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Enhanced low-threshold motor unit capacity during endurance tasks in patients with spinal muscular atrophy using pyridostigmine

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HIGHLIGHTS

- Surface electromyography (sEMG) signals from upper extremity muscles are recorded in 31 patients with spinal muscular atrophy (SMA) treated with pyridostigmine.
- sEMG signal frequency and amplitude dynamics reveal enhanced low-threshold motor unit (LT MU) capacity during execution of endurance tasks in individual patients.
- Ameliorating LT MU function is a potential therapeutic target to reduce fatigability in SMA.

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ABSTRACT

Objective: To investigate the electrophysiological basis of pyridostigmine enhancement of endurance performance documented earlier in patients with spinal muscular atrophy (SMA).
Methods: We recorded surface electromyography (sEMG) in four upper extremity muscles of 31 patients with SMA types 2 and 3 performing endurance shuttle tests (EST) and maximal voluntary contraction (MVC) measurements during a randomized, double blind, cross-over, phase II trial. Linear mixed effect models (LMM) were used to assess the effect of pyridostigmine on (i) time courses of median frequencies and of root mean square (RMS) amplitudes of sEMG signals and (ii) maximal RMS amplitudes during MVC measurements. These sEMG changes over time indicate levels of peripheral muscle fatigue and recruitment of new motor units, respectively.
Results: In comparison to a placebo, patients with SMA using pyridostigmine had fourfold smaller decreases in frequency and twofold smaller increases in amplitudes of sEMG signals in some muscles, recorded during ESTs ($p < 0.05$). We found no effect of pyridostigmine on MVC RMS amplitudes.
Conclusions: sEMG parameters indicate enhanced low-threshold (LT) motor unit (MU) function in upper-extremity muscles of patients with SMA treated with pyridostigmine. This may underlie their improved endurance.
Significance: Our results suggest that enhancing LT MU function may constitute a therapeutic strategy to reduce fatigability in patients with SMA.

Abbreviations: **ESBBT**, **endurance shuttle box and block test**; **ESNHPT**, **endurance shuttle nine hole peg test**; **ESTs**, **endurance shuttle tests**; **HT**, **high-threshold**; **LT**, **low-threshold**; **LMM**, **linear mixed model**; **MG**, **Myasthenia Gravis**; **MU**, **motor unit**; **MVC**, **maximal voluntary contraction**; **NMJ**, **neuromuscular junction**; **RMS**, **root mean square**; **sEMG**, **surface electromyography**; **SMA**, **spinal muscular atrophy**; **SMN**, **survival motor neuron**.

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1. Introduction

Hereditary proximal spinal muscular atrophy (SMA) is caused by homozygous deletion of the *survival motor neuron* (*SMN*) *1* gene. Insufficient SMN protein expression in tissues leads to intertwined abnormalities and reduced connectivity at multiple levels of the motor unit (MU), including α-motor neuron degeneration with selective motor deafferentiation, abnormal anatomy and function of the neuromuscular junction (NMJ), and atrophy and fatty infiltration of the associated musculature (Goulet et al., 2013; Lefebvre et al., 1995; Mentis et al., 2011; Otto et al., 2020; Wadman et al., 2012). Muscle weakness is most pronounced in proximal muscle groups of the extremities and in the more severe and early onset types in axial, respiratory and bulbar muscle groups. In addition, limited endurance for repetitive motor tasks, i.e. fatigability, has been identified as an important characteristic (Bartels et al., 2020, 2019; Montes et al., 2010; Stam et al., 2018).

Pyridostigmine is a neuromuscular excitation enhancer (Robb et al., 2011) and first-line of treatment for Myasthenia Gravis (MG) (Maggi and Mantegazza, 2011). Approximately half of the patients with SMA present with similar electrophysiological abnormalities as patients with MG during repetitive nerve stimulation, i.e. a pathological decrement (Meriggioli and Sanders, 2009; Wadman et al., 2012). Therefore, we recently examined the efficacy of pyridostigmine in patients with SMA types 2–4 in a placebo-controlled, double-blind, cross-over trial (Stam et al., 2022, 2018). We found a significant positive effect on endurance performance, while muscle strength and motor function remained stable (Stam et al., 2022). Specifically, patients showed a 70% reduced risk of endurance shuttle test (EST) failure under pyridostigmine. However, the electrophysiological basis of this finding remains unclear.

Surface electromyography (sEMG) is a widely used, non-invasive, research tool to study real-time electrophysiological events associated with muscle activation. As such, its use may advance our understanding of the mechanisms responsible for increased fatigability in patients with SMA (Beck et al., 2014; Bonato et al., 2001; Bosch et al., 2009, 2007; Eken et al., 2019; Linssen et al., 1993; Peeters et al., 2019; Qin et al., 2014; Rogers and MacIsaac, 2013). We recently reported MU reserve capacity in some, but not all, treatment naïve patients with SMA performing ESTs (Habets et al., 2021). The MU reserve capacity was estimated as the potential to increase activity of low-threshold (LT) and high-threshold (HT) Mus consecutively. LT Mus are small, more easily recruited and the most fatigue resistant units, used for relatively low forces over a prolonged time (Jones et al., 2005). HT MU are larger, faster and rapidly fatigable units, recruited for high force contractions or after exhaustion of LT units (Jones et al., 2005). The use of motor unit reserve capacity to prevent task failure is reflected in an increase of the sEMG amplitude during ongoing exercise (Habets et al., 2021). In addition, a decrease of the median sEMG frequency can be used as an index of developing muscle acidification (Habets et al., 2021), as soon as HT Mus are recruited. Importantly, both manifestations are representations of a muscle's attempt to prolong an ongoing task, but may appear independent from each other (Jones et al., 2005).

Here, we performed sEMG recording from working muscles in patients with SMA treated with pyridostigmine or placebo during execution of ESTs, to study electrophysiological processes determining fatigability. We hypothesized augmented reserve capacity of activated Mus during ESTs in patients with SMA using pyridostigmine.

2. Methods

2.1. Study design

Data was collected as part of a clinical trial on the efficacy of pyridostigmine in SMA (Stam et al., 2018). This investigator-initiated, mono-center, placebo-controlled study had a cross-over, double blinded design. The protocol consisted of five study visits within a timeframe of 22 weeks (Supplementary Figure S1). The screening visit was performed at the outpatient clinic of the University Medical Center Utrecht, The Netherlands, while follow-up visits took place either at the hospital or at home, depending on the participant's preference.

2.2. Subjects

We included patients with SMA types 2, 3a (onset of symptoms between 18 and 36 months) and 3b (onset after 36 months), ≥12 years of age, registered in the Dutch SMA registry (Wadman et al., 2017). The inclusion and exclusion criteria for this study have been previously described in detail. In short, we included patients with genetically confirmed SMA with predefined minimal and maximal motor scores to allow assessments of meaningful changes during treatment, without other relevant disorders, contraindications for the use of pyridostigmine or use of SMN-augmenting therapies (Stam et al., 2018). The latter category of SMA therapies were not yet reimbursed in the Netherlands at the time of this clinical trial.

2.3. Standard protocol approvals, registrations and patient consents

All participants and, in case of minors, their parents signed informed consent. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht and by the national Central Committee on Research Involving Human Subjects in the Netherlands. Trial registration number NCTO2941328 and EudraCT number 2011–004369-34.

2.4. Intervention procedures

Study medication (pyridostigmine or placebo) was taken four times per day, approximately 4 hours apart according to the drugs effect. The dose was gradually increased in the first week to minimize side-effects. The maximum dose, reached after one week, was 6 mg/kg/day. In case this dose was not well tolerated the highest tolerable dose was continued. Randomization and intervention procedures are described in detail elsewhere (Stam et al., 2018).

2.5. Endurance shuttle tests

To examine the effect of pyridostigmine use on muscle activation we used two ESTs: the endurance shuttle box and block test (ESBBT) for patients with proximal arm function and the endurance shuttle nine hole peg test (ESNHPT) for patients with only distal arm function (Bartels et al., 2020, 2019). The design and validation of the EST protocols have been published previously (Bartels et al., 2020, 2019). In short, we first determined the individual's maximum test intensity level by asking the subject to perform one cycle of an EST at maximum speed. Subjects then repeated the cycle of an EST at 75% of their maximum speed until they consecutively twice failed to complete a cycle within the defined time period, paced by auditory signals. Subjects were blinded for the maximal test duration of 20 minutes. We docu-

mented time to limitation (s) for each EST patients performed and compared this outcome to assess differences between pyridostigmine and placebo use. We standardized individual adjustments on table height and preferences on the distance between the participant and EST material in order to be able to compare data gathered at the hospital and at the participants home.

2.6. Maximal voluntary contraction

We measured maximal voluntary contraction (MVC) force of four muscle groups of the dominant arm (shoulder abduction, elbow flexion, wrist extension and hand grip) before the EST using a handheld dynamometer (CT 3001; C.I.T. Technics, Groningen, The Netherlands) following standardized procedures (Beenakker et al., 2001). We performed manual muscle testing of the same muscle groups in patients with overt muscle weakness (Medical Research Council (MRC) score for muscle strength < 4) (Hislop and Montgomery, 2002).

2.7. Surface electromyography

2.7.1. sEMG registration and electrode placement

We continuously recorded four bipolar sEMG signals during MVCs and ESTs at study visits 2 and 4, using a wireless Bio Radio (Great Lakes Neurotechnologies, Cleveland, Ohio, USA). We have previously described the sEMG registration and electrode placement procedures (Habets et al., 2021). We used self-adhesive Ag/AgCl Discs (3*M*^{TM} Red *Dot*^{TM}, 9 mm electrode, 18 mm gel, 50 mm disk), attached in overlap, with 34 mm center-to-center inter-electrode distance. Skin preparation procedures included removal of hair if necessary and rubbing and cleaning of the skin with alcohol (70% denatured ethanol incl. 5% isopropanol). We placed standard electrodes in a bipolar montage on four muscles, i.e. *m. deltoideus pars anterior* (shoulder abduction), *m. biceps brachii* (elbow flexion), *m. flexor digitorum superficialis* (hand grip), *m. extensor digitorum* (wrist extension), on the dominant side of the body, in parallel to myofiber direction using standard guidelines (Criswell, 2011; Hermens et al., 2000). We placed a reference electrode on the spina scapulae. Electrode wires were secured with tape on the skin to prevent cable motion artefacts.

2.7.2. Signal acquisition and processing

We used Biocapture software to measure real time muscle activation and raw sEMG data was processed using custom programs written in MATLAB R2016b (Habets et al., 2021). The sampling rate was 1000 samples/s and sampling resolution was 6 μV per least significant bit. We used a 250 Hz anti-aliasing filter and a 4th order Butterworth high pass bidirectional filter at 20 Hz. A 50 Hz notch filter removed power line noise of the signal. sEMG outcome parameters were median frequencies (Hz) and mean root mean square (RMS) amplitudes (V) determined per cycle of an EST (Konrad, 2005). The median frequency of a cycle was determined using the Fast Fourier Transformation. The mean RMS amplitude per cycle was determined over an overlapping moving window (100 samples). The sEMG outcome parameter of an MVC measurement was the maximal RMS amplitude (V), determined over 500 samples overlapping moving windows of the recorded signal.

2.8. Statistical analysis

We used a Wilcoxon signed rank test with continuity correction to analyze differences in time to limitation on the ESTs under pyridostigmine and placebo. RMS amplitude intercepts of the four muscles during performance of the two different ESTs (ESNHPT and ESBBT) were similar, meaning that both tests were performed at the same intensity. Therefore, we combined the sEMG outcome parameters measured during the ESNHPT and ESBBT for further analyses. RMS amplitudes were not normally distributed and therefore ln-transformed to meet assumptions for statistical analyses. For analyses of the sEMG parameters measured during the EST we first used linear models to provide individual slopes for the median frequencies (Hz) and the natural logarithm of RMS amplitudes (ln(V)) over time (s). Second, we used linear mixed effects models (LMM) to determine the effect of treatment period (1 or 2) adjusted for treatment (placebo or pyridostigmine) (fixed effect) on intra-individual clustering of median frequency slopes (Hz/s), and RMS amplitude slopes (ln(V)/s)). The random part was modelled with a random intercept per individual and an unstructured covariance matrix. Differences between treatment periods on MVC forces are examined using related-samples Wilcoxon signed rank tests. For the analyses of the MVC measurements we used LMM to determine the effect of treatment period (1 or 2) adjusted for treatment (placebo or pyridostigmine) (fixed effect) on intra-individual clustering of maximal RMS amplitudes (ln(V)). We used R statistics (R-3.4.3 for Windows with Rstudio v1.1.414) for all statistical analyses, with the "lme4" package for LMM (Bates et al., 2015). *P-values* of < 0.05 were considered significant.

3. Results

Thirty-two patients completed all study visits; in 31 high-quality sEMG data was collected. Of these, 16 performed the ESNHPT, 15 performed the ESBBT. Participant characteristics are summarized in Table 1. Time to limitation was more than two-fold longer on pyridostigmine compared to placebo (389 s (94–971) versus 149 s (96–467), respectively; median and IQR, *p = 0.003*), similar to previously reported results (Stam et al., 2022).

3.1. Efficacy of pyridostigmine on muscle activation

3.1.1. Median frequency slopes

We found a significant difference in downward slope of the median frequency over time between the placebo and pyridostigmine period in one of the interrogated upper extremity muscles. Specifically, the rate of decrease in median frequencies of the *m. biceps brachii* between the placebo and pyridostigmine period was significantly different (Fig. 1a and Supplementary Table S1). No significant effect of treatment period was found on the rate of decrease in median frequencies of the other muscles examined during the tests (Fig. 1a and Supplementary Table S1). The decrease in median frequencies of the *m. biceps brachii* was significantly smaller over time under pyridostigmine ($-14*10^{-3}$ Hz/s, 95% CI: [$-86*10^{-3}$ $58*10^{-3}$]) compared to placebo ($-58*10^{-3}$ Hz/s), 95% CI: [$-94*10^{-3}$ $23*10^{-3}$]), *p = 0.018* (Fig. 1b). An example dataset of median frequencies during the two treatment periods measured at the *m. biceps brachii* of an individual patient is shown in Fig. 1c.

3.1.2. RMS amplitude slopes

We found significant differences in upward slope of the RMS amplitude over time between the placebo and pyridostigmine period in two of the interrogated upper extremity muscles. Specifically, the rate of increase in RMS amplitudes of the *m. flexor-* and *extensor digitorum* between the placebo and pyridostigmine period were significantly different (Fig. 2a and Supplementary Table S1). No significant effect of treatment period was found on the rate of increase in RMS amplitudes of the other two muscles examined during the tests (Fig. 2a and Supplementary Table S1).The increase in RMS amplitudes of the *m. flexor digitorum* was significantly smaller over time under pyridostigmine ($11*10^{-4}$ ln(V)/s, 95% CI:

Table 1
Baseline characteristics.

Characteristics	EST (n = 31)
SMA sub-type, n	
Type 2:	15
Type 3a:	12
Type 3b:	4
Gender, m/f	9/22
Age, y, mean (SD)	35.2 (14.1)
MFM, mean (SD)	58.4 (19.5)
MVC force, N, median (min–max)	
m. deltoideus	
m. biceps brachii	17 (9–40)
m. flexor digitorum	16 (11.5–25)
m. extensor digitorum	8 (6–14.5)
	8 (4–22.5)
Missing sEMG data per visit (n-subjects)	
Visit 2	
Visit 4	1
	6

SMA = spinal muscular atrophy, subtype 3a = onset of clinical symptoms < 3yrs; subtype 3b = onset of clinical symptoms > 3yrs, *EST* = endurance shuttle test, *sEMG* = surface electromyography, *IQR* = interquartile range, *MFM* = motor function measure, *MVC* = maximal voluntary contraction.

[-10*10^{-4} 32*10^{-4}]) compared to placebo (24*10^{-4} ln(V)/s, 95% CI: [-13*10^{-4} 34*10^{-4}]), *p = 0.018* (Fig. 2b). Similarly, the increase in RMS amplitudes of *m. extensor digitorum* was significantly smaller over time under pyridostigmine (9*10^{-4} ln(V)/s, 95% CI: [-12*10^{-4} 31*10^{-4}]) compared to placebo (22*10^{-4} ln(V)/s, 95% CI: [11*10^{-4} 33*10^{-4}]), *p = 0.021* (Fig. 2c). Individual effects of treatment on all muscles for both sEMG parameters are shown in Supplementary Figure S2.

3.2. Efficacy of pyridostigmine on MVC forces

Maximal voluntary contraction forces of all muscle groups were not significantly different between the placebo and pyridostigmine period, *p > 0.05* (Table 2). In all four muscle groups, maximal RMS amplitudes measured during MVC force examination under pyridostigmine were similar to maximal RMS amplitudes measured during these examinations under placebo, *p > 0.05* (Supplementary Table S2).

4. Discussion

This study employed sEMG to examine the physiological mechanism of pyridostigmine on muscle activation during EST performance in patients with SMA types 2 and 3. Our results significantly show a smaller down going slope of the median frequency and a smaller up going slope of the RMS amplitude over time measured in part of interrogated upper extremity muscles. These effects both are explained by an enhanced performance of LT Mus during treatment, in accordance with the twofold longer endurance time on pyridostigmine compared to placebo. As expected, we found no significant effect of pyridostigmine on short time MVC. These findings reaffirm pyridostigmine efficacy on endurance performance, but not strength in patients with SMA (Stam et al., 2022).

4.1. Understanding the effect of pyridostigmine on endurance performance

Pyridostigmine is a commonly prescribed drug for patients with MG (Maggi and Mantegazza, 2011). Case studies and clinical experience, report a short term symptom relief as a consequence of daily intake of pyridostigmine in newly diagnosed patients and in patients with a mild form of MG (Maggi and Mantegazza, 2011; Skeie et al., 2010). There is, however, no evidence from placebo-controlled randomized clinical trials that explains the precise mechanism of action by which pyridostigmine reduces fatigability (Maggi and Mantegazza, 2011; Skeie et al., 2010). A decrement during repetitive nerve stimulation, with an absence of incrementing signals, was previously found in patients with SMA suggesting postsynaptic abnormalities of the excitation response at the NMJ (Wadman et al., 2012).

Here, pyridostigmine enhancement of endurance performance in SMA (Stam et al., 2022) was hypothesized to improve NMJ functionality and, as such, LT MU recruitment in SMA. Our sEMG results offer two possible explanations for the beneficial effect of pyridostigmine on endurance performance. First, a decline in median frequencies is typically indicative of decreased myofiber propaga-

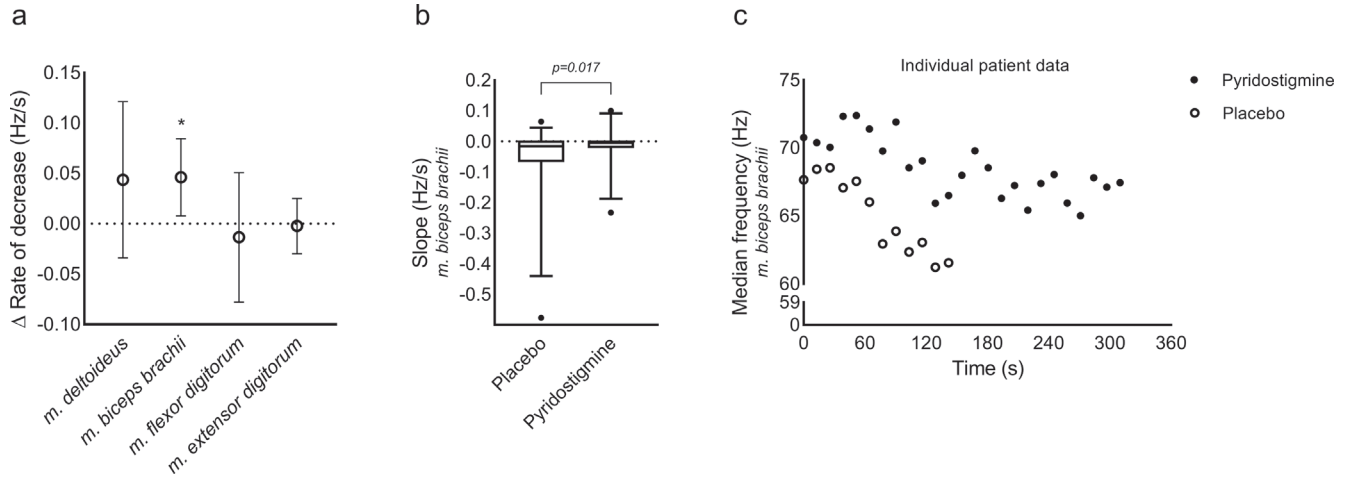

Fig. 1. Median frequency slopes measured during endurance shuttle tests (ESTs) performed by patients with spinal muscular atrophy (SMA) under treatment of placebo or pyridostigmine. A) Mean differences (pyridostigmine-placebo) and 95% confidence intervals in rate of decrease in median frequencies (Hz/s) of four muscles, *$p < 0.05$. Median frequencies measured in *m. biceps brachii* decreased significantly slower under pyridostigmine compared to placebo. B) Significant difference in median frequency slopes (Hz/s) of the *m. biceps brachii* between the placebo and pyridostigmine period. The boxplot shows the median, interquartile range and min/ max of the median frequency slopes. C) Slower decrease in median frequencies calculated from the surface electromyography signal of the *m. biceps brachii* combined with a longer exercise duration under pyridostigmine (solid dots) during the endurance shuttle box and block test in an individual patient with SMA compared to placebo (open dots).

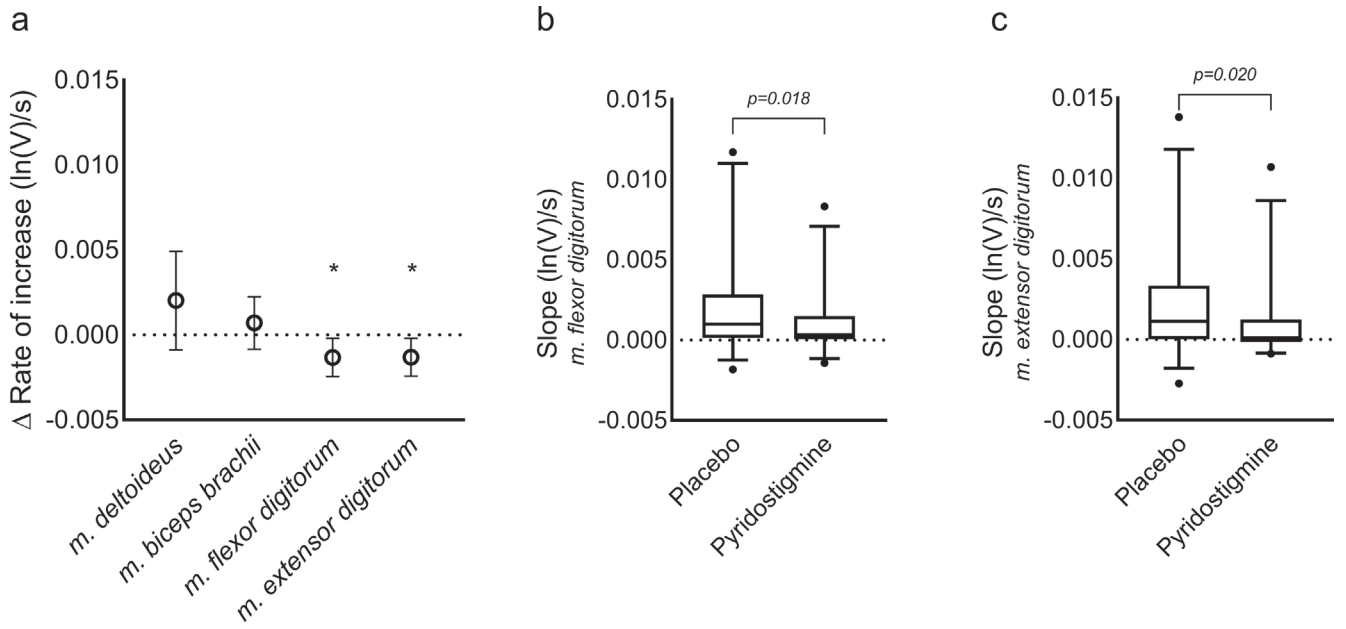

Fig. 2. Root mean square (RMS) amplitude slopes measured during endurance shuttle tests (ESTs) performed by patients with SMA under treatment of placebo or pyridostigmine. A) Mean differences (pyridostigmine-placebo) and 95% confidence intervals in rate of increase in RMS amplitudes (ln(V)/s) of four muscles, $^{*}p < 0.05$. RMS amplitudes measured in *m. flexor-* and *m. extensor digitorum* increased significantly slower under pyridostigmine compared to placebo. B-C) Significant difference in RMS amplitude slopes (ln(V)/s) of the *m. flexor digitorum* and *m. extensor digitorum* between the placebo and pyridostigmine period. The boxplot shows the median, interquartile range and min/ max of the median frequency slopes.

Table 2
Non-parametric related samples Wilcoxon signed rank test to examine difference between placebo and pyridostigmine period on Maximal Voluntary Contraction force.

Parameter	**MVC force (N)**		
EST	Placebo Median (IQR)	Pyridostigmine Median (IQR)	*p-value*
m. deltoideus (n = 7)	13 (33)	15 (25)	0.176
m. biceps brachii (n = 29)	16 (15)	18 (14)	0.078
m. flexor digitorum (n = 29)	8 (9)	9 (7)	0.890
m. extensor digitorum (n = 28)	7.5 (17)	7.5 (15)	0.930

EST = endurance shuttle test, *MVC* = maximal voluntary contraction, *sEMG* = surface electromyography, *IQR* = interquartile range.

tion velocity associated with muscle acidification (Linssen et al., 1993; Schmitz et al., 2012). We found a fourfold smaller decrease in median frequency in the *m. biceps brachii* under pyridostigmine (Fig. 1) indicating less recruitment of HT Mus associated with glycolytic myofibers to perform the physical task at the same power output (Ertl et al., 2016). Secondly, we found a twofold smaller increase in RMS amplitude in the *m. flexor-* and *extensor digitorum* under pyridostigmine (Fig. 2) indicating that neither activation of unrecruited HT Mus nor increasing firing rates of active Mus was necessary to perform the physical task (EST) for a longer duration (González-Izal et al., 2012; Henneman et al., 1974). These findings may be explained either by delayed onset of fatigue in the activated Mus or by the presence of unrecruited LT Mus (i.e., 'MU reserve') during task execution enabling MU rotation (Bawa and Murnaghan, 2009) in the presence of pyridostigmine.

In summary, we found evidence for pyridostigmine enhancement of neuromuscular excitation capacity, specifically in LT Mus. Additionally, visual inspection of individual patient data revealed that pyridostigmine affected sEMG parameter slopes in an important fraction of patients while no effect was found in others (Supplementary Figure S2). This observation is in agreement with the previously reported effect of pyridostigmine on endurance (Stam et al., 2022) and makes a strong case for personalized rather than one-size-fits-all therapeutic use of pyridostigmine suppletion.

4.2. Understanding the effect of pyridostigmine on muscle strength

We did not find any effect of pyridostigmine on MVC force. As discussed in the above, enhanced function of LT Mus appeared to be mainly responsible for the improved endurance performance under pyridostigmine, while the combined number and function of LT and HT Mus is more closely associated with muscle strength (Fitts, 1994). Current studies exploring muscle-directed therapy in SMA are mainly focussed on rescuing muscle strength because of the apparent higher vulnerability of HT Mus and associated white myofibers in SMA (Houdebine et al., 2019; Long et al., 2019; Pirruccello-Straub et al., 2018). Our present findings suggest that additional abnormalities in excitation capacity at the neuromuscular junction of LT Mus in SMA may be rescued by pyridostigmine suppletion. Pyridostigmine thus presents a potential therapy to complement muscle-directed therapies and existing SMN upregulatory therapies. Further research is needed to examine this hypothesis and to study the exact working mechanism of pyridostigmine on the NMJ in Mus of different myofiber types.

4.3. Applicability of sEMG as an outcome measure

The results of this study confirm the need for novel biomarkers to develop and expand the multisystemic treatment approach in SMA (Smeriglio et al., 2020; Yeo and Darras, 2020). SEMG results add to understanding functional changes in muscle activation. Here it adds to the finding of neurotransmission abnormalities in SMA already indicated by repetitive nerve stimulation (Wadman et al., 2012). Importantly, clinical applicability of the latter methodology, also included in this clinical trial on the efficacy of pyridostigmine (Stam et al., 2022), in SMA appeared to be less than straightforward, particularly in patients with contractures and excessive muscle weakness (Bartels et al., 2021; Stam et al., 2022). We therefore believe that sEMG in combination with phys-

ical tests, such as the ESTs, contribute to gaining further insight in the mechanisms underlying fatigability in SMA.

4.4. Limitations

In this explorative study, we used sEMG in a relatively small number of patients. Although the results provide more insight in the effect of pyridostigmine on muscle activation, future studies that encompass a larger number of patients have the potential to additionally provide a more complete picture of the differences between different types of SMA during separate ESTs, i.e. ESNHPT and ESBBT. In general, patients with SMA type 2 performed the ESNHPT and patients with SMA type 3a/b performed the ESBBT. Previous research (Wadman et al., 2012) showed a trend, but not a significant correlation, between NMJ dysfunction and SMA subtype. Patients with SMA type 2 and 3a showed an abnormal decrement in 60% of the patients compared to 33% in SMA type 3b. In line with these findings, larger effects of pyridostigmine in patients performing the ESNHPT may be expected in future studies.

The sEMG parameters explored in this study showed the effects of pyridostigmine in some, but not all, examined muscles (Supplementary Table S1). Median frequency and RMS amplitude slopes varied between muscles, which raises the question which underlying factors may influence these differences. Patients were allowed to use compensational strategies, to temporarily relieve certain muscles, because the ESTs were designed to represent daily activities. We suggest that this may have led to underestimations of our results. From experience we learned that patients used compensational strategies to relieve for example the *m. deltoideus,* in which no significant effects of pyridostigmine on sEMG parameters were found. To examine the working mechanism of pyridostigmine on specific muscle groups further, we suggest the use of low intensity movements with a smaller amount of degrees of freedom.

5. Conclusion

Patients with SMA treated with pyridostigmine reveal increased capacity of LT Mus, partly because of MU rotation resulting in improved endurance. No significant effects on sEMG variables during maximal muscle strength were detected, suggesting that pyridostigmine selectively affects LT Mus, associated with endurance performance. Improving LT MU capacity should further be explored as a therapeutic target in clinical care to reduce fatigability in patients with SMA.

Conflict of interest statement

BB obtained research grants from Prinses Beatrix Spierfonds and Stichting Spieren voor Spieren, both non-profit foundations. He is a member of the scientific advisory board of Scholar Rock. His employer receives fees for SMA-related consultancy activities. JALJ obtained a research grant from Prinses Beatrix Spierfonds, a non-profit foundation. WLP obtained grants from Prinses Beatrix Spierfonds, Stichting Spieren voor Spieren and Vriendenloterij. He is a member of the scientific advisory board of SMA Europe and has served as an ad hoc member of the scientific advisory boards of Biogen and Avexis and as a member of data monitoring committee for Novartis. All other authors report no conflicts of interest.

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Human and animal rights

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2023.06.024.

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