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Respiratory muscle training (RMT) in late-onset Pompe disease (LOPD): Effects of training and detraining

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ABSTRACT

Background: Determine the effects of a 12-week respiratory muscle training (RMT) program in late-onset Pompe disease (LOPD).
Methods: We investigated the effects of 12-weeks of RMT followed by 3-months detraining using a single-subject A-B-A experimental design replicated across 8 adults with LOPD. To assess maximal volitional respiratory strength, our primary outcomes were maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP). Effect sizes for changes in MIP and MEP were determined using Cohen's *d* statistic. Exploratory outcomes targeted motor function, and peak cough flow (PCF) was measured in the last 5 subjects.
Results: From pretest to posttest, all 8 subjects exhibited increases in MIP, and 7 of 8 showed increases in MEP. Effect size data reveal the magnitude of increases in MIP to be large in 4 ($d \geq 1.0$) and very large in 4 ($d \geq 2.0$), and effect sizes for increases in MEP were large in 1 ($d \geq 1.0$) and very large in 6 ($d \geq 2.0$). Across participants, pretest to posttest MIP and MEP increased by a mean of 19.6% ($sd = 9.9$) and 16.1% ($sd = 17.3$), respectively. Respiratory strength increases, particularly for the inspiratory muscles, were generally durable to 3-months detraining.
Conclusions: These data suggest our 12-week RMT program results in large to very large increases in inspiratory and expiratory muscle strength in adults with LOPD. Additionally, increases in respiratory strength appeared to be relatively durable following 3-months detraining. Although additional research is needed, RMT appears to offer promise as an adjunctive treatment for respiratory weakness in LOPD.

1. Introduction

Pompe disease (glycogen storage disease type II, acid maltase deficiency) is a progressive metabolic myopathy resulting from deficiency of the lysosomal enzyme acid alpha glucosidase (GAA). Although a single disease entity, it manifests clinically across a broad spectrum based on age of onset, progression rate, genetic mutation(s), and disease distribution [1]. Infantile-and late-onset Pompe disease (LOPD) are the two broad phenotypes recognized. The most severe form, classic infantile-onset Pompe disease, results from a virtual absence of GAA. In patients with LOPD, GAA deficiency is only partial. Age of onset and symptom severity in LOPD is associated with the residual amount of GAA present and varies according to factors such as the specific GAA gene mutations expressed [2]. The LOPD phenotype continues to be expanded and refined with recent data implicating features such as lingual weakness [3–5], ptosis [6,7], impaired gastric function [8], and cardiac involvement [9].

LOPD typically presents in adulthood with signs and symptoms related to progressive weakness in the lower limbs and trunk. Respiratory muscle weakness (involving both inspiratory and expiratory musculature) is common and is a primary cause of morbidity and mortality in LOPD. Respiratory weakness often first emerges when supine [10]. Sleep-disordered breathing is common, causing nocturnal hypoventilation, hypercapnia, and hypoxia. Non-invasive nocturnal ventilation is often required [11]. Cough adequacy may be affected by progressive respiratory weakness, threatening airway protection/secretion management and leading to recurrent respiratory infections

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and hospitalization. The respiratory muscles also make important non-ventilatory motor contributions to trunk stability and mobility [12,13]. Motor impairments are often first noted with walking, running, and activities of elevation against gravity (rising from the floor, standing from a chair, stair climbing) [14]. Over time, ambulation is often more severely affected and use of a wheelchair may be necessary.

Drug therapy for patients with LOPD is available in the form of enzyme replacement therapy (ERT). ERT provides an external form of GAA via intravenous infusion of alglucosidase alfa (Lumizyme®). A large multicenter double-blind, placebo-controlled study was conducted to assess the use of alglucosidase alfa for the treatment of LOPD over 18-months. The primary study endpoints were distance walked on the 6-min walk test (6MWT) and percent of predicted upright forced vital capacity (FVC). Secondary and tertiary outcomes included measures of volitional respiratory muscle strength: maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP). The results revealed increased walking distance on the 6MWT and stabilization of pulmonary function based on FVC. Improvements in MIP and MEP showed the effects of ERT on respiratory strength to be modest. For the most part, increases in respiratory strength were accomplished shortly after ERT initiation (12 to 26 weeks) and then maintained over the duration of the study [15]. However, despite treatment, up to about a third of patients have pulmonary decline [16] and the pulmonary benefits of ERT may lessen or deteriorate over time [17]. Additionally, respiratory weakness is often present at the time of diagnosis and upon initiation of treatment [15]. Thus despite treatment with ERT, respiratory weakness remains a critical obstacle to improving outcomes for patients with LOPD.

One approach to address respiratory muscle weakness is respiratory muscle training (RMT). RMT is accomplished using hand-held respiratory trainer devices to provide pressure-threshold resistance against inhalation (inspiratory muscle training [IMT]) and/or exhalation (expiratory muscle training [EMT]). Pressure-thresholds may be individualized and adjusted over time [18–22]. We first reported of the use of RMT in LOPD in 2011. Two adults with severe respiratory muscle weakness completed a clinical RMT regimen and demonstrated marked increases in inspiratory and expiratory muscle strength [20]. More recently, we described the effects of a 12-week RMT program in two pediatric survivors of infantile-onset Pompe disease with severe respiratory muscle weakness. Effect size data revealed one subject to have negligible to modest increases in inspiratory and expiratory strength; the other subject had very large increases in both inspiratory and expiratory strength [23].

Although the role of exercise in LOPD remains somewhat controversial, recent consensus guidelines support submaximal supervised exercise programs in patients with Pompe disease [1,24]. In a Pompe mouse model, exercise training is associated with improvements in motor function, aerobic capacity, and grip strength [25]. Human data suggest that supervised exercise programs in LOPD are well-tolerated, benefit muscular strength and possibly functional capacity, and improve pain and fatigue [20,26–28]. Such data support the notion that RMT and other forms of exercise may have clinical benefit for patients with LOPD.

We now report the results of this 12-week RMT program in 8 adults with LOPD using a single-subject experimental design replicated across subjects. Repeated measures of the primary outcome measures of MIP and MEP were obtained. MIP and MEP were selected as our primary outcome measures in order to assess the effects of RMT on respiratory strength in a non-invasive fashion. In addition, MIP and MEP are direct measures of respiratory strength and the effects of RMT which correlate highly with invasive measures in LOPD [10]. MIP and MEP were measured before RMT (pretest), after RMT (posttest), and after 3-months detraining (post-detraining). We expected increases in MIP and MEP to be at least large in magnitude ($d \geq 1.0$) from pretest to posttest, with peak MIP and MEP achieved at posttest and negligible declines post-detraining.

2. Materials and methods

2.1. Subjects

This research was approved by the Duke University Health System Institutional Review Board and written informed consent was obtained from each subject. This study was part of a larger investigation into the effects of RMT on respiratory muscle strength in subjects with infantile-onset Pompe disease or LOPD. Data from the two participants with infantile-onset were previously published [23]. The present manuscript presents the data from our 8 subjects with LOPD. We recruited subjects for participation through the Duke Pompe Disease Clinical and Research Program. Inclusion criteria included confirmed diagnosis of LOPD via enzyme activity showing deficient GAA activity (skin fibroblasts, muscle, or dried blood spot assay) and mutation analysis showing two GAA gene mutations; ability to participate in an intensive RMT research program; and ability to maintain a consistent amount of non-research related physical activity over the course of the study. Exclusion criteria included inability to perform the research protocol; medical problems that precluded meaningful study participation; inability to perform high-effort respiratory tasks; and profound respiratory weakness that would prevent RMT at the minimum pressure-threshold resistance offered by commercially available, FDA-approved RMT devices.

2.2. Experimental design

We used an A-B-A single-subject experimental design to allow for statistical analysis of effect size and control threats to internal and external validity [29–31]. The first A phase represents pre-RMT function (pretest), the B phase represents the 12-week RMT program, and the second A phase represents post-RMT function after 3 months of detraining (posttest to post-detraining). MIP and MEP were the primary dependent variables. Each day of assessment involved 4 discrete MIP/MEP test sessions comprising repeated measurements of both maneuvers. Pretest and posttest assessments were completed over 2 consecutive days; post-detraining assessments were completed over 1 day. We calculated effect sizes using Cohen's d to compare MIP and MEP at pretest, posttest, and post-detraining [32]. Additional exploratory data were also obtained at each assessment point to assess the effects of RMT on motor function including the 6MWT and timed function tests [33] (TFTs; time to walk 10 m, time from supine to standing, time to climb 4 stairs). Volitional peak cough flow (PCF) was measured in the last 5 participants (subjects 4–8).

2.3. Procedures

2.3.1. Measurement of primary dependent variables

MIP and MEP were measured using established guidelines [34,35]. Each day of assessment involved 4 discrete MIP/MEP test sessions comprising repeated measurements of both maneuvers. Each test session was comprised of the mean of the 3 best efforts. MIP/MEP test sessions were discontinued when 3 efforts of each maneuver were produced with ≤10% variability (maximum of 6 efforts). We enforced a 30-min rest period between test sessions. Test equipment included a calibrated digital pressure gauge (RPM-001, Micro Direct; Lewistown, ME), flanged mouthpiece, and nose clips. MIP was performed from forced residual volume; MEP from total lung capacity [34].

2.3.2. Other assessment procedures

At pretest, a pulmonologist with expertise in Pompe disease interviewed the patient, completed a physical exam, and interpreted spirometry results to ensure medical optimization for safe and meaningful study participation. We selected our exploratory outcomes to assess the effects of increases in respiratory strength on motor function and volitional cough (subjects 4–8 only). At pretest, posttest, and post-detraining, subjects completed the 6MWT [36] and TFTs [33] with the

supervision and instruction of a licensed physical therapist experienced in working with patients with LOPD. Additionally, we measured volitional peak cough flow (PCF) in subjects 4 through 8. PCF (L/s) during volitional cough was measured using a calibrated oral pneumotachograph system (MLT1000L, ADInstruments; Colorado Springs, CO).

2.3.3. Respiratory muscle training (RMT) therapy

Subjects participated in RMT therapy sessions approximately 45 min in duration every other week during the 12-week RMT program (6 sessions per subject). RMT therapy was initiated following completion of pretest on day 2 with a licensed speech pathologist trained by the PI who was not involved in any assessment activities (RMT clinician). RMT was individualized to provide inspiratory and expiratory pressure-threshold resistance based upon each subject's particular levels of inspiratory and expiratory strength; MIP and MEP were assessed at the beginning of each RMT therapy session. Both IMT and EMT were completed at 60–70% of MIP and MEP, respectively.

FDA-approved, commercially available, handheld inspiratory- and expiratory-type pressure-threshold RMT devices were used (Threshold PEP, Threshold IMT-Phillips Respironics, Andover, MA; EMST 150-Aspire Products, Gainesville, FL). In subjects 1 through 3, pressure-threshold resistance was established using the calibration markings on the RMT devices. In subjects 4 through 8, pressure-threshold resistance was determined using a 3-way gas pressure circuit comprising 1) an air delivery system to introduce positive or negative pressure, 2) the inspiratory or expiratory RMT device being calibrated, and 3) a differential atmospheric pressure gauge/meter (see Fig. 1). Pressure-threshold resistance is determined by introducing pressure until it is sufficient to overcome the spring-loaded valve on the device. The pressure gauge reading at this point indicates the pressure-threshold resistance and, as necessary, the spring-loaded valve is adjusted and the method repeated until the intended pressure-threshold resistance is obtained [23]. This approach allows for control over pressure-threshold resistance, a critical aspect of RMT "dose".

Subjects first completed 5 EMT repetitions at 70% of MEP while the RMT clinician trained subjects for successful repetitions and monitored their RMT tolerance. Subjects proceeded to complete 20 more repetitions with a brief period of rest after every 5 repetitions to complete the first set of 25 EMT repetitions. We intended that RMT repetitions be completed with a high degree of success in order to provide a sufficient training stimulus for respiratory strength increases. Therefore, if subjects were unable to perform at least 22 out of 25 EMT repetitions successfully, pressure-threshold resistance was reduced to 60% of MEP for the next set of EMT repetitions. Subjects next completed 25 IMT repetitions at 70% of MIP. If necessary, pressure-threshold resistance was reduced to 60% of MIP. RMT therapy alternated between EMT and IMT until 3 sets of 25 repetitions of each were completed with the clinician (150 total repetitions). Instructions for the home-based RMT program were reviewed. This included 3 sets of 25 repetitions of both IMT and EMT 5 days per week (repetitions completed during RMT therapy were counted toward this goal), maintenance of a treatment log to document adherence and accuracy with RMT, and a directive to discontinue RMT and contact the PI if negative side effects emerged such as pain.

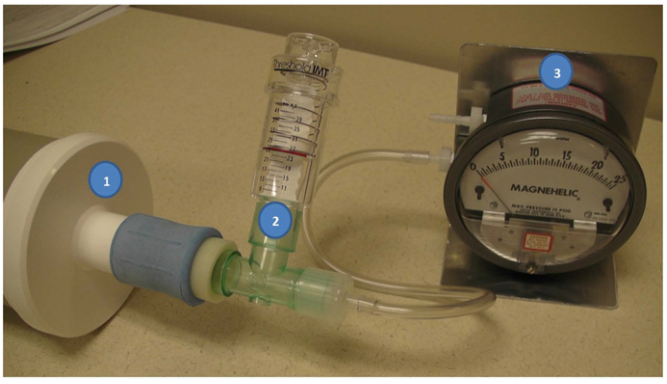

Fig. 1. RMT device calibration methodology. Accomplished using a 3-way gas pressure circuit comprising 1) an air delivery system to introduce positive or negative pressure, 2) the inspiratory or expiratory RMT device being calibrated, and 3) a differential atmospheric pressure gauge.

2.4. Data analysis

The magnitude of change from pretest to posttest, posttest to post-detraining, and pretest to post-detraining was determined for the primary outcome measures of MIP and MEP using Cohen's measure of effect size (d) as defined by Busk and Serlin's first equation for single-subject d [32]. Simply stated, d is obtained by subtracting the mean value of the first assessment from the mean of the second assessment, divided by the standard deviation of the first assessment. Conservative interpretation guidelines for effect size calculations were established in which $d < 0.6$ is negligible, $d \geq 0.6$ modest, $d \geq 1.0$ large, and $d \geq 2.0$ very large. Descriptive statistics are provided for the exploratory outcome measures and PCF.

3. Results

Subject demographic data are provided in Table 1. Eight subjects with LOPD participated in the study including 4 Caucasian males and 4 Caucasian females ranging from 41 to 69 years of age (mean = 49.3, sd = 8.4). All subjects were receiving ERT, with a mean treatment time of 39.9 months (sd = 21.3). Seven subjects ambulated without assistance; 1 required use of a cane. None of the subjects had a history of acute respiratory failure, though four did require home nocturnal ventilation (bilevel positive airway pressure [BiPAP]). The ERT regimens of each subject and all other aspects of their care remained consistent throughout the study.

Over 12-weeks, subjects were prescribed 9000 total RMT repetitions (4500 IMT and 4500 EMT). Individual subject adherence data are provided in Table 2. Across subjects, subjects completed a mean of 99% of their prescribed IMT repetitions (sd = 4.5) and a mean of 101% of their prescribed EMT repetitions (sd = 5.5).

The RMT program was generally well-tolerated, although there was 1 instance of a negative side effect. Subject 1 developed greater than mild thoracic pain between weeks 4 and 6 of the home-based RMT program. Although the informed consent and treatment protocol directed subjects to discontinue RMT in such event, this did not occur and so this thoracic pain was not discovered until the third RMT therapy session.

Due to limitations in the range of pressure-threshold resistance provided by the IMT device, the IMT regimen completed by Subjects 2 and 3 required modification. Subject 2 completed 2 weeks of IMT at 70% of MIP. At the second RMT therapy session, MIP was increased and the resistance range of the IMT device was insufficient to continue IMT at 60–70% of MIP. Therefore, IMT was completed at 50% of MIP for the remaining 10-weeks. Subject 3 completed EMT only.

3.1. Primary dependent variables

Changes in MIP/MEP over the 6-months of study participation are detailed for each individual subject in Appendix A (Subjects 1–8).

3.1.1. Maximum inspiratory pressure

3.1.1.1. Pretest to posttest. MIP increased in all 8 subjects (Table 3). Individual effect size data reveal the magnitude of these increases to be large in 4 ($d \geq 1.0$) and very large in 4 ($d \geq 2.0$) subjects. Across subjects, MIP increased by a mean of 19.6% (median = 18%); the mean effect size was d = 2.3 (median = 2.0).

Table 1
Subject demographic information.

Subject	Age	Ethnicity	Sex	Time on ERT at 1st study visit [months]	Ambulatory status	Nocturnal ventilation	Baseline MIP [cm H_20]	Baseline MEP [cm H_20]	FVC [l] (%predicted)	FEV1 [l] (%predicted)	PEF [l/s] (%predicted)
1	45	W	F	48	Ambulatory	Unknown	27	35	2.65 (79%)	2.29 (84%)	5.34 (82%)
2	47	W	F	10	Ambulatory	None	61	80	2.93 (70%)	1.95 (59%)	4.83 (64%)
3	47	W	M	12	Ambulatory	None (non-compliant CPAP)	87	121	3.92 (76%)	2.97 (73%)	7.06 (70%)
4	42	W	M	58	Ambulatory	BiPAP	48	86	2.73 (51%)	2.13 (50%)	7.43 (72%)
5	41	W	F	47	Ambulatory	BiPAP	32	52	1.70 (46%)	1.59 (53%)	4.97 (71%)
6	48	W	M	67	Ambulatory	BiPAP	23	83	1.39 (26%)	1.16 (28%)	3.95 (39%)
7	59	W	F	51	W/Assistance (Cane)	None	55	69	3.01 (77%)	2.01 (51%)	5.02 (71%)
8	65	W	M	26	Ambulatory	BiPAP	50	100	3.29 (76%)	2.47 (76%)	7.26 (86%)

W = white, F = female, M = male, ERT = enzyme replacement therapy, CPAP = continuous positive airway pressure, BiPAP = bilevel positive airway pressure, MIP = maximum inspiratory pressure, MEP = maximum expiratory pressure, cm H20 = centimeters water pressure, FVC = forced vital capacity, FEV1 = forced expiratory volume in 1 s, PEF = peak expiratory flow, l = liters.

3.1.1.2. Posttest to post-detraining. MIP increased or was unchanged in all 8 subjects (Table 4). Individual effect size data reveal the magnitude of these increases were negligible in 3 ($d < 0.6$), modest in 2 ($d \geq 0.6$), large in 1 ($d \geq 1.0$), and very large in 2 ($d \geq 2.0$) subjects. Across subjects, MIP increased by a mean of 12.1% (median = 8); the mean effect size was $d = 1.4$ (median = 0.8).

3.1.1.3. Pretest to post-detraining. MIP increased in all 8 subjects (Table 5). Individual effect size data reveal the magnitude of these changes to be large in 2 ($d \geq 1.0$) and very large in 6 ($d \geq 2.0$) subjects. Across subjects, MIP increased by a mean of 34.7% (median = 32.5); the mean effect size was $d = 3.8$ (median = 3.8).

3.1.2. Maximum expiratory pressure

3.1.2.1. Pretest to posttest. MEP increased in 7 of 8 subjects (Table 3). Effect size data reveal the magnitude of these increases to be large in 1 ($d \geq 1.0$) and very large in 6 ($d \geq 2.0$) subjects. Across subjects, MEP increased by a mean of 16.1% (median = 13.2); the mean effect size was $d = 1.8$ (median = 2.4).

3.1.2.2. Posttest to post-detraining. Out of 8 subjects, MEP increased in 3 and decreased in 5 (Table 4). Positive effect sizes were negligible in 1 ($d < 0.6$) and modest in 2 ($d \geq 0.6$) subjects. Negative effect sizes were modest in 2, large in 1, and very large in 2 subjects. Across subjects, MEP decreased by a mean of −1.0% (median = −2.5); the mean effect size was $d = -0.7$ (median = −0.5).

3.1.2.3. Pretest to post-detraining. MEP increased in 7 of 8 subjects (Table 5). Positive effect sizes were negligible in 1 ($d < 0.6$), large in 3 ($d \geq 1.0$), and very large in 3 ($d \geq 2.0$) subjects. Across subjects, MEP increased by a mean of 14.8% (median = 10.3); the mean effect size was $d = 1.4$ (median = 1.7).

Table 2
Subjects' adherence to prescribed RMT program.

Subject	Number IMT repetitions (% prescribed)	Number EMT repetitions (% prescribed)	Mean days per week of Exercise	% Accuracy IMT	% Accuracy EMT
1	4575 (102%)	4575 (102%)	5.08	100	96
2	4275 (95%)	4275 (95%)	5.18	100	100
3	–	4925 (109%)	5.08	–	100
4	4785 (106%)	4800 (107%)	5.42	95	100
5	4175 (93%)	4175 (93%)	4.75	94	100
6	4425 (98%)	4425 (98%)	5.00	90	99
7	4475 (99%)	4475 (99%)	5.00	95	100
8	4575 (102%)	4575 (102%)	5.08	100	100

4500 prescribed IMT repetitions, 4500 prescribed EMT repetitions. Days per week of exercise and percent accuracy are a combination of self-reported data and data collected in bimonthly RMT treatment sessions.

3.2. Exploratory outcomes

3.2.1. 6 min walk test (6MWT)

Pretest to posttest 6MWT improved in 5 of 8 subjects (Table 6); improvements ranged from 5.5% to 37.3% (median = 24.9). Across subjects, pretest to posttest 6MWT increased by a mean of 2.2% ($sd = 6.3$). Posttest to post-detraining 6MWT improved in 4 subjects and declined in 4 subjects (Table 7); improvements ranged from 0.4 to 9.3% (median = 3.5). Across subjects, posttest to post-detraining 6MWT increased by a mean of 1.0% ($sd = 4.3$). Pretest to post-detraining 6WMT improved in 6 of 8 subjects (Table 8); improvements ranged from 0.6% to 16.6% (median = 3.4). Across subjects, pretest to post-detraining 6MWT increased by a mean of 3.1% ($sd = 6.3$).

3.2.2. Timed function tests

3.2.2.1. Supine to stand. Pretest to posttest supine to stand improved in 4 of 7 subjects (posttest data not collected for subject 1; Table 6); improvements ranged from 10.9% to 53.6% (median = 25.25). Across subjects, pretest to posttest supine to stand improved by a mean of 13.4% ($sd = 23.7$). Posttest to post-detraining supine to stand declined in 4 of 6 subjects (posttest data not collected for subject 1, post-detraining data not collected for subject 4; Table 7); declines ranged from 8.2% to 53.8% (median = 19.4). Across subjects, posttest to post-detraining supine to stand declined by a mean of 33.7% ($sd = 38.0$). Pretest to post-detraining supine to stand improved or was unchanged in 4 of 7 subjects (Table 8); these changes ranged from 0% to 11.8% (median = 5.1). Across subjects, pretest to post-detraining supine to stand declined by a mean of 14.9% ($sd = 27.4$).

3.2.2.2. Stair climbing. Pretest to posttest stair climbing improved in 7 of 8 subjects (Table 6); improvements ranged from 2.9% to 41.9% (median = 16.7). Across subjects, pretest to posttest stair climbing improved by a mean of 15.0% ($sd = 14.2$). Posttest to post-detraining stair climbing improved in 5 of 7 subjects (post-detraining data not collected for subject 4; Table 7); improvements ranged from 1.6% to 17.9% (median = 8.8). Across subjects, posttest to post-detraining stair climbing declined by a mean of 7.0% ($sd = 37.0$). Pretest to post-detraining stair climbing improved in 6 of 7 subjects (Table 8); improvements ranged from 10.0% to 23.4% (median = 17.15). Across subjects, pretest to post-detraining stair climbing improved by a mean of 13.0% ($sd = 11.2$).

3.2.2.3. 10 m walk. Pretest to posttest 10 m walk improved or was unchanged in 6 of 8 subjects (Table 6); improvements ranged from 0% to 20.9% (median = 3.9). Across subjects, pretest to posttest 10 m walk improved by a mean of 3.4% ($sd = 9.1$). Posttest to post-detraining 10 m walk declined in 6 of 8 subjects (Table 7); declines ranged from

Table 3
Pretest to posttest data for primary outcomes: MIP and MEP (cm H_2O).

	S1	S2	S3	S4	S5	S6	S7	S8	Group mean (sd)
Pretest MIP	27	61	87	48	32	23	55	50	47.9 (20.9)
ΔMIP (cm H_2O)	+5	+3	+15	+17	+9	+6	+9	+5	+8.6 (5.0)
%Δ	+19	+5	+17	+35	+28	+26	+16	+10	+19.6 (9.9)
Effect size (*d*)	+2.4	+1.2	+5.1	+1.6	+2.8	+1.9	+1.7	+2.0	+2.3 (1.2)
Pretest MEP	35	80	121	86	52	83	69	100	78.3 (26.8)
ΔMEP (cm H_2O)	+13	+10	+14	+12	+10	−13	+7	+40	+11.6 (14.4)
%Δ	+37	+13	+12	+14	+19	−16	+10	+40	+16.1 (17.3)
Effect size (*d*)	+2.1	+3.0	+2.9	+2.6	+1.6	−4.6	+2.3	+4.6	+1.8 (2.7)

cm H_2O = centimeters water pressure, *d* = Cohen's d effect size statistic, Δ = change.

1.8% to 22.6% (median = −7.8). Across subjects, posttest to post-detraining 10 m walk declined by a mean of 5.5% (*sd* = 10.1). Pretest to post-detraining 10 m walk improved or was unchanged in 6 of 8 subjects (Table 8); these changes ranged from 0% to 5.8% (median = 3.2). Across subjects, pretest to post-detraining 10 m walk declined by a mean of −1.4% (*sd* = 7.8).

3.2.3. Peak cough flow (PCF)

Pretest to posttest PCF improved in 3 of 5 subjects (PCF measured in subjects 4 through 8 only; Table 6); improvements ranged from 8.0 to 34.0% (median = 17.9%). Across subjects, pretest to posttest PCF improved by a mean of 12.0% (14.3). Posttest to post-detraining PCF declined in 4 of 5 subjects; declines ranged from 2.1% to 18.4% (Table 7). Across subjects, posttest to post-detraining PCF declined by a mean of 6.1% (*sd* = 15.0). Pretest to post-detraining PCF improved in 3 of 5 subjects (Table 8); improvements ranged from 5.7% to 39.3% (median = 18.6). Across subjects, pretest to post-detraining PCF increased by a mean of 5.7% (*sd* = 24.4).

4. Discussion

Our results suggest that a supervised, individualized 12-week RMT regimen in individuals with LOPD is generally well-tolerated by subjects and results in large to very large increases in inspiratory and expiratory muscle strength. These respiratory strength increases were largely persistent following 3-months detraining, particularly increases in inspiratory strength. In addition, our exploratory outcomes also suggest that RMT-induced enhancements in respiratory strength may have benefits for some aspects of motor function and cough.

These data suggest that our 12-week RMT program may be a relatively inexpensive and useful adjunctive treatment for respiratory weakness in LOPD patients on ERT. Over 18-months, ERT in adults with LOPD has been shown to increase walking distance and stabilize pulmonary function. However, based on changes in MIP and MEP, the effects of ERT on respiratory muscle strength are modest in magnitude and most improvement occurs 12 to 26 weeks after treatment initiation [15]. Additionally, about one-third of patients have pulmonary decline despite treatment with ERT [16], many patients have respiratory weakness at diagnosis and thus upon treatment initiation [15], and the effects of ERT on pulmonary function may lessen or deteriorate after 2 to 3 years of treatment [17]. Therefore, progressive respiratory weakness and pulmonary decline remain critical obstacles to improving clinical outcomes in patients with LOPD.

These data are largely consistent with our previously reported findings in patients with Pompe disease. We first described the clinical implementation of RMT in 2 patients with LOPD; RMT was well-tolerated and substantial increases in respiratory muscle strength were observed in both participants [20]. Using the same experimental design and methodology as in the present report, we have also studied the effects of our 12-week RMT program in 2 children with infantile-onset Pompe disease. Pretest to posttest, 1 subject demonstrated negligible to modest increases in respiratory strength and the other subject demonstrated very large increases in respiratory strength [23].

In the absence of additional data regarding the effects of RMT in subjects with Pompe disease, the effects of RMT in others forms of muscle disease such as Duchene muscular dystrophy (DMD) may be informative. Koessler and colleagues studied the effects of 24-months of IMT in 27 patients with neuromuscular disorders including 18 with DMD [37]. Statistically significant increases in inspiratory strength were observed until plateau at 10 months and these increases were maintained over the remaining 14 months of IMT. Wanke and colleagues studied the effects of IMT in 15 subjects with DMD [38]. Ten subjects demonstrated increases in inspiratory strength after 1-month and continued IMT for 5 more months. Continued increases in inspiratory strength were present after 3- and 6-months of IMT. Gozal and Thiriet investigated the effects of 6-months of RMT in 21 undifferentiated participants with DMD or spinal muscular atrophy [39]. Statistically significant increases in inspiratory strength, expiratory strength, and "perceived respiratory load" were present after RMT. Overall, these data are consistent with our own findings that suggest RMT may result in increases in respiratory strength, even in the setting of progressive muscle disease [40].

To our knowledge, the only data regarding the durability of RMT-induced respiratory strength increases in Pompe disease come from our aforementioned report on the effects of 12-weeks of RMT in 2 children with infantile-onset Pompe disease. Similar to the present

Table 4
Posttest to post-detraining data for primary outcomes: MIP and MEP (cm H_2O).

	S1	S2	S3	S4	S5	S6	S7	S8	Group mean (sd)
Posttest MIP	32	64	102	65	41	29	64	55	56.5 (23.5)
ΔMIP (cm H_2O)	+7	+1	+3	+8	+9	+10	+1	0	+4.9 (4.1)
%Δ	+22	+2	+3	+12	+22	+35	+2	0	+12.1 (12.8)
Effect size (*d*)	+1.9	+0.5	+0.7	+0.9	+3.3	+3.6	+0.2	0	+1.4 (1.4)
Posttest MEP	48	90	135	98	62	70	76	140	89.9 (33.2)
ΔMEP (cm H_2O)	+4	−1	−12	−4	0	+5	−3	−8	−2.4 (5.7)
%Δ	+8	−1	−9	−4	0	+7	−4	−6	−1.0 (6.1)
Effect size (*d*)	+0.6	−0.3	−2.6	−0.8	0	+0.8	−1.1	−2.1	−0.7 (1.2)

cm H_2O = centimeters water pressure, *d* = Cohen's d effect size statistic, Δ = change.

Table 5
Pretest to post-detraining data for primary outcomes: MIP and MEP (cm H_2O).

	S1	S2	S3	S4	S5	S6	S7	S8	Group mean (sd)
Post-detraining MIP	39	65	105	73	50	39	65	55	61.4 (21.5)
ΔMIP (cm H_2O)	+12	+4	+18	+25	+18	+16	+10	+5	+13.5 (7.1)
%Δ	+44	+7	+21	+52	+56	+70	+18	+10	+34.7 (23.7)
Effect size (*d*)	+5.6	+1.6	+6.1	+2.4	+5.6	+5.1	+1.9	+2.0	+3.8 (2.0)
Post-detraining MEP	52	89	123	94	62	75	73	132	87.5 (28.2)
ΔMEP (cm H_2O)	+17	+9	+2	+8	+10	−8	+4	+32	+9.3 (11.7)
%Δ	+49	+11	+2	+9	+19	−10	+6	+32	+14.8 (18.3)
Effect size (*d*)	+2.8	+2.7	+0.2	+1.7	+1.6	−2.8	+1.3	+3.7	+1.4 (2.0)

cm H_2O = centimeters water pressure, *d* = Cohen's d effect size statistic, Δ = change.

data, respiratory strength increases were persistent after 3-months detraining. In fact, both subjects demonstrated peak inspiratory and expiratory strength at post-detraining assessment. Data regarding the durability of RMT-induced respiratory strength increases in other forms of muscle disease are also sparse. Wanke and colleagues reported that inspiratory strength increases were largely persistent after 6-months detraining, while Gozal and Thiriet found that inspiratory and expiratory strength increases were "rapidly reversible", returning to pretest values after 3-months detraining [38,39]. Although our finding that inspiratory and expiratory strength increases were largely persistent after 3-months detraining is encouraging, additional data are necessary to confirm this finding and establish long-term durability.

We selected our exploratory outcomes primarily to examine the effects of RMT on motor function using the 6MWT and TFTs (stair climbing, supine to stand, 10 m walk) to direct future research. These motor data are less robust than primary outcome measures and caution must be used in their interpretation. However, these data suggest that RMT may have had a positive influence on motor function. Overall, based on individual and group response, the direction of change suggests that motor function generally improved after RMT. However, the magnitude of change differed across these outcome measures. For example, although individual and group improvements were observed on the 6MWT and 10 m walk, these changes were generally relatively small in magnitude; improvements in supine to stand and stair climbing were also observed that were generally larger in their magnitude. Improved pulmonary function as a result of respiratory strength increases appears an unlikely explanation for improvements in supine to stand and stair climbing in particular. However, the respiratory muscles also make important non-ventilatory motor contributions [12,13]. For example, the muscles of the abdominal wall also aid trunk mobility and stabilization and are often prominently affected in LOPD [41]. Improvements in standing from supine and stair climbing may reflect the benefit of RMT-induced improvements in respiratory muscle strength on non-ventilatory motor performance. However, additional investigation is necessary to determine the effects of RMT on motor function in individuals with LOPD including determination of their clinical significance and mechanism of action.

Cough improvements were also suggested by the observed increases in PCF. Cough is dependent on both inspiratory and expiratory muscles to generate adequate airflow for airway protection and pulmonary hygiene. Using RMT to counteract respiratory weakness and enhance cough offers a plausible biological mechanism that warrants additional investigation.

Alternative explanations for our findings must be also considered. Considering that all 8 subjects were receiving ERT, one such explanation is that the observed respiratory strength increases were the result of drug treatment rather than RMT. However, this explanation appears unlikely due to the magnitude of respiratory strength increases observed with ERT and RMT. In the pivotal 18-month randomized, placebo-controlled clinical trial of alglucosidase alfa in LOPD, MIP and MEP were secondary outcome measures [15]. While MIP and MEP both increased in the treatment arm, this difference was only statistically significant for MEP. The authors report MIP and MEP data as percent predicted using reference values from Black and Hyatt [42]: from pretest to posttest, percent predicted MIP increased by 3.7% (from 40.0% to 43.7%) and percent predicted MEP increased by 3.1% (from 32.0% to 35.1%). If we use these reference values to interpret the present data, pretest to posttest percent predicted MIP increased by 9% (from 48% to 57%) and percent predicted MEP increased by 6% (from 42% to 48%). This suggests the magnitude of respiratory strength increases

Table 6
Pretest to posttest data for exploratory outcomes: 6MWT, TFTs, and PCF.

	S1	S2	S3	S4	S5	S6	S7	S8	Group mean (sd)
Pretest 6MWT (m)	393.5	412.5	551.0	378.8	409.3	451.9	302.0	513.8	426.6 (78.5)
Δ6MWT (m)	+24.9	+5.5	−11.0	−11.5	+29.8	−31.9	+37.3	+12.1	+6.9 (23.8)
%Δ	+6.3	+1.3	−2.0	−3.0	+7.3	−7.1	+12.4	+2.4	+2.2 (6.3)
Pretest Supine-stand (s)	10.2	7.6	3.3	11.6	6.2	5.5	13.8	5.0	7.9 (3.6)
ΔSupine-stand (s)[a]	DNT	+1.0	+0.1	−4.0	+0.3	−0.6	−7.4	−0.8	-1.6 (3.0)
%Δ[a]	DNT	+13.2	+3.0	−34.5	+4.8	−10.9	−53.6	−16	-13.4 (23.7)
Pretest Climb 4 stairs (s)	3.5	4.7	2.3	5.4	4.1	2.7	7.0	3.1	4.1 (1.6)
ΔClimb 4 stairs (s)[a]	−0.1	−0.5	−0.6	−0.9	−0.7	+0.1	−0.6	−1.3	-0.6 (0.4)
%Δ[a]	−2.9	−10.6	−26.1	−16.7	−17.1	+3.7	−8.6	−41.9	-15.0 (14.2)
Pretest Walk 10 m (s)	7.2	6.9	5.9	7.5	6.7	4.6	10.4	6.7	7.0 (1.6)
ΔWalk 10 m (s)[a]	+0.1	−0.6	−0.3	−0.2	0	+0.5	−0.2	−1.4	-0.3 (0.6)
%Δ[a]	+1.4	−8.7	−5.1	−2.7	0	+10.9	−1.9	−20.9	-3.4 (9.1)
Pretest PCF (L/s)	DNT	DNT	DNT	9.7	6.0	5.6	8.7	7.6	7.5 (0.6)
ΔPCF (L/s)	DNT	DNT	DNT	+3.3	0	+1.0	+0.7	0	+1.0 (1.4)
%Δ	DNT	DNT	DNT	+34	0	+17.9	+8	0	+12.0 (14.3)

Δ = change, m = meters, s = seconds, L/s = liters/s, DNT = did not test, standard deviations in parentheses.
[a] In timed functional tests, (−) represents improvement and (+) represents decline in function.

Table 7
Posttest to post-detraining posttest data for exploratory outcomes: 6MWT, TFTs, and PCF.

	S1	S2	S3	S4	S5	S6	S7	S8	Group mean (sd)
Posttest 6MWT (m)	418.4	418.0	540.0	367.3	439.1	420.0	339.3	525.9	433.5 (69.5)
Δ6MWT (m)	−4.0	+13.3	+2.3	−4.4	−20.7	+39.0	+13.0	−9.1	+3.7 (18.2)
%Δ	−1.0	+3.2	+0.4	−1.2	−4.7	+9.3	+3.8	−1.7	+1.0 (4.3)
Posttest Supine-stand(s)	DNT	8.6	3.4	7.6	6.5	4.9	6.4	4.2	5.9 (1.9)
ΔSupine-stand (s)[a]	DNT	+1.7	0	DNT	+3.5	+0.4	−6.5	+0.8	−2.2 (2.5)
%Δ[a]	DNT	+19.8	0	DNT	+53.8	+8.2	−101.6	+19	+33.7 (38.0)
Posttest Climb 4 stairs(s)	3.4	4.2	1.7	4.5	3.4	2.8	6.4	1.8	3.5 (1.5)
ΔClimb 4 stairs (s)[a]	−0.3	−0.6	+0.1	DNT	−0.1	−0.5	−0.1	+1.6	0 (0.7)
%Δ[a]	−8.8	−14.3	+5.9	DNT	−2.9	−17.9	−1.6	+89.9	+7.0 (37.0)
Posttest Walk 10 m (s)	7.3	6.3	5.6	7.3	6.7	5.1	10.2	5.3	6.7 (1.6)
ΔWalk 10 m (s)[a]	+0.9	+0.2	+0.1	+0.2	+0.9	−0.5	−0.2	+1.2	+0.4 (0.6)
%Δ[a]	+12.3	+3.2	+1.8	+2.7	+13.4	−9.8	−2.0	+22.6	+5.5 (10.1)
Posttest PCF (L/s)	DNT	DNT	DNT	13.0	6.0	6.6	9.4	7.6	8.4 (0.9)
ΔPCF (L/s)	DNT	DNT	DNT	−1.5	−1.0	+1.2	−0.2	−1.4	−0.6 (1.1)
%Δ	DNT	DNT	DNT	−11.5	−16.7	+18.2	−2.1	−18.4	−6.1 (15.0)

Δ = change, m = meters, s = seconds, L/s = liters/s, DNT = did not test, standard deviations in parentheses.
DNT all TFTs at Post-detraining in subject 4 due to subject refusal due to shoulder pain and need to avoid weight-bearing.
[a] In timed functional tests, (−) represents improvement and (+) represents decline in function.

associated with RMT is two to three times larger than those associated with ERT. Additionally, increases in respiratory muscle strength with ERT are largely achieved 12 to 26 weeks after treatment initiation [15]. As seen in Table 2, the duration of ERT in the present research ranged from 10 to 67 months with a median of 47.5 months. It is likely that our participants had received their maximum benefit from ERT in terms of respiratory strength by the time of their RMT participation.

Another alternative explanation of our findings is the possibility that observed increases in MIP and MEP were due to learning effects. In order to minimize learning effects, repeated trials of each maneuver are typically completed. For measurement of either MIP or MEP, standard guidelines recommend the completion of 3 maneuvers that vary by less than 20% [34]; a more recent update recommended the completion of 5 maneuvers [35]. To control for learning effects, we obtained repeated measures of MIP and MEP during multiple test sessions produced with minimal variability. Specifically, each MIP and MEP test session comprised at least 3 and no more than 6 repetitions of each maneuver. Testing was discontinued when subjects produced three values with <10% variability or after six efforts regardless of variability. At both pretest and posttest, subjects completed 8 MIP/MEP test sessions over 2 days (4/day) or a minimum of 24 MIP and 24 MEP maneuvers during each of these assessments. Although these repeated measures appear sufficient to mitigate concerns regarding learning effects in the present research, future research should explore the use of non-volitional measures of respiratory strength (electrical/magnetic phrenic nerve stimulation), as well as supplementary volitional measures (sniff nasal inspiratory pressure).

Table 8
Pretest to post-detraining data for exploratory outcomes: 6MWT, TFTs, and PCF.

	S1	S2	S3	S4	S5	S6	S7	S8	Group mean (sd)
Post-detraining 6MWT (m)	414.4	431.3	542.3	362.9	418.4	459.0	352.3	516.8	437.2 (67.1)
Δ6MWT (m)	+20.9	+18.8	−8.7	−15.9	+9.1	+7.1	+50.3	+3.0	+10.6 (20.3)
%Δ	+5.3	+4.6	−1.6	−4.2	+2.2	+1.6	+16.7	+0.6	+3.1 (6.3)
Post-detraining supine-stand (s)	9.0	10.3	3.4	DNT	10.0	5.3	12.9	5.0	8.0 (3.5)
ΔSupine-stand (s)[a]	−1.2	+2.7	+0.1	DNT	+3.8	−0.2	−0.9	0	+0.9 (1.9)
%Δ[a]	−11.8	+35.5	+3.0	DNT	+61.3	−3.6	−6.5	0	+14.9 (27.4)
Post-detraining climb 4 stairs (s)	3.1	3.6	1.8	DNT	3.3	2.3	6.3	3.4	3.4 (1.4)
ΔClimb 4 stairs (s)[a]	−0.4	−1.1	−0.5	DNT	−0.8	−0.4	−0.7	+0.3	−0.5 (0.4)
%Δ[a]	−11.4	−23.4	−21.7	DNT	−19.5	−14.8	−10.0	+9.7	−13.0 (11.2)
Post-detraining walk 10 m (s)	8.2	6.5	5.7	7.5	7.6	4.6	10.0	6.5	7.1 (1.6)
ΔWalk 10 m (s)[a]	+1.0	−0.4	−0.2	0	+0.9	0	−0.4	−0.2	+0.1 (0.6)
%Δ[a]	+13.9	−5.8	−3.4	0	+13.4	0	−3.8	−3.0	+1.4 (7.8)
Post-detraining PCF (L/s)	DNT	DNT	DNT	11.5	5.0	7.8	9.2	6.2	7.9 (0.9)
ΔPCF (L/s)	DNT	DNT	DNT	+1.8	−1.0	+2.2	+0.5	−1.4	+0.4 (1.6)
%Δ	DNT	DNT	DNT	+18.6	−16.7	+39.3	+5.7	−18.4	+5.7 (24.4)

Δ = change, m = meters, s = seconds, L/s = liters/s, DNT = did not test, standard deviations in parentheses.
DNT all TFTs at Post-Detraining in subject 4 due to subject refusal due to shoulder pain and need to avoid weight-bearing.
[a] In timed functional tests, (−) represents improvement and (+) represents decline in function.

Although these data suggest that RMT may be a useful adjunctive treatment to ERT to address respiratory weakness in patients with LOPD, additional research is needed in several key areas before advancing to a more robust efficacy trial. One important need is to test the effects of RMT in LOPD against a placebo control (i.e., sham-RMT). This is particularly important considering that medical device treatments may have more pronounced placebo effects than inactive oral medications [43]. Future research in this area should include a randomly assigned sham-RMT control group. Additionally, the optimal repertoire of outcome measures to capture the functional benefits of respiratory strength increases must continue to be refined. Data from our exploratory outcomes provide support for continued investigations on the effects of RMT on motor function and cough. Outcome measures that provide insight into the effects of RMT on diurnal and nocturnal ventilation and respiration also appear to warrant future study.

Although RMT was generally well-tolerated, there was a negative side effect in subject 1 characterized by greater than mild thoracic pain. The explanation for this response is not entirely clear. However, it is possible that the pressure-threshold resistance provided by this subject's RMT devices was higher than intended, as we did not calibrate for pressure-threshold resistance in subjects 1 to 3. Additionally, this subject, along with other participants, self-reported completing more repetitions than prescribed (see Table 2). Future research must also continue to refine control over RMT dose, including pressure-threshold resistance and number of repetitions. This also underscores the need for supervision to assess response and tolerance of RMT in patients with LOPD.

Although our data appear promising, our study is limited by a small sample size and lack of a control group. While single-subject designs are a powerful experimental approach for early phase clinical research, such designs, by definition, lack a control group. Our research was also limited by current RMT device technology. For example, we developed concerns about the accuracy of the pressure-threshold resistance delivered using the calibration markings on the RMT devices. Laboratory bench tests confirmed discrepancies between intended and delivered pressure-threshold resistance. In response, we developed a calibration system (see Fig. 1) to establish pressure-threshold resistance for inspiratory and expiratory RMT that was used in Subjects 4 through 8 [23]. The IMT devices we used also had limitations in the range of pressure-threshold resistance available, impacting the RMT regimens of Subjects 2 and 3. Improvements in FDA-approved, commercially available RMT devices will be necessary to maximize the safety and efficiency of RMT.

5. Conclusions

Overall, these data provide indication that RMT may offer benefit for the treatment of respiratory muscle weakness in adults with LOPD. Based on changes in MIP and MEP from pretest to posttest, inspiratory strength increases were large to very large in all 8 subjects and expiratory strength increases were large to very large in 7 subjects. These respiratory strength increases, particularly inspiratory strength gains, were largely persistent after 3-months detraining. Furthermore, RMT-induced strength increases may have benefitted motor function and cough. Future research should test RMT against a placebo-control and continue to refine the optimal outcome measures to capture functional benefits of RMT.

Competing interests

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

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List of abbreviations

RMT	respiratory muscle training
LOPD	late-onset Pompe disease
MIP	maximum inspiratory pressure
MEP	maximum expiratory pressure
PCF	peak cough flow
GAA	alpha glucosidase
ERT	enzyme replacement therapy
6WMT	6-min walk test
FVC	forced vital capacity
IMT	inspiratory muscle training
EMT	expiratory muscle training
TFTs	timed functional tests
BiPAP	bilevel positive airway pressure
DMD	Duchenne muscular dystrophy

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Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2015.09.003.

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