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Effects of respiratory muscle training (RMT) in children with infantile-onset Pompe disease and respiratory muscle weakness

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Abstract.
PURPOSE: Respiratory muscle weakness is a primary therapeutic challenge for patients with infantile Pompe disease. We previously described the clinical implementation of a respiratory muscle training (RMT) regimen in two adults with late-onset Pompe disease; both demonstrated marked increases in inspiratory and expiratory muscle strength in response to RMT. However, the use of RMT in pediatric survivors of infantile Pompe disease has not been previously reported.
METHOD: We report the effects of an intensive RMT program on maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP) using A-B-A (baseline-treatment-posttest) single subject experimental design in two pediatric survivors of infantile Pompe disease. Both subjects had persistent respiratory muscle weakness despite long-term treatment with alglucosidase alfa.
RESULTS: Subject 1 demonstrated negligible to modest increases in MIP/MEP (6% increase in MIP, $d = 0.25$; 19% increase in MEP, $d = 0.87$), while Subject 2 demonstrated very large increases in MIP/MEP (45% increase in MIP, $d = 2.38$; 81% increase in MEP, $d = 4.31$). Following three-month RMT withdrawal, both subjects maintained these strength increases and demonstrated maximal MIP and MEP values at follow-up.
CONCLUSION: Intensive RMT may be a beneficial treatment for respiratory muscle weakness in pediatric survivors of infantile Pompe disease.

Keywords: Glycogen storage disease type II, muscle, skeletal, neuromuscular diseases, rehabilitation, breathing exercises

1. Introduction

Pompe disease (glycogen storage disease type II) is a rare autosomal recessive neuromuscular disease. It occurs in approximately 1 of 40,000 births due to a deficiency of the lysosomal enzyme acid alpha glucosidase (GAA) [1]. The most severe phenotype, infantile-onset Pompe disease, results from an almost com-

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plete lack of GAA. Historically, the natural course of infantile-onset Pompe disease has resulted in death prior to one year of age [2–4].

The advent of treatment with enzyme replacement therapy (ERT) with alglucosidase alfa (Myozyme™) has resulted in marked improvements in overall and ventilator-free survival, and cardiac disease [5–12]. However, respiratory muscle involvement is often severe by the time of diagnosis [13], and at least 50% of individuals with infantile-onset Pompe disease still eventually require the need for mechanical ventilation [14]. Thus, despite treatment with ERT, respiratory muscle response is often suboptimal. Respiratory muscle weakness and associated morbidity and mortality remain a primary therapeutic challenge for patients with this condition.

It has long been recognized that the respiratory muscles respond to strength training regimens in a manner similar to other spinal musculature. That is, when an appropriate training stimulus is provided over time, increases in respiratory muscle strength can be established. Although methods vary, respiratory muscle training (RMT) is generally accomplished with handheld, pressure-threshold respiratory trainer devices, as seen in Fig. 1. These devices provide a training stimulus in the form of pressure-thresholds against inspiration (inspiratory muscle training [IMT]), expiration (expiratory muscle training [EMT]), or both (RMT). Pressure-thresholds may be individualized and adjusted over time [15–19].

We previously described the clinical implementation of a RMT regimen in two adults with late-onset Pompe disease. Both patients demonstrated marked increases in inspiratory and expiratory muscle strength in response to RMT [17]. However, the use of RMT in pediatric survivors of infantile Pompe disease has not, to our knowledge, been previously described. We report the results of an intensive 12-week RMT program in two subjects with infantile Pompe disease and substantial associated respiratory muscle weakness. We used a single-subject experimental design replicated across subjects. Based upon our experience in adults with Pompe disease, our *a priori* hypotheses were that our RMT regimen would result in: 1) increased respiratory muscle strength as measured via maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP), 2) changes that were at least large in magnitude ($d \geqslant 1.00$), and 3) maximal increases in respiratory muscle strength at posttest, followed by decreases in MIP/MEP due to detraining effects from posttest to three-month follow-up.

2. Materials and methods

2.1. Subjects

Two subjects diagnosed with infantile-onset Pompe disease participated in the research. Subject 1 was a 6.5 year-old white male who initiated ERT at 2 months of age via biweekly infusions of alglucosidase alfa at 20 mg/kg every other week. At approximately 36 months of age, dosage was increased to 40 mg/kg every other week. Subject 2 was a 5.75 year-old white female who began ERT infusions at 7 months of age at 20 mg/kg every other week. At a little over 12 months of age, dosage was increased to 40 mg/kg every other week. The ERT regimens of both subjects and all other aspects of their care remained consistent throughout their participation in RMT research, including ongoing physical therapy. These participants were the only pediatric subjects from a larger investigation into the effects of RMT in Pompe disease. The study was approved by the Duke University Health System Institutional Review Board, and informed consent was obtained from the parent or guardian of each subject after explaining the purpose and procedures of the study.

2.2. Experimental design

We used an A-B-A single-subject experimental design in order to allow for statistical analysis of effect size while controlling for threats to internal and external validity [20–22]. MIP and MEP were selected as co-primary dependent variables due to their non-invasive nature and high correlation with invasive measures of respiratory strength [23]. During the first A phase, repeated observations of MIP and MEP were obtained over two days (i.e., baseline). During the B phase, an intensive 12-week RMT regimen was implemented that required the completion of 9,000 total prescribed repetitions. During this time, subjects were seen for RMT therapy sessions every other week. Immediately following 12-weeks of RMT, repeated measures of MIP and MEP were obtained. During the second A stage, repeated observations of MIP and MEP were again obtained over two days (i.e., posttest). Additionally, RMT withdrawal was completed for three months to assess detraining effects. Effect sizes to compare performance at baseline, posttest, and follow-up were determined using Cohen's d [24]. Additional descriptive data were obtained at baseline, posttest, and follow-up including pulmonary function testing, assessment of gross motor function, and measurement of peak cough flow (PCF; Subject 2 only).

2.3. Procedures

2.3.1. Measurement of primary dependent variables

During baseline and posttest, a total of eight measures of both MIP and MEP were obtained over two consecutive days of assessment (i.e., four per day). During withdrawal, one measure each of MIP and MEP were obtained at one- and two-months to monitor detraining effects. At three-month follow-up, four measures of MIP and MEP were obtained over a single day. Each of these single data points represents multiple trials with minimal variability. That is, MIP and MEP maneuvers were both completed a minimum of three times with less than 10% variability. If this could not be achieved, a maximum of six trials were completed to prevent excessive fatigue regardless of variability. The mean of the three measures that exhibited the highest values and the least amount of variability served as one data point. A 30-minute rest period was enforced following each MIP and MEP measurement session during which time subjects remained in their wheelchairs. Measurements were completed using a standard protocol and calibrated digital pressure gauge (RPM-001, Micro Direct; Lewistown, ME) by a trained clinician not otherwise involved in the research. A flanged mouthpiece and nose clips were used. Subjects were presented with standard verbal instructions and verbal encouragement was provided to elicit best performance. Subjects completed maximum inhalation from forced residual volume during measurement of MIP and maximum exhalation from total lung capacity during measurement of MEP [25].

2.3.2. Other testing procedures

A pulmonologist with expertise in infantile-onset Pompe disease performed a patient/family interview and physical examination during baseline to ensure medical optimization for safe and meaningful participation in the study, including interpretation of upright spirometry performed twice during baseline and posttest and once at follow-up. The 6 Minute Walk Test (6MWT) [26] was completed under the supervision of a licensed physical therapist with experience in working with patients with infantile-onset Pompe disease at baseline, posttest, and follow-up. Additionally, by the time of enrollment of Subject 2, the study had been amended to allow measurement of PCF at baseline, posttest, and follow-up. A calibrated oral pneumotoachograph with a flanged mouthpiece and nose clips was used (MLT1000L, ADInstruments; Colorado Springs, CO) to measure PCF during volitional cough. Three PCF measures were obtained during each day of baseline, posttest, and follow-up testing; thus, PCF was obtained a total of six times each during baseline and posttest, and three times at follow-up (Subject 2 only). Measures of PCF were obtained by a trained clinician not otherwise involved in the research.

2.3.3. Respiratory muscle training (RMT) therapy

One MIP and MEP data point were established every other week during the treatment phase prior to RMT therapy. Over the 12-weeks of treatment, subjects were seen for 30–60 minute therapy sessions every other week (six RMT therapy sessions/subject). These therapy sessions allowed for provision of progressive resistance in terms of inspiratory and expiratory pressure-thresholds, review of self-reported adherence and accuracy of performance with the home-based RMT regimen, an opportunity to problem solve challenges that interfered with home-based RMT, and safety and tolerance of RMT in terms of adverse events and side effects.

RMT was initiated following completion of baseline assessment at the end of day two via handheld pressure-threshold respiratory trainer devices set to provide a pressure-threshold of 60–70% of MIP and MEP. During RMT therapy sessions, subjects first completed 5 sets of 5 repetitions of EMT for a total of 25 repetitions at 70% pressure-threshold. Following 25 repetitions, subjects were queried regarding negative side effects and accuracy of performance was determined. Targeted performance was 88% or greater accuracy in performing EMT successfully with negative side effects that were no worse than mild in severity. If this was not achieved over the first 25 EMT repetitions at 70% pressure-threshold, this was reduced to 60% pressure-threshold for the next set of 25 repetitions. Subjects next repeated these procedures with 5 sets of 5 repetitions of IMT for a total of 25 repetitions. EMT and IMT alternated in this manner until 75 total repetitions of each were completed. Instructions for the home-based RMT program were reviewed at the end of each visit which prescribed 75 repetitions of both IMT and EMT (3 sets of 25) five days per week for a total of 750 RMT repetitions weekly (375 IMT repetitions, 375 EMT repetitions). The subjects' parents/guardians were trained to determine successful versus unsuccessful repetitions during RMT therapy visits. They were provided with home-based treatment logs to document adherence and accuracy and these were returned at the next RMT therapy session.

Commercially available pressure-threshold respiratory trainer devices that provide a calibrated training

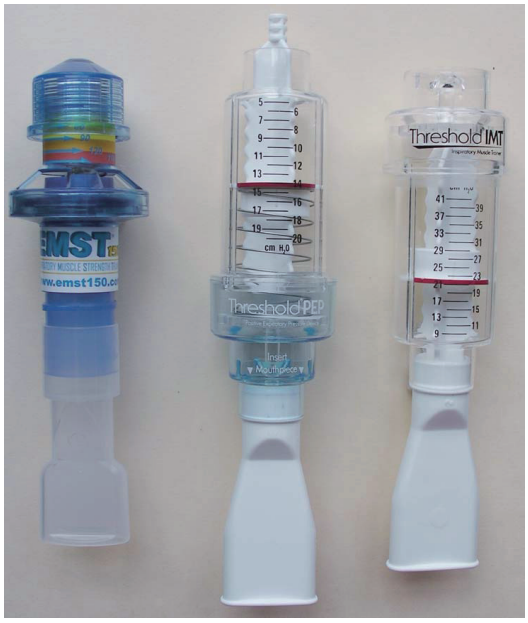

Fig. 1. Commercially available pressure-threshold respiratory trainer devices used in the present research (Threshold PEP, Threshold IMT-Phillips Respironics, Andover, MA; EMST 150-Aspire Products, Gainesville, FL). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/PRM-140294)

stimulus were used (Fig. 1; Threshold PEP, Threshold IMT-Phillips Respironics, Andover, MA; EMST 150-Aspire Products, Gainesville, FL). In Subject 1, pressure-thresholds were established by setting the devices to the target value using the device calibration markings. However, over the course of the larger research study, concerns over the calibration accuracy of the prescribed pressure-thresholds emerged based on testing in our laboratory. We therefore developed a novel system for the hand-calibration of pressure-thresholds with these devices comprising three principal components to create a gas pressure circuit: 1) an air delivery system to introduce positive or negative pressures, 2) a differential atmospheric pressure meter, and 3) the respiratory trainer device that requires calibration. Depending on whether and IMT or EMT trainer was being calibrated, positive or negative pressure was applied via a 3L syringe. The pressure-threshold was determined based on the positive or negative differential atmospheric pressure necessary to overcome the air seal and set accordingly. This method of hand-calibration was used for Subject 2.

2.4. Data analysis

The magnitude of change between baseline and posttest, posttest and follow-up, and baseline and follow-up for the primary endpoints of MIP and MEP was determined using a variation of Cohen's measure of effect size (d) [24]. Simply stated, d is obtained by subtracting the mean of the first session from the mean of the second session, divided by the standard deviation of the first session. Conservatively, we established an effect size < 0.6 to be negligible, $\geqslant 0.6$ modest, $\geqslant 1.0$ large, and $\geqslant 2.0$ very large. Descriptive statistics are also provided.

3. Results

Subject 1 and Subject 2 each completed 12 weeks of RMT targeting 4,500 repetitions of IMT and 4,500 repetitions of EMT for a total cumulative goal of 9,000 RMT repetitions during the B phase. Subject 1 completed a total of 3,605 IMT repetitions and 3,605 EMT repetitions for an overall total of 7,210 RMT repetitions, demonstrating an overall adherence rate of 80% with the prescribed RMT regimen. Subject 2 completed a total of 3,525 IMT repetitions and 3,450 EMT repetitions for an overall total of 6,975 RMT repetitions, demonstrating an overall adherence rate of 78% with the prescribed RMT program. On average, both subjects completed their home therapy programs approximately 4 days per week with a mean percent accuracy of greater than 90% with both IMT and EMT.

3.1. Subject 1

3.1.1. Maximum inspiratory pressure

Based on values established in healthy controls, predicted MIP for Subject 1 was 73.5 cm H_2O [27]. As seen in Fig. 2, Subject 1's mean MIP was 17 cm H_2O at baseline, 18 cm H_2O at posttest, and 26 cm H_2O at follow-up. Between baseline and posttest, MIP increased 6%, from 17 to 18 cm H_2O ($d = 0.25$). From posttest to follow-up, MIP increased 44%, from 18 to 26 cm H_2O ($d = 1.57$). When comparing baseline and follow-up, MIP increased a total of 53%, from 17 to 26 cm H_2O ($d = 2.25$), over the entire duration of the study. Percent predicted MIP was 23% at baseline, 24% at posttest, and 35% at follow-up.

3.1.2. Maximum expiratory pressure

Predicted MEP for Subject 1 was 85.67 cm H_2O [27]. As seen in Fig. 2, Subject 1's mean MEP was 16 cm H_2O at baseline, 19 cm H_2O at posttest, and 20 cm H_2O at follow-up. Between baseline and posttest, MEP increased 19%, from 16 to 19 cm H_2O ($d = 0.87$).

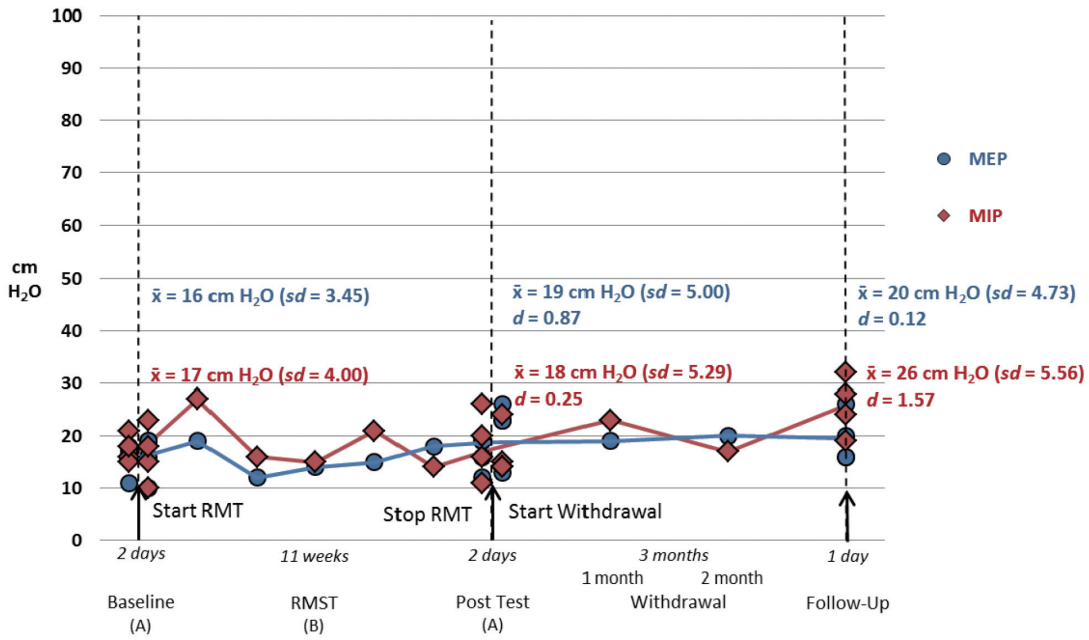

Fig. 2. Subject 1's inspiratory and expiratory muscle strength as measured by maximum inspiratory strength (MIP) and maximum expiratory muscle strength (MEP) throughout research study and associated effect sizes. (cm H2O: centimeters water pressure; x? mean; sd: standard deviation; RMT: respiratory muscle training, MIP: maximum inspiratory pressure; MEP: maximum expiratory pressure (MEP); *d*: Cohen's *d* [effect size]; BL: baseline; F/U: follow-up). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/PRM-140294)

From posttest to follow-up, MEP increased 5%, from 19 to 20 cm H_2O ($d = 0.20$). When comparing baseline and follow-up, MEP increased a total of 25% ($d = 1.16$), from 16 to 20 cm H_2O, over the entire duration of the study. Percent predicted MEP was 19% at baseline, 22% at posttest, and 23% at follow-up.

3.1.3. Descriptive data

3.1.3.1. Spirometry

Mean upright FVC was 1.07 L at baseline (70% predicted; REF = 1.53 L), 1.25 L at posttest (82% predicted), and 1.39 L at follow-up (88% predicted; REF = 1.58L). From baseline and posttest, mean FVC increased 17% and an 11% increase was seen between posttest and follow-up. Overall, when comparing baseline and follow-up, FVC increased 30% over the entire study duration and percent predicted FVC increased 18%, from 70 to 88%.

Mean upright FEV1 was 0.96 L at baseline (71% predicted; REF = 1.35L), 1.00 L at posttest (74% predicted), and 1.19 L at follow-up (93% predicted; REF = 1.28). Between baseline and posttest, mean FEV1 increased 4% and a 19% increase was seen between posttest and follow-up. Overall, when comparing baseline and follow-up, FEV1 increased 24% over the entire study duration, from 0.96 to 1.19 L.

3.1.3.2. 6 Minute walk test

Distance walked on the 6MWT was 233.6 m at baseline, 120.0 m at posttest, and 159.0 m at follow-up. From baseline to posttest, distance walked declined 113.6 m (49%), with the subject stopping at posttest at 4 minutes and 52 seconds due to fatigue. Between posttest and follow-up, distance walked increased 39 m (33%). However, overall, when comparing baseline to follow-up, distance walked on the 6MWT decreased 74.6 m (32%), from 233.6 m to 159.0 m over the entire study duration.

3.2. Subject 2

3.2.1. Maximum inspiratory pressure

Predicted MIP for Subject 2 was 51.6 cm H_2O [28]. As seen in Fig. 3, Subject 2's mean MIP was 22 cm H_2O at baseline, 32 cm H_2O at posttest, and 34 cm H_2O at follow-up. Between baseline and posttest, MIP increased 45%, from 22 to 32 cm H_2O ($d = 2.38$). From posttest to follow-up, MIP further increased 6%, from 32 to 34 cm H_2O ($d = 1.16$). When comparing baseline to follow-up, MIP increased a total of 55%, from 22 to 34 cm H_2O ($d = 2.86$), over the total study duration. Percent predicted increased from 43% at baseline to 62% at posttest and 66% at follow-up.

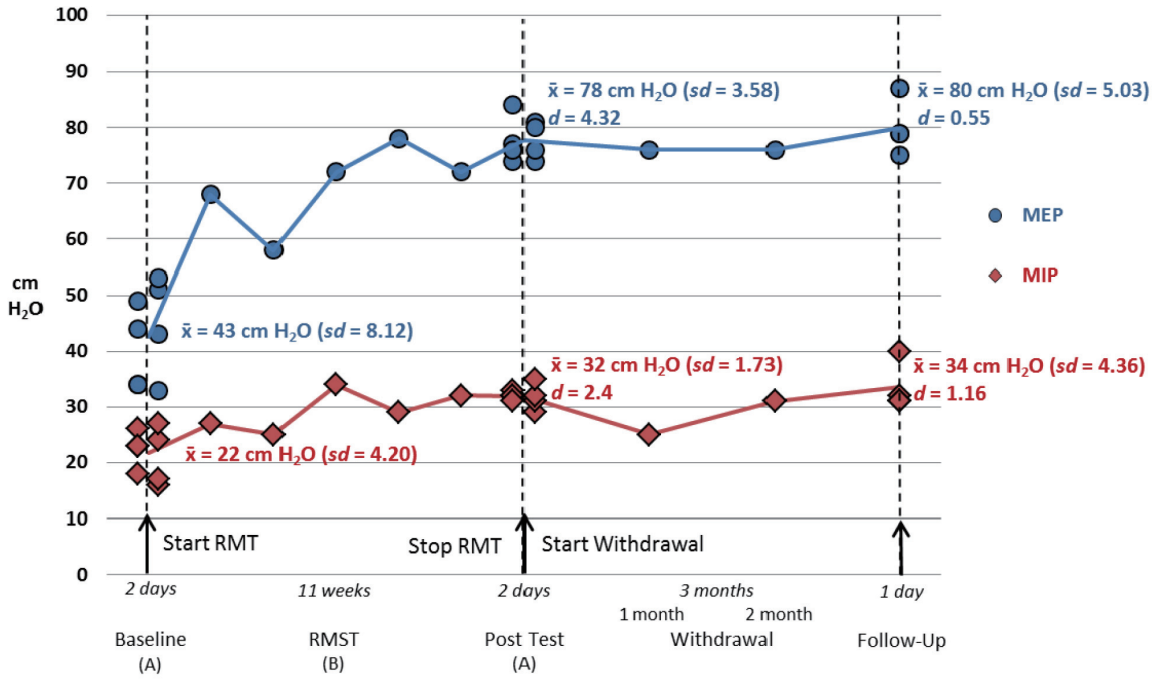

Fig. 3. Subject 2's inspiratory and expiratory muscle strength as measured by maximum inspiratory strength (MIP) and maximum expiratory muscle strength (MEP) throughout research study and associated effect sizes. (cm H2O: centimeters water pressure; x? mean; sd: standard deviation; RMT: respiratory muscle training, MIP: maximum inspiratory pressure; MEP: maximum expiratory pressure (MEP); d: Cohen's d [effect size]; BL: baseline; F/U: follow-up). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/PRM-140294)

3.2.2. Maximum expiratory pressure

Predicted MEP for subject 2 was 52.8 cm H_2O [28]. As seen in Fig. 3, mean MEP was 43 cm H_2O at baseline, 78 cm H_2O at posttest, and 80 cm H_2O at follow-up. Between baseline and posttest, MEP increased 81%, from 43 to 78 cm H_2O ($d = 4.31$). From posttest to follow-up, MEP further increased 3%, from 78 to 80 cm H_2O ($d = 0.59$). When comparing baseline to follow-up, MEP increased a total of 86%, from 43 to 80 cm H_2O ($d = 4.56$), over the total study duration. Percent predicted increased from 81% at baseline to 147% at posttest and 152% at follow-up.

3.2.3. Descriptive data

3.2.3.1. Spirometry

Mean upright FVC was 0.82 L at baseline (75% predicted; REF = 1.09L), 0.81 L at posttest (74% predicted), and 0.92 L at follow-up (85% predicted). Between baseline and posttest, FVC decreased 1% and a 14% increase was seen between posttest and follow-up. Overall, when comparing baseline and follow-up, FVC increased 12% over the entire study with percent predicted FVC increasing from 75 to 85%.

Mean upright FEV1 was 0.74 L at baseline (75% predicted; REF = 0.99L), 0.79 L at posttest (80% predicted), and 0.86 L at follow-up (87% predicted). Between baseline and posttest, FEV1 increased 7% and a 9% increase was seen between posttest and follow-up. Overall, when comparing baseline and follow-up, FEV1 increased 16% over the entire study duration, from 0.74 to 0.86 L.

3.2.3.2. 6 Minute walk test

Distance walked on the 6MWT was 30 m at baseline, 62.4 m at posttest, and 74.9 m at follow-up. The subject used a supportive walker (Rifton Pacer) during all assessments. Between baseline and posttest, distance walked increased 32.4 m (108%) and a 12.5 m increase (20%) was exhibited between posttest and follow-up. Overall, when comparing baseline and follow-up, distance walked on the 6MWT increased by 150% from 30 m to 74.9 m, over the total study duration.

3.2.3.3. Peak cough flow

Mean PCF was 2.92 L/sec ($sd = 0.51$) at baseline, 3.51 L/sec ($sd = 0.30$) at posttest, and 3.44 L/sec ($sd = 0.50$) at follow-up. Between baseline and

posttest, PCF improved by 0.59 L/sec (20%) and a 0.07 L/sec decline (2%) was seen between posttest and follow-up. Overall, when comparing baseline to follow-up, mean PCF increased 0.52 L/sec (18%), from 2.92 to 3.44 L/s over the entire study duration.

4. Discussion

Our *a priori* hypotheses were that our 12-week RMT regimen would: 1) result in increased respiratory muscle strength as measured via MIP and MEP, 2) the magnitude of these changes would be at least large ($d \geqslant 1.00$); and 3) maximal increases in respiratory muscle strength would be achieved at posttest, followed by decreases in respiratory muscle strength due to detraining effects associated with withdrawal at three-month follow-up.

Regarding our first hypothesis, RMT was indeed associated with increases in respiratory strength between baseline and posttest, though there was considerable variability across the two participants. Negligible to modest respiratory strength increases were seen in Subject 1 (6% increase in MIP and a 19% increase in MEP, respectively) and remarkably large increases in respiratory strength were noted in Subject 2 (45% increase in MIP and an 81% increase in MEP, respectively). Our second hypothesis was true at posttest in Subject 2 only. The effect sizes in Subject 1 were negligible to modest (inspiratory strength effect size: $d = 0.25$; expiratory strength effect size: $d = 0.87$), while Subject 2 demonstrated very large effect sizes (inspiratory strength effect size: $d = 2.38$; expiratory muscle strength effect size: $d = 4.31$).

The data, which inform our third hypothesis, were unexpected. Rather than the detraining effects and negative effect sizes from posttest to follow-up that might be expected with RMT withdrawal, both subjects maintained and even increased their respiratory muscle strength at three-month follow-up. In fact, both subjects demonstrated their peak MIP/MEP values at follow-up, after three-month RMT withdrawal. Over the entire study duration, Subject 1 increased inspiratory strength by 53% ($d = 2.25$) and expiratory strength by 25% ($d = 1.16$), corresponding to very large and large effect sizes, respectively. Subject 2's response surpassed that of Subject 1, with inspiratory strength increases of 55% ($d = 2.86$) and expiratory strength increases of 86% ($d = 4.56$), corresponding to very large effect sizes.

Overall, these Phase I data provide initial indication that RMT may offer benefit for the treatment of respiratory muscle weakness in pediatric survivors of infantile Pompe disease. Comparative data on the effects of RMT in children with infantile-Pompe disease are not available, though results from our previous case study of two adult patients with late-onset Pompe disease may be informative. Similar to the children in the present report, these adults had each received long-term ERT and had persistent baseline respiratory weakness. Over 16–32 weeks of IMT, inspiratory muscle strength increased 73 to 74% and expiratory muscle strength increased 31 to 48% over 12–22 weeks of EMT [17]. These previous clinical data support the notion that RMT may result in increases in respiratory muscle strength in individuals with Pompe disease.

Our data are also consistent with previous reports on the use of RMT in subjects with other forms of neuromuscular disease, such as Duchene muscular dystrophy (DMD) and spinal muscular atrophy (SMA). For example, Wanke and colleagues [29] employed IMT in 15 individuals with DMD ages 10–24 years. Inspiratory strength was measured following one, three, and six months of IMT and again after six-month withdrawal. In ten of 15 participants, strength improvements were seen at one month and IMT continued for six months. Continued strength increases were observed after three and six months of IMT. Moreover, these strength enhancements were generally persistent following six-month withdrawal. Koessler et al. [30] studied IMT in 27 patients with DMD or SMA over 24 months. These subjects demonstrated large, statistically significant increases in inspiratory muscle strength over the first ten months of IMT. At that time, plateau was achieved, though no decline in inspiratory muscle strength was evident over the course of the additional 14 months of training. Gozal and Thiriet [31] employed a RMT program in 21 children with DMD or SMA with a mean age of 12 years. Statistically significant increases in both inspiratory and expiratory muscle strength, as well as decreases in self-perceived effort when overcoming inspiratory and expiratory pressure-thresholds, were demonstrated. Overall, these and other studies suggest that RMT may effect increases in respiratory muscle strength, even in the setting of progressive neuromuscular disease [32].

Although previous data in patients with neuromuscular disease have suggested that retention of respiratory strength increases is possible following RMT withdrawal [29], we predicted a decline of respiratory muscle strength from posttest to follow-up due to detraining effects. Detraining, or the principle of

reversibility, states that discontinuing training causes a partial or complete reversal of effects [33]. Previous data have demonstrated detraining effects after one- to three- month withdrawal in healthy and disordered populations following RMT-induced respiratory muscle strength enhancements [31,34–38]. However, in the present study, both subjects maintained, and even slightly increased, their respiratory muscle strength over three-month withdrawal. Certainly, further exploration of the detraining effects associated with the discontinuation of RMT regimens requires much additional study [39].

The persistence of the respiratory muscle strength increases exhibited by our subjects may also be related to methodological differences across studies, including important aspects of the training regimen related to the intensity of RMT. Although intensity can be challenging to define in behavioral treatments, Warren and colleagues [40] provide a model in which cumulative intervention intensity is defined as the dose $\times$ dose frequency $\times$ total intervention duration. Therefore, the cumulative intervention intensity of our RMT regimen was a dose of 150 repetitions (75 of IMT, 75 of EMT) per day $\times$ dose frequency of 5x/week $\times$ a total intervention duration of 12 weeks for a cumulative intervention intensity of 9,000 total RMT repetitions (4,500 repetitions of both IMT and EMT). This cumulative intervention intensity is markedly increased in comparison to most previous RMT research. For example, the cumulative intervention intensity in the study from Baker and colleagues [34] was either 500 or 1000 EMT repetitions, depending on randomization allocation. Chiara and colleagues [35] similarly targeted 960 EMT repetitions. These studies are typical of the intervention intensities encountered in the RMT literature. In contrast, our cumulative intervention intensity was at least nine times greater. This increased cumulative intervention intensity may provide at least partial explanation for the lack of detraining effects observed in these two subjects over the course of three-month withdrawal.

The benefits of increased training intensity in strength training regimens are well-recognized. In the RMT literature, Topin et al. [41] reported that RMT was dose-dependent in terms of both pressure-thresholds and the number of repetitions. More studies are needed to determine the effects of the multiple training variables related to intensity (pressure-thresholds, number of repetitions, frequency of repetitions, total intervention duration) that may be manipulated to influence RMT outcomes. The effects of these variables may differ depending on the population of interest and intended outcomes. Ultimately, however, the optimal intensity of a RMT regimen is presently unknown and requires additional research.

We intended for the overall intensity of our RMT regimen to be greater than reported in prior research studies. Our rationale for this was due, at least in part, to the severity of the respiratory weakness present in patients with Pompe disease. This increased intensity may have also fostered generalization of motor learning to respiration independent of the training regimen to facilitate experience-dependent plasticity over time [42]. It is well-established that motor skill learning results in functional and structural cortical and subcortical reorganization associated with long-term retention of motor skills [43]. Long-term motor learning may also provide an explanation for the lack of detraining effects demonstrated by these subjects during withdrawal. Future research is warranted in order to determine the mechanism(s) of strength increases with RMT, including the central and peripheral neuromotor adaptations that occur over time.

The overall intensity of our RMT regimen was also increased by the combined use of both IMT and EMT to comprehensively address respiratory weakness. Although data are limited, combined RMT may be superior to IMT or EMT in isolation [15,18,44]. Subjects in the present study may have benefitted from the combination of IMT and EMT to comprehensively address respiratory muscle weakness.

The very young age of the present participants also merits discussion. Upon entry into the study, these two subjects were 6.5 and 5.75 years of age. Based on review of the literature, these subjects appear to be some of the youngest individuals with neuromuscular disease to ever participate in a RMT research study. In prior studies with patients with neuromuscular disease in which the age range of participants is described, the youngest experimental participants were between 7 and 10 years of age [29,31,44–46]. However, age did not appear to substantially interfere with our subjects' adherence to the intensive home RMT program that required 9,000 total repetitions over 12-weeks, as Subjects 1 and 2 had adherence rates of 80 and 78%, respectively. While both subjects had supportive and encouraging caregivers, this level of adherence is comparable, though slightly reduced, to the 90% or better adherence demonstrated by most adult Pompe research subjects we have studied.

The negative consequences of respiratory muscle weakness in neuromuscular disease are well-docum-

ented. Inspiratory muscle weakness leads to chronic alveolar hypoventilation and increasingly severe respiratory disease, including chronic and acute respiratory failure. Expiratory muscle weakness leads to decreased strength of the cough, which causes an inadequate pulmonary toilet. Current treatments for respiratory weakness in Pompe disease and other neuromuscular diseases are compensatory (e.g., mechanical ventilation). Such treatments do not address underlying respiratory muscle weakness. Considering that respiratory muscle weakness is the primary cause of morbidity and mortality in Pompe disease, treatments that directly target and treat respiratory weakness have clear clinical appeal, though the functional implications require further study. In the present study, changes in the primary outcome variables of MIP and MEP did not appear to be strongly associated with changes in FVC, FEV1, and 6MWT. Although both subjects continued their participation in physical therapy during their involvement, a worsening in performance on the 6MWT was noted in Subject 1. During this period, the subject underwent a great deal of growth (57th percentile to the 81st percentile in height), which may have negatively influenced his ambulation.

Although not measured in Subject 1, PCF data from Subject 2 provide direction for further study. In this subject, mean PCF increased 20% from baseline to posttest, from 2.92 to 3.51 L/s. Following three-month withdrawal, mean PCF remained 18% increased in comparison to baseline. These findings suggest that cough strength may be enhanced with RMT in Pompe disease, as has been reported in other conditions [47].

Other explanations for the present findings must be considered. Possible alternative explanations include learning effects associated with MIP and MEP testing and subject maturation. With regard to potential learning effects, every attempt was made to control for this. Subjects received training regarding MIP and MEP testing before testing was initiated. A standard set of instructions was provided during each trial. Additionally, each data point used to represent MIP and MEP throughout the study involved a minimum of three trials with $\leqslant 10\%$ variability (with a maximum of 6 trials regardless of variability). Thirty minute enforced rest breaks were also used between each MIP/MEP testing session. Overall, these controls appear sufficient to mitigate concerns regarding learning effects. Maturation effects also appear unlikely to explain the strength enhancements exhibited by these subjects due to the progressive course of infantile Pompe disease, even when treated.

Interactions between RMT and ERT must also be considered. For example, it is known that ERT provides an improved therapeutic response in type I (slow-oxidative) versus type II (fast-oxidative glycolytic) muscle fibers [48]. Additionally, some research suggests that conversions between type I and II muscle fibers are possible with certain manipulations of training variables [49]. Such adaptations to muscle fiber composition could be hypothesized to interact with the glycogen clearing properties of ERT. However, the RMT regimen we utilized is a strength training approach, which appears more likely to increase the proportion of type II versus type I muscle fibers. Therefore, improved response to ERT due to a shift of muscle fiber composition to a greater proportion of type I fibers with RMT appears to be an unlikely explanation for the strength gains exhibited by these subjects. Nevertheless, the possibility of unknown interactions between RMT and ERT to increase respiratory muscle strength must be acknowledged and is an important consideration for future research in this area.

In conclusion, two pediatric survivors of infantile-onset Pompe disease demonstrated increases in inspiratory and expiratory muscle strength following an intensive 12-week RMT regimen. In one subject these changes were negligible to modest in magnitude and in the other they were very large. Increases in MIP and MEP were maintained and slightly increased over three-month RMT withdrawal and both subjects exhibited their peak inspiratory and expiratory muscle strength at follow-up after three-month withdrawal. Both children demonstrated good adherence with the intensive RMT regimen without evidence of adverse events or side effects. Larger studies to confirm these findings and provide indication of the expected response in children with Pompe disease, as well as with other neuromuscular disorders, are needed. Future research in this area must investigate the effects of increased inspiratory and expiratory muscle strength on ventilation and cough. Additionally, improved understanding of the short- and long-term neural and muscular adaptations associated with RMT is needed to maximize response and promote long-term retention.

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Conflict of interest

HNJ has received research/grant support and honoraria from Genzyme Corporation.

LEC has received honoraria from Genzyme Corporation of Sanofi, has participated in research supported by Genzyme Corporation of Sanofi, and is a member of the Pompe Registry Board of Advisors for Genzyme Corporation of Sanofi. PSK has received research/grant support and honoraria from Genzyme Corporation and is a member of the Pompe and Gaucher Disease Registry Advisory Board for Genzyme Corporation. KDC, TM, KS, RR, JS, MC, LM, RMK and LM have no conflicts of interest to disclose.

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