European Journal of Applied Physiology
https://doi.org/10.1007/s00421-021-04647-y

ORIGINAL ARTICLE

Effects of NMES pulse width and intensity on muscle mechanical output and oxygen extraction in able-bodied and paraplegic individuals

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Received: 17 September 2020 / Accepted: 15 February 2021

Abstract

Purpose Neuromuscular Electrical Stimulation (NMES) is commonly used in neuromuscular rehabilitation protocols, and its parameters selection substantially affects the characteristics of muscle activation. Here, we investigated the effects of short pulse width (200 µs) and higher intensity (short-high) NMES or long pulse width (1000 µs) and lower intensity (long-low) NMES on muscle mechanical output and fractional oxygen extraction. Muscle contractions were elicited with 100 Hz stimulation frequency, and the initial torque output was matched by adjusting stimulation intensity.

Methods Fourteen able-bodied and six spinal cord-injured (SCI) individuals participated in the study. The NMES protocol (75 isometric contractions, 1-s on–3-s off) targeting the knee extensors was performed with long-low or short-high NMES applied over the midline between anterior superior iliac spine and patella protrusion in two different days. Muscle work was estimated by torque–time integral, contractile properties by rate of torque development and half-relaxation time, and vastus lateralis fractional oxygen extraction was assessed by Near-Infrared Spectroscopy (NIRS).

Results Torque–time integral elicited by the two NMES paradigms was similar throughout the stimulation protocol, with differences ranging between 1.4% ($p = 0.877$; able-bodied, mid-part of the protocol) and 9.9% ($p = 0.147$; SCI, mid-part of the protocol). Contractile properties were also comparable in the two NMES paradigms. However, long-low NMES resulted in higher fractional oxygen extraction in able-bodied (+36%; $p = 0.006$).

Conclusion Long-low and short-high NMES recruited quadriceps femoris motor units that demonstrated similar contractile and fatigability properties. However, long-low NMES conceivably resulted in the preferential recruitment of vastus lateralis muscle fibers as detected by NIRS.

Keywords Functional electrical stimulation · NMES · Spinal cord injury · Muscle oxygen extraction · NIRS

Communicated by Nicolas place.

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Published online: 03 March 2021

Introduction

Neuromuscular Electrical Stimulation (NMES) is commonly used in neuromuscular rehabilitation protocols and can be applied either in isometric conditions (Shields and Dudley-Javoroski 2006) or during functional movements (Momeni et al. 2019). In particular, after spinal cord injury (SCI), NMES-based training can minimize the loss of muscle mass (Shields and Dudley-Javoroski 2006; Crameri et al. 2004), increase muscle strength (Crameri et al. 2004) and improve cardiovascular function (Gibbons et al. 2016), thus contributing to improve motor function recovery and overall health. The NMES-elicited motor unit activation pattern is still uncertain, and different mechanisms are proposed. Some suggest that during NMES, the recruitment of motor units occurs in the superficial portion of the muscle, in close proximity to the stimulation pads (Vanderthommen et al. 2002), and this activation decreases in the deeper portion of the muscle (Vanderthommen et al. 2002). Other studies proposed that in the muscle region reached by the depolarization wave, the motor unit recruitment order is random and nonselective, with activation of both fast and slow types of motor units (Gregory and Bickel 2005; Bickel et al. 2011).

The selection of NMES parameters affects the characteristics of muscle activation (Bickel et al. 2011; Maffiuletti 2010; Bergquist et al. 2011), which are important to optimize NMES-promoted neuromuscular adaptations. However, some of these aspects are also not completely understood. Previous work using magnetic resonance imaging suggested that increments in NMES intensity result in a linear increase of force generation because of the progressive amount of motor units activated, maintaining constant the ratio between force generated and amount of muscle activated (i.e., muscle specific tension) (Gorgey et al. 2006; Bickel et al. 2004, 2011; Hillegass and Dudley 1999; Adams et al. 1993). Pulse width also plays an important role in determining muscle activation characteristics. Larger pulse widths require lower NMES intensity to activate peripheral motor nerves and achieve a desired force output. Increases in pulse width up to approximately 600 µs consistently lead to increased force production, while longer pulses do not necessarily result in further force increment (Bickel et al. 2011). Applying NMES over the muscle belly at longer pulse width, while selecting equal stimulation intensity and frequency, promoted a force increment that was greater than the increase in the amount of muscle activated, resulting in an increased specific tension (Gorgey et al. 2006). The interpretation of these findings was that longer pulse widths activated preferentially fast-twitch (and fast-fatigable) muscle fibers, which generate greater force than slow twitch fibers after controlling the size of the fibers (Bodine et al. 1987), thus explaining the increased specific tension. Interestingly, another study reported that the application of stimulation over the muscle belly with 30 Hz stimulation frequency, long pulse width and lower intensity resulted in less muscle fatigue (i.e., less torque decrement) compared to short pulse width and higher intensity set to elicit a matched mechanical output (25% MVC) during intermittent contractions (Jeon and Griffin 2018). These findings suggested that long pulse width and lower intensity (long-low) NMES led to less torque decrement because of preferential recruitment of smaller, fatigue-resistant muscle fibers. However, the corresponding effects of reciprocal NMES pulse width and intensity manipulation on muscle oxygen extraction remain unknown. Also, it is still unclear whether the positive effects of long-low NMES compared to short pulse width and higher stimulation intensity (short-high), selected to elicit moderate-level torque output, persist when high stimulation frequency is applied. In fact, differences in mechanical output elicited by 30 Hz or 100 Hz NMES protocols have been reported (Papaiordanidou et al. 2014; Gorgey et al. 2009).

In the present study, we aimed at investigating further the effects on oxygen fraction extraction of two NMES paradigms applied on the muscle belly and obtained by manipulating pulse width (short—200 µs, or long—1000 µs) and intensity (higher or lower), which was set to elicit a matched torque output, while maintaining a fixed stimulation frequency (100 Hz). We hypothesized that long-low NMES would promote greater total muscle work and higher muscle fractional oxygen uptake during the 5-min intermittent stimulation protocol because of extra muscle activation persisting after the end of NMES (Arpin et al. 2019a), and/or because of a preferential activation of smaller motor units that present greater oxidative capacity and fatigue resistance. We tested this hypothesis on able-bodied as well as SCI individuals, as these last: (i) present a compromised muscle oxidative function and a shift toward the fast-fatigable phenotype (Shields 2002), and (ii) may respond differently than able-bodied to NMES parameters modulation (Nickolls et al. 2004). Hence, while SCI individuals would particularly benefit from an increased oxygen extraction when using NMES for rehabilitation interventions, they may respond differently than able-bodied individuals to the proposed NMES protocols.

Materials and methods

A total of 14 able-bodied subjects (11 males and 3 females) recruited at the School of Sport Sciences (University of Udine, Italy) and 6 male individuals with clinically motor complete SCI recruited at the Kentucky Spinal Cord Injury

Research Center (Louisville, KY, USA), participated in this study. The experimental protocol was in accordance with the declaration of Helsinki, and was approved by the Institutional Review Boards of each research site: at the University of Udine (Italy) for the able-bodied participants (9/IRB DAME_17), and at the University of Louisville for the SCI participants (IRB #17.0135). Before the study began, the purpose and objectives were carefully explained to each subject and written informed consent was obtained.

In able-bodied participants, mean age was 24 ± 5 (years), stature was 1.78 ± 0.12 (m) and body mass was 72.6 ± 11.2 (kg), with a resulting BMI of 22.8 ± 2.0 (kg m^{-2}); also, adipose tissue thickness (ATT) at the vastus lateralis (see below, "NIRS data acquisition muscle" section) was 10 ± 5 (mm). Subjects were healthy, moderately active and had no history of orthopedic and neurological injuries. SCI participants presented a mean age of 32 ± 11 (years) and time since injury equal to 3.0 ± 1.1 (years) (range 2.0 to 5.6 years); their mean stature was 1.82 ± 0.09 (m), body mass was 80.5 ± 16.5 (kg), the resulting BMI was 24.2 ± 4.6 (kg m^{-2}), and ATT was 19 ± 8 (mm). The International Standards for Neurological Classification of Spinal Cord Injury (Burns et al. 2012) was used for classifying the injury using the ASIA (American Spinal Injury Association) Impairment Scale (AIS). The neurological level of injury ranged between C4 and T2; four individuals presented a clinically sensory and motor complete injury (graded A), and two individuals a motor complete and sensory incomplete injury (graded B).

Experimental protocol

All participants visited the laboratory twice, with the two experimental sessions scheduled between 1 and 4 days apart. Subjects were asked to refrain from any strenuous activity 24 h prior to the testing. The total duration of each visit was between 1 and 1.5 h (Fig. 1).

On day 1, after anthropometric measurements, participants were seated on a dynamometer. Able-bodied subjects (but not SCI individuals) performed non-fatiguing maximal voluntary contractions (MVC) of knee extensors aimed at assessing maximal torque output (Fig. 1b). Research participants were then asked to relax during the NMES recruitment curve with long pulse width (1000 µs) (Fig. 1b, c). After 10 min of recovery, the stimulation protocol resulting in 75 elicited muscle contractions at a fixed stimulation intensity (NMES protocol) with long pulse width (1000 µs) was performed.

On day 2, after the MVC attempts, which were performed to compare the participants' neuromuscular status with Day 1, subjects underwent the NMES recruitment curve and subsequently the NMES protocol with short pulse width (200 µs) and higher intensity (short-high; Fig. 1d, e). Long pulse NMES was always tested on Day 1 because it elicited higher torque output than short pulse at a given stimulation intensity, and the maximum torque output during the recruitment curve was the parameter required for SCI individuals to set the torque target during the NMES protocol. Muscle fractional oxygen extraction was investigated via the Near Infrared Spectroscopy technique (NIRS).

Maximal torque output

Able-bodied participants performed MVCs of the right knee extensors while sitting on the isometric dynamometer previously described by Rejc et al. (2010). Briefly, subjects were seated with their legs hanging vertically down, and with hips and knees flexed at 90°. A strap connected to a fixed attachment instrumented with a force sensor was secured around the right (dominant) ankle to perform the isometric knee extensions. Movements of the trunk and leg were minimized using a crossover shoulder strap and a strap around the ankle (5 cm proximal to the malleoli). All participants had previous extensive familiarization with this experimental setup.

An initial warm-up was performed, during which the participants were encouraged to generate between 20 and 30 contractions, each of them lasting approximately 3 s, at a self-selected and increasing intensity. After a rest period of 3 min, subjects were asked to perform a maximal isometric knee extension lasting approximately 6 s. Three MVC attempts were performed, with a 5-min rest in between attempts, and the contraction that resulted in the highest peak force was considered for further analysis. All data were collected as a force output and then transformed in torque data during off-line analysis. To calculate the torque value in each subject, force values were multiplied by the force lever arm which was the distance between the center of the knee joint and the 5 cm proximal to the superior malleoli of the ankle where the center of the force cell (AM C3, Laumas Elettronica, Italy; Sensitivity: 2.2mv/V ± 10%) was placed. Peak torque (MVC-T_{peak}) was defined by a 0.5 s moving average window.

In SCI subjects, the highest torque generated during NMES recruitment curve with long pulse width was considered as the maximal elicited torque output.

NMES recruitment curve

The relationship between stimulation intensity and peak torque exerted (i.e., recruitment curve) (Arpin et al. 2019b) was assessed by delivering NMES (Digitimer DS7A, Hertfordshire, UK) to the knee extensors through two surface electrodes (size: 5 × 10 cm; Axelgaard Manufacturing Co., Ltd., Fallbrook, CA; Fig. 1e). The distal end of the proximal electrode was placed at 50% of the distance from the anterior superior iliac spine to the patella protrusion, and the distal end of the distal electrode was placed at 10% of

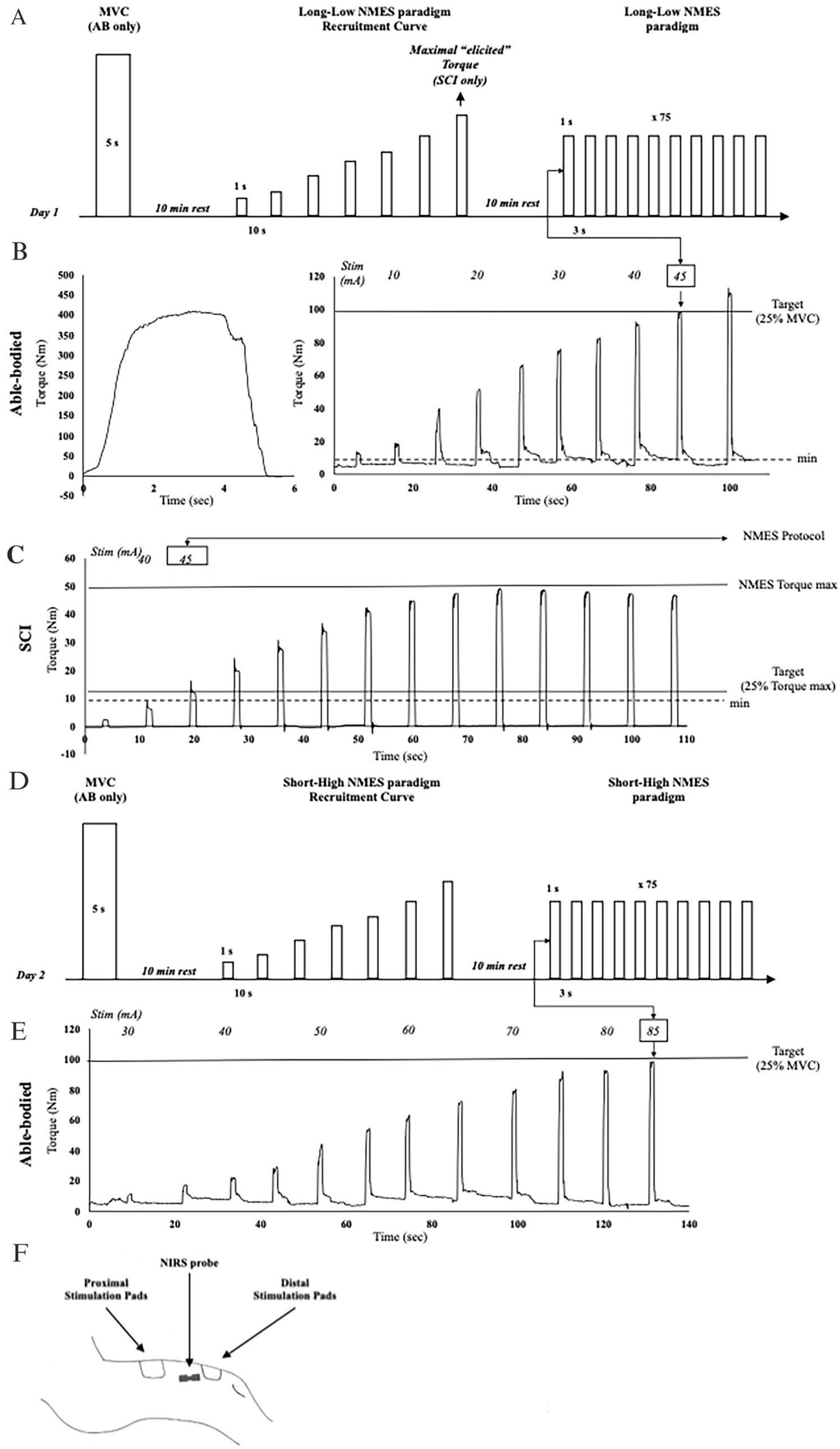

Fig. 1 Representation of the experimental protocol. **a–c** Maximal Voluntary Contractions (MVC, for able-bodied only), neuromuscular electrical stimulation (NMES) recruitment curve and NMES protocol (75 contractions, 1-s on–3-s off, fixed stimulation intensity) with long-low NMES parameters (1000 µs pulse width) was performed on Day 1. **d–e** MVC (for able-bodied only), NMES recruitment curve and NMES protocol with short-high NMES parameters (200 µs pulse width) was performed on Day 2. **f** Schematics of NMES electrodes and near infrared spectroscopy (NIRS) probe placement. NMES Torque Max: maximum torque elicited by NMES during the recruitment curve in spinal cord-injured (SCI) individuals. Stim (mA): NMES stimulation intensity. The NMES recruitment curve determined the stimulation intensity to be applied during the NMES protocol, which was the lowest intensity eliciting torque output equal or greater than the torque target (i.e., 25%MVC for able-bodied; 25% NMES Torque Max for SCI). Min: minimum initial absolute torque output to be elicited during NMES protocol

the distance between the patella protrusion and the anterior superior iliac spine (Arpin et al. 2019a). One-second stimulation trains with a constant frequency and voltage of 100 Hz and 400 V, respectively, were delivered every 10 s. The initial stimulation intensity was 5 mA, and it increased by 5 mA for every subsequent stimulation until either the recruitment curve reached a plateau (able-bodied $n=0$; SCI $n=4$), the participant requested to stop the stimulation attributed to discomfort (able-bodied $n=14$ with long pulse width, and $n=9$ with short pulse width; SCI $n=0$), or the maximum amplitude of the stimulator (100 mA) was reached (able-bodied $n=0$ with long pulse width, and $n=5$ with short pulse width; SCI $n=2$). The maximum NMES intensity achieved by able-bodied was 85.0 ± 14.8 mA (range 100–60 mA) with short pulse width and 50.0 ± 14.3 mA (range 70–20 mA) with long pulse width.

Able-bodied participants performed NMES assessments on the same isometric dynamometer used for MVC, while individuals with SCI used a Biodex dynamometer (Biodex Inc., Shirley, NY), which was set to mimic the configuration of that used by able-bodied subjects. Torque data were recorded by custom LabVIEW software (National Instrument Inc., Austin, TX) and sampled at 1 kHz. LabChart 8 (ADInstruments) was used to low-pass filter at 10 Hz all torque data and for the subsequent analysis. Peak torque for each NMES-induced muscle contraction was defined as the maximum torque value reached during each stimulation train (Arpin et al. 2019a).

NMES protocol

During the NMES protocol, the stimulation intensity was constant (i.e., was never modified throughout the entire stimulation protocol), and was carefully selected based on the NMES recruitment curves data to achieve the same initial torque output with both stimulation paradigms of 1000 µs pulse width and lower stimulation intensity (long-low) or 200 µs pulse width and higher intensity (short-high). In particular, we aimed at inducing moderate-level muscle contractions that would elicit meaningful muscle oxygen extraction throughout the duration of the NMES protocol. Based on preliminary observations, we aimed at eliciting an initial torque target equal to 25% of the peak torque generated during MVC for able-bodied subjects (Fig. 1b), which is also consistent with previous literature (Jeon and Griffin 2018), or to the 25% of the peak torque elicited during the recruitment curve for SCI individuals (Fig. 1c). However, we also observed that 10 Nm was the minimum initial torque output that would allow an accurate analysis of oxygen extraction throughout the 5-min NMES protocol. Hence, based on the recruitment curves data, we selected NMES intensities aimed at eliciting 25% MVC torque as initial target for all able-bodied subjects, which always resulted in absolute torque output higher than 10 Nm. On the other hand, for 2 of the 6 SCI individuals, 25% of the maximum peak torque elicited during recruitment curve resulted in an absolute torque output lower than 10 Nm. For these 2 subjects, NMES intensity was selected to elicit 10 Nm as initial torque target during the NMES protocol, so that reliable oxygen extraction data could be obtained.

The NMES protocol lasted 5 min and consisted in 75 muscle contractions delivered with a 4-s duty cycle (1 s on–3 s off). Torque output was recorded during the entire NMES protocol. Since during isometric contractions no mechanical work is performed, the torque–time integral (TTI) of each NMES-elicited muscle contraction was calculated to estimate muscle work (Porcelli et al. 2016). In particular, onset and offset of each NMES-elicited contraction were defined considering a torque threshold equal to the baseline (calculated between 650 and 150 ms prior to the delivery of NMES) + 3 standard deviations. TTI was calculated during the 1-s on-phase of muscle contraction (TTI_{on}) and during the entire 4-s duty cycle (TTI_{all}). Mean Torque expressed as percentage of peak torque (Mean Torque, $\%T_{peak}$), rate of torque development computed over the time windows 0–50 ms (RTD 0–50 ms) and 0–100 ms (RTD 0–100 ms), and half-relaxation time (1/2 Relax Time), defined as the time elapsed from the peak torque to 50% peak of the elicited contraction, were also assessed. These variables were determined for the initial 5 muscle contractions (start), the 36th–40th contractions (mid), the last 5 muscle contractions (end), and all 75 contractions (tot).

NIRS data acquisition

Near InfraRed Spectroscopy (NIRS) data were recorded during the NMES protocol. A continuous wave NIRS probe (Portalite; Artinis, The Netherlands), with sampling rate at 10 Hz, was positioned on the muscle belly of right *vastus lateralis* after the skin was properly shaved and cleaned with alcohol preparation. Adipose tissue thickness was measured at the site of NIRS placement using a manual caliper (GIMA, Italy). Oxygenation changes in the *vastus lateralis* muscle were evaluated as described in a paper by our group (Porcelli et al. 2012). The instrument estimates micromolar (µM) changes in oxygenated hemoglobin (Hb) and myoglobin (Mb) concentrations ([oxy(Hb + Mb)], and in deoxygenated Hb and Mb ([deoxy(Hb + Mb)]), with respect to an initial value arbitrarily set equal to zero and obtained during the resting condition preceding the test. [deoxy(Hb + Mb)] is relatively insensitive to changes in blood volume (Grassi and Quaresima 2016) and has been considered an estimate of skeletal muscle fractional oxygen extraction (ratio between oxygen consumption and oxygen delivery) (Porcelli et al. 2019). A "physiological calibration" of [deoxy(Hb + Mb)] values was performed by obtaining a transient ischemia of the limb 10 min after the recruitment curve. In particular, a blood pressure cuff was placed at the root of the thigh, and inflated at a pressure of 300 Torr to occlude both venous and arterial blood flow. The occlusion lasted approximately 3 min, which was the time sufficient to reach a plateau in the [deoxy(Hb + Mb)] curve (Grassi and Quaresima 2016). The maximal fractional oxygen extraction of the skeletal muscle was calculated as the amplitude of the difference between [deoxy(Hb + Mb)] values obtained from the baseline and the [deoxy(Hb + Mb)] value at the end of the occlusion procedure (Δ[deoxy (Hb + Mb)]).

The average in Δ[deoxy (Hb + Mb)] was analyzed using a moving window of 4 s, to evaluate the oxygen extraction during each duty cycle for the total duration of the protocol (75 contractions; HHb_{75}). The same analysis was performed also for the total(Hb + Mb), to estimate microvascular blood volume changes within the muscle during exercise.

Statistics

All results are expressed as mean and standard deviation (SD). Normal distribution of the data was tested using the Kolmogorov–Smirnov test. Maximal voluntary isometric contraction, stimulation intensity and electric charge, torque–time integral for the on-phase (TTI_{on}) and for the entire duty cycle (TTI_{all}), mean torque, RTD and 1/2 Relax time were analyzed considering the 5 contractions at the beginning (start), mid (mid), and final (end) part of the NMES protocol as well as for the total (tot) duration of the protocol (75 contractions). Δ[deoxy (Hb + Mb)] (HHb_{75}) and Δ[tot(Hb + Mb)] were analyzed for the total duration of the NMES protocol. These variables obtained with long-low or short-high NMES paradigms were statistically compared using Paired t test using GraphPad Prism 7.0 with significance set at $p < 0.05$.

Results

Able-bodied subjects generated similar MVC of knee extensors on Day 1 and Day 2 (317 ± 87 Nm and 326 ± 110 Nm, respectively; $p = 0.664$). As reported in Fig. 2 for representative able-bodied and SCI individuals, NMES-elicited muscle contractions resulted in an initial substantial decrease in torque output, followed by a more stable torque exertion during the second part of the NMES protocol (Fig. 2a, c, e, g). Also, muscle fractional oxygen extraction of vastus lateralis showed an initial steep increase, occurring approximately within the first 5 contractions, followed by a more consistent trend throughout the stimulation protocol (Fig. 2b, d, f, h).

Muscle mechanical output elicited by short-high and long-low NMES

The approach implemented in this study to match the initial torque output for long-low and short-high NMES paradigms while maintaining a fixed stimulation frequency was successful. In fact, TTI_{on_start} and Mean $Torque_{start}$ elicited by the two NMES paradigms were similar in able-bodied participants ($p = 0.202$ and $p = 0.327$, respectively) as well as in SCI participants ($p = 0.432$ and $p = 0.951$, respectively; Table 1). To match the initial torque output, higher stimulation intensities were used when short-high NMES was applied, both for able-bodied (82.5 ± 15.2 mA vs 43.2 ± 12.0 mA; $p < 0.001$, respectively) and SCI subjects (96.7 ± 24.8 mA vs 54.2 ± 17.2 mA; $p < 0.001$). Electric charge, determined as the product of stimulation intensity and pulse width, was 17 ± 3 and 43 ± 12 μC ($p < 0.001$) for short-high and long-low NMES paradigms, respectively, in able-bodied, and 19 ± 5 and 54 ± 17 μC ($p = 0.001$) for short-high and long-low stimulation paradigms, respectively, in the SCI group.

During the initial 5 contractions of the NMES protocol, the selected NMES intensity elicited a Mean $Torque_{start}$ of 24.0 ± 8.2%MVC and 25.3 ± 9.4%MVC ($p = 0.327$) at short-high and long-low NMES, respectively, in able-bodied subjects. In the SCI group, the initial 5 contractions of the NMES protocol generated a Mean $Torque_{start}$ of 38.8 ± 10.8%T_{peak} with short-high NMES, and 38.8 ± 10.2%T_{peak} with long-low NMES ($p = 0.951$). Mean Torque elicited by the two stimulation paradigms during the mid and final part of the NMES protocol were also similar, both for able-bodied and SCI participants (Table 1).

When considering the stimulation on-phase, the torque–time integral generated during the mid (TTI_{on_mid}) and final (TTI_{on_end}) portion of the NMES protocol by short-high and long-low paradigms were very similar, with non-significant differences ranging between 1.4% ($p = 0.877$; TTI_{on_mid}, able-bodied) and 9.9% ($p = 0.147$; TTI_{on_mid}, SCI) (Table 1). Also, when the entire duty cycle was considered, differences in TTI ranged between 0.7% ($p = 0.888$; TTI_{all_mid}, able-bodied) and 4.4% ($p = 0.520$; TTI_{all_end}, able-bodied) (Table 1).

Similarly, the total muscle work (as estimated by TTI_{on_tot} and TTI_{all_tot}) showed comparable values between short-high and long-low NMES paradigms in both groups, with differences ranging between 0.1% ($p = 0.885$; TTI_{all_tot}, able-bodied) and of 6.1% ($p = 0.363$; TTI_{on_tot}, SCI) (Table 1, Fig. 3).

The RTD calculated for the time windows 0–50 ms and 0–100 ms was also similar for the short-high and long-low stimulation parameters when considering the initial, mid, and final part of the NMES protocol, as well as the entire protocol, in both groups (Table 2). Similarly, ½ Relax Time was comparable between short-high and long-low

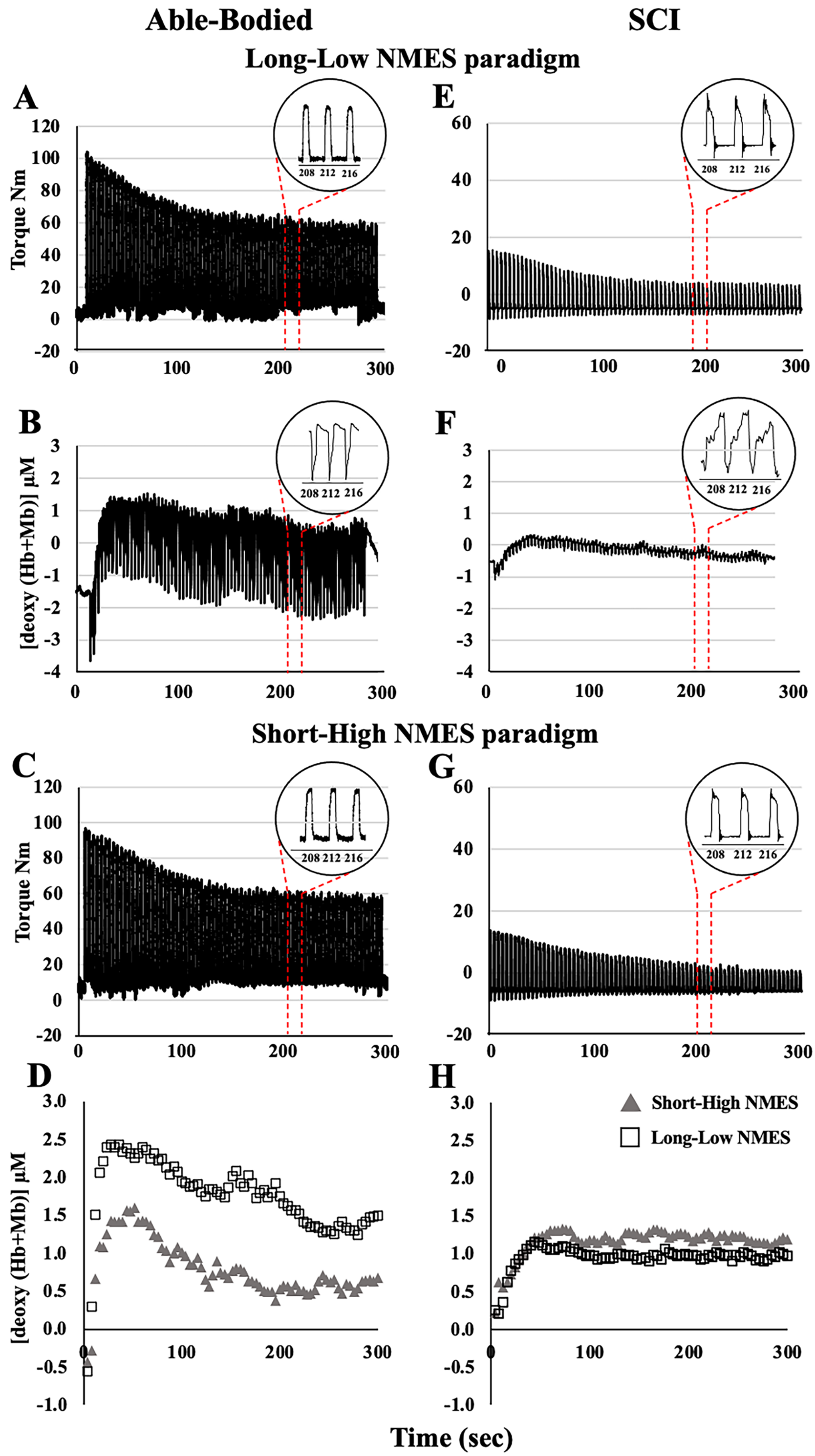

Fig. 2 Raw torque and [deoxy(Hb + Mb)] near infrared spectroscopy (NIRS) data from representative able-bodied (**a–c**) and spinal cord-injured (SCI) (**e–g**) individuals during long-low (1000 µs pulse width) and short-high (200 µs pulse width) NMES. Mean [deoxy(Hb + Mb)] values for each contraction elicited by short-high (gray triangle) and long-low (empty square) NMES parameters are shown (**d–h**)

Table 1 Mechanical output of knee extensors and vastus lateralis oxygen extraction elicited by short-high and long-low NMES paradigms for able-bodied and spinal cord-injured (SCI) individuals

	Able-bodied				SCI			
	Short-High	Long-Low	Diff. (%)	*p* value	Short-High	Long-Low	Diff. (%)	*p* value
TTI_{on_start} (Nm s)	136 ± 43	148 ± 51	9.2	0.202	37 ± 10	35 ± 8	5.0	0.432
TTI_{on_mid} (Nm s)	93 ± 45	92 ± 50	1.4	0.877	20 ± 5	18 ± 4	9.9	0.147
TTI_{on_end} (Nm s)	83 ± 40	81 ± 49	1.6	0.876	15 ± 4	14 ± 5	0.2	0.981
TTI_{on_tot} (Nm s)	1473 ± 645	1494 ± 727	1.5	0.856	333 ± 80	313 ± 70	6.1	0.363
TTI_{all_start} (Nm s)	430 ± 144	448 ± 163	4.2	0.455	78 ± 19	75 ± 18	4.5	0.211
TTI_{all_mid} (Nm s)	273 ± 108	271 ± 111	0.7	0.888	40 ± 7	39 ± 12	3.2	0.739
TTI_{all_end} (Nm s)	230 ± 190	240 ± 103	4.4	0.520	28 ± 6	29 ± 12	3.9	0.782
TTI_{all_tot} (Nm s)	4467 ± 1634	4472 ± 1756	0.1	0.985	677 ± 130	667 ± 191	1.5	0.856
Mean $Torque_{start}$ (% T_{peak})	24.01 ± 8.21	25.36 ± 9.43	5.2	0.327	38.73 ± 10.77	38.84 ± 10.20	0.3	0.951
Mean $Torque_{mid}$ (% T_{peak})	15.93 ± 6.03	15.75 ± 5.79	1.7	0.699	21.14 ± 4.87	20.58 ± 5.65	2.7	0.596
Mean $Torque_{end}$ (% T_{peak})	13.57 ± 5.30	13.98 ± 5.47	3.0	0.621	14.02 ± 2.10	14.76 ± 2.08	5.2	0.482
Mean $Torque_{tot}$ (% T_{peak})	17.11 ± 6.23	17.16 ± 6.28	0.2	0.959	23.26 ± 5.71	23.22 ± 5.65	0.1	0.978
HHb (% Isc)	29.47 ± 13.69	39.96 ± 13.51	35.6	0.006*	19.18 ± 9.50	16.59 ± 7.67	15.6	0.507

Values are mean ± standard deviation. TTI_{on}: Sum of the Torque Time Integral assessed for the 1-s muscle contraction; TTI_{all}: Sum of the Torque Time Integral assessed for the 4-s duty cycle (1-s NMES on and 3-s NMES off); Mean Torque: Mean torque expressed as percent of the peak torque generated during maximal voluntary contraction (able-bodied) or NMES recruitment curve (SCI); HHb (% isc): Δ deoxygenated Hemoglobin and Myoglobin as percentage of ischemia. TTI_{on}, TTI_{all} and Mean Torque are reported for the initial 5 contractions (start; 1st–5th contractions), mid (mid; 36th–40th contractions), and the last 5 contractions (end; 71st–75th contractions), as well as for all 75 (tot) muscle contractions

*Significant difference by Paired *t* test

NMES paradigms both in able-bodied and in SCI participants, with non-significant differences ranging between 2.1% ($p = 0.268$; able-bodied, ½ Relax $Time_{tot}$) and 15.5% ($p = 0.300$; SCI, ½ Relax $Time_{start}$) (Table 2).

Muscle fractional oxygen extraction

While mechanical output of knee extensors elicited throughout the 5-min NMES protocol by long-low and short-high parameters was comparable, average (Fig. 3c, d) time course of NIRS data showed that long-low NMES promoted higher vastus lateralis fractional oxygen extraction in able-bodied but not in SCI participants. HHb_{75} measured by the Δ[deoxy (Hb + Mb)] in percentage of physiological calibration, was significantly higher during long-low NMES compared to short-high (+ 10% ischemia, $p = 0.006$; Fig. 3c) in able-bodied participants. Conversely, HHb_{75} was not significantly different (-3% ischemia, $p = 0.507$; Fig. 3d) between muscle contractions elicited by long-low and short-high NMES paradigms in SCI individuals. At the same time, total (Hb + Mb) was similar in able-bodied subjects (6.49 ± 0.88 vs 6.74 ± 0.97 μMol, $p = 0.760$) and SCI participants (7.06 ± 1.71 vs 6.87 ± 1.86 μMol, $p = 0.920$) for short-high and long-low NMES paradigms, respectively.

Discussion

In the present study, we investigated the effects of two NMES paradigms resulting from the manipulation of pulse width (long—1000 μs, or short—200 μs) and intensity (higher or lower), which was set to elicit an initial matched torque output, on muscle mechanical output of knee extensors and fractional oxygen extraction of the vastus lateralis during an intermittent stimulation protocol. Contrary to our hypothesis, all muscle mechanical output variables assessed during the 5-min NMES protocol were similar between long-low and short-high NMES paradigms. On the other hands, higher fractional oxygen extraction was promoted by long-low in able-bodied subjects only.

Muscle mechanical output

The two NMES paradigms tested in the present study (long-low and short-high) elicited muscle contractions resulting in similar Mean Torque, TTI_{on}, RTD and ½ Relax Time throughout the 5-min intermittent NMES protocol (Tables 1, 2). These findings suggest that both NMES paradigms recruited a population of quadriceps femoris motor units with similar contractile and fatigability properties.

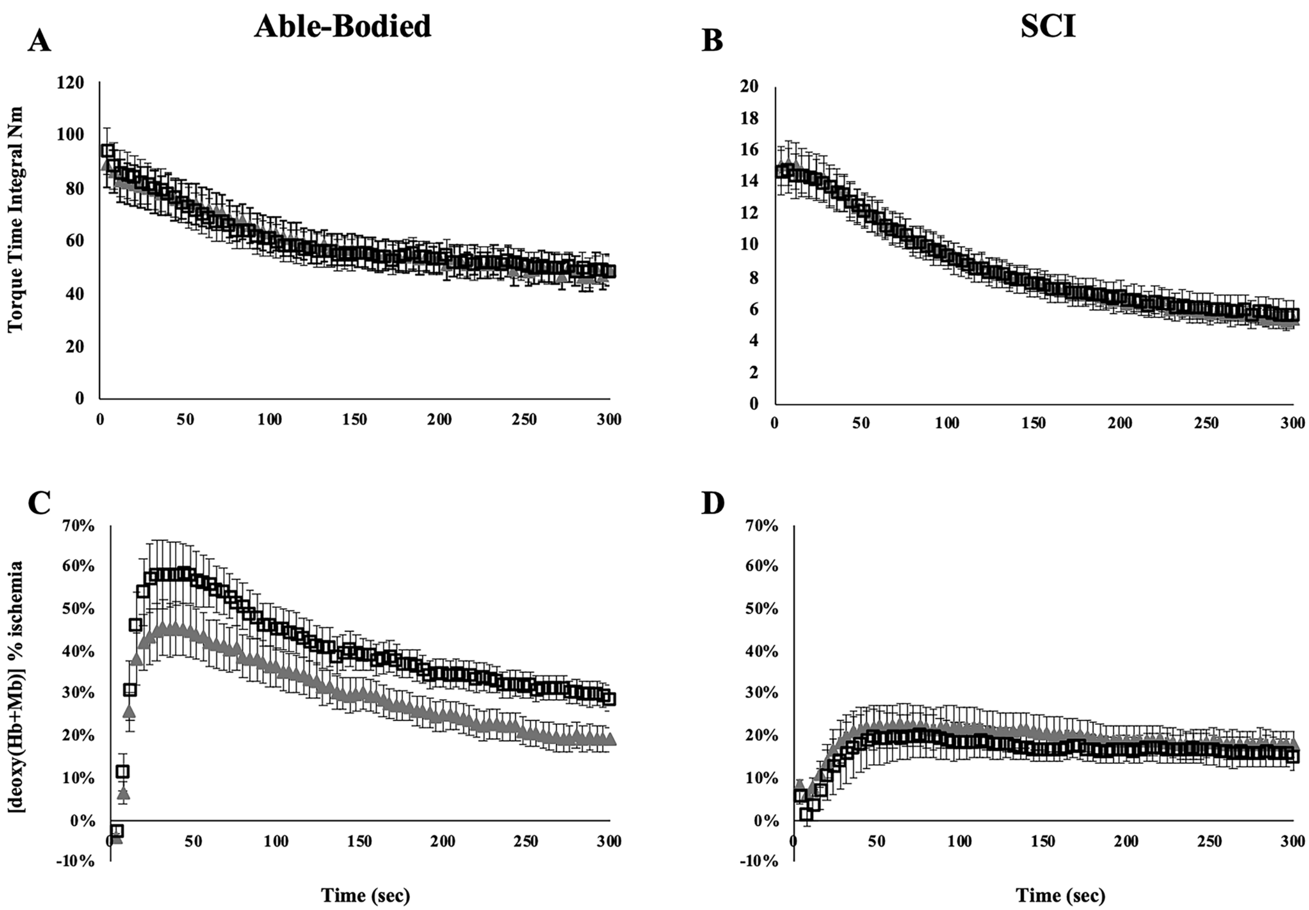

Fig. 3 Mean values in Torque–Time Integral (Nm) (during the 1-s contraction period) and [deoxy(Hb + Mb)] (% ischemia) for short-high (gray triangle) and long-low (empty square) NMES parameters in able-bodied (**a, c**) and Spinal Cord Injury subjects (**b, d**)

Table 2 Contractile properties of knee extensors during muscle contractions elicited by short-high and long-low NMES paradigms for able-bodied and spinal cord-injured (SCI) individuals

	Able-bodied				SCI			
	Short-High	Long-Low	Diff. (%)	*p* value	Short-High	Long-Low	Diff. (%)	*p* value
RTDstart 0–50 ms (Nm s^{-1})	504 ± 181	474 ± 176	6.3	0.459	33 ± 5	33 ± 7	6.2	0.406
RTDmid 0–50 ms (Nm s^{-1})	314 ± 122	281 ± 145	11.6	0.261	21 ± 7	21 ± 4	3.5	0.826
RTDend 0–50 ms (Nm s^{-1})	281 ± 129	258 ± 165	9.0	0.450	17 ± 5	17 ± 5	3.1	0.819
RTDtot 0–50 ms (Nm s^{-1})	345 ± 134	311 ± 156	10.7	0.316	23 ± 4	23 ± 5	0.2	0.977
RTDstart 0–100 ms (Nm s^{-1})	464 ± 145	497 ± 156	7.1	0.281	63 ± 13	63 ± 14	0.2	0.961
RTDmid 0–100 ms (Nm s^{-1})	310 ± 117	314 ± 137	1.4	0.875	40 ± 11	39 ± 8	1.5	0.815
RTDend 0–100 ms (Nm s^{-1})	278 ± 111	285 ± 139	2.6	0.789	33 ± 8	33 ± 11	0.3	0.970
RTDtot 0-100 ms (Nm s^{-1})	332 ± 118	342 ± 134	2.8	0.722	44 ± 9	43 ± 9	1.5	0.708
1/2 Relax $Time_{start}$ (msec)	45.34 ± 2.61	47.49 ± 5.03	4.7	0.071	124.17 ± 53.05	107.50 ± 18.64	15.5	0.300
1/2 Relax $Time_{mid}$ (msec)	70.46 ± 14.08	68.53 ± 10.39	2.7	0.517	142.50 ± 24.24	126.67 ± 10.33	12.5	0.077
1/2 Relax $Time_{end}$ (msec)	71.33 ± 10.91	69.16 ± 10.74	3.0	0.292	128.33 ± 23.17	119.17 ± 27.10	7.7	0.451
1/2 Relax $Time_{tot}$ (msec)	65.28 ± 9.08	63.89 ± 7.72	2.1	0.268	130.83 ± 24.95	117.33 ± 13.76	11.5	0.070

Values are mean ± standard deviation. RTD: rate of torque development calculated for the time windows 0–50 ms and 0–100 ms; 1/2 Relax Time: half relaxation time. RTD and 1/2 Relax Time are reported for the initial 5 contractions (start; 1st–5th contractions), mid (mid; 36th–40th contractions), and the last 5 contractions (end; 71st–75th contractions), as well as for all 75 (tot) muscle contractions

However, long-low and short-high NMES may have led to muscle fatigue via different mechanisms (Martin et al. 2016). In particular, intracellular mechanisms (number of attached cross-bridges or reduced sensitivity to calcium ions) may be more responsible for the reduced force output during short-high NMES, while the decreased number of active motor units may play a greater role in force reduction observed with long-low NMES.

The comparable muscle mechanical output elicited by the two NMES paradigms in this study is not in agreement with a similar study (Jeon and Griffin 2018), which demonstrated less decrement of torque output with long-low NMES application as compared to short-high NMES when stimulation was delivered at 30 Hz. It is possible that higher (100 Hz) NMES frequency may cancel out the positive effects of long-low NMES as compared to short-high NMES on torque output generation that are observed at lower (i.e., 30 Hz) frequencies (Gorgey et al. 2009). Repetitive high frequency stimulation may increase the excitability threshold of active intramuscular axonal branches close to the stimulation intensity, resulting in a greater chance of not recruiting the related motor units (Papaiordanidou et al. 2014). It is also possible that high NMES frequency may have led to an extent of neuromuscular propagation failure that was similar for both NMES paradigms (Boyas and Guével 2011; Bergquist et al. 2017), contributing to the similar muscle mechanical output observed in this study throughout the intermittent NMES protocol. Furthermore, neuromuscular propagation failure was shown to be a primary determinant of muscle torque decrement when NMES was applied over the muscle belly (as in the present study), but not when it targeted the nerve trunk or it was interleaved between muscle belly and nerve trunk (Bergquist et al. 2017). However, a limit of the present study is that we did not assess neurophysiological variables (i.e., M-wave) to support or rule out this hypothesis.

In this study, the total muscle work, estimated by the torque–time integral for the entire 4-s duty cycle (TTI_{all}), was also comparable between long-low and short-high NMES (Table 1). We previously observed that long-low NMES favored the generation of EMG activity that persisted after the end of NMES (Arpin et al. 2019a) during a similar intermittent NMES protocol in SCI individuals. However, here we did not detect any significant extra torque output favored by long-low NMES. This may be due, at least partially, to the fact that the moderate (i.e., 25%MVC) torque output targeted in this study required stimulation intensities that favor the recruitment of motor units via efferent pathways (Bergquist et al. 2011, 2012).

Muscle fractional oxygen extraction

While comparable muscle mechanical output of knee extensors was elicited by the two NMES paradigms implemented in this study, suggesting similar types and proportion of activated motor units, we found that long-low NMES promoted greater vastus lateralis oxygen extraction in able-bodied subjects (Fig. 3). NIRS technique is considered a valid methodology to assess metabolism during exercise and estimate concentration changes in deoxygenated/oxygenated hemoglobin and myoglobin (Grassi and Quaresima 2016). For example, NIRS has been previously applied to evaluate fractional oxygen extraction in healthy individuals during constant work load (Grassi and Quaresima 2016), to assess the effects of prolonged disuse (Porcelli et al. 2010), as well as in individuals with SCI (Gollie et al. 2017). In the present study, we analyzed changes in [deoxy(Hb + Mb)] during NMES-elicited muscle contractions to overcome possible limitations derived from increased muscle blood flow to the skin. Indeed, [deoxy(Hb + Mb)] is considered a more reliable estimator of muscle fractional oxygen extraction during muscle contraction (Grassi and Quaresima 2016).

On the other hand, NIRS can assess a relatively small portion of the muscle (approximately 2–6 cm^3) with a depth penetration of half of the distance between light source and detector (Grassi and Quaresima 2016). Also, in the present study, the torque output resulted from the contribution of the four muscles of the quadriceps femoris. It is, therefore, plausible that long-low and short-high NMES applied over the midline between anterior superior iliac spine and patella protrusion elicited preferential activation of different portions of the quadriceps femoris muscle, and that long-low promoted the preferential recruitment of vastus lateralis muscle fibers to result in the higher oxygen extraction detected by the NIRS probe. Previous observations support the view that NMES can activate distinct portions of the quadriceps femoris, even with variability between subjects (Adams et al. 1993). Nonetheless, portions of the muscle in close proximity to the stimulating pads are preferentially activated (Fouré et al. 2019). In particular, the short-high NMES may have recruited a greater amount of motor units that were outside of the NIRS probe detection field (Gorgey et al. 2006). In SCI individuals, vastus lateralis fractional oxygen extraction was similar for long-low and short-high NMES protocols (Fig. 3). This is conceivably due to the fact that SCI leads to extensive skeletal muscle atrophy (Durozard et al. 2000; Shah et al. 2006; Gorgey and Dudley 2007), thus reducing the possibility to activate different portions of the quadriceps femoris muscle with different NMES parameters targeted to elicit a matched torque output. A limit of the present study is the use of one NIRS probe, which did not allow to assess the oxygen extraction of the other two superficial muscles of the quadriceps femoris (vastus medialis and rectus femoris). This could have better elucidated the effects of the different NMES parameters on the preferentially activated portions of the quadriceps femoris.

In conclusion, the long-low and short-high NMES paradigms applied at high frequency (100 Hz) in this study elicited similar muscle work and contractile properties of knee extensors, suggesting that both sets of parameters recruited quadriceps femoris motor units with similar contractile and fatigability properties. Also, the higher vastus lateralis fractional oxygen extraction detected by the NIRS probe in able-bodied individuals when long-low NMES parameters were applied suggests that long-low NMES conceivably resulted in the preferential recruitment of vastus lateralis muscle fibers.

Acknowledgements We thank all research participants for their valuable contribution to this study. We also thank Dr. Sara Wagers (medical oversight), advocates, research nurses, and physiotherapists for additional support to the spinal cord-injured research volunteers. The study was supported by Fondazione Pietro Pittini (Italy), and by the Leona M. and Harry B. Helmsley Charitable Trust.

Author contributions ER, FG, SH and SL contributed to conception and design of the study. FG, ER, SS, AW and TR contributed to data collection. FG, ER, MF contributed to data analysis. ER, FG, NG, MF, SP and SL contributed to the initial interpretation of results. FG and ER designed the figures. FG, ER and SL wrote the first draft of the manuscript. All authors contributed to data interpretation and writing the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest No conflicts of interest, financial or otherwise, are declared by the authors.

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