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The nature of respiratory muscle weakness in patients with late-onset Pompe disease

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Abstract

Late-onset Pompe disease (LOPD) causes myopathy of skeletal and respiratory muscles, and phrenic nerve pathology putatively contributes to diaphragm weakness. The aim of this study was to investigate neural contributions to diaphragm dysfunction, usefulness of diaphragm ultrasound, and involvement of expiratory abdominal muscles in LOPD. Thirteen patients with LOPD (7 male, 51±17 years) and 13 age- and gender-matched controls underwent respiratory muscle strength testing, ultrasound evaluation of diaphragm excursion and thickness, cortical and cervical magnetic stimulation (MS) of the diaphragm with simultaneous recording of surface electromyogram and twitch transdiaphragmatic pressure (twPdi; $n=6$), and MS of the abdominal muscles with recording of twitch gastric pressure (twPgas; $n=6$). The following parameters were significantly reduced in LOPD patients versus controls: forced vital capacity ($p<0.01$), maximum inspiratory and expiratory pressure (both $p<0.001$), diaphragm excursion velocity ($p<0.05$), diaphragm thickening ratio (1.8±0.4 vs. 2.6±0.6, $p<0.01$), twPdi following cervical MS (12.0±6.2 vs. 19.4±4.8 cmH_2O, $p<0.05$), and twPgas following abdominal muscle stimulation (8.8±8.1 vs. 34.6±17.1 cmH_2O, $p<0.01$). Diaphragm motor evoked potentials and compound muscle action potentials showed no between-group differences. In conclusion, phrenic nerve involvement in LOPD could not be electrophysiologically confirmed. Ultrasound supports assessment of diaphragm function. Abdominal expiratory muscles are functionally involved in LOPD.

Keywords: Late-onset Pompe disease; Respiratory muscle strength; Phrenic nerves; Diaphragm; Phrenic nerve conduction studies.

1. Introduction

Late-onset Pompe disease (LOPD) is an autosomal-recessive lysosomal storage disease caused by deficiency of the α−1,4-glucosidase enzyme [1]. This causes accumulation of glycogen in various tissues but mainly in skeletal muscle, leading to myofibrillar dysfunction, muscle atrophy and lipodystrophic changes [1]. Clinical hallmarks of the condition include a limb-girdle pattern of muscle weakness and respiratory muscle dysfunction, both resulting in functional disability and reduced life span [2–5].

Enzyme replacement therapy (ERT) has been available since 2006 and may improve or stabilize motor function and vital capacity [6], but some patients still progress to significant respiratory muscle weakness and develop nocturnal hypoventilation requiring home ventilatory support [7]. In clinical practice, assessment of respiratory muscle function follows standard recommendations [8], and forced

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vital capacity (FVC), maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP) and peak cough flow (PCF) in addition to blood gas analysis are indispensable for medical indication for non-invasive ventilation (NIV) or mechanical cough assistance [8,9].

In LOPD, respiratory muscle weakness has mainly been attributed to myopathic involvement of the diaphragm itself, which is supported by both functional and imaging studies [10–13], but expiratory abdominal wall muscles may also be affected. Interestingly, accumulation of glycogen has also been found in cervical motor neurons and central nervous system (CNS) neurons suggesting that neuronal pathology may contribute to respiratory muscle dysfunction [14,15]. These findings have recently been discussed as a potential limitation of current ERT strategies, which do not specifically target the CNS [16,17]. However, electrophysiological studies to verify phrenic nerve pathology in patients with LOPD are lacking. Therefore, the present study investigated phrenic nerve conduction properties following posterior cervical magnetic stimulation (CEMS). This technique has been shown to be less painful for patients than electrical stimulation of the phrenic nerves [18–20]. Resulting EMG responses are referred to as compound muscle action potentials (CMAP) which can be analyzed in terms of amplitude and latency, and help to differentiate axonal damage from demyelination. MS of the diaphragm can also be performed transcranially yielding motor evoked potentials that allow assessment of central conductivity of the inspiratory pathway [21,22].

Evaluation of pulmonary function by FVC and respiratory muscle strength by MIP, MEP and PCF as described above is highly dependent on co-operation and effort, which are difficult to control for [8]. Non-volitional assessment of diaphragm strength can be achieved when CEMS of the phrenic nerves is combined with invasive measurement of the resulting twitch transdiaphragmatic pressure (twPdi) using esophageal and gastric balloon catheters [8,23]. This technique has been applied only once in patients with LOPD, showing that twPdi correlates with supine FVC and MIP [24].

Magnetic resonance imaging (MRI) has been proposed for quantification of diaphragm involvement in LOPD [10,11,13], but technical prerequisites may not always be available. For this reason, our study evaluated diaphragm morphology and function by ultrasound. As shown in healthy subjects, diaphragm thickening ratio (DTR) and diaphragm velocity during a voluntary sniff maneuver may be specifically useful to assess diaphragm function [25].

MS of the abdominal expiratory muscles along with recording of twitch gastric pressure (twPgas) has been introduced for non-volitional assessment of expiratory muscle strength [26]. In LOPD, substantial involvement of the abdominal wall muscles has been shown by computer tomography [10], but to date, studies applying MS of the abdominal nerve roots have not been published. One previous study found weak correlation between the MEP and both twPdi and maximum voluntary Pdi without specifically reporting gastric pressures [24].

The aim of this study was to comprehensively evaluate diaphragm strength and respiratory muscle function in LOPD patients by combining spirometry with phrenic nerve conduction studies, diaphragm ultrasound, and non-volitional assessment of twPdi and twPgas following MS of the diaphragm or the abdominal muscles, respectively.

2. Methods

2.1. Study participants

This cross-sectional case-control study was conducted from November 2017 to October 2018. Ethical approval was obtained from the local ethics committee (Ethikkommission der Ärztekammer Westfalen-Lippe und der WWU Münster). All patients provided written informed consent prior to enrolment in the study. This study was part of a wider project investigating respiratory muscle strength and function in neuromuscular disorders and chronic obstructive pulmonary disease (ClinicalTrials.gov Identifier: NCT03032562). Only adult patients with genetically proven LOPD and ongoing ERT were recruited. Control subjects were matched for age, gender, and body mass index (BMI) (age difference <5 years and variation in BMI <3 kg/m^2).

2.2. Clinical evaluation

For assessment of neurological disability, the Walton and Gardner-Medwin Scale (WGMS) was applied, which has been used in clinical studies on LOPD [27]. The WGMS score ranges from 0 to 10, with a higher score indicating more disability. The Medical Research Council dyspnea scale was used for self-assessment of breathlessness [28,29]. The 6 min walking test (6-MWT) was performed to quantify exercise performance [30].

2.3. Spirometry

Lung function tests were performed according to standard recommendations using an electronic spirometer (Vitalograph 3000^{TM}, Vitalograph, Hamburg, Germany) [8]. Participants were encouraged to perform a maximum effort towards their individual FVC and forced expiratory volume (FEV) in the upright position. At least five consecutive tests were performed until the best result was achieved and showed <10% variation from the preceding test. FVC was expressed as percentage of the predicted value [8,31]. MIP and MEP were obtained using a handheld electronic manometer (MicroRPM^{TM}, CareFusion, Baesweiler, Germany) equipped with a flanged mouthpiece. The best of three attempts was recorded if variability to the preceding test was <10%, and pathological cut-off values were obtained from the literature [32]. All measurements were performed using a nasal clip to prevent leakage of air.

2.4. Carbon dioxide monitoring

Daytime blood gas analysis (with samples drawn from the earlobe which was arterialized using nonivamid/nicoboxil ointment) and transcutaneous nocturnal capnometry (Sentec, Therwil, Switzerland) had been performed in all LOPD patients between 1 and 90 days prior to the study procedures.

2.5. Diaphragm ultrasound

A portable ultrasound machine (LOGIQ S8 -XD clear, GE Healthcare, London, United Kingdom) with a 3.5 MHz convex transducer was used for assessment of diaphragm excursions, and a 10 MHz linear transducer was used for evaluation of diaphragm thickness. Measurements were performed on the right hemidiaphragm with the subject in the supine position. Diaphragm excursions were evaluated during tidal breathing, at total lung capacity (TLC) and during a voluntary sniff maneuver, with the 3.5 MHz probe subcostally positioned between the mid-clavicular and anterior axillary line (Figure S1A). Diaphragm excursion velocity was assessed during tidal breathing and voluntary sniff only (Figure S1B). Diaphragm thickness was measured as the vertical distance between the pleural and peritoneal layer at both TLC and functional residual capacity (FRC) (Figures S1C and S1D) with the probe positioned in the posterior axillary line between the eighth and tenth intercostal space. The DTR was calculated as thickness at TLC divided by thickness at FRC.

2.6. Cortical and cervical magnetic stimulation of the diaphragm

Diaphragm surface electromyogram was recorded bilaterally (Dantec 2000™, Dantec Medical, Skovlunde, Denmark). Electrodes were placed in the seventh intercostal space with the reference electrodes positioned cranially to the xiphoid process (16 cm inter-electrode distance). The ground electrode was placed around the right wrist. Magnetic stimulation was performed using a MagPro Compact™ magnetic stimulator equipped with a 12 cm C-100 circular coil (MagVenture, Willich, Germany). Stimulus duration was 0.1 ms, and stimulus intensity was 100% of the magnetic flux density (2 Tesla). For CEMS, the coil was placed at C7 and then moved up towards C6 until the highest reproducible CMAP amplitude was obtained. For cortical MS (COMS), the magnetic coil was placed above Cz´ [33]. At least five stimuli were delivered in order to achieve the highest possible amplitude showing $<10\%$ variation from the preceding two stimulations. To avoid twitch potentiation, stimuli were separated by at least 40 s. Stimulation at FRC was determined by visual observation of abdominal movements. Figure S2 displays representative CMAP following cortical (Figure S2A) and cervical (Figure S2B) stimulation at FRC. Central motor conduction time (CMCT) was calculated by subtraction of peripheral latency following CEMS from overall response latency following COMS.

2.7. Transdiaphragmatic pressure recordings following volitional inspiratory maneuvers and CEMS of the phrenic nerves

Esophageal and gastric pressure (Pes and Pgas) were simultaneously measured using balloon catheters (Cooper Surgical, Trumbull, CT, USA) transnasally inserted into the stomach and the distal esophagus [34]. Balloon catheters were connected to a differential pressure transducer (DPT-100™, Utah Medical Products, Athlone, Ireland) and carrier amplifier (AD Instruments, Oxford, UK), and Pgas, Pes and Pdi (defined as Pes – Pgas) were continuously displayed using LabChart™ software (Fig. S3A). Pdi measurements were performed during a voluntary sniff and Mueller maneuver (i.e. maximal inspiration against an occluded airway).

Subjects were encouraged to achieve maximum deflection of the Pdi curve. After participants had learned and practiced the maneuvers several times, five measurements were recorded for each and the best result achieved was taken for analysis (Figure S3B). Volitional maneuvers were separated by 5 min of quiet breathing. Twitch Pdi (twPdi), calculated as twPes – twPgas, was simultaneously recorded with diaphragm CMAP following CEMS of the phrenic nerves as described above.

2.8. Gastric pressure recordings following voluntary cough and magnetic stimulation of the abdominal muscles

For recording of Pgas during forced expiration, subjects were instructed to repeatedly perform a voluntary cough with 5 min between single maneuvers. After careful instruction, five attempts were recorded with visual feedback to the patient in order to obtain maximum deflection of the Pgas curve (Figure S3D). The best result achieved was kept for further analysis.

The abdominal nerve roots were magnetically stimulated at the tenth vertebra [26]. Surface electrodes were bilaterally placed near the lower costal margin 16 cm from the xiphoid process. Stimulus duration was 0.1 ms, and stimulus intensity was 100% of the maximum magnetic output as previously described [35]. Again, stimulation at FRC was determined by visual observation of abdominal movements. Twitch Pgas following stimulation at FRC at TH10 is shown in Fig. S3C.

2.9. Statistical analyses

All analyses were performed using Sigma Plot™ software (Version 13.0, Systat Software GmbH, Erkrath, Germany). Results are expressed as mean and standard deviation for parametric data, and median and interquartile range (IQR) for continuous variables with a skewed distribution. Categorical variables are expressed as percentages, unless otherwise specified. Differences between groups were analyzed using the unpaired *t*-test or the Mann-Whitney U test as appropriate.

Table 1
Demographic and basic lung function data in LOPD patients and controls.

	LOPD patients ($n=13$)	Healthy volunteers ($n=13$)	p-value
Male, n (%)	7 (54)	7 (54)	n.s.
Age, years	50.5 ± 15.9	49.9 ± 14.4	n.s.
BMI, kg/m²	24.5 ± 3.9	24.9 ± 2.3	n.s.
6MWD, m	408±175	–	–
MRC dyspnea score	2.1 ± 1.4	–	–
Lung function data			
FVC, L	2.7 ± 1.2	4.5 ± 1.3	<0.01
FVC,% predicted	64.3 ± 21.3	110.5 ± 18.7	<0.01
PEF, L/sec	5.1 ± 1.5	8.3 ± 2.5	<0.01
PEF,% predicted	67.3 ± 18.3	104.6 ± 25.0	<0.01
Maximum inspiratory pressure, cmH_2O	37.5 ± 18.5	97.5 ± 26.6	<0.01
Maximum exspiratory pressure, cmH_2O	74.2 ± 26.7	130.1 ± 26.1	<0.01

Data are presented as mean and standard deviation or number of patients (%).
6MWD, 6 min walking distance; BMI, body mass index; FEV_1, forced expiratory volume after 1 s; FVC, forced vital capacity; n.s., not significant; PEF, peak expiratory flow.

Table 2
Baseline characteristics for individual patients.

Patient ID	Age	Sex	Age at symptom onset (years)	Age at diagnosis (years)	Age at ERT start (years)	Duration of ERT (years)	MRC-DS	WGMS
1	42	M	38	39	39	3	1	3
2 ♦	36	M	childhood	28	29	7	4	5
3	33	F	adolescence	25	25	8	3	2
4 ♦	63	F	20	34	51	12	2	7
5 ♦	73	F	55	64	64	9	4	6
6 ♦	58	M	childhood	56	56	2	2	4
7	62	F	15	59	61	1	4	5
8 ♦	77	M	51	71	76	1.5	3	6
9	45	F	34	35	35	10	1	6
10	22	M	10	19	20	2	1	2
11 ♦	52	F	30	41	41	9	1	3
12	59	M	childhood	50	55	4	1	3
13 ♦	35	M	adolescence	33	33	2	2	4
Mean		–	–	42.6	42.2	5.4	2.3	4.3
SD		–	–	15.4	14.7	3.7	1.2	1.6

ERT, enzyme replacement therapy; F, female; M, male; MRC-DS, MRC dyspnea scale; SD, standard deviation; WGMS, Walton Gardner Medwin Scale.
♦ non-invasive ventilation for nocturnal hypoventilation. Patient 4 was wheelchair-bound.

Differences in categorical data were compared using the χ^2 –test. For all analyses, a p-value of <0.05 was considered statistically significant. For graphical illustrations, GraphPad Prism 7™ (GraphPad Software, San Diego, California) was used.

3. Results

3.1. Subjects

Thirteen patients with LOPD (age 51±6 years, 7 male, BMI 25.0 ± 4 kg/m²) and 13 healthy control subjects (age 50±15 years, 7 male, BMI 25.1 ± 2 kg/m²) were enrolled (Table 1). Spirometry, phrenic nerve conduction studies following CEMS and diaphragm ultrasound were performed in all subjects. Invasive measurement of Pgas and Pes was refused by 7 LOPD patients leaving a cohort of 6 patients in whom full data sets were available. In all patients, diagnosis of Pompe disease was confirmed by both genetic and enzyme activity testing. Individual baseline characteristics of LOPD patients are shown in Table 2. All patients were receiving ERT according to standard recommendations (biweekly, 20 mg/kg body weight). Age at symptom onset ranged from late childhood to 55 years. Duration of ERT ranged from 1 year to 12 years. Mean WGMS score was 4.3 ± 1.7 with 4 patients requiring walking aids ($n=3$) or wheelchair assistance ($n=1$). Mean MRC breathlessness grade was 2.1 ± 1.4. Seven patients were receiving nocturnal NIV for treatment of sleep-related hypoventilation.

3.2. CO_2 measurements, spirometry, and bedside tests of respiratory muscle strength

None of the patients showed daytime hypercapnia (data not shown), and in non-ventilated individuals, nocturnal hypercapnia had recently been excluded by transcutaneous capnometry (maximum transcutaneous carbon dioxide tension [$p_{tc}CO_2$] 44±3 mmHg, mean $p_{tc}CO_2$ 39±3 mmHg). In patients with LOPD ($n=13$), FVC, MIP and MEP were

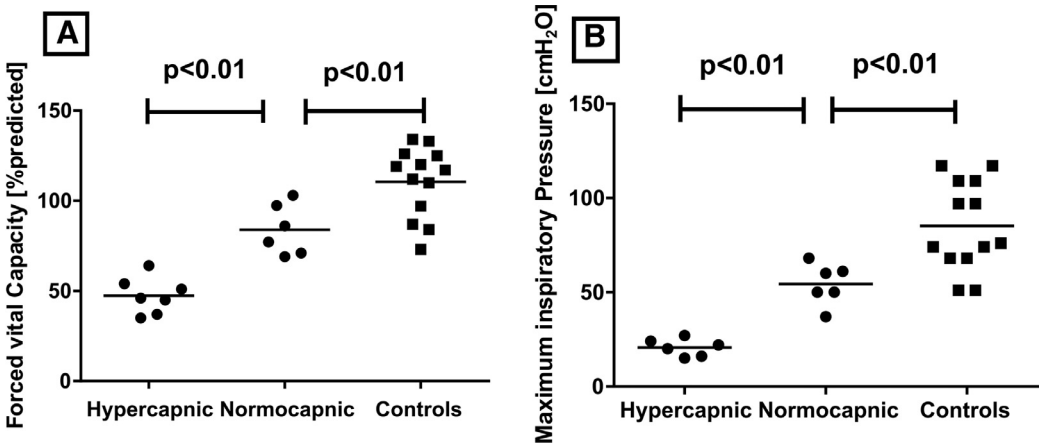

Fig. 1. Forced vital capacity (A) and maximum inspiratory pressure (B) in hypercapnic (left panels) and normocapnic late-onset Pompe disease (LOPD) patients (mid panels) and healthy controls (right panels).

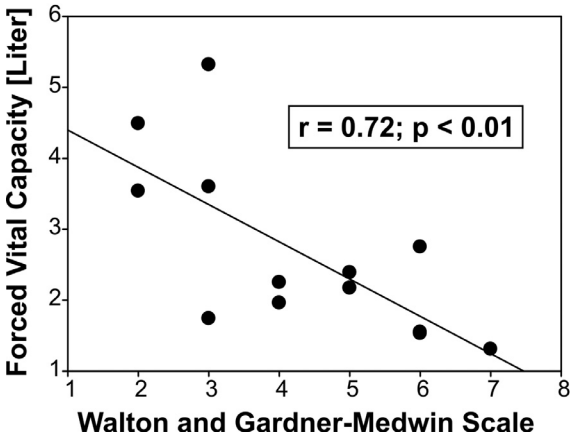

Fig. 2. Correlation between forced vital capacity and clinical disease severity (WGMS) in patients with late-onset Pompe disease.

significantly reduced compared with control subjects (Table 1, all $p<0.01$). Both FVC and MIP were significantly lower in LOPD patients receiving home ventilatory support than in non-ventilated patients (Fig. 1A and B). FVC ($r=-0.72$; $p<0.01$) and MIP ($r=-0.59$; $p<0.03$) were correlated with clinical disease severity based on the WGMS score, but not with the MRC dyspnea scale score (Fig. 2).

3.3. Diaphragm ultrasound

Whereas diaphragm thickness could be measured in all subjects, quantitative assessment of diaphragm excursion was hampered by an insufficient acoustic window in a subset of patients and controls (Table 3). Diaphragm excursion amplitude during deep breathing was significantly reduced (by 42%, $p=0.01$) in LOPD patients versus controls (Table 3; Fig. 3). Excursion velocity during voluntary sniff and DTR were also significantly lower in LOPD patients than in controls (Table 3). When LOPD patients were stratified by the presence of nocturnal NIV, no significant between-group differences were found with regard to DTR, excursion velocity, and excursion amplitude. Diaphragm ultrasound parameters did not significantly correlate with the MRC dyspnea scale score or the WGMS score, but FVC was significantly correlated with DTR ($r=0.62$; $p=0.02$).

3.4. Diaphragm motor evoked potentials and phrenic nerve conduction studies following magnetic stimulation

In all study participants ($n=26$), reproducible EMG responses were recorded following both CEMS and COMS of the diaphragm. Motor evoked potentials and CMAP showed no significant side-to-side difference regarding latency and amplitude (Table 4). According to reference values reported in the literature and compared with control subjects in this study, the latency and amplitude of diaphragm CMAP and motor evoked potentials were normal (Table 4).

3.5. Transdiaphragmatic pressure recordings following volitional inspiratory maneuvers and CEMS of the phrenic nerves

Following a voluntary sniff maneuver, Pdi was significantly reduced in LOPD patients ($n=6$) compared with control subjects (Table 5). Twitch Pdi amplitude following CEMS of the phrenic nerves was significantly lower in LOPD patients than in healthy volunteers (Table 5, Fig. 4A). Twitch Pdi was not statistically related to MIP and upright FVC (data not shown).

In-depth analysis of the twPdi curve showed that the area under the curve (AUC) was significantly reduced in LOPD patients versus controls (median 1.16 [IQR 0.65–1.71] vs. 3.62 [1.75–4.99] $cmH_2O{\cdot}sec$, $p<0.05$), possibly due to a decrease in both amplitude and maximum rate of relaxation (-67.8 ± 35 vs. -134.2 ± 74.0 $cmH_2O/msec$; $p=0.07$). The latter is defined as the negative peak of the pressure derivative as a function of time, and measures the initial part of the pressure decay [36,37]. Maximum rate of contraction (MRC), defined as the steepest slope of the inclining

Table 3
Diaphragm ultrasound measures in LOPD patients and control subjects.

	LOPD patients	Controls	*p*-value
Diaphragm excursion			
Amplitude during tidal breathing, cm	1.4±0.6 ($n=10$)	1.4±0.5 ($n=11$)	n.s.
Velocity during tidal breathing, cm/sec	1.3±0.5 ($n=10$)	1.0±0.4 ($n=11$)	n.s.
Amplitude during voluntary sniff, cm	1.9±0.7 ($n=8$)	2.3±1.7 ($n=9$)	n.s.
Velocity during voluntary sniff, cm/sec	4.5±1.4 ($n=8$)	6.4±2.3 ($n=9$)	0.03
Amplitude at TLC, cm	4.3±1.9 ($n=9$)	7.4±2.6 ($n=10$)	0.01
Diaphragm thickness			
at FRC, cm	0.13±0.06 ($n=13$)	0.21±0.05 ($n=13$)	<0.01
at TLC, cm	0.21±0.07 ($n=13$)	0.55±0.20 ($n=13$)	<0.01
DTR	1.8±0.4 ($n=13$)	2.6±0.6 ($n=13$)	<0.01

Data are presented as mean and standard deviation. DTR, diaphragm thickening ratio, FRC, functional residual capacity; max., maximum; n.s., not significant; TB, tidal breathing; TLC, total lung capacity. Quantitative assessment of diaphragm excursion was hampered by an insufficient acoustic window in a subset of patients and controls.

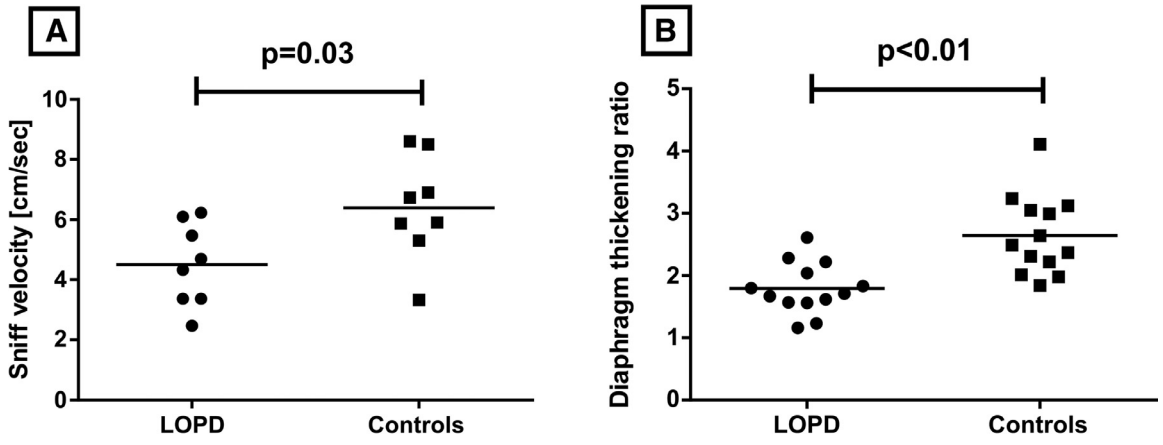

Fig. 3. Sniff velocity (A) and diaphragm thickening ratio (B) in patients with late-onset Pompe disease (left panels) and controls (right panels).

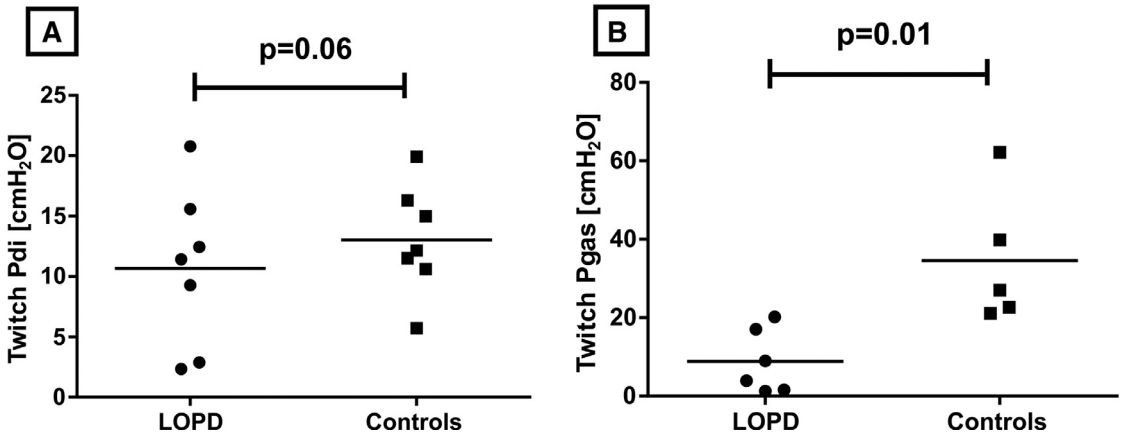

Fig. 4. Inspiratory and expiratory muscle strength in late-onset Pompe disease (LOPD) patients and controls. Values of twitch transdiaphragmatic pressure (Twitch Pdi) following cervical stimulation of the phrenic nerve roots at functional residual capacity (i.e. inspiratory muscle strength) in patients with late-onset Pompe disease (left panels) and in controls (right panels) [**A**]. Values of twitch gastric pressure (Twitch Pgas) following stimulation of the abdominal muscle roots at functional residual capacity (FRC) (i.e. expiratory muscle strength) in patients (left panels) and controls (right panels) [**B**].

twPdi curve) did not significantly differ between patients and controls (354±290 vs. 439±354 cmH_2O/msec; $p=0.65$). Twitch Pdi showed no correlation with parameters reflecting clinical disease severity (i.e. MRC dyspnea scale and WGMS).

3.6. Gastric pressure recordings following voluntary cough and magnetic stimulation of the abdominal muscles

Twitch Pgas was significantly reduced (by 74%, $p<0.01$) in LOPD patients ($n=7$) compared with healthy volunteers

Table 4
Magnetic phrenic nerve conduction studies in LOPD patients and control subjects.

	LOPD patients ($n=12$)	Controls ($n=12$)	p-value
Cortical MS at FRC			
Right-sided latency, msec	18.57±1.85	19.15±3.15	n.s.
Right-sided amplitude, mV	0.83±0.84	0.60±0.59	n.s.
Left-sided latency, msec	19.26±2.78	19.30±3.18	n.s.
Left-sided amplitude, mV	0.92±0.98	0.76±0.53	n.s.
Cervical MS at FRC			
Right-sided latency, msec	5.11±1.42	4.64±1.42	n.s.
Right-sided amplitude, mV	0.24±0.10	0.31±0.28	n.s.
Left-sided latency, msec	5.08±0.76	4.96±1.34	n.s.
Left-sided amplitude, mV	0.23±0.14	0.35±0.32	n.s.
Right-sided CMCT, msec	13.45±2.33	14.51±3.49	n.s.
Left-sided CMCT, msec	14.18±3.03	14.33±4.02	n.s.

Data are shown as mean and standard deviation. CMCT, central motor conduction time, FRC, functional residual capacity; MS, magnetic stimulation; n.s., not significant. One patient with LOPD denied magnetic stimulation.

(Table 5 and Fig. 4B), but Pgas following voluntary cough was not (Table 5). Twitch Pgas was not associated with the functional dysphagia score, the WGMS score, or the MRC dyspnea scale score (data not shown). Furthermore, twPgas and MEP showed no significant correlation ($r=0.743$ $p=0.09$).

4. Discussion

This observational study comprehensively explored respiratory muscle strength, phrenic nerve and diaphragm function in adult patients with genetically proven LOPD receiving long-term ERT. Two major findings can be summarized: Firstly, magnetic nerve conduction studies suggest that diaphragm involvement in LOPD is not attributable to impaired conductivity of the phrenic nerves or the central inspiratory pathway. Secondly, MS of the lower thoracic nerve roots confirms significant weakness of the expiratory abdominal muscles in patients with LOPD.

Respiratory muscle weakness is common in LOPD and gradually increases with disease progression [2–4,6,9–11,24,38,39]. However, its age of manifestation and natural course appear to be highly variable [2–4]. ERT has been shown to slightly improve average FVC for several years [6,7,39] but treatment response is heterogeneous [6,39]. It has been suggested that not only diaphragm myopathy but also neurogenic pathology may contribute to respiratory muscle weakness. In fact, glycogen accumulation has been reported in cervical motor neurons and CNS neurons [14,15,40,41], which would explain dysfunction of the phrenic nerves and even impairment of central breathing control. Currently approved ERT does not specifically target the CNS. This could be one explanation for the observation that improvement, or stabilization, of respiratory function by ERT cannot be achieved in up to one-third of patients, or is not sustained after several years of treatment [42]. Electrophysiological studies to verify neurogenic contributions to diaphragm weakness in LOPD are still lacking. Our study is the first to investigate phrenic nerve conduction and motor evoked potentials of the diaphragm in this condition. Although it is a limitation that the study enrolled only 13 individuals with LOPD,

Table 5
Invasively-obtained inspiratory and expiratory muscle strength data in LOPD patients and control subjects.

Subject ID	Inspiratory tests			Expiratory tests		
	twPdi (cmH_2O)	Sniff Pdi (cmH_2O)	MIP (cmH_2O)	twPgas (cmH_2O)	coughPgas (cmH_2O)	MEP (cmH_2O)
Patients						
1[§]	9.3	42.3	50	3.9	49.9	78
2	20.8	8.4	22	1.3	27.4	68
3	15.6	47.0	37	9.0	118.3	55
6	2.4	–	27	1.6	–	65
9	11.4	34.0	60	17.1	78.2	85
10	12.4	37.0	61	20.1	57.5	106
Mean ± SD	12.0±5.6	33.7±13.4	42.8±15.3	8.8±7.4	66.3±30.7	76.2±16.4
Control subjects (age [years], sex)						
1 (42, M)	20.4	107.9	74	62.2	111.3	100
2 (37, M)	15.7	96.9	117	27.0	79.4	176
3 (34, F)	16.5	57.4	51	22.7	58.4	72
4 (60, M)	27.1	–	68	–	–	115
5 (45, F)	14.4	51.5	117	21.1	68.5	110
6 (24, M)	22.2	123.8	143	39.9	172.1	162
Mean ± SD	19.4±4.4	87.5±28.4	95.0±32.6	34.6±15.3	97.9±41.1	122.5±35.8
p-value*	<0.05	<0.01	<0.01	<0.01	n.s.	<0.05

Data are shown as mean and standard deviation. CoughPgas, gastric pressure following a forced cough; F, female; M, male; MEP, maximum expiratory pressure; MIP, maximum inspiratory pressure; SD, standard deviation; n.s., not significant; Sniff Pdi, transdiaphragmatic pressure following a voluntary sniff maneuver; twPdi, transdiaphragmatic pressure following supramaximal cervical stimulation of the phrenic nerves; twPgas, gastric pressure following stimulation of the lower thoracic nerve roots.

[§] patient ID according to Table 2.

* Mann–Whitney U test for comparison between patients and controls.

it should be noted that electrophysiological findings were entirely normal in all patients. This speaks against any functional involvement of the phrenic nerves even in patients with significant diaphragm weakness and nocturnal NIV. In addition, we showed that central conductance of the inspiratory pathway was unaffected. The latter finding does not contradict the observation that central hypercapnic response may be decreased in LOPD patients, contributing to daytime hypercapnia beyond mere diaphragm dysfunction [4]. Further studies are required to confirm our results in larger patient samples, aiming to clarify whether accumulation of glycogen in phrenic nerve motor neurons has any electrophysiological correlate in LOPD patients. Notably, both the extent and impact of neuronal pathology are of particular interest in patients with infantile-onset Pompe disease, in whom long-term ERT has led to a new phenotype with nervous system manifestations previously unknown [17].

It is striking that between LOPD patients and healthy controls, group differences were much greater concerning volitional tests of respiratory muscle strength than with regard to twPdi measurements following CEMS of the phrenic nerves. For example, mean MIP and maximum volitional Pdi were more than 60% lower in LOPD patients than in controls, but the difference in mean twPdi at FRC was 40% only. Since CMCT and phrenic nerve conduction were normal in all subjects, our finding can be explained by decreased voluntary activation of the diaphragm, which might be subjected to alterations of central respiratory control [43]. Alternatively, maximum effort in volitional tests of respiratory muscle strength may be lower in patients than in healthy subjects, rendering non-volitional measurements indispensable for reliable evaluation.

In LOPD, respiratory muscle weakness is mainly attributable to diaphragm dysfunction, leading to impaired inspiration and hypercapnia as well as weakness of forced expiration or cough. Morphologic changes spare accessory inspiratory muscles such as the external intercostals [10]. The significance of diaphragm involvement is underlined by the fact that supine volume drop of FVC is a clinical hallmark of LOPD [24,44]. Our study confirms that FVC reduction is associated with the presence of (nocturnal) hypercapnia and the need for home ventilatory support as well as clinical disease severity [4]. Therefore, sonographic evaluation of diaphragm function and morphology is both promising and feasible for routine clinical purposes. Compared with healthy controls, LOPD patients showed significant reduction of excursion amplitude during deep breathing, excursion velocity during voluntary sniff and DTR, the latter two probably best reflecting inspiratory muscle strength [25].

Twitch Pdi represents a selective test of diaphragm action in response to magnetic stimuli. Detailed analysis of the twPdi curve allows further investigation of contractile and relaxation properties of the diaphragm [45]. Diaphragm contraction and relaxation are both crucial for normal respiration and adaptation to changes in respiratory load. MRC reflects the steepest slope or the "speed" of the inclining twPdi, whereas the maximum rate of relaxation (MRR) represents the initial part of the pressure decline. Our study provides these data in LOPD patients for the first time, showing that both MRC and MRR were reduced compared to controls. The fact that only the difference in MRR achieved statistical significance possibly reflects that diaphragm dysfunction due to myopathic tissue damage [10,11,44] is mainly related to impaired muscle relaxation. Further studies are needed to investigate this aspect in larger patient samples.

A recent whole-body MRI study showed that there is significant atrophy of the abdominal wall muscles in LOPD [10,11]. The present study confirms expiratory muscle dysfunction by means of magnetic stimulation of the lower thoracic nerve roots and invasive recording of twPgas. MEP was decreased by 44% in LOPD patients ($n=13$) versus controls, confirming the findings of a previous study [24], and twPgas showed even greater reduction (by 74%, $n=7$). Whereas MEP functionally reflects various expiratory muscles (diaphragm, internal intercostal and abdominal muscles), the protocol used in this study allowed more selective assessment of abdominal muscle function. There is still controversy about the significance of expiratory muscle strength testing in lung and neuromuscular diseases [35]. MEP is a feasible bedside marker to exclude expiratory muscle weakness if measured values are clearly normal [32]. However, for patients with borderline values or difficulties in performing forced expiration, twPgas following MS of the abdominal muscles might be useful to distinguish weakness from normality [35].

Despite the comprehensive protocol applied in this study, several limitations have to be acknowledged. Firstly, the number of patients enrolled was small. For this reason, we combined a case-control design with our initial, cross-sectional approach, to minimize the influence of age and gender on inspiratory pathway conductivity. Secondly, inter- and intra-observer variability may have affected the COMS and CEMS results in particular. We aimed to address this by extensive training and by performing up to five tests per patient until variability was $<10\%$. Finally, threshold testing for magnetic output was not performed. Instead, the same magnetic field output (100%) was used in every subject to achieve reproducible CMAP amplitudes in patients and control subjects.

In conclusion, LOPD is associated with significant inspiratory and expiratory muscle weakness. The latter comprises functional impairment of abdominal wall muscles which can be detected by MS of the lower thoracic nerve roots and invasive recording of twPgas. However, diaphragm dysfunction remains the mainstay of respiratory muscle weakness in LOPD, and standard tests of respiratory muscle strength can easily be supplemented by ultrasound. Motor evoked potentials of the diaphragm and phrenic nerve conduction studies provide no evidence for neurogenic contributions to diaphragm weakness in patients with LOPD.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2019.06.011.

References

[1] Reuser AJ, Kroos MA, Hermans MM, Bijvoet AG, Verbeet MP, Van Diggelen OP, et al. Glycogenosis type II (acid maltase deficiency). Muscle Nerve 1995;3:S61–9.

[2] Hagemans ML, Winkel LP, Van Doorn PA, Hop WJ, Loonen MC, Reuser AJ, et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. Brain 2005;128:671–7. Available from: https://academic.oup.com/brain/article/128/3/671/693003.

[3] Hagemans MLC, Hop WJC, Van Doom PA, Reuser AJJ, Van Der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. Neurology 2006;66:581–3. Available from: http://n.neurology.org/content/66/4/581.long.

[4] Berger KI, Chan Y, Rom WN, Oppenheimer BW, Goldring RM. Progression from respiratory dysfunction to failure in late-onset Pompe disease. Neuromuscul Disord 2016;26:481–9. Available from: https://www.nmd-journal.com/article/S0960-8966(16)30038-4/fulltext.

[5] Schuller A, Wenninger S, Strigl-Pill N, Schoser B. Toward deconstructing the phenotype of late-onset Pompe disease. Am J Med Genet C Semin Med Genet 2012;160C:80–8. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ajmg.c.31322.

[6] van der Ploeg AT, Clemens PR, Corzo D, Escolar DM, Florence J, Groeneveld GJ, et al. A randomized study of alglucosidase alfa in late-onset Pompe's disease. N Engl J Med 2010;362:1396–406. Available from: https://www.nejm.org/doi/10.1056/NEJMoa0909859?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dwww.ncbi.nlm.nih.gov.

[7] Regnery C, Kornblum C, Hanisch F, Vielhaber S, Strigl-Pill N, Grunert B, et al. 36 months observational clinical study of 38 adult Pompe disease patients under alglucosidase alfa enzyme replacement therapy. J Inherit Metab Dis 2012;35:837–45. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1007/s10545-012-9451-8.

[8] American Thoracic Society/European Respiratory Society ATS/ERS statement on respiratory muscle testing. Am J Respir Crit Care Med 2002;166:518–624. Available from: https://www.atsjournals.org/doi/abs/10.1164/rccm.166.4.518.

[9] Johnson EM, Roberts M, Mozaffar T, Young P, Quartel A, Berger KI. Pulmonary function tests (maximum inspiratory pressure, maximum expiratory pressure, vital capacity, forced vital capacity) predict ventilator use in late-onset Pompe disease. Neuromuscul Disord 2016;26:136–45. Available from: https://www.nmd-journal.com/article/S0960-8966(15)00797-X/fulltext.

[10] Gaeta M, Barca E, Ruggeri P, Minutoli F, Rodolico C, Mazziotti S, et al. Late-onset Pompe disease (LOPD): correlations between respiratory muscles CT and MRI features and pulmonary function. Mol Genet Metab 2013;110:290–6. Available from: https://www.sciencedirect.com/science/article/pii/S1096719213002254?via%3Dihub.

[11] Gaeta M, Musumeci O, Mondello S, Ruggeri P, Montagnese F, Cucinotta M, et al. Clinical and pathophysiological clues of respiratory dysfunction in late-onset Pompe disease: new insights from a comparative study by MRI and respiratory function assessment. Neuromuscul Disord 2015;25:852–8. Available from: https://www.nmd-journal.com/article/S0960-8966(15)00716-6/fulltext.

[12] Mogalle K, Perez-Rovira A, Ciet P, Wens SCA, Van Doorn PA, Tiddens HAWM, et al. Quantification of diaphragm mechanics in Pompe disease using dynamic 3D MRI. PLoS ONE 2016;11:e0158912. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0158912.

[13] Wens SCA, Ciet P, Perez-Rovira A, Logie K, Salamon E, Wielopolski P, et al. Lung MRI and impairment of diaphragmatic function in Pompe disease. BMC Pulm Med 2015;15:54. Available from: https://bmcpulmmed.biomedcentral.com/articles/10.1186/s12890-015-0058-3.

[14] DeRuisseau LR, Fuller DD, Qiu K, DeRuisseau KC, Donnelly WH Jr, Mah C, et al. Neural deficits contribute to respiratory insufficiency in Pompe disease. Proc Natl Acad Sci U S A 2009;106:9419–24. Available from: https://www.pnas.org/content/106/23/9419.long.

[15] Martin JJ, de Barsy T, Van Hoof F, Palladini G. Pompe's disease: an inborn lysosomal disorder with storage of glycogen. A study of brain and striated muscle. Acta Neuropathol 1973;23:229–44. Available from: https://www.ncbi.nlm.nih.gov/pubmed/4511788.

[16] Hordeaux J, Dubreil L, Robveille C, Deniaud J, Pascal Q, Dequéant B, et al. Long-term neurologic and cardiac correction by intrathecal gene therapy in Pompe disease. Acta Neuropathol Commun 2017;5:66. Available from: https://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-017-0464-2.

[17] Spiridigliozzi GA, Heller JH, Kishnani PS. Cognitive and adaptive functioning of children with infantile Pompe disease treated with enzyme replacement therapy: long-term follow-up. Am J Med Genet Part C Semin Med Genet 2012;160C:22–9. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ajmg.c.31323.

[18] Zifko U, Remtulla H, Power K, Harker L, Bolton CF. Transcortical and cervical magnetic stimulation with recording of the diaphragm. Muscle Nerve 1996;19:614–20. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/(SICI)1097-4598(199605)19:5%3C614::AID-MUS9%3E3.0.CO;2-E.

[19] Similowski T, Fleury B, Launois S, Cathala HP, Bouche P, Derenne JP. Cervical magnetic stimulation: a new painless method for bilateral phrenic nerve stimulation in conscious humans. J Appl Physiol 1989;67:1311–18. Available from: https://www.physiology.org/doi/abs/10.1152/jappl.1989.67.4.1311.

[20] Lissens MA. Electrodiagnostic evaluation of the respiratory muscles. Crit Rev Phys Rehabil Med 2010;22:91–101. Available from: https://doi.org/10.1615/critrevphysrehabilmed.v22.i1-4.80.

[21] Lissens MA. Motor evoked potentials of the human diaphragm elicited through magnetic transcranial brain stimulation. J Neurol Sci 1994;124:204–7. Available from: https://www.jns-journal.com/article/0022-510X(94)90327-1/pdf.

[22] Khedr EM, Trakhan MN. Localization of diaphragm motor cortical representation and determination of corticodiaphragmatic latencies by using magnetic stimulation in normal adult human subjects. Eur J Appl Physiol 2001;85:560–6. Available from: https://link.springer.com/article/10.1007%2Fs004210100504.

[23] Hamnegård CH, Wragg SD, Mills GH, Kyroussis D, Polkey MI, Bake B, et al. Clinical assessment of diaphragm strength by cervical magnetic stimulation of the phrenic nerves. Thorax 1996;51:1239–42. Available from: https://thorax.bmj.com/content/51/12/1239.long.

[24] Prigent H, Orlikowski D, Laforet P, Letilly N, Falaize L, Pellegrini N,

et al. Supine volume drop and diaphragmatic function in adults with Pompe disease. Eur Respir J 2012;39:1545–6. Available from: https://erj.ersjournals.com/content/39/6/1545.long.

[25] Cardenas LZ, Santana PV, Caruso P, Ribeiro de Carvalho CR, Pereira de Albuquerque AL. Diaphragmatic ultrasound correlates with inspiratory muscle strength and pulmonary function in healthy subjects. Ultrasound Med Biol 2018;44:786–93. Available from: https://www.umbjournal.org/article/S0301-5629(17)32464-X/fulltext.

[26] Polkey MI, Luo Y, Guleria R, Hamnegard CH, Green M, Moxham J. Functional magnetic stimulation of the abdominal muscles in humans. Am J Respir Crit Care Med 1999;160:513–22. Available from: https://www.atsjournals.org/doi/full/10.1164/ajrccm.160.2.9808067?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed.

[27] Angelini C, Semplicini C, Ravaglia S, Moggio M, Comi GP, Musumeci O, et al. New motor outcome function measures in evaluation of late-onset Pompe disease before and after enzyme replacement therapy. Muscle Nerve 2012;45:831–4. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/mus.23340.

[28] Hajiro T, Nishimura K, Tsukino M, Ikeda A, Koyama H, Izumi T. Analysis of clinical methods used to evaluate dyspnea in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;158:1185–9. Available from: https://www.atsjournals.org/doi/full/10.1164/ajrccm.158.4.9802091?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed.

[29] Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. Chest 1988;93:580–6. Available from: https://journal.chestnet.org/article/S0012-3692(16)30335-X/fulltext.

[30] Crescimanno G, Modica R, Lo Mauro R, Musumeci O, Toscano A, Marrone O. Role of the cardio-pulmonary exercise test and six-minute walking test in the evaluation of exercise performance in patients with late-onset Pompe disease. Neuromuscul Disord 2015;25:542–7. Available from: https://www.nmd-journal.com/article/S0960-8966(15)00105-4/fulltext.

[31] Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40:1324–43. Available from: https://erj.ersjournals.com/content/40/6/1324.long.

[32] Evans JA, Whitelaw WA. The assessment of maximal respiratory mouth pressures in adults. Respir Care 2009;54:1348–59. Available from: http://rc.rcjournal.com/content/54/10/1348.short.

[33] Seeck M, Koessler L, Bast T, Leijten F, Michel C, Baumgartner C, et al. The standardized EEG electrode array of the IFCN. Clin Neurophysiol 2017;128:2070–7. Available from: https://www.sciencedirect.com/science/article/pii/S1388245717304832?via%3Dihub.

[34] Baydur A, Behrakis PK, Zin WA, Jaeger M, Milic-Emili J. A simple method for assessing the validity of the esophageal balloon technique. Am Rev Respir Dis 1982;126:788–91. Available from: https://www.atsjournals.org/doi/abs/10.1164/arrd.1982.126.5.788.

[35] Polkey MI, Lyall RA, Green M, Nigel Leigh P, Moxham J, Leigh PN, et al. Expiratory muscle function in amyotrophic lateral sclerosis. Am J Respir Crit Care Med 1998;158:734–41. Available from: https://www.atsjournals.org/doi/full/10.1164/ajrccm.158.3.9710072?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed.

[36] Misuri G, Lanini B, Gigliotti F, Iandelli I, Pizzi A, Bertolini MG, et al. Mechanism of CO(2) retention in patients with neuromuscular disease. Chest 2000;117:447–53. Available from: https://journal.chestnet.org/article/S0012-3692(15)48636-2/fulltext.

[37] Similowski T, Yan S, Gauthier AP, Macklem PT, Bellemare F. Contractile properties of the human diaphragm during chronic hyperinflation. N Engl J Med 1991;325:917–23. Available from: https://www.nejm.org/doi/10.1056/NEJM199109263251304?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dwww.ncbi.nlm.nih.gov.

[38] van der Beek NA, van Capelle CI, van der Velden-van Etten KI, Hop WCJ, van den Berg B, Reuser AJ, et al. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. Mol Genet Metab 2011;104:129–36. Available from: https://www.sciencedirect.com/science/article/pii/S1096719211001995?via%3Dihub.

[39] Schneider I, Hanisch F, Müller T, Schmidt B, Zierz S. Respiratory function in late-onset Pompe disease patients receiving long-term enzyme replacement therapy for more than 48 months. Wien Med Wochenschr 2013;163:40–4. Available from: https://link.springer.com/article/10.1007%2Fs10354-012-0153-5.

[40] Turner SM, Hoyt AK, ElMallah MK, Falk DJ, Byrne BJ, Fuller DD. Neuropathology in respiratory-related motoneurons in young Pompe (Gaa) mice. Respir Physiol Neurobiol 2016;227:48–55. Available from: https://www.sciencedirect.com/science/article/pii/S1569904816300143?via%3Dihub.

[41] Falk DJ, Todd AG, Lee S, Soustek MS, ElMallah MK, Fuller DD, et al. Peripheral nerve and neuromuscular junction pathology in Pompe disease. Hum Mol Genet 2015;24:625–36. Available from: https://academic.oup.com/hmg/article/24/3/625/2900865.

[42] Toscano A, Schoser B. Enzyme replacement therapy in late-onset Pompe disease: a systematic literature review. J Neurol 2013;260:951–9. Available from: https://link.springer.com/article/10.1007%2Fs00415-012-6636-x.

[43] Similowski T, Duguet A, Straus C, Attali V, Boisteanu D, Derenne JP. Assessment of the voluntary activation of the diaphragm using cervical and cortical magnetic stimulation. Eur Respir J 1996;9:1224–31. Available from: https://erj.ersjournals.com/content/9/6/1224.long.

[44] Carlier RY, Laforet P, Wary C, Mompoint D, Laloui K, Pellegrini N, et al. Whole-body muscle MRI in 20 patients suffering from late onset Pompe disease: involvement patterns. Neuromuscul Disord 2011;21:791–9. Available from: https://www.nmd-journal.com/article/S0960-8966(11)00918-7/fulltext.

[45] Esau SA, Bellemare F, Grassino A, Permutt S, Roussos C, Pardy RL. Changes in relaxation rate with diaphragmatic fatigue in humans. J Appl Physiol 1983;54:1353–60. Available from: http://www.physiology.org/doi/10.1152/jappl.1983.54.5.1353.