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Motor unit reserve capacity in spinal muscular atrophy during fatiguing endurance performance

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HIGHLIGHTS

- Surface EMG signals from extremity muscles are recorded in a large cohort (n = 70) of spinal muscular atrophy (SMA) patients.
- EMG signal amplitude during endurance testing is strongly inversely correlated to SMA phenotype, e.g. the highest amplitudes in SMA type 2.
- Individual patients with SMA show motor unit reserve capacity providing a target for pharmacological and/or exercise therapy.

ABSTRACT

Objective: To investigate the availability of any motor unit reserve capacity during fatiguing endurance testing in patients with spinal muscular atrophy (SMA).
Methods: We recorded surface electromyography (sEMG) of various muscles of upper- and lower extremities of 70 patients with SMA types 2–4 and 19 healthy controls performing endurance shuttle tests (ESTs) of arm and legs. We quantitatively evaluated the development of fatigability and motor unit recruitment using time courses of median frequencies and amplitudes of sEMG signals. Linear mixed effect statistical models were used to evaluate group differences in median frequency and normalized amplitude at onset and its time course.
Results: Normalized sEMG amplitudes at onset of upper body ESTs were significantly higher in patients compared to controls, yet submaximal when related to maximal voluntary contractions, and showed an inverse correlation to SMA phenotype. sEMG median frequencies decreased and amplitudes increased in various muscles during execution of ESTs in patients and controls.
Conclusions: Decreasing median frequencies and increasing amplitudes reveal motor unit reserve capacity in individual SMA patients during ESTs at submaximal performance intensities.
Significance: Preserving, if not expanding motor unit reserve capacity may present a potential therapeutic target in clinical care to reduce fatigability in individual patients with SMA.

1. Introduction

Spinal muscular atrophy (SMA) is caused by a deficiency of survival motor neuron (SMN) protein due to the homozygous deletion of the *SMN* 1 gene (Lefebvre et al. 1995). SMA has a broad spectrum of severity, ranging from neonatal respiratory insufficiency and death (type 1), inability to walk independently

Abbreviations: ESBBT, endurance shuttle box and block test; ESNHPT, endurance shuttle nine hole peg test; ESTs, endurance shuttle tests; ESWT, endurance shuttle walk test; MVC, maximal voluntary contraction; RMS, root mean square; sEMG, surface electromyography; SMA, spinal muscular atrophy; SMN, survival motor neuron.

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(type 2) and problems with or loss of ambulation (type 3), to mild impairments in adults (type 4) (D'Amico et al., 2011). Degeneration of α-motor neurons is the pathological hallmark of the disease, but other components of the motor unit, such as the neuromuscular junction and the myofiber itself, are also affected (Mercuri et al. 2012; Shababi et al. 2014; Yeo and Darras 2020). With respect to the former, studies in rodent models and humans have reported aberrant neuromuscular junction development and abnormal function of postsynaptic acetylcholine receptors, resulting in neuromuscular transmission impairments (Arnold et al. 2004; Kariya et al. 2008; Kong et al. 2009; Wadman et al. 2012; Goulet et al. 2013). With respect to the myofiber, human muscle biopsies and SMA mouse models revealed altered skeletal muscle differentiation, growth and metabolism (Ripolone et al. 2015; Miller et al. 2016; Yeo and Darras 2020). The most prominent clinical characteristic of SMA is progressive muscle weakness leading to limitations in motor function (Mercuri et al. 2016; Chabanon et al. 2018; Wadman et al. 2018). In addition, fatigability, defined as a decline in physical endurance performance during repetitive motor tasks, has emerged as a common, but sparsely examined, symptom in SMA, affecting activities of daily life (Montes et al. 2010; Kluger et al. 2013; Stam et al. 2018; Bartels et al. 2019).

A repetitive upper and lower body motor task, tailored specifically to test fatigability in SMA (endurance shuttle tests (ESTs) (Bartels et al. 2019)), recently demonstrated abnormal fatigability during walking, proximal- and distal arm function in up to 85% of patients with SMA, compared to no fatigability in healthy controls (Bartels et al. 2020). Importantly, significantly higher endurance in disease controls (Limb girdle muscular dystrophy, Becker muscular dystrophy and Duchenne muscular dystrophy), compared to SMA, found in this previous study, indicated that fatigability was not secondary to weakness, but a specific feature of SMA (Bartels et al. 2020).

Surface electromyography (sEMG) recordings from muscles during endurance testing may provide insight into the mechanisms underlying fatigability in physical performance (Linssen et al. 1993; Bonato et al. 2001; Bosch et al. 2007, 2009; Travis et al. 2011; Rogers and MacIsaac 2013; Beck et al. 2014; Qin et al. 2014). Specifically, fatigability is evidenced by a shift towards lower median frequencies of the sEMG signal, concomitant with a decline in the amplitude (root mean square (RMS)) of the sEMG signal (Fitts 1994; Dimitrova and Dimitrov 2003; Konrad 2005). A transient rise in RMS, preceding the decline in RMS, is typically observed in healthy subjects (Dimitrova and Dimitrov 2003; Konrad 2005). This phenomenon is commonly thought to reflect recruitment of motor unit reserve capacity to prevent task failure. This reserve capacity can be present both in terms of still unrecruited (usually larger) motor units (Henneman et al. 1974), as well as in terms of 'rate coding', i.e. the possibility to increase the firing rate of active motor units (González-Izal et al. 2012). Montes et al. (2014) previously reported that there is little or no such reserve capacity in SMA.

Here, we further investigated motor unit reserve capacity in SMA using continuous sEMG recording from upper and lower extremity muscles during ESTs execution in patients with SMA types 2–4 and healthy controls. We tested two specific hypotheses: 1) patients with SMA would perform the ESTs at higher levels of muscle electrical activation, but submaximal to maximal voluntary contractions (MVC), compared to healthy controls, and 2) patients with SMA show reserve capacity during fatiguing ESTs. Rejection of the latter hypothesis would reveal failing use of existing reserve capacity as performance limiting factor, identifying a potential patient-specific therapeutic target in SMA.

2. Methods

2.1. Subjects

Data was collected as part of a cross-sectional study on fatigability in SMA (Bartels et al. 2020). We invited patients with SMA types 2, 3a, 3b, and 4, registered in the Dutch SMA registry (www.treatnmd.eu/patientregistries), to participate in this study. The SMA classification system (i.e. type 1–4) is based on the age of onset and the best of two achieved motor milestones reported in medical records or by patient reports. All had a confirmed homozygous deletion of the SMN1 gene. We recruited healthy controls through the HU University of Applied Sciences, the University Medical Center Utrecht, and the subject's social network of family, friends, and schoolmates. Inclusion criteria were: 1) aged between 8 and 60 years, 2) able to follow test instructions, and 3) able to perform and repeat, at least once, the physical tasks involved in execution of each of the ESTs described below. Exclusion criteria were: 1) history of a disorder which affects the neuromuscular junction function, 2) use of medication that affects neuromuscular junction function, and 3) other medical problems that could influence ESTs results.

2.2. Standard protocol Approvals, registrations and patient consent

All participants and their parents (if they were under 18 years of age) signed informed consent. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht in the Netherlands (NL48715.041.14).

2.3. Study design

The study consisted of three visits within approximately 6 weeks. At the first visit, we documented baseline characteristics and subjects performed a practice test (Bartels et al. 2020). At the second and third visits, participants performed ESTs and retests at the participant's home, or at the exercise laboratory in our hospital, depending on the subject's preference. Visits two and three were separated by at least one week of rest (Bartels et al. 2020).

2.4. Endurance shuttle tests

We used three different, recently validated endurance shuttle tests to assess fatigability: the endurance shuttle nine hole peg test (ESNHPT) for distal arm function, the endurance shuttle box and block test (ESBBT) for proximal arm function, and the endurance shuttle walk test (ESWT) for leg function (Bartels et al. 2019, 2020). The execution of these ESTs has been described in detail elsewhere (Bartels et al. 2019). Briefly, we first determined the individual's maximum test intensity level by asking him/her to perform one cycle of an endurance test at maximum speed (i.e. one cycle of: putting nine pins in holes for the ESNHPT, transporting 10 blocks from a bin over a partition into an adjacent bin for the ESBBT, walking 10 meters for the ESWT) (Bartels et al. 2019). During execution of the ESTs, participants repeated the cycle at 75% of their maximum speed until they twice consecutively failed to complete a cycle within the defined time period, paced by auditory signals. Participants were not informed about the maximal duration of the test. During the pilot phase of the development of the ESTs, the maximal duration was 10 minutes (Bartels et al. 2019). Thereafter, this was adjusted to 20 minutes. Participants performed at least one of the ESTs, always keeping the sequence of tests in the same order: ESNHPT, ESBBT and ESWT. A resting period of at least 30 minutes was taken between two tests to allow full recovery.

2.4.1. Maximal voluntary contractions

We measured MVCs of muscles (Fig. 1A, 1B) prior to each EST using a handheld dynamometer (CT 3001; C.I.T. Technics, Groningen, The Netherlands) in combination with the break test, according to standardized procedures (Beenakker et al. 2001).

We used manual muscle testing in patients with overt muscle weakness (Medical Research Council (MRC) score for muscle strength < 5) (Hislop and Montgomery 2002). Contractions lasted for approximately three seconds. We maintained standardized starting positions in a fixed sequence, proximal to distal.

2.5. sEMG registration

We used sEMG at visit 2 during the ESNHPT and ESBBT and at visit 3 during the ESWT. Muscle electrical activation was continuously measured with wireless Bio Radio (Great Lakes Neurotechnologies, Cleveland, Ohio, USA) bipolar four channel sEMG during both MVCs and the ESTs. Each cycle performed during an EST was marked manually online in the sEMG signal.

2.5.1. Electrode placement

We used self-adhesive Ag/AgCl Discs (3*M*^{TM}Red *Dot*^{TM},0.9 mm electrode, 1.8 mm gel, 50 mm disc) with 20 mm inter-electrode distance. Skin preparation procedures included removal of hair, if necessary, and rubbing and cleaning the skin with alcohol (70% denatured ethanol incl. 5% isopropanol). We placed standard electrodes on muscles of upper and lower extremities using standard guidelines (Hermens et al. 2000; Criswell 2011). For the ESNHPT/ESBBT: *m. deltoideus pars anterior* (one finger distal and anterior to the acromion), *m. biceps brachii* (1/3 on the line from fossa cubit to medial acromion), *m. flexor digitorum superficialis* (1/4 between wrist and elbow on the area where the greatest movement is felt while the subject flexes his/her fingers), and *m. extensor digitorum superficialis* (1/4 between wrist and elbow on palpable muscle mass while the subject extends his/her fingers). For the ESWT: *m. rectus femoris* (1/2 on the line from anterior spina iliaca superior to the superior part of the patella), *m. biceps femoris* (1/2 on the line from ischial tuberosity to the lateral epicondyle of the tibia), *m. tibialis anterior* (1/3 on the line from the tip of the fibula to the medial malleolus), and *m. gastrocnemius* (1/3 on the line from the head of the fibula to the heel). Electrodes were placed on the dominant side of the body parallel to the direction of the fibers. Reference electrodes were placed on the spina scapulae and spina iliaca anterior superior for the ESNHPT and ESBBT, and ESWT, respectively. Wires were secured to the skin with tape to prevent cable movement artifacts.

2.5.2. Signal acquisition and processing

We used Biocapture software, at a sampling rate of 1000 samples/s and amplified with a gain of 1000, to measure real time muscle electrical activation during the ESTs. The sampling resolution was 6 μV per least significant bit. An anti-aliasing filter, set to 250 Hz, was implemented in the recording system. Raw sEMG data were detrended offline, high pass filtered bidirectionally with a fourth order Butterworth filter at 20 Hz, and filtered with a 50 Hz notch filter to remove power line noise. Lastly, the sEMG signal was rectified using custom programs written in MATLAB R2016b. Markers were manually checked for presence and position. The mean root mean square (RMS) amplitude per cycle was calculated over an overlapping moving window (100 samples). We calculated the median frequency of the power spectrum, determined in Hertz (Hz), using Fast Fourier Transformation for every single cycle of the ESTs. Maximum RMS amplitudes of the MVCs were calculated for an overlapping moving window of 500 samples. RMS amplitudes per cycle were normalized to the MVC of the corresponding muscle to determine exercise intensity over the EST. Performances at intensities below 100% are referred to as submaximal. Raw RMS amplitudes were used to determine time-related changes.

2.6. Statistical analysis

The first ten minutes of all sEMG signals were analyzed. First, we aimed to assess the overall group differences between patients with SMA type 2, type 3a, type 3b/4, and controls performing ESNHPT and ESBBT. Due to a smaller sample size, the overall group difference between all patients with SMA and controls performing ESWT was assessed. Mean differences in muscle electrical activation, as quantified by the median frequencies or the natural logarithm of RMS (lnRMS) amplitudes, were estimated using linear mixed effects models for the four muscles described above for the ESNHPT, ESBBT, and the four muscles for the ESWT. lnRMS amplitudes were processed as described by Duan (1983) to reduce back-transformation bias. The relationship between lnRMS amplitudes and group (SMA type 2, type 3a, type 3b, and controls) was assessed, for every muscle, by Spearmans's correlation. Secondly, we assessed whether muscle electrical activation changed over time (i.e. over the course of the 10-minute endurance test) and whether the effect over time differed between patients with SMA and controls. We constructed a linear mixed effect model with group, time, and their interaction as fixed effects, and a random intercept and slope for time per individual. An unstructured covariance type was chosen. All statistical analyses were performed using SPSS and the level of significance was set at 0.05.

3. Results

In total, 70 patients with SMA and 19 healthy controls participated in this study. Participant characteristics per EST are summarized in table 1. Age and gender were similar in patients with SMA

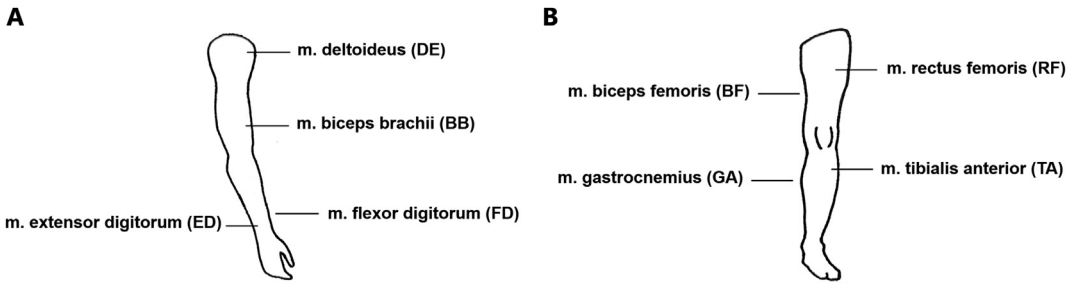

Fig. 1. Schematic muscle representation. A: Schematic representation of upper extremity muscle, B: Schematic representation of lower extremity muscle, DE = *m. deltoideus*, BB = *m.* biceps *brachii*, FD = *m. flexor digitorum*, ED = *m. extensor digitorum*, RF = *m. rectus femoris*, BF = *m. biceps femoris*, TA = *m. tibialis anterior*, GA = *m. gastrocnemius.*

Table 1
Participant characteristics.

	ESNHPT		ESBBT		ESWT	
Characteristics	**SMA (n = 66)**	**Controls (n = 19)**	**SMA (n = 45)**	**Controls (n = 16)**	**SMA (n = 17)**	**Controls (n = 16)**
SMA subtype (n)		-		-		-
type 2:	34		14		0	
type 3a:	14		13		2	
type 3b:	18		17		14	
type 4:	0		1		1	
Gender (m:f)	28:38	9:10	24:21	6:10	12:5	8:8
Age, y, mean (SD)	26.9 (14.0)	23.0 (9.6)	26.8 (13.7)	23.2 (7.8)	28.8 (11.9)	24.0 (9.8)
HFMSE, mean (SD)	18.5 (22.0)	-	29.6 (22.8)	-	54.8 (7.2)	-
Strength (n): median (N) (min–max)	DE (30): 40.5 (2.0–38.5) BB (65): 22.5 (3.5–47.0) FD (63): 15.0 (1.0–167.0) ED (65): 7.5 (0.5–149.0)	DE: 132.0 (40–243.5) BB: 213.5 (83.0–361.5) FD: 167.0 (57.5–274.5) ED: 127.0 (46.5–196.0)	DE (33): 41.5 (6.5–122.0) BB (45): 39.0 (12.0–356.0) FD (45): 40.0 (1.5–171.5) ED (45): 17.0 (3.5–149.5)	DE: 136.5 (40.0–244.0) BB: 219.8 (76.5–386.0) FD: 147.5 (53.5–297.5) ED: 117.0 (46.0–186.0)	RF (17): 25.5 (16.5–201.0) BF (17): 117.0 (26.5–237.0) TA (17): 215.5 (52.5–319.0) GA (17): MRC 5	RF (13): 359 (262–437) BF (13): 275 (123–343) TA (13): 316 (213–364) GA (17): MRC 5
Test drop-out rate (%)		0		13		0
Type 2:	71		93		-	
Type 3a:	50		85		50	
Type 3b/4:	17		44		36	
Time to limitation (n): median (s) (IQR)	P1 (10): 600 (548) P2 (56): 639 (1001)	P1 (13): 600 (0) P2 (6): 1200 (0)	P1 (6): 272 (412) P2 (39): 194 (1096)	P1 (13): 600 (0) P2 (3): 579 (-)	P1 (2): 600 (0) P2 (15): 861 (846)	P1 (11): 600 (0) P2 (5): 1200 (-)
Excluded RMS-MVC (n) *	24	4	5	1	5	5

ESNHPT = endurance shuttle nine hole peg test, ESBBT = endurance shuttle box and block test, ESWT = endurance shuttle walk test, SMA = spinal muscular atrophy; subtype 3a: clinical symptoms < 3yrs; subtype 3b: clinical symptoms > 3yrs, HFMSE = Hammersmith functional motor scale expanded, DE = *m. deltoideus,* BB = *m. biceps brachii,* FD = *m. flexor digitorum,* ED = *m. extensor digitorum,* RF = *m. rectus femoris,* BF = *m. biceps femoris,* TA = *m. tibialis anterior,* GA = *m. gastrocnemius,* MRC = Medical Research Council score for muscle strength, *IQR = interquartile range,* P1 = protocol 600 seconds, P2 = protocol 1200 seconds, RMS-MVC = root mean square amplitude normalized to maximal voluntary contraction, *Number of muscles excluded due to inadequate MVC measurement.

and healthy controls: 1) ESNHPT: age: $p = 0.173$, gender: $p = 0.712$, 2) ESBBT: age: $p = 0.198$, gender: $p = 0.287$, and 3) ESWT: age: $p = 0.206$, gender: $p = 0.241$). Test drop-out rate in subgroups of SMA, during all ESTs, varied between 17% in SMA type 3b/4 during ESNHPT, and 93% in SMA type 2 during ESBBT (table 1). Results of muscle electrical activation measured with sEMG in different muscles are described per EST below.

3.1. Median frequency dynamics

Significantly higher median frequencies in SMA compared to controls may indicate that larger motor units are recruited at onset of an EST (Fitts 1994; Dimitrova and Dimitrov 2003; Konrad 2005). A subsequent decrease in median frequencies during test performance would indicate: 1) muscle acidification, associated with fatiguing, fast-twitch, anaerobic muscle fibers, already recruited at onset; and 2) synchronization of motor unit firings (Fitts 1994; Dimitrova and Dimitrov 2003; Konrad 2005). Results of the linear mixed model analyses are listed in Supplementary Tables S1, S2, S3.

ESNHPT We found significantly higher median frequencies of *m. extensor digitorum* in patients with SMA types 2 and 3a compared to healthy controls (Fig. 2A). Median frequencies decreased in *m. deltoideus* of all patient groups, whereas healthy controls showed an increase in median frequencies (Fig. 2B).

ESBBT We found significantly higher median frequencies of *m. biceps brachii*, *m. flexor digitorum* and *m. extensor digitorum* in patients with SMA types 2 and type 3b/4, compared to healthy controls (Fig. 3A). Similar findings are shown in *m. biceps brachii* and *m. extensor digitorum* of patients with SMA type 3a (Fig. 3A). Median frequencies showed a stronger decrease in various muscles of all patient groups, compared to healthy controls (Fig. 3B).

ESWT Median frequencies of sEMG recordings from *m. rectus femoris*, *m. biceps femoris*, *m. tibialis anterior and m. gastrocnemius*, at onset of the ESWT, were similar in patients with SMA and in controls (Fig. 4A). We found a smaller decrease in median frequency of *m. biceps femoris* and *m. tibialis anterior*, and an increase for *m. gastrocnemius* over time, in patients with SMA compared to controls (Fig. 4B).

3.2. RMS amplitude dynamics

RMS amplitudes, normalized to the highest amplitude determined during MVC of the corresponding muscle, indicate the level of exercise intensity on a scale from 0 to 100%. Significantly higher amplitudes in SMA, compared to controls, would indicate the recruitment of larger motor units at onset to perform an EST (Fitts 1994; Dimitrova and Dimitrov 2003; Konrad 2005). An increase in RMS amplitude during test performance would indicate increased firing rates, motor unit synchronization, and recruitment of additional larger motor units not activated at onset of the task, to prevent task failure as a result of fatigability (Fitts 1994; Dimitrova and Dimitrov 2003; Konrad 2005). Results of the linear mixed model analyses are listed in Supplementary Tables S1, S2, S3.

ESNHPT All subjects performed the ESNHPT at an intensity submaximal to their MVC (8–60% MVC) (Fig. 2C). These exercise intensities were strongly inversely correlated to SMA phenotype in all muscles, $r_S(1269) = -0.764$ (*m. deltoideus*), $r_S(1396) = -0.775$ (*m. biceps brachii*), $r_S(1358) = -0.803$ (*m. flexor digitorum*), and $r_S(1322) = -0.811$ (*m. extensor digitorum*), all $p < 0.001$. Patients with SMA type 3a showed a significant increase in RMS amplitude of *m. deltoideus* and *m. flexor digitorum* over time, compared to a decrease in controls (Fig. 2D).

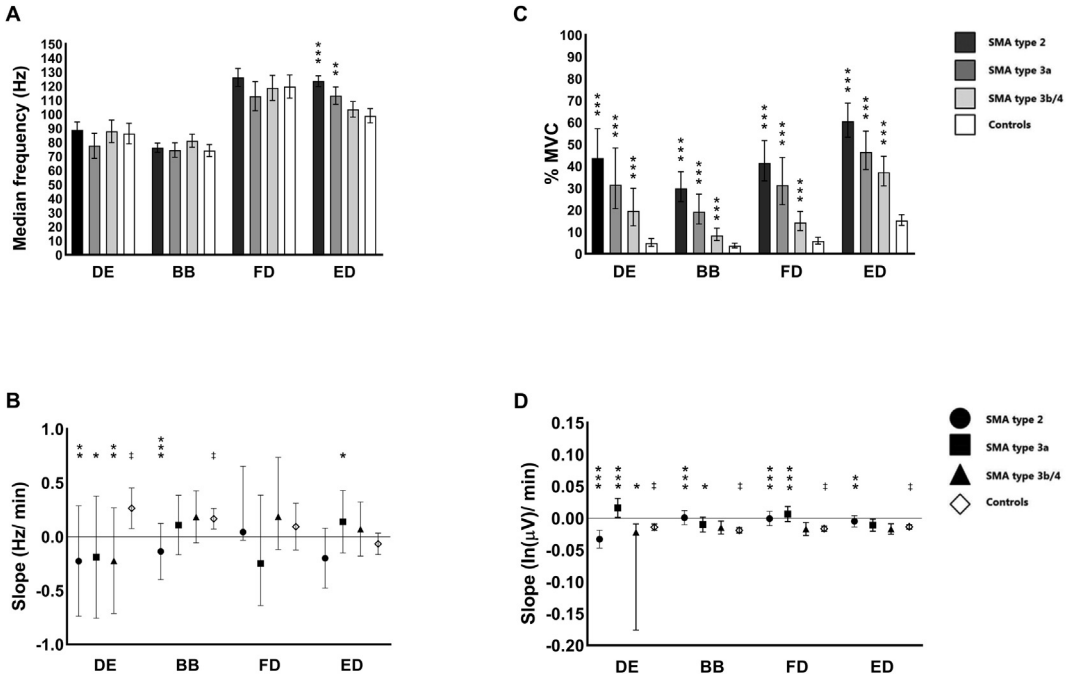

Fig. 2. Endurance shuttle nine hole peg test (ESNHPT). A: Median frequency at onset, B: Slope median frequency over time, C: Exercise intensity at onset, D: Slope amplitudes over time, error bars indicate upper and lower limits, */**/*** = significantly different from controls ($p < .05/p < .01/p < .001$), ‡ = significantly different from zero ($p < .05$), DE = *m. deltoideus*, BB = *m.* biceps *brachii*, FD = *m. flexor digitorum*, ED = *m. extensor digitorum*.

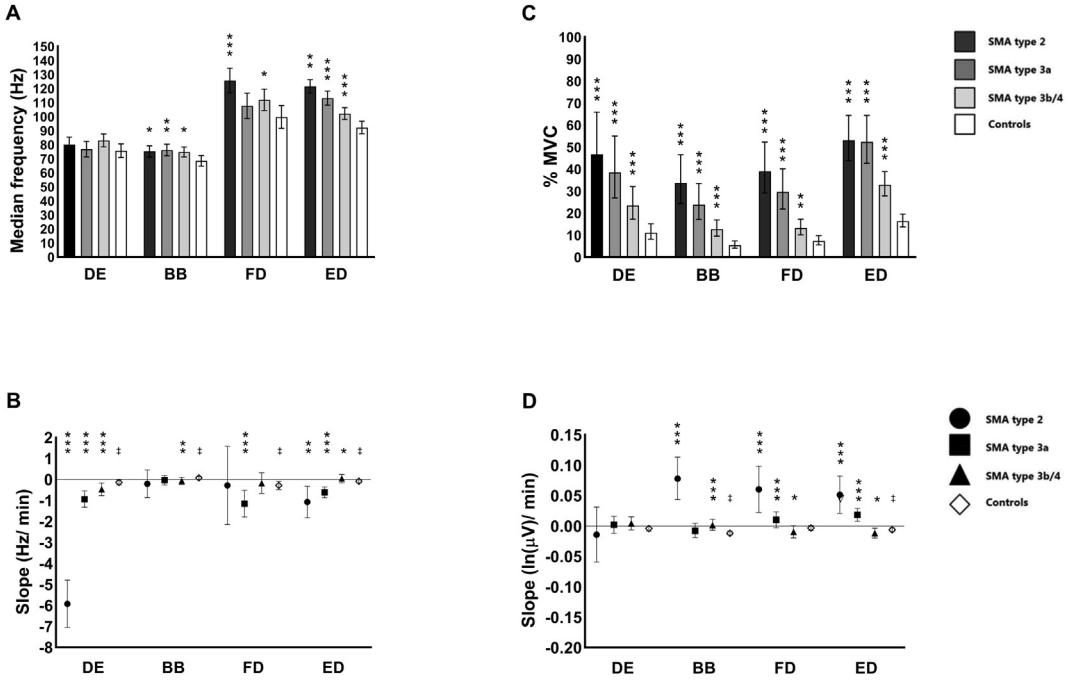

Fig. 3. Endurance shuttle box and block test (ESBBT). A: Median frequency at onset, B: Slope median frequency over time, C: Exercise intensity at onset, D: Slope amplitudes over time, error bars indicate upper and lower limits, */**/*** = significantly different from controls ($p < .05/p < .01/p < .001$), ‡ = significantly different from zero ($p < .05$), DE = *m. deltoideus*, BB = *m. biceps brachii*, FD = *m. flexor digitorum*, ED = *m. extensor digitorum*.

ESBBT All subjects performed the ESBBT at an intensity submaximal to their MVC (13–53% MVC) (Fig. 3C). These exercise intensities were again inversely correlated to SMA phenotype, $r_S(2062) = -0.549$ (*m. deltoideus*), $r_S(2117) = -0.645$ (*m. biceps brachii*), $r_S(2001) = -0.693$ (*m. flexor digitorum*), and $r_S(2057) = -0.773$ (*m. extensor digitorum*), all $p < 0.001$. We found a significantly larger increase in RMS amplitude over time in *m. biceps brachii*, *m. flexor digitorum*, and *m. extensor digitorum* of various patients with SMA, compared to healthy controls (Fig. 3D).

ESWT All patients performed the ESWT at an intensity submaximal to their MVC (41–82% MVC) (Fig. 4C). Patients with SMA showed an equal or smaller decrease in RMS amplitude of the sEMG signal from, respectively, *m. rectus femoris*, *m. gastrocnemius and m. tibialis anterior*, compared to controls (Fig. 4D). At the indi-

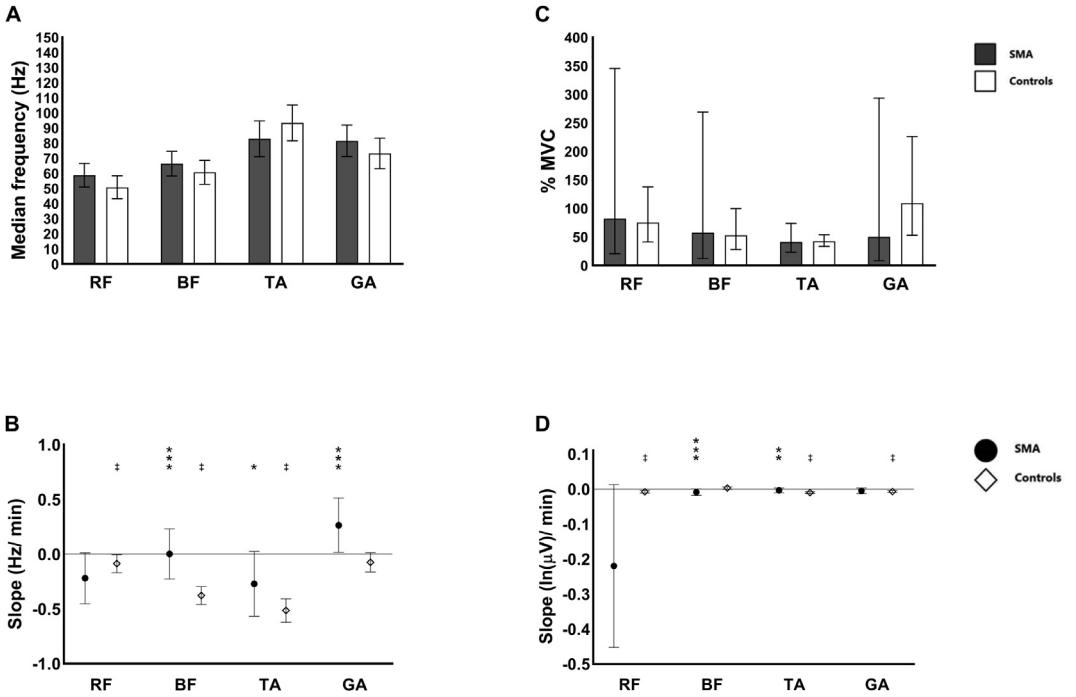

Fig. 4. Endurance shuttle walk test (ESWT). A: Median frequency at onset, B: Slope median frequency over time, C: Exercise intensity at onset, D: Slope amplitudes over time, error bars indicate upper and lower limits, */**/*** = significantly different from controls ($p < .05/p < .01/p < .001$), ‡ = significantly different from zero ($p < .05$), RF = *m. rectus femoris*, BF = *m. biceps femoris*, TA = *m. tibialis anterior*, GA = *m. gastrocnemius*.

vidual level, we observed both patients and healthy controls with increasing or decreasing RMS amplitudes over time (Supplementary Fig. S4).

4. Discussion

This study applied sEMG to investigate motor unit reserve capacity during execution of three quantitative endurance shuttle tests in patients with SMA types 2–4. Our results show decreasing median frequencies and rising RMS amplitudes during fatiguing submaximal endurance performance in some, but not all, patients with SMA, indicating the availability of motor unit reserve capacity in upper and lower extremities in individual patients. Current therapeutic approaches in SMA are principally aimed at restoring survival motor neuron (SMN) protein expression to prevent motor neuron loss (Yeo and Darras 2020). However, others have argued that therapy design in SMA should also consider targeting other components of the motor units – i.e., the neuromuscular junction and skeletal muscle itself (Shababi et al. 2014; Yeo and Darras 2020; Zhou et al. 2020). Our current finding of motor unit reserve capacity in SMA suggests that boosting this reserve, for example, using exercise training, may be a target for such combined treatment approaches.

All ESTs were executed at submaximal intensity, normalized to MVCs (Fig. 2C, 3C, 4C). As such, the original EST design criterion of ''a submaximal test protocol of repetitive activities over a longer period'' (Bartels et al. 2019), was met in all subjects. The relative exercise intensities of ESNHPT and ESBBT were found to be inversely related to SMA phenotype. This finding is in agreement with a recent study that measured trunk muscle electrical activation during unsupported sitting in patients with SMA (Peeters et al. 2019). Specifically, the authors reported three-fold higher activation levels in trunk muscles of patients with SMA types 2 and 3, during execution of a reaching task or a daily task, compared to controls.

Our sEMG recordings from muscles of the arm and leg in patients, during EST execution, showed a range of (patho)physiological trends in the time courses of median frequencies and RMS amplitudes. Progressive decrement in median frequency of the sEMG signal during execution of a physical task is commonly thought to be indicative of muscle acidification (Linssen et al. 1993). We observed such a decrease in median frequency over time in sEMG recordings of *m. deltoideus* of the shoulder during ESNHPT, of *m. deltoideus*, *m. flexor digitorum* and *m. extensor digitorum* of the arm during ESBBT, and of *m. rectus femoris* and *m. tibialis anterior* of the leg during ESWT execution. Conversely, any concomitant progressive increase in RMS amplitude would be indicative of motor unit reserve capacity progressively recruited to prevent task failure (Dimitrova and Dimitrov 2003; Konrad 2005). This phenomenon was indeed observed in sEMG recordings of *m. deltoideus* and *m. flexor digitorum* during ESNHPT, and of the *m. biceps brachii*, *m. flexor digitorum* and *m. extensor digitorum* during ESBBT execution, respectively. As such, these results support the hypothesis under investigation, that patients with SMA types 2–4 are able to recruit motor unit reserve capacity during fatiguing motor tasks.

Montes et al. (2014) previously observed a decrease in RMS amplitude of sEMG signals recorded from leg muscles in SMA type 3 patients performing the six-minute walking test, and attributed their finding to a limited, if not absent, motor unit reserve capacity in SMA (Montes et al., 2014). Here, we observed a similar manifestation of fatigability of the leg muscles in patients with SMA performing the ESWT, with an overall mean decrease in median frequency and RMS amplitude in *m. rectus femoris* and *m. tibialis anterior*. However, at the individual level, increases in RMS amplitudes over time were found in some lower extremity muscles, indicating the availability of motor unit reserve capacity in leg muscles during the ESWT in individual patients (Supplementary Fig. S4).

We found the largest changes in median frequencies and RMS amplitudes over time in patients with SMA type 2 during the

ESBBT (Fig. 3B-D). For example, we measured a change in median frequency of 6 Hz per minute in *m. deltoideus* of these patients. Although experimental setup and subject conditions are not fully comparable, a previous study, examining muscle fatigue and shoulder injury in the physically demanding car industry, reported a change of this magnitude in median frequency only after more than 120 minutes of executing a repetitive task (Ferguson et al., 2013). Since no reference values for fatigability during cyclic dynamic tasks exist in the literature, the absolute magnitude of change in median frequency and RMS amplitude over time should be interpreted with caution.

MVC determination for muscle of the human leg, using a hand-held dynamometer, is generally considered to be reliable and valid; i.e. in *m. rectus femoris* in young healthy subjects (Lee et al. 2012). However, we had difficulty accurately determining MVC of leg muscles in healthy controls, and in the *m. gastrocnemius* of the lower leg in patients. Here, maximal recorded strength may well have reflected physical strength of the individual handling the dynamometer rather than maximal strength of the leg muscle of the test subject (Marmon et al. 2013). This may have led to underestimation of MVC, and thereby to overestimation of exercise intensity of ESWT for these muscles in these subjects. Another complication for true normalization of effort of leg muscles, is the fact that MVC determination and ESWT execution were performed in a different body position (i.e. supine/sitting vs. upright); for example, a study in healthy subjects reports diverse EMG-length relations for the *m. rectus femoris* muscle at varying knee-joint angles (Hahn 2011). This may have contributed further to variance of strength and sEMG activation during ESWT. The large range of estimated exercise intensities in *m. rectus femoris* and *m. biceps femoris* muscles in patients with SMA (Fig. 4C), may additionally reflect variation in muscle strength between individuals (table 1).

This study has confirmed exercise intolerance at submaximal exercise intensity in patients with SMA types 2–4, while sEMG recordings showed pattern heterogeneities over time between individuals. Such variability suggests that a multifactorial causal base underlies fatigability in SMA types 2–4. A possible cause of fatigability may be dysfunction of the neuromuscular junction, with a prevalence of 40–50% in patients with SMA (Wadman et al. 2012). If this is the case, one might expect to find constant sEMG median frequencies, concomitant with decreasing amplitudes in every other patient. However, our results did not show this, a possible explanation being individual compensational strategies during ESNHPT and ESBBT execution. Movements, such as lateral bending of the trunk and elevation of the shoulder, were allowed. The reported patterns of muscle electrical activation should, therefore, be interpreted as a component of executing a complex motor task rather than as an indication of isolated muscle performance.

Lastly, oxidative capacity of skeletal muscle in SMA may also contribute to the clinical presentation of early fatigability. Various mouse and human studies have reported evidence of mitochondrial dysfunction in SMA (Boyd et al., 2017; Miller et al., 2016; Ripolone et al., 2015). Specifically, Miller et al. (2016) analysed the transcriptome of spinal motor neurons in a transgenic pre-symptomatic SMA mouse model and found altered mitochondrial function. Ripolone et al. (2015) reported down-regulated mitochondrial biogenesis in quadriceps and paraspinal muscle biopsy samples from patients with SMA types 1–3. Clearly, more research into this particular subject matter is warranted, to establish whether or not oxidative abnormalities, found in SMA mouse models and muscle biopsies, may contribute to exercise intolerance in human SMA. Dynamic in vivo ^{31}P Magnetic Resonance Spectroscopy of muscles, engaged in execution of a physical task, offers a well-established and non-invasive method for evaluating in vivo muscle mitochondrial function in neuromuscular diseases (Meyerspeer et al. 2020).

5. Conclusion

In the present study, individual patients with SMA types 2, 3 and 4 demonstrate some motor unit reserve capacity during execution of fatiguing endurance tasks, yet present with exercise intolerance during submaximal activities in daily life. Preserving, if not expanding this reserve capacity, may, therefore, present a potential therapeutic target in clinical care for some patients with SMA types 2–4.

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Declaration of Competing 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.2020.11.044.

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