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Short communication

Altered activation of the diaphragm in late-onset Pompe disease

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ABSTRACT

Pompe disease is an inherited neuromuscular disorder that affects respiratory function and leads to dependence on external ventilatory support. We studied the activation of the diaphragm using bilateral phrenic magnetic stimulation and hypothesized that diaphragm compound muscle action potential (CMAP) amplitude and evoked transdiaphragmatic pressure (Twitch P_{DI}) would correlate to disease severity. Eight patients with late onset Pompe disease (LOPD, aged 14–48 years) and four healthy control subjects completed the tests. Maximal Twitch P_{DI} responses were progressively reduced in patients with LOPD compared to control subjects (1.4–17.1 cm H_2O, $p < 0.001$) and correlated to voluntary functional tests ($p < 0.05$). Additionally, CMAP amplitude (mA) was lower in the patients who used nighttime or fulltime ventilatory support, when compared to controls and patients who used no ventilatory support ($p < 0.005$). However, the normalized (%peak) Twitch P_{DI} and CMAP responses were similar between patients and controls. This suggests a loss of functional phrenic motor units in patients, with normal recruitment of remaining motor units.

1. Introduction

Pompe disease is a rare neuromuscular disorder characterized by a defect in the gene that encodes acid alpha-glucosidase (GAA), the enzyme responsible for lysosomal breakdown of glycogen (reviewed in (Fuller et al., 2013)). As a result, glycogen accumulates in the tissues of patients, resulting in altered neuromuscular structure and function. Neural accumulation of glycogen has been observed from post-mortem patient specimens of the cervical cord (DeRuisseau et al., 2009). Additionally, abnormal spontaneous EMG activity (Corti et al., 2015) and impaired NM transmission may be present (Kassardjian et al., 2015). However, there is limited information on both the electrophysiological responses to maximal phrenic stimulation and clinical estimates of ventilatory motor function in patients with Pompe disease.

In the late-onset form of Pompe, patients may retain ambulation into adulthood, yet preferential diaphragm weakness is a prevalent and serious feature that elevates the risk for respiratory failure (Pellegrini et al., 2005). Specifically, the capacity to generate tidal volume gradually decreases with respiratory muscle weakness (Fuller et al., 2013; Mah et al., 2010). Existing evidence from the murine model of Pompe disease and patients suggests that the changes in the phrenic neuromotor drive and motor output of the diaphragm over time may distinguish milder from more severe ventilatory dysfunction (Fuller et al., 2013).

Using twitch transdiaphragmatic pressure (Twitch P_{DI}) as an index of diaphragm muscle function and the evoked compound muscle action potential (CMAP) responses to reflect neuromuscular activity, we sought to evaluate the neuromuscular properties that contribute to phrenic motor dysfunction in late onset Pompe disease. The hypothesis was that Twitch P_{DI} and CMAP responses to phrenic stimulation would be significantly lower in patients than in unaffected, age-matched control subjects, in accordance to their functional requirements for external ventilatory assist.

2. Material and methods

2.1. Subjects.

Individuals were eligible to participate if they were at least 12 years of age, medically stable, and either had a confirmed diagnosis of late onset Pompe disease or were an unaffected control subject. Subjects were ineligible if they had a cardiac pacemaker or metal implants in the head or chest, other than dental fillings. The University of Florida Institutional Review Board approved the study procedures, and written informed consent was obtained for participation.

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2.2. Clinical respiratory muscle tests

After a minimum 15-min rest, seated forced vital capacity and maximal inspiratory pressure were tested in accordance to ATS/ERS criteria. Three consistent maximal efforts were typically achieved within 5–6 trials, and of these, the best effort was recorded.

2.3. Magnetic stimulation of the phrenic nerves

The right and left phrenic nerves were stimulated simultaneously with custom 43 mm double coils powered by two stimulation units (Magstim 200, UK), using the anterolateral approach described by Mills (Mills et al., 1996). Subjects swallowed two solid state pressure transducers (Millar, USA) and esophageal and gastric placement was confirmed. Additionally, sEMG electrodes (Medi-Trace 200 Series Electrodes, Covidien) were placed in the configuration described by Verin et al. (2002). Respiratory bands at the chest and abdomen recorded the breathing pattern. After catheter placement, subjects rested a minimum of 10 min, then twitch simulations were administered at least 30 s apart. Five satisfactory stimulations were obtained at end-exhalation, at 40, 50, 60, 70, 80, 90, and 100% of stimulator output. Magnetic stimulation has been utilized extensively to evaluate human motoneuron excitability and found to elicit consistent EMG responses within a session (Martin et al., 2009).

2.4. Data analysis

EMG was sampled at 10 kHz, band-pass filtered at 3–1000 Hz, and respiratory parameters sampled at 1 kHz (PowerLab S30-16, ADInstruments). Data were analyzed off-line using Lab Chart Pro v7.2 (ADInstruments, Colorado Springs). For each intensity of stimulation, the three stimulations that yielded the highest P_{DI} were averaged and reported. These three stimulations were used for subsequent analysis of the CMAP. The right and left CMAP were similar and thus were averaged together. The Twitch P_{DI} (cm H_2O) and peak-to-peak CMAP amplitude (mA) responses were also normalized to the percentage of the value achieved at 100% stimulator output (%peak). At each stimulation intensity, the Twitch P_{DI} and peak-to-peak CMAP amplitudes (both absolute and %peak) were averaged to construct recruitment curves. The effects of stimulator output settings and group assignment were contrasted with two-way ANOVA and Tukey's post-hoc tests. Group differences in the onset latency and duration of the CMAP at 100% stimulator output were calculated with independent *t*-tests. Strength of association between the evoked and voluntary maneuvers was evaluated with Pearson's correlation. Statistical analysis was completed using GraphPad Prism 5.0, using a significance threshold of $p < 0.05$. The mean and standard deviation of the data are reported.

3. Results

3.1. Sample

Eight patients with later onset Pompe disease and four control subjects volunteered to participate. Patients had diverse ages, ambulatory status, and ventilatory function (Table 1). The mean age of the patients (34.2 ± 13.3 years) was matched to the control group (32.5 ± 8.3 years, $p = 0.917$), and body mass index did not differ between the patients (22.7 ± 5.2 kg/m^2) and controls (22.4 ± 2.3 kg/m^2, $p = 0.85$). Three patients reported they did not use ventilatory assistance ("no assist"), three reported the use of overnight non-invasive support ("nighttime assist"), and two subjects reported the routine use of non-invasive support overnight plus at least six hours of daytime support ("full-time assist"). All of the patients who did not use daytime ventilatory support remained ambulatory in the community, with one exception (P3). This patient previously used nighttime support, but discontinued the therapy prior to the study.

3.2. Voluntary tests

The mean forced vital capacity of the patients was 56 (±34) % of expected values for age, gender, height, and ethnicity. In contrast, control subjects achieved 95 (±9) % of the expected FVC. MIP averaged 40.1 (±17.1) cm H_2O in the patients, and 98.8 (±20.6) cm H_2O in the control subjects.

3.3. Evoked responses

3.3.1. Entire Sample.

Absolute Twitch P_{DI} output was lower in patients at every level of stimulation output, when compared to unaffected controls ($p < 0.001$, Fig. 1A). Likewise the absolute peak to peak CMAP amplitude was lower in patients ($p < 0.001$, Fig. 1B) and appeared to be more variable. In all subjects, the P_{DI} and CMAP both increased significantly as the stimulator output settings were raised ($p < 0.001$). Although the evoked responses were clearly reduced in the patient sample (Fig. 1A and B), the normalized Twitch P_{DI} and CMAP recruitment (i.e., expressed as %peak) was indistinguishable between patients and controls (NS, Fig. 1C and D).

3.3.2. Phrenic neuromuscular function stratified by level of respiratory support

We separately compared stimulation responses between the no assist patients, the nighttime-assist patients, fulltime-assist patients, and the controls. A significant group effect was observed for both the absolute Twitch P_{DI} ($p < 0.001$) and CMAP amplitude ($p < 0.001$) responses. Compared to the controls, Twitch P_{DI} was significantly lower in no-assist patients, and continued to significantly worsen as the level of ventilatory assist increased (Fig. 2A). In addition, the CMAP of nighttime- and fulltime-assist patients was significantly smaller than the no-assist patients and healthy controls (Fig. 2B). There was a significant interaction between stimulation output and %peak Twitch P_{DI} ($p < 0.001$). In fulltime assist, Twitch P_{DI} at 40% and 50% peak was lower than all of the other groups, and 60% peak Twitch P_{DI} was lower than control subjects (Fig. 2C). In nighttime assist, 40% peak Twitch P_{DI} was lower than no-assist patients (Fig. 2C). There was a significant group main effect for %peak CMAP ($p < 0.001$), where responses were significantly lower in fulltime-assist users than the other patients or controls (Fig. 2D). In general, Twitch P_{DI} and CMAP responses of the full-time assist patients were severely diminished and could not be consistently detected at stimulation intensities <80% of maximum output.

3.3.3. Timing of evoked responses

At the highest stimulator output, the onset latency of the CMAP response did not differ between the patients (6.9 ± 1.5 ms) and controls (6.1 ± 2.6 ms, $p = 0.54$). While CMAP duration varied among the patients (42.8 ± 20.1 ms), it did not significantly differ from the controls (35.2 ± 6.2 ms, $p = 0.48$).

3.4. Associations between evoked and voluntary responses

Maximal Twitch P_{DI} was significantly and positively correlated with FVC ($r = 0.862$, $p < 0.01$) and MIP ($r = 0.768$, $p < 0.05$). Additionally, the correlation between FVC and MIP was highly significant ($r = 0.935$, $p < 0.005$). Maximal CMAP amplitude did not correlate with Twitch P_{DI} or any of the clinical tests.