing due to respiratory muscle weakness. QMUS and hEIM may offer a quick, portable assessment of muscle offered in the clinic or at home.

Practical limitations aside, MRI does have the ability to detect pre-clinical muscle involvement. Muscle MRI using T1 weighted imaging and 3-point Dixon imaging have been used to identify pre-symptomatic muscle involvement in childhood and adult onset Pompe disease. In a series of 34 patients, it was noted that strong correlations were present between fatty fraction of muscle as measured by 3-point Dixon imaging and tests of muscle function. [12] The correlations were good, but not as strong for T1 weighted MRI. More recently, muscle diffusion tensor imaging (mDTI) has been used to detect pre-symptomatic change in LOPD. The changes seen with mDTI preceded fatty replacement of the muscle and the authors felt this might reflect intracellular debris and enlarged lysosomes [22]. Additionally, the degree of non-fatty muscle on MRI (contractile cross-sectional area) has been noted to have better correlations with strength than the fat fraction of muscle [29].

The ability of MRI to monitor change in LOPD is of significant interest. [20] analyzed trunk and thigh MRI in 12 patients with infantile onset Pompe disease, focusing on muscles of the trunk, pelvic girdle and thighs. Despite ERT, they found early involvement of the quadriceps and more rapid progression of changes in the thigh musculature as compared to the gluteal and trunk muscles [20]. However, a recent retrospective review demonstrated an increase in fatty infiltration of the psoas muscle in 13 ERT-treated patients after a mean of 39 months, but no change in seven of those patients imaged at a mean of 63 months. The authors interpreted this to mean that ERT may provide long term stability in patients with LOPD [17].

4.4. Study limitations

COVID-19 had a significant negative impact upon this longitudinal study. Patients were recruited and data collected from an annual institutional LOPD patient meeting, which drew participants from throughout the United States. At the April 2020 meeting, 8 of 25 patients were scheduled to complete their last yearly visit. Unfortunately, the meeting was cancelled due to the pandemic. In the absence of any plan to host an in-person 2021 event, a decision was reached to halt the study in August 2020 and begin data analysis. This likely diminished the study's ability to detect change in QMUS or EIM measures over time. Additionally, it should be noted that a period of 1–2 years may be too short a time period to detect change in LOPD, even under ideal circumstances.

Using MRC scores failed to provide significant information in regard to the relationship between muscle measures and strength. This was likely due to the lack of sensitivity using a 5-point scale. Quantitative muscle assessment (QMA) might have provided more detailed information for analysis. This will be considered in future studies.

Finally, an important limitation is the lack of muscle MRI for comparison to QMUS and hEIM results. QMUS and hEIM have not been validated against MRI measures in LOPD. QMUS EI and hEIM fat % need to be compared with quantitative measures of fatty muscle infiltration on MRI. Only five of the patients in the current study had ever undergone muscle MRI, but the none were performed within 3 years of study enrollment. Future studies should incorporate direct comparison with contemporaneous muscle MRI measures of fat fraction.

5. Conclusions

Although a small study with follow-up impacted but the COVID-19 pandemic, some promising findings were noted in the current study. Both QMUS and hand-held EIM had high inter-rater reliability, making them good candidates for further investigation as measures of muscle health and disease progression. Additionally, EIM had meaningful correlations with patient functional measures, making it a promising tool for further assessment of muscle health in LOPD. QMUS shared some of these correlations as well. Hand-held EIM may be deployed into patients' homes to allow them to track muscle health between clinic visits. This may also reduce travel burden, not only for clinical care, but for those individuals participating in research studies.

Most all patients in this trial were receiving ERT over the 2 years they were studied. Their health and function measures were stable during that time, which is extremely encouraging. However, QMUS was able to detect worsening EI in the vastus lateralis over the study period, along with a reduction in forearm flexor thickness. hEIM did not detect any significant changes, although the small size of the study and pandemic-related dropout rate limits any firm conclusions.

In summary, larger longitudinal studies looking for change over time might be streamlined by focusing QMUS measurements on the vastus lateralis and forearm flexors, while hEIM may be used as a surrogate measure of patient function that can be performed at home. However, further validation with muscle MRI is a necessary next step.

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