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"Long and short-term effects of targeted Neuromuscular Electrical Stimulation on the lower limb muscle function in healthy adults and elderlies"

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Abstract

Neuromuscular Electrical Stimulation (NMES) consists in the use of an electrical current to elicit a muscle contraction and is used in rehabilitation or training protocols to restore muscle function. The parameters used to evoke a muscle contraction can be manipulated, and the manipulation changes not only the muscle recruitment pattern but also the muscle metabolism. However, it is hypothesized that during an electrically elicited muscle contraction, the recruitment pattern of Motor Units is the different from the one occurring during a voluntary contraction. This represents a limitation to the use of the NMES mainly because of an excessive fatigue and metabolic demand occurring in the muscle involved. At the same time the combination of high stimulation frequency and wide pulse width was found to be effective in allowing the recruitment of muscle fibers via afferent fibers and restoring at least partially the activation pattern of a voluntary contraction. Also, NMES can be used to potentiate both twitch and voluntary muscle contractions by positively affecting muscle contractile characteristics. This effect is of particular importance not only in the context of performance improvement but also in elderly population. In fact, during ageing the body goes through multiple modifications, among which a decrease in muscle mass, and selective atrophy of type II muscle fibers. The possibility to enhance muscle performance and explosiveness can prevent the risk of falling and increase the quality of life during daily living activities.

For these reasons, in the present thesis we focused on *i*) investigating the effects of modifying stimulation parameters of muscle function and metabolism; *ii*) comparing the effects of two conditioning contraction protocols on muscle contractile properties; *iii*) studying the effects of two training modalities on muscle quality, strength, explosiveness, physical capacities and balance in elderlies. We demonstrated that NMES delivered at high frequency, low amplitude and wide pulse

width, can generate higher fractional oxygen extraction in healthy adults (+36%; p=0.006), as compared to a high frequency, high amplitude and short pulse NMES paradigm. The same result was not exhibited in spinal cord injured individuals (+15.6%; p=0.507). Moreover, we demonstrated that a NMES-elicited conditioning contraction protocol can enhanced (+38%) explosive contractile characteristics (i.e. early phase of the Rate of Torque development, RTD_0-50ms) of a following voluntary explosive contractions as compared to a voluntary conditioning contraction protocol (p=0.027). Finally, we evaluated the effects of two training interventions performed on lower limb of 19 healthy elderlies. In particular, the EXP group performed a resistance training with the combination of a NMES-elicited conditioning contraction and a following explosive voluntary contraction, while the CTRL group performed a resistance training composed of solely explosive voluntary contractions. We demonstrated that both the EXP and the CTRL group showed an increased in muscle architecture parameters, MVC and physical capacities. However, the NMES-elicited conditioning contraction increased contractile characteristics of the voluntary contraction more than what exhibited by the CTRL group that performed only voluntary contractions with differences ranging between +54% (p=0.003, RTD_0-50ms) and +24% (p=0.038, RTD_0-200ms) at the beginning of the training intervention.

In conclusion, NMES is effective to produce higher oxygen utilization when delivered at high frequency, low amplitude, and long pulse width. This paradigm is also effective in enhancing contractile characteristics of a voluntary explosive contraction in healthy adults. Lastly, when the focus is the increase in muscle explosiveness, a training paradigm combining NMES and voluntary contractions represents a valid alternative for elderlies.

List of publications

Gonnelli F, Rejc E, Giovanelli N, Floreani M, Porcelli S, Harkema S, Willhite A, Stills S, Richardson T, Lazzer S. Effects of NMES pulse width and intensity on muscle mechanical output and oxygen extraction in able-bodied and paraplegic individuals. Eur J Appl Physiol. 2021 Jun;121(6):1653-1664. doi: 10.1007/s00421-021-04647-y. Epub 2021 Mar 3. PMID: 33656575.

Gonnelli F, Giovanelli N, Floreani M, Bravo G, Parpinel M, D'Amuri A, Brombo G, Dalla Nora E, Pišot R, Šimunič B, Pišot S, Biolo G, di Girolamo FG, Situlin R, Passaro A, Lazzer S. Physical capacities and leisure activities are related with cognitive functions in older adults. J Sports Med Phys Fitness. 2022 Jan;62(1):131-138. doi: 10.23736/S0022-4707.21.11599-3. Epub 2021 Mar 17. PMID: 33728840.

Mesbah S, **Gonnelli F**, Angeli CA, El-Baz A, Harkema SJ, Rejc E. Neurophysiological markers predicting recovery of standing in humans with chronic motor complete spinal cord injury. Sci Rep. 2019 Oct 9;9(1):14474. doi: 10.1038/s41598-019-50938-y. PMID: 31597924; PMCID: PMC6785550.

D'Alleva M, **Gonnelli F**, Vaccari F, Boirie Y, Montaurier C, Thivel D, Isacco L, Vermorel M, Lazzer S. Energy cost of walking and body composition changes during a 9-month multidisciplinary weight reduction program and 4-month follow-up in adolescents with obesity. Appl Physiol Nutr Metab. 2021 Sep 13:1-9. doi: 10.1139/apnm-2021-0273. Epub ahead of print. PMID: 34516928.

Under Revision

Gonnelli F, Rejc E, Floreani M, Lazzer S. Effects of NMES-elicited versus voluntary low-level conditioning contractions on explosive knee extensions – Submitted to the Journal of Musculoskeletal and Neural Interactions

Abstract Published at International Congress

Federica Gonnelli, Enrico Rejc, Mirco Floreani, Stefano Lazzer, NMES-elicited Low-level Conditioning-Contractions Affect Explosive Performance of Knee-Extensors, BMES 2021, Oral Presentation.

Federica Gonnelli, Enrico Rejc, Mirco Floreani, Stefano Lazzer, Effects of electrically-evoked or voluntary low-level conditioning contractions on explosive, isometric knee extensions, ECSS 2021, Oral Presentation.

Federica Gonnelli, Enrico Rejc, Mirco Floreani, Nicola Giovanelli, Stefano Lazzer, Neuromuscular Electrical Stimulation At Long Pulse Duration Is Associated With Higher Muscle Oxygen Utilization, ACSM 2019, Poster Presentation.

Samineh Mesbah, **Federica Gonnelli**, Enrico Rejc, Susan Harkema, Ayman El-baz, Predictive Modeling to Assess the Effectiveness of Epidural Stimulation Parameters that Promote Standing in Individuals with Severe Spinal Cord Injury, KSCHIRT 2019, Poster Presentation.

Samineh Mesbah, **Federica Gonnelli**, Claudia Angeli, Ayman El-Baz, Susan Harkema, Enrico Rejc. Spectral Analysis of Lower Limb EMG Activity in Individuals with Motor Complete SCI during Standing with Epidural Stimulation, ISSPIT 2018, Poster Presentation.

Samineh Mesbah, **Federica Gonnelli**, Enrico Rejc, Susan Harkema, Ayman El-baz, Frequency Analysis of EMG Signals of Individuals with Spinal Cord Injury: Comparison Between FFT, STFT and Wavelet Methods, BMES 2018, Poster Presentation.

Abstract Presentations

During the PhD period, the candidate participated in different congresses presenting abstracts related with the projects described in the following thesis.

In particular, the candidate participated as the presenting author in the Congress of American College of Sports Medicine 2020, Congress of the European College of Sport Science 2021 and the Congress of the Biomedical Engineering Society 2021.

Introduction

The following PhD thesis is divided into four main chapters, each representing a project that was carried during the different periods of the doctorate.

In the first chapter the attention is focused on the manipulation of the Neuromuscular electrical stimulation (NMES) parameters and on muscle response to the modifications of the stimulation characteristics.

Then, on the second chapter the attention is shifted towards the effects of different types of conditioning contraction protocols. In particular, the effects on muscle contraction characteristics will be discussed comparing an electrically induced conditioning contraction protocol and a voluntary one. Also, central and peripheral fatigue topics will be discussed.

The third chapter of the present thesis will be developed on the topics of population aging and muscle adaptations in elderlies. Thus, the evaluation of the local population of elderlies will be reported along with the recent findings from the comparison of a classical resistance training with a novel modality which integrates a NMES conditioning contractions and voluntary contractions.

On the fourth and last chapter of the thesis, additional collaborations performed during the PhD will be briefly introduced.

At the end of each chapter the related scientific production will be attached (refer to List of Publications).

Chapter 1

1.1 Use and application of Neuromuscular Electrical Stimulation

Electrical current can be used to elicit muscle contractions in animal and human muscles, both to evaluate its neuromuscular function and as a rehabilitation and training method (Maffiuletti 2010; Martin et al. 2004). Stimulation techniques are widely used not only in the orthopedic contest (i.e. after surgeries, or fracture), but also in the case of patients with chronic or refractory heart failure, or even following a stroke (Harris et al. 2003; Fitzgerald et al. 2003; Glinsky et al. 2007). Firstly, it is important to consider that the electrical activation signal does not generate action potentials at the muscle fibers level but rather in intramuscular axons, thus differing from a voluntary contraction. This characteristic is of rather importance when trying to evaluate muscle behaviors during an electrically elicited muscle contraction (Enoka et al. 2019).

The so called Neuromuscular Electrical Stimulation (NMES) can be delivered in different ways, for example during functional movements or under isometric condition (Shields and Dudley-Javoroski 2006; Momeni et al. 2019). Moreover, the stimulation can be delivered at the peripheral level (i.e. on the femoral nerve) or with multiple pads directly placed above the muscle belly. Finally, stimulation wave characteristics can be specifically selected to modulate muscle contraction. However, changing only one of the above-mentioned stimulation parameters can deeply influence muscle activation patterns.

Isometric conditions are preferentially selected in rehabilitation protocols to improve strength after musculotendinous injuries or evaluate muscle characteristics, especially in wider muscle mass like the knee extensors, because both the intensity and muscle length can be controlled by the operator (Allen et al. 2018). Using an isometric contraction type is also particularly convenient to easily evaluate muscle mechanical work, which is the result of the force produced by the muscle itself multiplied by its level arm (i.e. the distance between the joint and the point of force application) and again for the total duration of the contraction. On the other hand, functional electrical stimulation (FES) is performed in rehabilitation protocols with the goal to restore limb movements. For example, FES can be delivered to perform a sequence of muscle contractions to perform a task like pedaling or walking, but also to help grasp objects (i.e. key, toothbrush ecc) in people with spinal cord injuries (Marquez-Chin and Popovic 2020; Crameri et al. 2002).

The stimulation site can also influence activation patterns. It is thought that, during NMES performed directly on the muscle belly, Motor Units (MUs) in the superficial portion of the muscle are preferentially recruited, because they are in close proximity with the stimulation pads (Vanderthommen et al. 2002). At the same time, activation decreases in the deeper portion of the muscle. In a study from Mesin and Merletti, the authors evaluated that at a depth of 10 mm in the muscle from the stimulation site, current density decays by 10% of the value applied to the skin (Mesin and Merletti 2008). On the other hand, during stimulation performed on the peripheral nerve tract, it is suggested that also deeper MUs are recruited.

Finally, stimulation wave characteristics can be changed to modulate muscle contraction behaviors (Maffiuletti 2010). The characteristics that can be modified are stimulation frequency (Hz), amplitude (mA), pulse width (μs) and stimulation duration (sec).

Increasing stimulation frequency (>80 Hz) will produce an increment in the excitability of axonal branches therefore limiting the number of recruited MUs over time (Papaiordanidou et al. 2014). While selecting lower stimulation frequency (i.e 30Hz) may reduce this effect preventing, to some extent, neuromuscular propagation failure (Bergquist et al. 2017; Boyas and Guével 2011). 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). At the same time, increasing stimulation amplitude will generate an increase in the muscle force generated, because more MUs are recruited even in deeper portion of the muscle (Gorgey et al. 2006). Therefore, a progressive linear increase in force generation and muscle activated produced by higher stimulation amplitude, will increase muscle tension (Bickel et al. 2011). It has been demonstrated in a study by Adams et al. (2013) that when high stimulation intensities were selected in order to generate a strong muscle contraction (75-100% MVC), magnetic resonance imaging indicated that the recruitment pattern involved different portions of the muscle. Pulse width can also be modified to produce different muscle responses. When larger pulse widths are selected, muscle force generation will increase in a linear manner up until 600 μs. Selecting even higher stimulation pulse width, without modifying other parameters, will not produce a further increase in muscle force production (Bickel et al. 2011). Selecting longer (or shorter) stimulation duration will also affect muscle contractions. In fact, when stimulation is held constant for a few seconds and amplitude is not modified, it is possible to evaluate a constant force decrement over time. For example, stimulation delivered on the muscle belly with longer pulse width, as compared to shorter one, can increase specific tension suggesting a preferential recruitment of fast-fatigable MUs in the muscle (Gorgey et al. 2006).

This considered, when multiple parameters are modified, interpretation of the contraction characteristics is even more complex. However, it is suggested that, generally, an electrically evoked muscle contraction differs from a voluntary contraction. In fact, during a voluntary contraction muscle fibers and MUs follow the Hennemann principle. Therefore, depending on the task that is performed, small and low-fatiguing MUs are recruited first, followed by larger and high-fatiguing one. This principle is suggested to be reverse, at least to some degree, during a conventional NMES paradigm using short pulse width, low frequency, and relatively low stimulation amplitudes. An excellent review paper by Maffiuletti (2010) presented a table which is indicated to synthetize the main differences between a voluntary contraction and an electrically evoked one.

Table 1: Summary of the main differences in recruitment pattern of Motor Units during a voluntary *vs* a NMES muscle contraction (from Maffiuletti et al. 2010)

Voluntary contraction	NMES contraction
Temporal	
Asynchronous	Synchronous
Spatial	
Dispersed	Superficial (close to the electrodes)
Rotation is possible	Spatially fixed
Quasi-complete (even at the maximum)	Largely incomplete (even at the maximum)
Orderly	
Yes, selective (slow to fast)	No, nonselective/random/ disorderly (slow and fast)
Consequence	
Partially fatiguing	Extremely fatiguing

Therefore, it is important to state that, even though the use of NMES is beneficial, like in the case of rehabilitation protocols or to produce a functional movement after spinal cord injuries, it also presents some downsides. In fact, when the muscle is activated by means of an electrical stimulus, it is suggested that MUs are recruited in a random order. This will result in depolarization of both fast and slow muscle fibers, resulting in a less functional activation pattern (Gregory and Bickel 2005; Bickel et al. 2011).

Also, apart from MUs activation pattern, when both pulse width and stimulation amplitude are manipulated to produce the same muscle output, different mechanisms can be responsible for the muscle response and muscle fatigue development. In fact, during stimulation at short pulse width, fatigue could be developed due to intracellular mechanisms while, when selecting higher pulse width concurrently with a reduced stimulation amplitude, the decreased number of engaged MUs could be responsible for the same muscle force decrement (Martin et al. 2016).

1.2 Central Activation Pattern

As stated before, manipulating stimulation characteristics can produce different muscle behaviors in its activation pattern. It has been suggested that, under certain circumstances, the muscle can be activated via central or peripheral pathways. In fact, it was suggested previously that, when selecting long stimulation pulse width (i.e. 1000 µs) together with high stimulation frequency (i.e. 100Hz), it is possible to facilitate the activation of spinal motor neurons via afferent pathways, resulting in an initial recruitment of smaller, fatigue-resistant MUs. In fact, the evoked action potentials can have a double propagation direction. Not only the depolarization wave can partially propagate orthodromically (in the physiological propagation direction), but it can also partially travel in the opposite antidromic direction (Yamada and Nagata 2012). The same mechanisms can be evaluated when investigating the Hoffmann reflex (H-reflex). Studies on the H-reflex highlight that, when the stimulating electrodes are attached to the skin close to a peripheral nerve or muscle belly it is possible, with a single stimulus, to elicit action potentials in sensory axons and eventually motor axons. However, it is suggested that the contribution of central pathways can differ when NMES is applied over a nerve trunk compared with over a muscle belly. In a study from Bergquist AJ (2012), H-reflex and M-wave (the purely motor response) generated via stimulation applied on the muscle belly or the peripheral nerve tract was compared. During stimulation performed over the nerve as compared to the muscle, H reflexes were two to three times larger, and the asynchronous activity was recorded regardless of stimulation location. Thus, it is possible that an electrical stimulus that engages also spinal motor neurons via afferent pathways in an orthodromic direction will reproduce a more physiological recruitment order (Dean et al. 2007). As mentioned above, because afferent pathways are activated, this activation pattern is also referred to as Central activation pattern. This phenomenon has been observed during a train of electrical stimuli not only in healthy subjects but also in individuals with a spinal cord injury (Collins 2007; Arpin et al. 2019). Collins et al. (2007) first demonstrated that an NMES burst delivered using a subthreshold intensity above the tibialis anterior and triceps surae, elicited action potentials in sensory axons and evoked a muscle response that ranged between 2 and 10% MVC. To further demonstrate the involvement of afferent pathways, a nerve block was performed, and the motor response was abolished (Lagerquist et al. 2009; Blouin et al. 2009).

On the other hand, with the term "peripheral activation" we refer to activation of muscle fibers without the engagement of central structure (i.e. afferent fibers). Therefore, it is important to consider that a central activation pathway, that is more physiological, could prevent to some extent the fatigue component in the muscle that is stimulated.

Moreover, it is important to highlight that, when stimulation is delivered at high frequency and at long pulse duration to engage large sensory diameter afferents, stimulation intensity needs to remain low. This way, because type Ia afferents have a lower rheobase and longer strength-duration time constant, low stimulation amplitude is needed to depolarize those fibers and prevent antidromic collusion. The evoked depolarization waive can then recruit motoneurons within the spinal cord in a reflexive manner and following the size principle (Veale et al. 1973; Collins et al. 2001).

Another characteristic that seems of key importance when trying to engage a central pattern contraction mechanism is stimulation duration. At first, it was demonstrated that a 20sec stimulation train was necessary to produce a contraction by central mechanisms (Dean et al. 2007). However, previously Collins et al (Collins et al. 2001, 2002) demonstrated that a 7 sec stimulation train is also effective to discharge spinal motoneurons in the spinal cord. More recently, it was also demonstrated that a stimulation train of 0.5 sec is also sufficient to elicit a central activation in knee extensors and plantar flexor muscle groups in a population of subject with a spinal cord injury (Arpin et al. 2019).

1.3 Muscle Metabolism during electrically evoked protocols

Along with activation pattern, it is of key importance to also consider the type of metabolism that is involved during an electrically elicited muscle contraction.

Previously, it was demonstrated that when short pulse width (<600μs) is selected, because of the preferential MUs recruitment pattern that is involved, muscle fibers that are preferentially activated are type II with low oxidative capacity (McNeil et al. 2006). For example, when NMES is delivered at 25Hz stimulation frequency and 50μs pulse width on plantar flexors, tissue saturation was higher than during a voluntary contraction generating equal muscle output (50%MVC). Different levels of tissue saturation suggest that MUs that are preferentially recruited during the electrically evoked bout were the ones less capable of utilizing oxygen in order to resynthesized ATP (McNeil et al. 2006). Also, when comparing an electrically induced muscle contraction (25Hz and 250μs pulse width) with a voluntary contraction, the latter will induce an increased metabolic demand for an identical mechanical level (10% MVC) (Vanderthommen et al. 2002). More specifically, interleaved ^{1}H- and ^{31}P-NMR spectroscopy highlighted a larger increase in Pi-to-phosphocreatine ratio, increase pH and myoglobin saturation during an NMES bout as compared to an equivalent voluntary task. This suggests a different pattern in fibers recruitment, with preferential involvement of type II muscle fibers during an NMES bout performed to engage peripheral pathways (Vanderthommen et al. 2002).

As mentioned above, when NMES is performed to recruit MUs via peripheral pathways, it is possible that an excessive metabolic demand is generated due to the synchronous activation of fibers in a spatially fixed order (Jubeau et al. 2015).

Another example is represented by an NMES bout delivered at 50Hz and 250μs pulse width at relatively low muscle output (10% MVC) during which metabolic interleaved ^{1}H- and ^{31}P-NMR spectroscopy was used to measure Pi and PCr ratio, pH and myoglobin desaturation. During the NMES bout, the Pi to PCr ratio, and pH and myoglobin saturation decreased significantly, reaching values comparable with a voluntary contraction performed at 6 times the one generated with NMES (i.e. 50-60% MVC) (Vanderthommen et al. 2003). This suggests that, at least for these specific stimulation parameters, motor units are mainly recruited via efferent pathways, activating preferentially type IIa/b muscle fibers with lower oxidative capacity strongly impacting the metabolism involved in the elicited contraction. Similarly, when a force equal to 20% MVC was generated with an electrical stimulus, an increased Pi/PCr ratio and decreased pH were identified as compared to a voluntary contraction performed to match the muscle mechanical work of the NMES bout (Vanderthommen et al. 1999). This further suggests that, when peripheral pathways are preferentially engaged during an NMES bout, it causes an exaggerated metabolic demand to develop muscle contraction as compared with a voluntary contraction (Vanderthommen et al. 1999).

Furthermore, interesting results have been achieved when comparing two NMES paradigms and a voluntary one by mean of ^{31}P magnetic resonance spectroscopy (Wegrzyk et al. 2015). In particular, an NMES task performed at short pulse duration (50μs) and at low frequency (25Hz) was compared with an NMES task performed at long pulse width (1000μs) and at high frequency (100Hz). The three protocols were applied on plantar flexor muscles in order to elicit 10% of MVC. Authors highlighted that a higher metabolic demand (ΔPCr) was evaluated for the stimulation performed at low frequency and short pulse width as compared to the voluntary task. While a stimulation performed at high frequency and long pulse width shown a lower metabolic demand than the low frequency and short pulse width paradigm but similar to the voluntary one (Wegrzyk et al. 2015).

Therefore, when selecting a stimulation paradigm for re-training and rehabilitation purposes it is important to not only carefully evaluate the underneath muscle activation patterns, but also the consequences for the type and degree of metabolism involved.

1.4 Aim of Project 1

Taken into considerations all these factors, in the first project of the present PhD, the main objective was to compare the effects of two NMES muscle contraction protocols on oxygen fractional extraction of knee extensors in healthy adults (n=14) and in participants with a spinal cord injury (SCI) (n=6). The Near Infrared Spectroscopy (NIRS) Technique was used to evaluate oxygenated and deoxygenated myoglobin in the region of interest which was the *vastus lateralis* muscle.

The two NMES protocols were selected in order to manipulate solely one parameter which is pulse width, while maintaining a fixed stimulation frequency (100Hz). Pulse width selected were 200µs and 1000µs (i.e. short and long pulse width). Also, in order to compare the two protocols, the stimulation intensity was carefully adjusted in order to generate a match in the muscle mechanical output. It was hypothesized that a long pulse width (1000µs) and higher stimulation intensity of 100Hz would promote a higher muscle fractional oxygen uptake than the short pulse width protocol because of the partial activation of smaller MUs

1.5 Main Results of Project 1

The main results from the first project of this PhD were:

i) The two NMES paradigms elicited equal mean torque and muscle mechanical work throughout the whole task (i.e. muscle fatigue) both in healthy and in spinal cord injured participants, even though absolute force production was lower in SCI subjects;

ii) Muscle contractile properties, calculated as the Rate of Torque Decrement (RTD) and the Half Relaxation Time was equal in the two NMES paradigms and in both groups;

iii) NMES performed by selecting long pulse width (1000μs) produced higher muscle fractional oxygen extraction than the short pulse width (200μs) protocol in healthy participants but not in SCI ones.

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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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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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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 $\pm$ 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

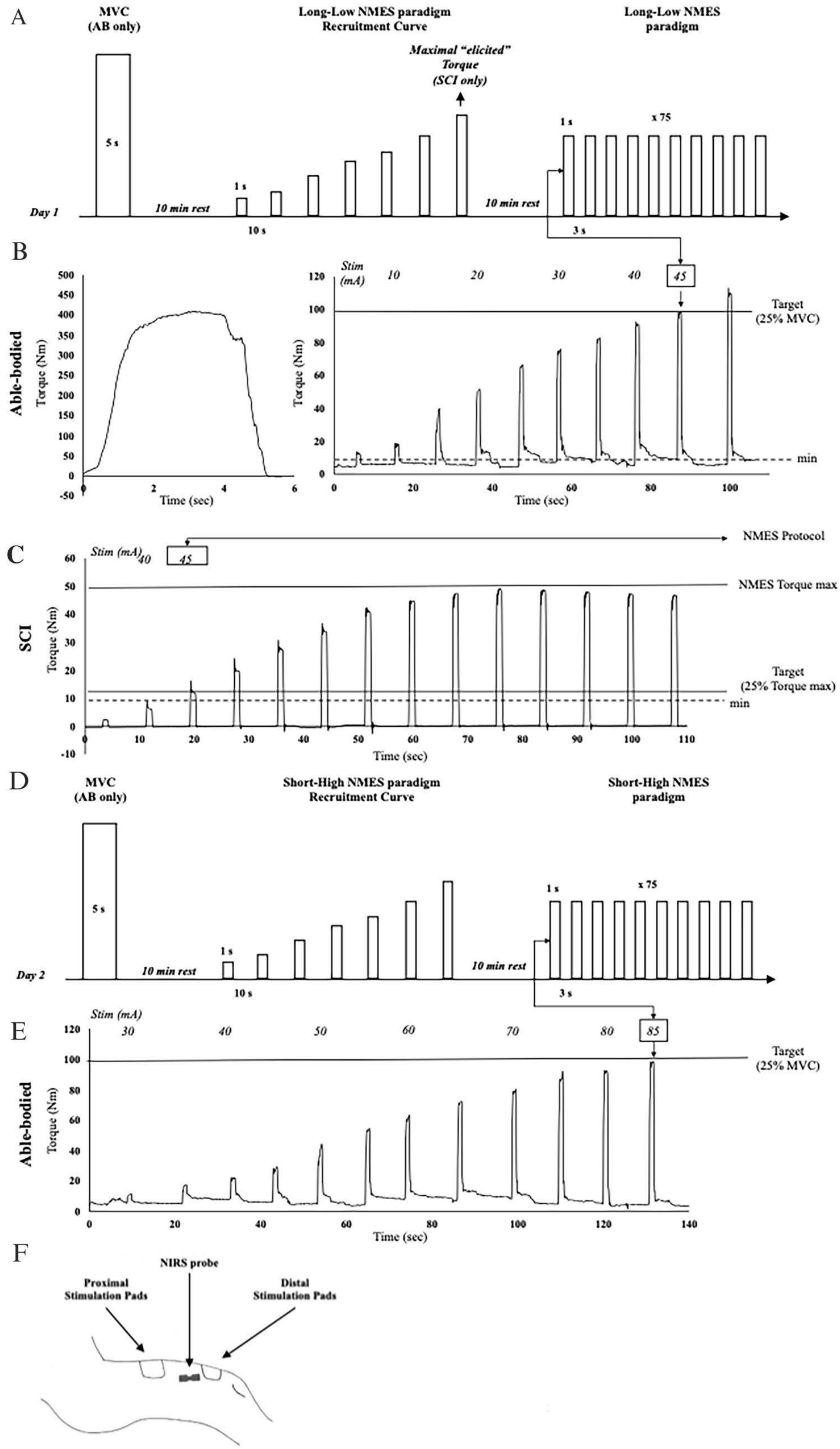

Fig. 1 Representation of the experimental protocol. **a–c** Maximal Voluntary Contractions (MVC, for able-bodied only), neuromuscular electrical stimulation (NMES) recruitment curve and NMES protocol (75 contractions, 1-s on–3-s off, fixed stimulation intensity) with long-low NMES parameters (1000 µs 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

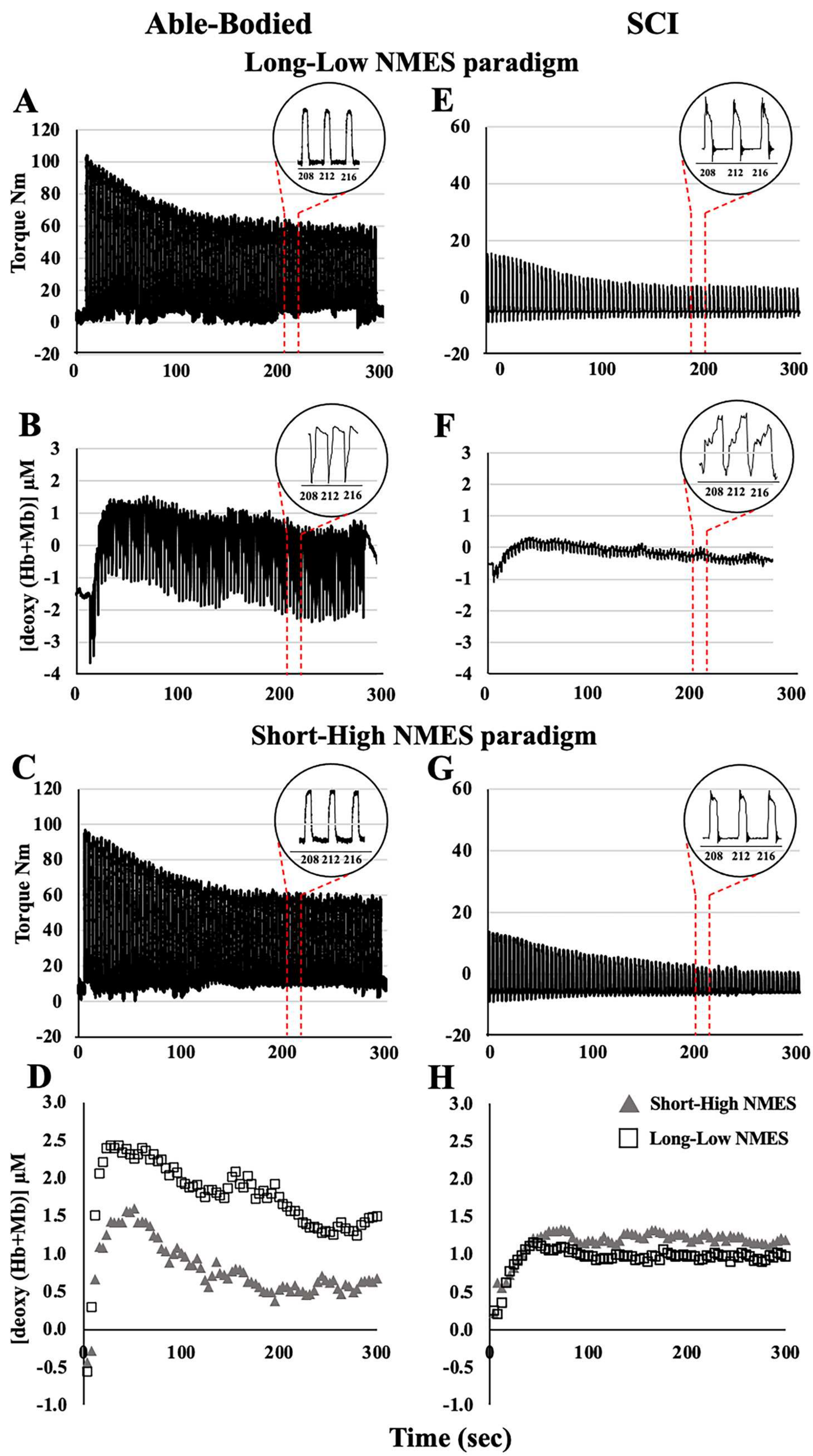

Fig. 2 Raw torque and [deoxy(Hb + Mb)] near infrared spectroscopy (NIRS) data from representative able-bodied (**a–c**) and spinal cord-injured (SCI) (**e–g**) individuals during long-low (1000 µs pulse width) and short-high (200 µs pulse width) NMES. Mean [deoxy(Hb + Mb)] values for each contraction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

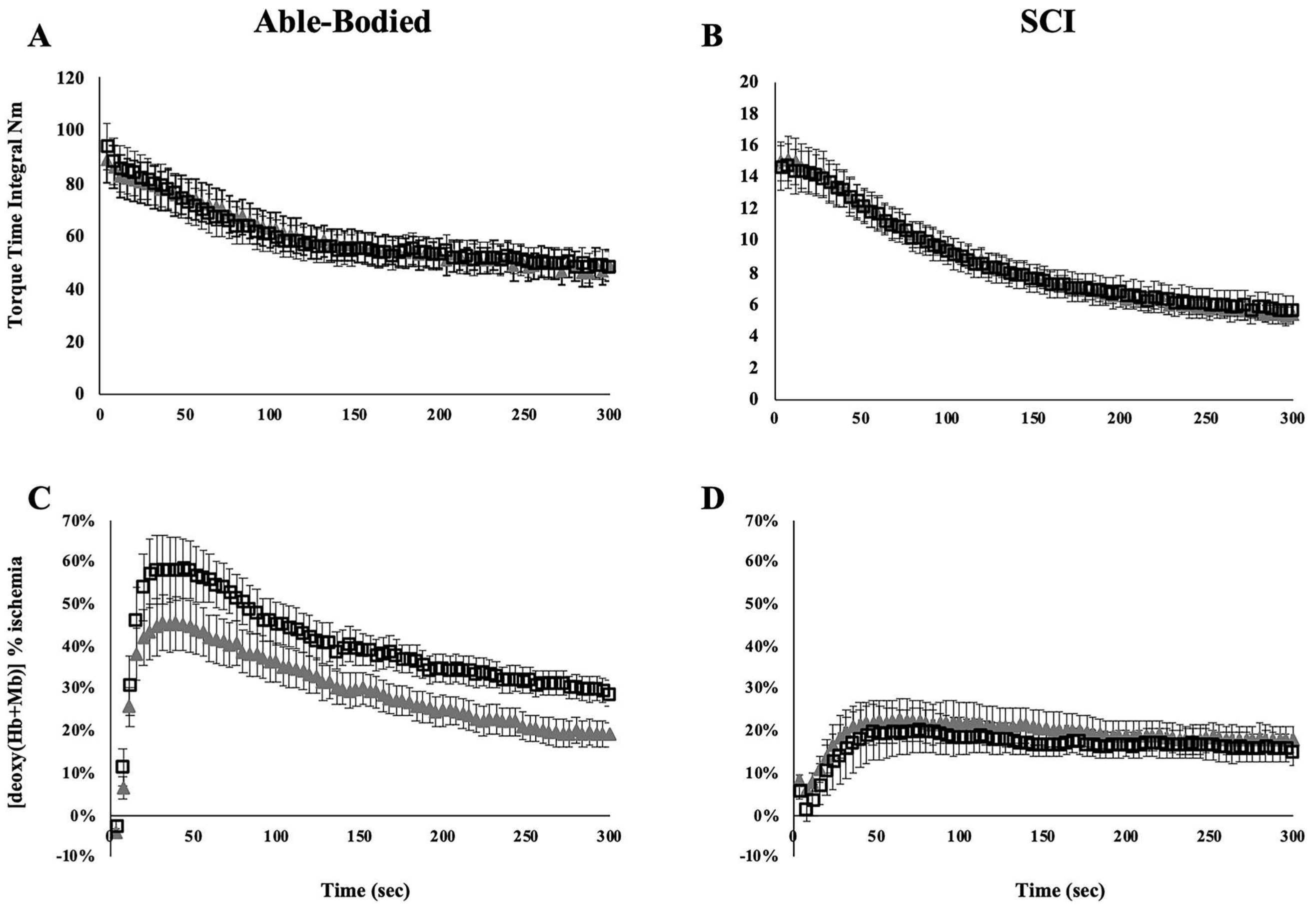

Fig. 3 Mean values in Torque–Time Integral (Nm) (during the 1-s contraction period) and [deoxy(Hb + Mb)] (% ischemia) for short-high (gray triangle) and long-low (empty square) NMES parameters in able-bodied (**a, c**) and Spinal Cord Injury subjects (**b, d**)

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.

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Compliance with ethical standards

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

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Chapter 2

2.1 Conditioning contractions can influence muscle performance

Activation history can affect the properties of the involved muscle. This is the example of a conditioning contraction, which can influence either positively or negatively a following muscle contraction of the same district. The positive effect produced on the muscle is also called Postactivation Potentiation (PAP) and can produce a sharp enhancement of the contractile properties such as Peak Torque, twitch Peak Torque, and Rate of Force Development (RFD) (Hodgson et al. 2005; Sale 2002). On the other hand, activation history that negatively affects muscle performance is referred to as a fatiguing muscle contraction which eventually impairs muscle performance (Stone et al. 2008). An increase in the contractile properties can be produced by a maximal or submaximal voluntary contraction produced under either concentric, eccentric, or isometric conditions (Mitchell and Sale 2011). After a conditioning contraction, it is possible to evoke a twitch potentiation usually manifested as a shortening of twitch contraction and half-relaxation times (Vandervoort et al. 1983; Hamada et al. 2000), but also as an increase in twitch rate of torque development (Baudry and Duchateau 2004). Moreover, potentiation can be produced also via an electrical stimulus (Krarup 1981; Vandervoort et al. 1983). In the case of a voluntary conditioning contraction, intensity ranges between 100% and 75% of a maximal voluntary contraction (MVC), while contraction duration ranges between 10 and 5 seconds (Sale 2004; Sweeney and Stull 1990; Smith et al. 2014). After a conditioning contraction that is performed by means of an MVC, twitch potentiation is maximal immediately after the conditioning stimulus and then tends to decline over time. However, its effect can be seen even 5 to 10 min after the conditioning event (Hamada et al. 2000).

The mechanisms involved in the potentiation effect are related to calcium release and spinal excitation. In fact, during a conditioning contraction, myosin regulatory light chains are phosphorylated causing an increased sensitivity to Ca^{+2} ions in the actin-myosin complex. Therefore, during a subsequent contraction, Ca^{+2} release in the sarcoplasmic reticulum increases effectiveness and force production of the twitch contraction (Sweeney et al. 1993; Grange et al. 1993). Additionally, it is possible that a strong muscle contraction performed prior to an explosive exercise would produce an increased synaptic excitation at the spinal cord level, therefore producing an increase in post synaptic potentials and thus an increase in force production (Rassier and MacIntosh 2002). However, the following proposed mechanism is mainly involved in the case of complex training strategies.

As mentioned before, preceding a muscle contraction with a conditioning contraction can also produce fatigue in the muscle itself, thus preventing the positive outcomes of PAP effect. Fatigue can develop not only at high percentage of MVC, but also during a submaximal contraction. In fact, during a sustained low level muscle contraction (i.e. around 5-30% MVC), both central and peripheral fatigue can increase in the muscle, therefore preventing its optimal performance (Taylor and Gandevia 2008). When fatigue is accumulated in the muscle, in order to maintain the same muscle output, it is necessary for the subject to intensify the effort and increase the firing rate of MUs and/or involve new ones. However, when a further increase or maintenance in the descending drive is not possible, it leads eventually to muscle fatigue (Smith et al. 2007; Søgaard et al. 2006).

It is also important to consider that the PAP effect is influenced not only by muscle contraction characteristics, but also by fiber type content of the involved muscle. In fact, type II muscle fibers, with higher explosive characteristics, are strongly affected by potentiation as compared to muscles with a predominancy of type I fibers (Sweeney et al. 1993; Grange et al. 1993).

Related to this, another aspect that needs to be considered is that it is possible to produce potentiation not only via a voluntary conditioning contraction but also by an electrical stimulus (Smith et al. 2014). This method can even produce higher potentiation effects than a voluntary contraction. As mentioned above, type II muscle fibers are more sensitive to potentiation than type I fibers, and the latter are the main target during an NMES task. When fast type fibers are recruited during an electrical stimulus at a low force level, they can produce an even higher force than a voluntary contraction performed at the same force level (Feiereisen et al. 1997; Smith et al. 2014). During a relatively long (7 sec) low-level (25% MVC) NMES bout, potentiation effect can reach even an increase of more that 110% in the peak twitch torque in comparison to rest. In the study from Requena, the same level of potentiation was not produced by a voluntary conditioning contraction, performed at the same muscle output, as the electrically evoked conditioning contraction (Requena et al. 2008). Another study from Binder-Macleod et al. (2002) performed a stimulation task selecting frequencies of 14 Hz or higher, and demonstrated that, in order to evoke a positive potentiation effect, the number of electrical pulses delivered to the muscle was the principal factor.

Moreover, applying a voluntary conditioning contraction, rather than evoked tetanus, may affect potentiation in the following ways: peripherally, by influencing myosin phosphorylation and other intramuscular processes; or centrally, by modifying the motor nervous system behaviors near the level of the neuromuscular junction and at cortical level (Smith et al. 2014).

For these reasons, it is important to also evaluate that a conditioning contraction can have a negative effect on muscle performance causing fatigue occurrence bot that the peripheral and central level. In fact, during an electrical stimulus the supraspinal structures are not involved in the task, thus preventing the fast development of supraspinal (central) fatigue which might negatively affect potentiation of the following voluntary contraction (Gandevia et al. 1996; D'Amico et al. 2020). It has been observed that following both brief and sustained voluntary contractions corticomotor excitability is reduced and this effect can last even for 20 minutes after the contraction (Teo et al. 2012; Sacco et al. 1997). At the same time, failure to activate the muscle was highlighted even for a maximal contraction sustained for 2 minutes or performed repeatedly at 1-min intervals (Taylor et al. 1996; Babault et al. 2006). Therefore, supraspinal and spinal voluntary control pathways can be overcome by an electrical stimulus, which can induce a potentiation effect but without engaging those exquisitely central structures responsible for central fatigue (Smith et al. 2014).

However, we reported a lack of strong evidence regarding the use of a submaximal isometric contraction (voluntary or electrically induced) in rehabilitation or strength training protocols.

2.2 Central and Peripheral Fatigue

We can affirm that, during a conditioning contraction, potentiation and fatigue are two sides of the same coin. The concept of potentiation was introduced in the previous paragraph, therefore, before moving to the project's details, it is important to briefly contextualize also the two aspects of fatigue: central and peripheral muscle fatigue.

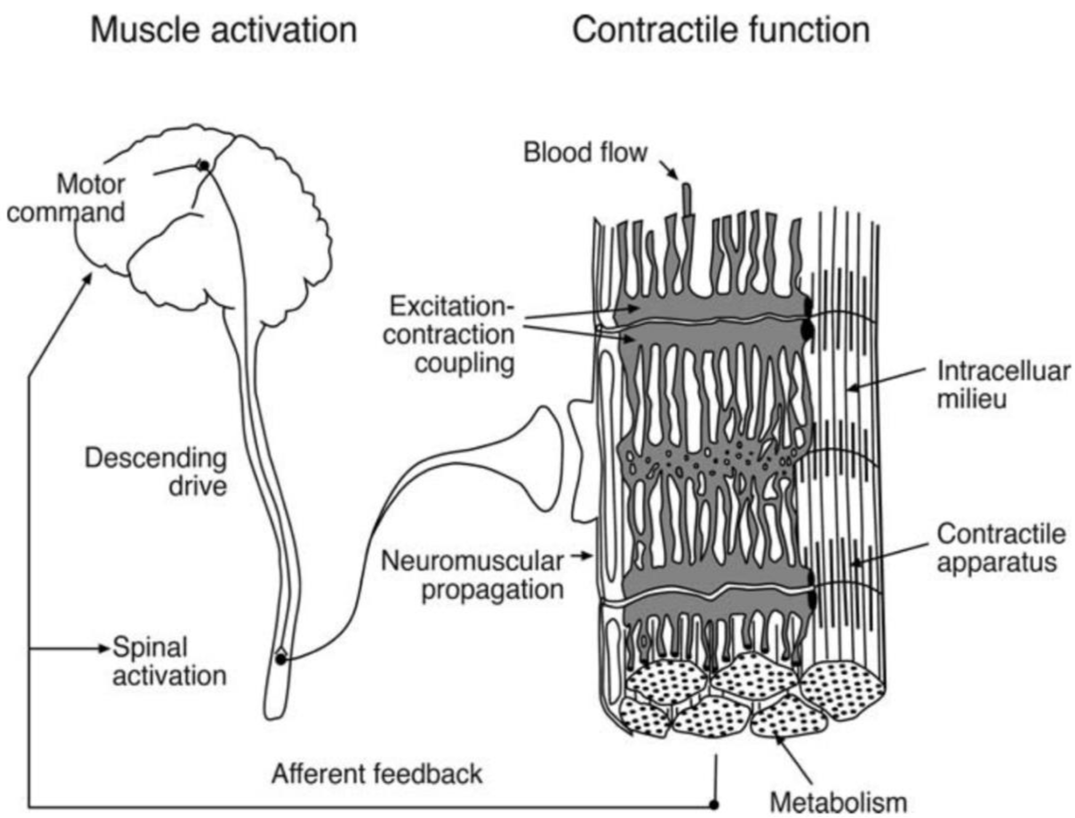

Figure 1.2: Schematical representation of fatigue development sites that is divided into two main categories: central (muscle activation) and peripheral (contractile function). From Mosso A et al. 1904, Fatigue.

As previously mentioned, muscle fatigue is induced by a task/exercise and typically is exhibited by a decrease in the maximal voluntary muscle force or even by the inability of the subject to maintain the required task and eventually reaching total exhaustion. The reduction in muscle output can be triggered by peripheral factors that happen between the neuromuscular junction and the muscle cells. At the same time, the central nervous system is unable to drive the motoneurons in order to maintain the desired muscle output (Gandevia 2001). Voluntary activation during a maximal contraction is usually evaluated via the twitch interpolated technique and gives an insight of the ability of the central nervous system to drive the maximal activation of the muscle. Therefore, it is widely used as an index of central fatigue (Gandevia 2001). Similarly, Transcranial magnetic stimulation over the motor cortex is also used to evaluate differences in cortical excitability and decline in supraspinal drive. Central fatigue can also be described as a subjective sense of fatigue and the related signal to the motoneuron pools during the fatiguing task is initiated even before a reduction in the exerted muscle force is detectible (Carpentier et al. 2001; Farina et al. 2004). At the same time the central nervous system receives sensory feedbacks coming from the periphery which are transmitted by type III and IV afferent fibers and contribute to the perception of fatigue (Martin et al. 2008). Therefore, it is not easy to consider in a separate manner both the decline in muscle output triggered in the muscle itself and fatigue sensation (Taylor and Gandevia 2008). At the same time, fatigue and the underneath adjustments vary depending on the task that is performed (Enoka and Duchateau 2008). For example, blood glucose, temperature, arousal level and even mood are modulating factors of perceived fatigue but can also modulate voluntary activation which will produce a declined performance (also called performance fatigability) (Nybo 2003; Enoka and Duchateau 2016). At the same time, type III and IV afferent fibers influence those central factors during a high intensity exercise and thus contribute to perceived fatigability (Kennedy et al. 2015).

For these reasons, it is not always easy, or even possible, to completely distinguish between the two sources of fatigue, especially during sustained low-level muscle contractions. Therefore, in the following project, some inferences on muscle potentiation (described in the previous chapter) and muscle central or peripheral fatigue will be made. However, we would like to remind that the fundamental purpose of the following project was not the detection of the degree and level of fatigue, but rather the source or muscle performance potentiation.

2.3 Aim of Project 2

Considering the above introduction and the findings from the first project, in the second project of this PhD we focused on evaluating the effects of a voluntary and a NMES-elicited conditioning contraction protocols on a subsequent voluntary explosive muscle contraction of knee extensors. It was hypothesized that an electrically evoked conditioning contraction would enhance explosive characteristics (i.e. increased RFD) of a following voluntary contraction to a higher extent than a voluntary conditioning contraction performed at the same muscle output.

The results from this study will be useful in the future to implement rehabilitation protocols or to design a new training paradigm in elderly population.

2.4 Main Results of Project 2

The main result from the second project of this PhD were:

i) Applying an electrically induced *vs* voluntary generated conditioning contraction enhances (+38%) neuromuscular characteristics (i.e. RTD) of an isometric voluntary contraction of knee extensors at 70% MVC . Explosive characteristics were evaluated by means of the RTD, calculated in the 0-50ms and 0-100ms time windows;

ii) The Time to reach the Peak Torque of the explosive voluntary contraction was significantly lower (-11%) when the contraction was preceded by an electrically evoked conditioning contraction instead of a voluntary one;

Effects of NMES-elicited versus voluntary low-level conditioning contractions on explosive knee extensions

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Running Title: NMES conditioning contraction effects on explosive knee extension

Key words: Rate of Torque development, Explosive contraction, Electrical Stimulation, Afferent pathways

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Abstract

OBJECTIVES: Electrically or voluntary conditioning-contractions (CC) can be used to effect contractile properties of a subsequent explosive contraction (EC). Here, we aimed at comparing the effect of neuromuscular-electrical-stimulation (NMES) vs voluntary CC performed prior to explosive contractions of the knee extensors.

METHODS: A 10 sec NMES CC (100Hz, 1000μs, 10% MVC) was performed followed by an isometric EC of the knee extensors and was compared with a voluntary contraction (VOL CC) mimicking the NMES CC. Explosive contraction was performed with the goal to reach the target (70% MVC) as quickly as possible.

RESULTS: All the parameters related with the explosive contractions' muscle-output were similar between protocols (difference ranging from 0.23%, Mean Torque; to 0.24%, Time to Target). Time to Peak Torque was lower when preceded by NMES (-12.7%, p=0.019). Interestingly, the RTD 0–50 ms_EC was 37.3% higher after the NMES compared with the VOL CC protocol.

CONCLUSION: Explosive contraction was potentiated by an NMES CC as compared with a voluntary CC. This may be due to reduction in descending drive that has been shown to occur even with low-level voluntary efforts. These results could be used to implement rehabilitation or training protocols that also includes conditioning contractions.

Introduction

The neuromuscular system can be conditioned by its activation history. For example, active warm-up leading to an increase in muscle temperature can improve short-term performance by influencing neural properties such as the transmission rate of nerve impulses, muscular properties such as force-velocity relationship or muscle stiffness, and by enhancing bioenergetics mechanisms [1].

Also, post-activation potentiation resulting in acute enhancement of contractile properties (i.e. peak torque and rate of torque development) can be promoted by brief, high-intensity conditioning muscle contractions (CC). The type of muscle contraction (isometric or dynamic) does not seem to influence potentiation characteristics [2]. Potentiation can be achieved by electrically-evoked muscle contractions as well as maximal and submaximal voluntary contractions [3, 4]. When potentiation is achieved voluntarily, the contraction level usually ranges between 100% and 75% MVC, with a duration ranging between 5 and 10 seconds [5-7]. Mechanisms that are involved in potentiation effects are linked to calcium release. In fact, after a conditioning contraction sensitivity to Ca^{+2} is increased because of the phosphorylation of myosin regulatory light chains by the myosin light chain kinase [8, 9].

However, neuromuscular system activations preceding a given effort may also result in fatigue and impaired muscle output. This phenomenon can occur even during submaximal muscle contractions, during which both central and peripheral fatigue can be accumulated in the muscle [10]. For example, during a sustained low level muscle contraction at 5-30% MVC, when active muscle fibers fatigued subject needs to increase its voluntary effort and engage more motor units and/or increase firing rate. A possible contributor for central fatigue is a decreased descending drive from cortical structure [11, 12].

Neuromuscular electrical stimulation (NMES) might have also higher effect on potentiation as compared to a voluntary low-level muscle contraction. In fact, during NMES-elicited muscle contraction, type II muscle fibers contribute to a greater extent to force generation. Larger MUs are primarily recruited via efferent pathways during the electrical stimulus and have high force generation capacity as compared to a voluntary contraction at the same low level muscle output [13, 14]. Therefore, NMES has been implemented to generate conditioning contractions at lower muscle force output. For example, findings from Requena et al. [15] show that isometric peak twitch torque of knee extensors are potentiated (+117% compared to rest) after a 7 sec electrically evoked contraction at 25% MVC as compared to a voluntary contraction at the same muscle torque. A possible upside of using NMES for conditioning contractions preceding voluntary explosive efforts is that supraspinal voluntary neural drive to the muscle is not involved, and this may have a positive impact on how supraspinal fatigue would affect the subsequent explosive efforts. Another property of NMES applied with long pulse width, high frequency, low amplitude and long duration (long-low NMES) is that it may also lead to spinal circuitry and motor neuron activation via afferent pathways, contributing to a more physiological recruitment order of motor units [16, 17].

In this study, we aimed at assessing the effects of voluntary or long-low NMES-elicited conditioning contractions on the subsequent voluntary explosive muscle contractions performed to simulate a training intervention. We hypothesized that long-low NMES-elicited conditioning contractions would promote better contractile properties of the voluntary explosive efforts as compared to voluntary conditioning contractions.

Materials and Methods

Subjects

A total of 20 subjects (15 males and 5 females) recruited at the School of Sport Sciences (University of Udine, Italy) participated in this study. Mean age was 26 ± 7 (years), stature was 1.79 ± 0.08 (m) and body mass was 75.3 ± 13.8 (kg), with BMI equal to 23.5 ± 3.0 ($kg \cdot m^{-2}$). Subjects were healthy, moderately active and had no history of orthopedic and neurological injuries. The experimental protocol was conducted in accordance with the declaration of Helsinki, and was approved by the Institutional Review Boards of University of Udine (Italy) (9/IRB DAME_17).

Before the study began, the purpose and objectives of the study were carefully explained to each subject and written informed consent was obtained.

Experimental procedures

All participants visited the laboratory twice, and each visit was separated by at least 48 h. Subjects were asked to refrain from any strenuous activity 24 h before each testing day. Each experimental session lasted between 1 and 1.5 h (Figure 1).

During the first experimental session, anthropometric measurements preceded the assessment of maximal voluntary contraction (MVC) of knee extensors. After a 10-minute break, research subjects underwent the NMES recruitment curve to assess the relationship between NMES amplitude and torque output of knee extensors. After additional 10 minutes of rest, the experimental protocol consisting of NMES-elicited conditioning contractions interleaved by voluntary explosive knee extensions (NMES-CC protocol) was performed. During the second experimental session, MVC of knee extensors was retested to assess the neuromuscular status and compare it with the first experimental session. After 10 minutes of rest, the experimental protocol including voluntary conditioning contractions interleaved by voluntary explosive knee extensions (VOL CC protocol) was performed. VOL CC protocol was always performed on the second experimental session in order to optimize the matching of the torque output generate by the NMES-elicited conditioning contractions.

Anthropometric measurements

Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board. Body mass index (BMI) was calculated as BM (kg) · stature^{-2} (m).

Maximal voluntary contraction of knee extensors

Participants performed MVCs of the right knee extensors while sitting on the isometric dynamometer previously described by Rejc and colleagues [18]. Hips and knees were flexed at 90° and a crossover shoulder strap and a strap around the ankle (5 centimeters proximal to the malleoli) were set in order to minimize movements of the trunk and leg.

During the initial warm up each participant was instructed to generate between 20 and 30 four seconds contractions, at a self-selected and increasing intensity. After a 3-minute rest period, participants were asked to perform a maximal isometric knee extension of approximately 6 seconds. Three MVC attempts were performed, separated by a 5-minutes 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 join 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. 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.

To evaluate muscle activation, electromyography (EMG) activity was recorded from the vastus lateralis (VL), rectus femoris (RF) and biceps femoris (BF). First, the skin was shaved, rubbed with abrasive paste, and cleaned with a paper towel. Then, pre-gelled surface EMG electrodes (type N-00-S/25, Ambu A/S, Denmark) were placed, with an interelectrode distance of 20mm, at the midpoint between the anterior superior iliac spine and the superior portion of the patella for the RF muscle, at the two-third of the distance between the anterior superior iliac spine and the lateral portion of the patella for the VL muscle and at the midpoint between the ischial tuberosity and the lateral epicondyle of the tibia for the BF muscle [19]. A four-channel electromyography system was used (EMG100C, BIOPAC Systems, Inc., USA; Low Pass Filter: 500 Hz; High Pass Filter: 10 Hz; Noise

Voltage (10–500 Hz): 0.2 lV (rms); Zin: 2 M ohm; CMRR: 110 dB). Data was sampled at 2kHz using a data acquisition system (MP100, BIOPAC Systems, Inc., USA) and processed using MatLab2016. EMG activity of each muscle was assessed by calculating the Root-mean-square (RMS) applying a 0.5 sec overlapping moving window and then expressed as a percentage of the maximal EMG activity during the MVC (RMS %MVC).

NMES recruitment curve

After at least 10 minutes from the end of MVC testing, the relationship between NMES amplitude and peak torque exerted (i.e. recruitment curve) [20] was assessed. Stimulation pads (size: 5×10 cm; Axelgaard Manufacturing Co., Ltd., Fallbrook, CA) were positioned above the quadriceps muscle belly with the distal portion placed at 50% and 10% of the distance between the anterior superior iliac spine and the superior margin of the patella, for proximal and distal pads respectively [21]. Then, an electrical stimulator (Digitimer DS7A, Hertfordshire, UK; Maximal Voltage 400V) was used to deliver a 1 second monophasic positive rectangular waveform with 1000µs pulse width at constant frequency and voltage of 100 Hz and 400 V, respectively. Stimulation trains were delivered to the muscle every 10 seconds. NMES was applied starting with a stimulation amplitude of 5 mA, and increasing it by 5 mA for every subsequent stimulation until a minimum torque equal to 10% of Peak Torque was elicited and either the participant requested to stop the stimulation because of discomfort or the recruitment curve reached a plateau.

NMES-conditioning contraction protocol

On the first testing day participants performed voluntary explosive contractions preceded by long-low NMES-elicited conditioning contractions.

This experimental protocol was performed 10 minutes after the NMES recruitment curve. NMES CC protocol consisted of twelve 10-sec NMES-elicited contractions, with 20 sec in between each NMES CC. NMES frequency and amplitude were maintained at 100 Hz and 1000 us, respectively; amplitude was selected in order to initially elicit a torque output equal to 10% MVC based on the recruitment curve described above. Based on preliminary observations, the first 3 NMES CCs were delivered with the goal of priming the neuromuscular system and activating the spinal circuitry via afferent pathways. NMES CC and voluntary explosive knee extensions were interleaved from the fourth to twelfth NMES CC. In particular, participants were instructed to perform the voluntary explosive knee extension immediately after the end of NMES CC, aiming at reaching the target of 70% MVC as fast as possible, and maintaining it for 3 sec. Real-time visual feedback of torque output was provided.

Onset and offset of each 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. Peak Torque was calculated by applying a 0.5 sec moving window for explosive voluntary contractions (Peak Torque_EC). Mean Torque was calculated for both NMES-elicited conditioning contractions (Mean Torque_CC) and for voluntary explosive muscle contractions (Mean Torque_EC) by the Mean Torque value from the beginning to end of each contraction. Torque-time integral (TTI) was calculated to estimate muscle work of the electrically induced conditioning contractions (TTI_CC) and of the explosive voluntary contractions (TTI_EC). Rate of torque development of the explosive voluntary contraction was computed over the time windows 0–50 ms (RTD 0–50 ms_EC) and 0–100 ms (RTD 0–100 ms_EC). EMG values were used to assess any possible markers of afferent pathways activation (RMS VL_marker; RMS RF_marker; RMS BF_marker) during the NMES CC protocol. In particular, only for the first 3 conditioning contractions performed without a following explosive contraction, the RMS marker analysis was carried by selecting a 4 second time window 0.5 sec after the end of the NMES-elicited conditioning contraction. Then, the RMS marker values were compared to RMS baseline values. Also, EMG values were used to assess muscle electrical activation during the explosive voluntary contractions (RMS VL_EC; RMS RF_EC; RMS BF_EC).

Voluntary-conditioning contraction protocol

During the second experimental session, participants initially performed MVC of knee extensors in order to compare their neuromuscular status with the first experimental day. After 10 minutes of rest, participants performed the VOL CC protocol, which mirrored the NMES CC described above; the only difference was that conditioning contractions were performed voluntarily (and not by NMES). In particular, participants were asked to perform VOL CC matching the Mean Torque evoked by NMES during the first experimental session. As for the NMES CC protocol, EMG values were used to assess muscle electrical activation during the explosive voluntary contractions (RMS VL_$_{EC}$; RMS RF_$_{EC}$; RMS BF_$_{EC}$) during the VOL CC protocol.

Statistics

All results are expressed as mean and standard deviation (SD). Normal distribution of the data was tested using the Kolmogorov–Smirnov test. Sphericity (homogeneity of covariance) was verified by the Mauchly's test. When the assumption of sphericity was not met, the significance of the F-ratios was adjusted according to the Greenhouse–Geisser procedure. The comparison between the NMES CC and VOL CC protocols parameters of Peak Torque (Peak Torque_$_{EC}$), Mean Torque (Mean Torque_$_{CC;}$ Mean Torque_$_{EC}$), Torque Time Integral (TTI_$_{CC;}$ TTI_$_{EC}$), Rate of torque development over the time windows 0–50 ms (RTD 0–50 ms_$_{EC}$) and 0–100 ms (RTD 0–100 ms_$_{EC}$), RMS VL_marker, RMS RF_marker, RMS BF_marker, RMS VL_$_{EC}$, RMS RF_$_{EC}$, RMS BF_$_{EC}$ were performed by applying a paired T-test using GraphPad Prism 7.0 with significance set at $p \leq 0.05$.

Results

Subjects generated similar MVC of knee extensors in the two experimental sessions, (227±69 and 226±70; p=0.395) indicating a similar neuromuscular status. As reported in Figure 2 for a representative subject, during explosive contractions participant was always capable to reach the individual Target, while NMES-elicited conditioning contractions resulted in a decrement in muscle torque. On the contrary, during the voluntary conditioning contractions, muscle output was stabile within a single contraction and between the 12 contractions.

During the NMES and VOL CC protocols, Mean Torque_CC and TTI_CC of the conditioning contractions was equivalent, indicating that participants were able to perform identical muscle mechanical output ($p>0.05$) during the two testing days (Table 1). Also, explosive contractions performed during the two protocols generated similar Mean Torque_EC, Peak Torque_EC and TTI_EC ($p>0.05$).

Also, during voluntary explosive contractions, the Time to Peak_EC was significantly lower during the NMES CC protocol then the VOL CC one, with a percentage difference of 11% ($p=0.019$). However, the Time to Target was similar between protocols ($p>0.05$) with a difference of 6%.

Interestingly, the RTD 0–50 ms_EC which is the rate of torque increment calculated in the earliest phased of contraction, was significantly higher (+38%) for the NMES CC protocol than the VOL CC one ($p=0.027$) (Table 1; Figure 2). On the other hand, the RTD 0–100 ms_EC calculated by also considering the later contraction phase, failed to reach the significant level ($p=0.082$) with a difference of 26% between the NMES and VOL protocol.

Interestingly, during the NMES and VOL CC, muscle activation was equivalent indicating that participants had identical muscle output during the two testing days (Table 1). At the same time, we evaluated any possible marker of afferent pathways activation mechanisms during the NMES CC protocol only. However, no differences were evaluated in the RMS VL_marker, RMS RF_marker and RMS BF_marker values as compared to baseline ($p=0.357$, $p=0.719$ and $p=0.417$ respectively). More specifically, RMS VL_marker was 2 ± 2 %MVC, RMS RF_marker 4 ± 6%MVC and RMS BF_marker 2 ± 3 %MVC.

Discussion

In the present study we compared the effects of applying an electrically induced or voluntary generated conditioning contraction on explosive characteristics of an isometric voluntary contraction of knee extensors at 70% MVC; When comparing the different nature of conditioning contractions on a following isometric explosive contraction of knee extensors, the early explosive characteristics of the contraction were impaired by a voluntary stimulus as compared to an electrically induced one performed at the same muscle mechanical work.

NMES elicited vs Voluntary Conditioning Contractions Protocol

During the VOL CC protocol, performed on the second experimental day, muscle mechanical output from both explosive contractions and conditioning contractions was matched with the results from the previous testing day (NMES protocol). Potentiation was evaluated by mean of RTD in the early (50ms) and late phase (100ms) of the explosive contraction. RTD is considered to give valuable information on the neuromuscular system, particularly in the early phase [22], because it's influenced by neural mechanisms and motor unit's activation [23].

It is possible that the enhanced RTD 0–50 $ms_{\_EC}$ evaluated after a conditioning contraction performed electrically was the result of a potentiated status that enabled to overcome those spinal and supra-spinal pathways involved in central fatigue mechanisms. On the other hand, these mechanisms are well involved during a sustained voluntary contraction even at relatively low-level force [5, 24]. It has been described previously that, after a tetanic electrically evoked conditioning contraction of *tibialis anterior*, RTD of a ballistic contraction is increased as compared to a conditioning stimulus performed voluntary [5].

It is important to consider that fatigue is also induced by an electrical stimulus, but mainly involving peripheral structures. Therefore, it is plausible that an electrically evoked conditioning contraction was able to initiate a Ca^{2+} release in the sarcoplasmic space and induce potentiation over the following voluntary explosive contraction overcoming the development of peripheral fatigue [4, 5]. However, when considering the later phase of RTD (0-100ms time window), no significant difference was evaluated (p=0.082) between the NMES CC and VOL CC protocols. This result might reflect the preserved mechanical properties of the muscles, that are not influenced by an increase neural drive [25]. At the same time, similar Time to Peak$_{_EC}$ is considered and indicator of preserved muscle contractile properties [22]. On the other hand, it is important to highlight that, in the present study, Time to Target$_{_EC}$ was significantly lower during the NMES CC protocol compared with the VOL CC one (-6%). This might be explained by differences in RTD in the very early phase of contraction and by a tendence for this parameter to remain higher during the NMES CC protocol also in the later phase of contraction (RTD 0–100 ms$_{_EC}$).

As mentioned above, another aspect that needs to be considered is central fatigue occurrence. In fact, it is possible not only that a conditioning contraction performed electrically can enhance a following explosive contraction, but also that a voluntary conditioning contraction can limit potentiation due to increase central fatigue at spinal and supraspinal level. Even though central fatigue is developed more slowly during submaximal force level, as in this case, it is possible that afferents fibers (small diameters type III and IV afferents) are engaged in sustained contraction thus limiting cortical output and therefore voluntary activation [10, 26].

Even if in the present study participants were always able to reach the required muscle output (70% MVC), it is plausible that in the VOL CC protocol, contraction explosiveness could have been affected by an ongoing development of central fatigue to the muscle caused by repeated activation of the cortico-spinal pathways both during sustained low level muscle contractions and explosive contractions. Similar results were highlighted by D'Amico et al. [27] on a hand muscle Voluntary activation and motoneuron excitability were affected by a voluntary fatiguing task rather than an electrically evoked one. Moreover, it is important to highlight that, even though a long-low NMES paradigm was applied, the enhanced explosiveness was not produced by activation of afferent pathways. In fact, we were not able to detect any marker of spinal circuitry involvement as evaluated by the RMS values after the end of the NMES elicited conditioning contraction. One possible mechanism for this result is the high interindividual variability in our participants to the specific type of long-low NMES paradigm. This might be explained by peripheral factors (i.e. intrinsic muscle properties) such as Ca^{2+} release, and sensitivity and/or phosphorylation of myosin light chain [28, 29].

Limitations

The present study has some limitations. First it is important to considered that inferences on muscle fatigue occurrence were made without indeed evaluating the phenomenon. For future studies, voluntary activation might be evaluated by the interpolated-twitch technique and cortical activation be using the transcranial magnetic stimulation. Second, it is not possible to precisely identify the source of the enhanced explosiveness during the NMES CC protocol because no evidence of afferent activation pattern were evident.

Conclusion

In conclusion we demonstrated that an isometric explosive contraction of knee extensors was potentiated by an electrical evoked conditioning contraction performed at submaximal force level, as compared with a voluntary conditioning contraction.

The present results could be used in those training paradigms during which it is of key importance for athletes to generate higher level of muscle force as quickly as possible. Also, finding a way to enhance muscle explosive performance and maintain higher volume of muscle work could be used to design new strategies for training in elderly population. In fact, the ability to rapidly generate higher level of muscle force is also essential in frail population to reduce risk of falls.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

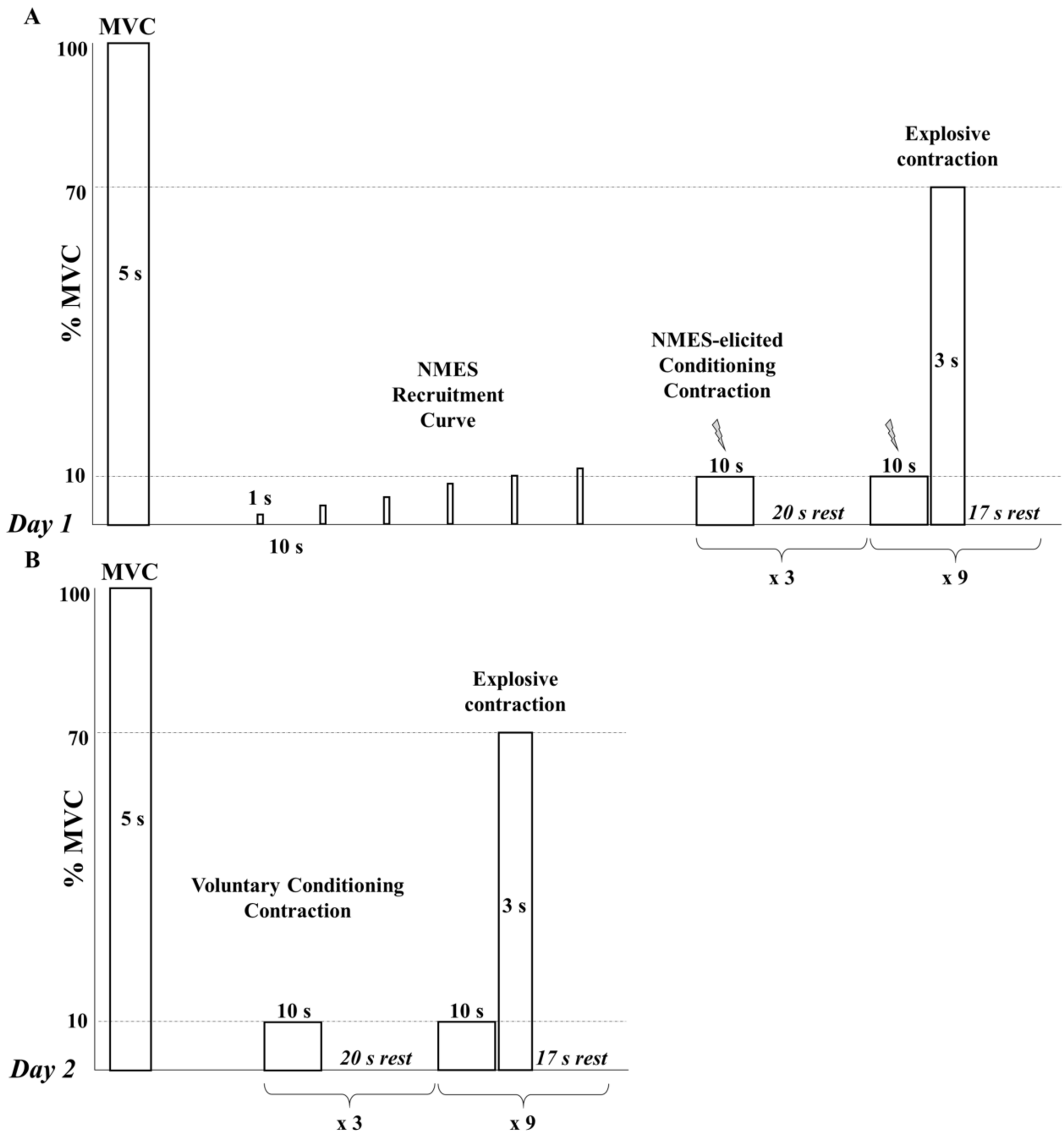

Figure 1: Schematic representation of the experimental protocol

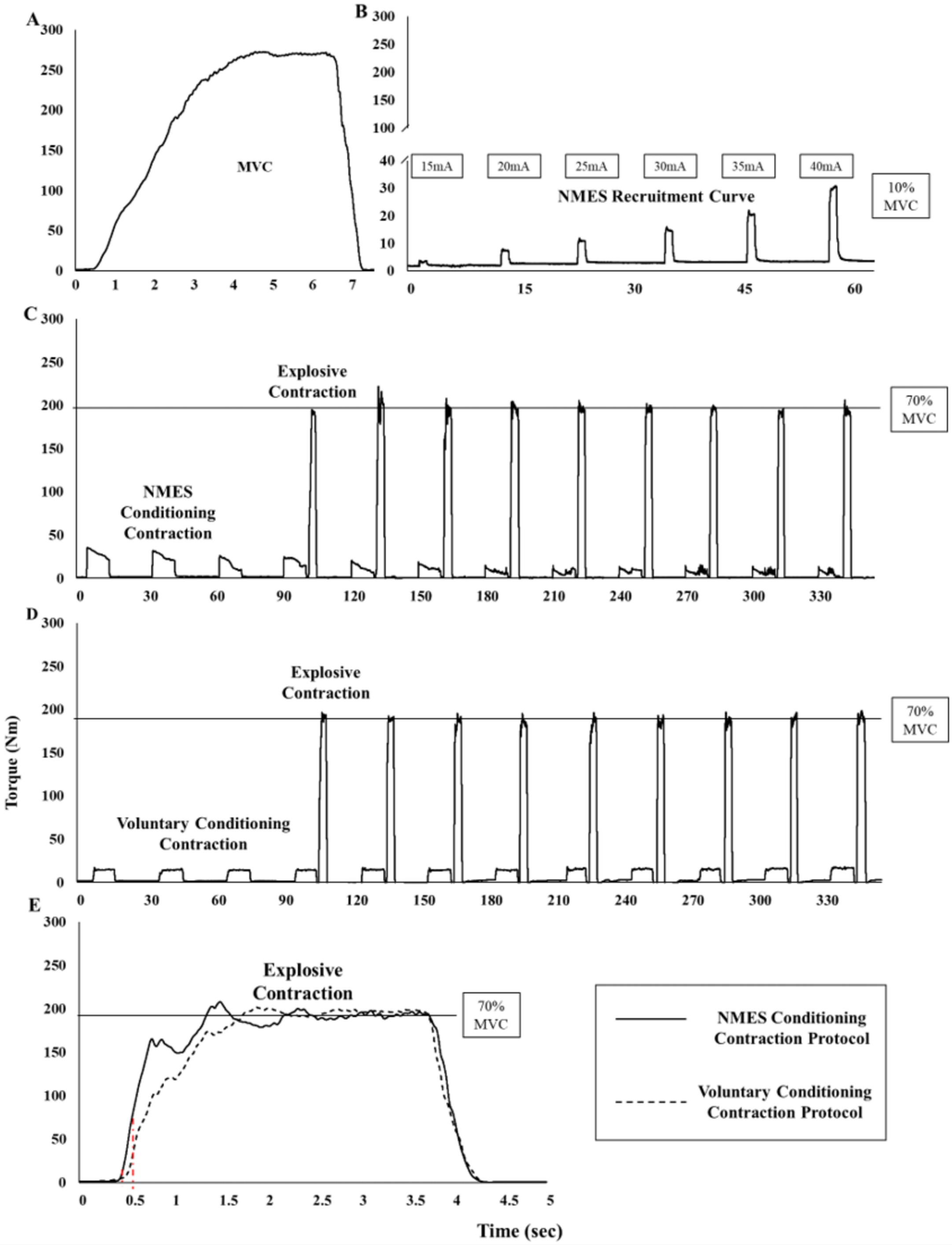

Figure 2: Raw data from one representative subject during the NMES conditioning contraction protocol and the voluntary conditioning contraction one. Maximal Voluntary Contraction and NMES recruitment curve are also depicted.

Table 1: Muscle mechanical output and contractile properties of the knee extensors and *vastus lateralis (VL)*, *rectus femoris (RF)* and *biceps femoris (BF)* electromyographic amplitude during voluntary explosive isometric contractions (EC) and conditioning contractions (CC) generated during the electrical stimulation (NMES) CC protocol or the voluntary (VOL) CC protocol.

	NMES CC	VOL CC	p value
Mean Torque_EC (Nm)	129.6 ± 39.4	129.9 ± 39.0	0.920
Mean Torque_CC (Nm)	10.1 ± 4.8	10.0 ± 4.4	0.553
Peak Torque_EC (Nm)	166.5 ± 50.5	166.9 ± 50.6	0.870
TTI_EC (Nm·s)	4687 ± 1488	4709 ± 1451	0.673
TTI_CC (Nm·s)	1227 ± 580	1205 ± 535	0.197
Time to Peak_EC (sec)	1.76 ± 0.38	1.98 ± 0.41	0.019*
Time to Target_EC (sec)	0.98 ± 0.38	1.04 ± 0.48	0.632
RTD 0-50ms_EC (Nm·s-1)	216 ± 194	135 ± 160	0.027*
RTD 0-100ms_EC (Nm·s-1)	325 ± 243	241 ± 236	0.082
RMS VL_EC (%MVC)	66 ± 23	65 ± 29	0.833
RMS RF_EC (%MVC)	63 ± 23	61 ± 17	0.534
RMS BF_EC (%MVC)	24 ± 29	19 ± 14	0.460

TTI: Torque Time Integral; RTD: Rate of Torque Development calculated in the 0-50ms and 0-100ms time windows; RMS: Root Mean Square
EC: Explosive Contraction; CC: Conditioning Contraction;
Values are mean ± standard deviation. N = 20 research subjects.

* Significant difference by Paired *t* test

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Chapter 3

3.1 Aging and muscle adaptations

With aging the body undergoes numerous modifications such as modification in body composition, decreased bone density, loss of muscle quality and mass (sarcopenia) which together contribute to physical limitations (Goodpaster et al. 2006). This process can then escalate into decreased life quality, increased risks of developing concurrent pathologies (i.e. cardiovascular or metabolic diseases), requiring hospitalization and eventually premature death (Tieland et al. 2018). These processes need to be carefully considered also in relation to the continuous and exponential increment of the global elderly population.

Even though the topic of aging requires the use of a multifactorial perspective, one aspect that influences many others is the role of the muscle apparatus in the elderlies. In fact, not only the loss of strength but also decreased motor coordination and excitation-contraction coupling, inevitably reduce general physical performance (Tieland et al. 2018; Renganathan et al. 1997). We can confidently say that one important process of aging is the link between reduced muscle performance and total loss of muscle mass, the so-called sarcopenia. Along with reduced muscle mass during aging there is also a reduction of motor units, change in muscle fiber type composition and decrease in neuromuscular drive. Consequently, these components can affect muscle force and velocity, produced strength during movements and thus reduce performance (Reid et al. 2012).

Nevertheless, skeletal muscle has not only a key role in performance, but it also contributes to maintain a health status during the lifespan. In fact, the muscle apparatus is essential in maintaining the glucose homeostasis, because it is the primary site of glucose uptake from the blood and, with the liver, it represents the physical storage of glycogen (Otto-Buczkowska and Dryżałowski 2016). For these reasons, a disrupt of metabolic homeostasis at the muscle level can provoke obesity, metabolic syndrome, and insulin resistance (Stump et al. 2006).

With age, it was shown that muscle mass can decline approximately by 0.4% each year in men and women this decline can go even beyond the 0.7% after the age of 75, but during inactivity the rate of muscle mass decline is even accelerated (Mitchell et al. 2012). As a result of the loss in muscle mass, strength is also negatively affected and this ranges between 0.3% to even 4.2% per day (Wall et al. 2013). Thus, it appears clear that when episodes of prolonged muscle disuse develop with high frequency, overall physical performance is strongly affected and can increase the risk of disabilities (Fielding et al. 2011). The reduction in muscle mass is mainly attributed to the total loss in muscle fibers. As an example, it was reported that vastus lateralis muscle size can be 18% smaller in the elderlies as compared to young adults (Lexell et al. 1988). Along with total muscle mass, muscle fibers also undergo a reduction in size, but this phenomenon involves only type II fibers. In fact, it seems that type II fibers are 10 to 40% smaller in the elderlies than in healthy adults. On the other hand, type I fibers are substantially maintained with aging (Larsson et al. 1978). Type II muscle fibers are fast type fibers, which are recruited during high intensity activities and thus, a specific decline in this fibers' population, may also produce a decrease in the ability to perform daily life activates such as lifting objects, fast walking or standing up from a chair. Therefore, a loss of type II muscle fibers will also negatively affect muscle power output along with the possibility of producing force rapidly, and this decline seems to be even faster than the loss of strength (De Vito et al. 1999).

One of the main determinants of muscle loss is the downregulation of protein turnover in the skeletal muscle, which provokes an imbalance between new protein synthesis and protein breakdown (Koopman and van Loon 2009). More specifically, fiber atrophy is attributed to an impairment in protein homeostasis, while denervation and remodeling of motor units are the causes of fiber loss per se.

Another parameter that is impaired during aging is muscle quality, which is the ratio between force production per unit of muscle area. An animal study on rats' planta-flexor elicited a muscle contraction via an electrical stimulus. By doing so they excluded the variable of neural impairment and found that in aged rats there was a reduction of 34% in muscle quality as compared with young rats. It was suggested that a possible determinant of this reduction, could be a disruption in processes related with excitation-contraction coupling (Russ et al. 2012).

3.2 Aging and resistance training

Considering muscle quality and quantity decrease, it is also important to state that skeletal muscle in the elderly population can also be reconditioned. To initiate a hypertrophy adaptation with a following increase in strength, resistance training is the most effective intervention in the elderly population (Rosendahl et al. 2006; Fiatarone et al. 1994).

One determinant to regain muscle mass in elderlies is the number of myofibers present in a muscle. Therefore, hypertrophy adaptations are possible but limited in an elderly population (Hunter et al. 2004). To produce a strength increment and a hypertrophy response, high intensity of more than 75% of maximal strength capacity is required and the training should be performed at least 3 times a week for 8-12 weeks (Steib et al. 2010; Petrella and Chudyk 2008). For each proposed exercise usually the training program should consist of 3 to 4 sets and about 10 repetitions (Mayer et al. 2011). As a hypertrophy response, it has been studied that in the skeletal muscle myofibers can increase their size up to 62% following a resistance training 2-3 times a week for 9 to 52 weeks. Along with the increase in muscle strength, it has been reported also an increased performance in walking performance, the ability to rise from a sitting position and overall mobility (Orr et al. 2008). In particular, an increase in muscle strength can be seen especially in the first weeks of intervention as a response from neural adaptations (Aagaard and Andersen 2010). Moreover, it was demonstrated that, in general, a strength-focused training intervention performed 2 or 3 times a week for 20 to 30 minutes is effective in lowering the risk for cardiovascular disorder, diabetes, and osteoporosis (Liu and Latham 2011; Daniels et al. 2008).

NMES can also be particularly useful to promote muscle adaptation when used as a training modality. In fact, NMES can mediates neuroplasticity and regulate neurotrophic factors that promote new formations of neuromuscular junctions and axonal sprouting (Guo et al. 2021). We also stated before that NMES can affect muscle metabolism and in fact it was demonstrated that electrically induced contractions can increase the level of oxidative enzymes, capillarization as well as mediate reorganization in muscle fibers (Bell et al. 2000). Evidence from morphological characteristics of the muscle also demonstrated that various type of NMES interventions were effective in evoking adaptations in total muscle size (Karlsen et al. 2020; Sillen et al. 2013). Thus, the use of NMES should be considered in healthy older adults as a way to counteract muscle atrophy.

Taken together these considerations on healthy aging and resistance training, we focused on analyzing a large cohort of elderlies in the north-east region of Italy and Slovenia by including information on physical performance, cognitive capacity, and quality of life (attached at the end of this chapter). Subsequently, we developed a new training modality which was administered in a selected group of healthy elderlies (>65 yo). In particular, the effects of two resistance training interventions on lower limbs was evaluated. One group (the experimental group) was trained by using the combination of an electrically induced conditioning contraction and a voluntary explosive contraction, since we evaluated the positive effects of an NMES conditioning contraction on explosive characteristics in healthy adults (refer to Project 2). The second group (control group) was trained by using a classic modality composed of only voluntary explosive contractions.

Here, we report the results and discussion of the third and last experimental project of this PhD, which along with the previous introduction will develop a scientific article to be submitted in the near future.

3.3 Methods

3.3.1 Subjects

Nineteen healthy, older participants (11 males and 8 females) agreed to take part in the research project and the whole group completed the training program successfully. All participants had a full medical history and physical examination in order to test their eligibility to the study protocol. Exclusion criteria were: <65 of age; body Mass Index (BMI) >26; having any cardiovascular or neurological conditions, having one or more implanted electrical devices, having fractures at lower limb, using psychotropic drugs. Anthropometric characteristics of the subjects are summarized in Table 1. Participants were randomly split into two groups, the experimental group (EXP) (6 males and 4 females) and the control group (CTRL) (5 males and 4 females). The experimental protocol was conducted in accordance with the declaration of Helsinki, and was approved by the Institutional Review Boards of University of Udine (Italy) (038/IRB DAME_2021). Before the study began, the purpose and objectives of the study were carefully explained to each subject and written informed consent was obtained.

3.3.2 Experimental Protocol

Subjects were asked to complete 6 weeks, three times a week, of an explosive resistance training performed on the knee extensors muscle group and two batteries of tests, which were performed before and after the completion of the training period. The EXP group performed the combination of a NMES-elicited conditioning contractions and explosive isometric voluntary contractions. The CTRL group performed a classic explosive training composed of solely explosive isometric voluntary contractions. The training was performed on a custom-bult isometric dynamometer previously described by Rejc and colleagues (Rejc et al. 2010). Hips and knees were flexed at 90°, a crossover shoulder strap and a strap around the ankle (5 centimeters proximal to the malleoli) were set in order to minimize movements of the trunk and leg. The training was performed on the right limb, which was the dominant leg for all participants. The tests held were the following: anthropometric and body composition measurements, morphological muscle characteristic of the thigh (trained leg only), maximal voluntary isometric force and maximal explosive power tests (both limbs), balance performance (trained leg only), physical performance evaluation and explosive characteristics evaluation during isometric contractions (trained leg only). Morphological muscle characteristic of the thigh and explosive characteristics evaluation during isometric contractions were evaluated only on the trained limb. All the other evaluations were performed on both limbs. The maximal voluntary isometric force were evaluated at the beginning of each week to evaluate any modifications in the maximal exerted force and to adjust the training load accordingly.

3.3.3 Training Protocol

The training protocol for both groups consisted of a warm-up, resistance training (i.e. central part) and a cool down. Each session lasted approximately 60 minutes.

The training protocol for the EXP group was conducted as following: firstly, the subject was seated on the isometric dynamometer and was asked to perform an initial warm up during which each participant was instructed to generate between 20 and 30 four-second contractions, at a self-selected and increasing intensity. After a 3-minute rest period, participants were asked to perform a maximal isometric knee extension of approximately 6 seconds. Then, the ratio between stimulation amplitude and peak torque exerted (i.e. recruitment curve) (Gonnelli et al. 2021) was assessed. The recruitment phase was separated by the MVCs attempts by at least 10 minutes. Stimulation pads (size: 5×10 cm; Axelgaard Manufacturing Co., Ltd., Fallbrook, CA) were positioned above the quadriceps muscle belly with the distal portion placed at 50% and 10% of the distance between the anterior superior iliac spine and the superior margin of the patella, for proximal and distal pads respectively (Arpin et al. 2019). Then, an electrical stimulator (Digitimer DS7A, Hertfordshire, UK; Maximal Voltage 400V) was used to deliver a 1-second monophasic positive rectangular waveform with 1000µs pulse width at constant frequency and voltage of 100 Hz and 400 V, respectively. Stimulation trains were delivered to the muscle every 10 seconds. NMES was applied starting at a stimulation amplitude of 5 mA, and increasing it by 5 mA for every subsequent stimulation until a minimum torque equal to 10% of Peak Torque was elicited and either the participant requested to stop the stimulation because of discomfort or the recruitment curve reached a plateau.

After a rest period of 5 minutes and after the end of the recruitment curve, the training protocol was initiated. The training for the EXP group consisted of 4 sets of 12 repetitions, with the last nine repetitions performed using the combination of a NMES-elicited conditioning contraction and a voluntary explosive contraction. Each cycle was separated from the following one by a 3-minute rest period. The first 3 NMES conditioning contractions performed at the selected stimulation amplitude, previously identified during the recruitment phase, in order to activate the neuromuscular system and the central mechanisms of the elicited contraction. Then, at the end of the 4th electrically elicited contractions, after full muscle relaxation, the participant was instructed to perform a voluntary explosive muscle contraction of the knee extensors in order to reach the individual 70% MVC (Target) as fast as possible and to maintain the Target for 3 sec. The same sequence was repeated for the remaining contractions and a total of 9 explosive contractions preceded by an NMES conditioning contraction were performed. This protocol was selected because it was found to be effective in increasing explosive characteristics of the neuromuscular system in healthy adults (refer to Chapter 2 and Project 2).

The cool down phase consisted of pedaling on a bike at 80rpm for 5 minutes at a self-selected intensity.

The training protocol for the CTRL group was performed as the one from the EXP group, except for the NMES-elicited conditioning contractions. More specifically, 4 cycles of 9 repetitions of the voluntary explosive contraction at the individual 70% MVC were performed.

For both groups, and only for the first and second week of the training period, 3 cycles instead of 4 were performed with the goal to reach a smoother adaptation in the skeletal muscle system and prevent any fatigue impairment.

3.4 Measurements

3.4.1 Anthropometric characteristics

Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board. The body mass index (BMI) was calculated as BM (kg) · stature^{-2} (m).

3.4.2 Morphological properties of the thigh muscles

To evaluate muscle morphological characteristics a B-mode ultrasound probe (Esaote Biomedica, AU3Partner, Florence, Italy) was used to investigate the cross-sectional area (CSA) of the vastus lateralis and rectus femoris muscles, and muscle thickness (MT), fascicle length (FL) and pennation angle (PA) of the vastus lateralis muscle. The subject was asked to lay supine on a medical bed where the femoral length, which is the distance between the greater trochanter and the mid patellar point, was measured. CSA was collected at the 50% of the femoral length using a 7.5-MHz linear-array transducer. The Region of Interests (ROIs) were identified laterally, from the borders of vastus lateralis muscle, and medially, until the borders of the rectus femoris. Then the CSA of vastus lateralis (CSA VL) and rectus femoris (CSA RF) were computed with the V-Pan function built in the Ultrasound device. Then, muscle thickness, Pennation angle and fascicle length were measured at the 50% of the femoral length and at the mid-point of the vastus lateralis muscle belly (Narici et al., 1996).

3.4.3 Maximal Voluntary Contractions

Participants performed MVCs of the right and left (non-trained leg) knee extensors while sitting on the isometric dynamometer. After the warm-up and a 3-minute rest period, participants were asked to perform a maximal isometric knee extension of approximately 6 seconds. Three MVC attempts were performed, separated by a 5-minutes rest in between attempts, and the contraction that resulted in the highest peak force was considered for further analysis. All data was 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 join 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. Torque was 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.

3.4.4 Maximal explosive power of the lower limbs

Explosive power of lower limb was assessed by means of the Explosive Ergometer (EXER), previously described by (2009). More specifically, the EXER device is formed by a metal frame that supports a rail inclined at 20° from the ground. A seat is attached to the rail and is free to slide on a carriage. At the starting position, the seat faces two force plates (LAUMAS PA 300, Parma, Italy) and is also attached to a tachometer (LIKA SGI, Vicenza, Italy). The total moving mass of the EXER (seat and carriage together) is equal to 31.6 kg. Force and velocity analog outputs were sampled at 1000 Hz using a data acquisition system (MP100; BIOPAC Systems, Inc., Goleta, CA, USA).

During the test, the subject is seated and is instructed to maximally accelerate himself allowing the carriage to slide on the rail. In such a way, it is possible to record the force reaction performed by the feet on the force plate and the peak velocity reached by the system.

The subject was seated on the carriage seat, secured by a safety harness fastened around the shoulders and abdomen, with his arms on the handlebars. Two mechanical blocks were used to set the distance between the seat and the force platforms, so that the knee angle at rest was 110°. The blocks also prevented any countermovement and, consequently, any recovery of elastic energy during the pushing phase. The tests were performed by asking the subject to push with both legs (EXP_DL), and then with each leg separately (EXP_R and EXP_L). The instantaneous power was calculated from the product of instantaneous force and velocity values, the attempt with the greatest peak power was then refrained for further analysis. For statistical analysis the Peak Power was normalized by body weight.

3.4.5 Physical performance evaluations

Physical performance was evaluated using the Short Physical Performance Battery (SPPB), the Time Up and Go Test (TUG) and the 6 Minutes Walking Test (6MWT).

The SPPB test is composed of 3 separate tests which are the balance test, the 4 meters walking course and the sit to stand test. During the balance test the participant was instructed to maintain a standing position with their feet as close together as possible, then in a semi-tandem position, and finally in a tandem position for at least 10 seconds. During the 4-meters walking course the participant was asked to cover the distance at an usual pace. Finally, in the sit to stand test the participant was asked to stand five times from a seated position in a chair without using their arms. Then, during the TUG test the participant was asked to stand up from a chair, walk a distance of 3 meters, turn around and sit on the chair as in the starting position. During the 6MWT each participant was instructed to cover as much distance as possible at a self-selected speed in 6 minutes.

3.4.6 Balance performance

The balance test was performed by measuring ground reaction forces on one Kistler force plate (model: 9286A; Winterthur, Switzerland) and BioWare® software was used for each leg. The sampling frequency of the platform was set at 100 Hz.

The test consisted in maintaining an upright standing position for 30 sec by using the right-trained leg. The center of pressure (COP) displacement was used and the main output parameter of the plate. Then, the COP ellipse area (COP_Area) was calculated, wherein the ellipse set of the data is used to quantify 95% of the total area formed by the COP trajectory covered by the AP and ML directions. Then, the COP path length (COP_Length) was calculated as the total distance traveled by COP during the test time (Prieto, T.E. 1996).

3.4.7 Neuromuscular characteristics of voluntary explosive contractions

To evaluate neuromuscular characteristics of knee extensors the first and last training session were analyzed in both EXP and CTRL groups. In particular, onset and offset of each contraction were defined considering a torque threshold equal to the baseline (calculated between 650 and 150 ms prior to the contraction initiation) + 3 standard deviations. Peak Torque was calculated by applying a 0.5 sec moving window for explosive voluntary contractions (Peak Torque_EC). Mean Torque was calculated for voluntary explosive muscle contractions (Mean Torque_EC) by the Mean Torque value from the beginning to end of each contraction. Torque-time integral (TTI) was calculated to estimate muscle work of the explosive voluntary contractions ($TTI_{\_EC}$). Rate of torque development of the explosive voluntary contraction was computed over the time windows 0–50 ms (RTD 0–50 ms), 0–100 ms (RTD 0–100 ms) 0–200 ms (RTD 0–200 ms).

3.5 Statistical Analysis

All results are expressed as mean and standard deviation (SD). Normal distribution of the data was tested using the Kolmogorov–Smirnov test. Sphericity (homogeneity of covariance) was verified by the Mauchly's test. When the assumption of sphericity was not met, the significance of the F-ratios was adjusted according to the Greenhouse–Geisser procedure.

Differences in baseline data of physical anthropometric characteristics, morphological properties of the thigh, MVC of right and left limb, maximal explosive power, surface EMG recordings, physical performance tests, balance test on force plate and neuromuscular characteristics of the explosive contractions were analyzed by Unpaired t-test.

To assess differences in the data after the completion of the 6 weeks training period between groups, statistical analysis was performed by applying two-way repeated measure ANOVA using GraphPad Prism 7.0 with significance set at $p \leq 0.05$ and with 2 main predictors variables (group, time and their interactions).

3.6 Results

3.6.1 Measurements at baseline

All the nineteen participants recruited completed the training period of 6 weeks composed of a total of 18 sessions. No dropouts after the start of the training period were experienced.

Baseline values of age, stature, body mass, body mass index, thigh muscle characteristics were not different among the two groups and are described in Table 1.3. Also, maximal voluntary contraction, explosive power, physical capacities, and balance measures are summarized were not different among the two groups in Table 2.3. Neuromuscular characteristics and muscle mechanical output results are summarized in Table 3.3. It is interesting to notice that the RTD calculated in the 0-50, 0-100 and 0-200ms were all significantly higher (+54%, +46%, +34% respectively) in the EXP group than in the CTRL one.

3.6.2 Measurements after the training intervention

After 6 weeks of specific explosive resistance there was no difference in anthropometric characteristics between the two groups (Weight and BMI). No time, group or interaction was found in CSA_RF and CSA_VL (p values ranging from 0.973 and 0.404) (Table 4.3). MT in the vastus lateralis muscle show significant time effect with an increase of +17% in the EXP group and -1% in the CTRL one (p=0.030) but no, group or interaction effect. On the other hand, PA showed no time or group effect but interaction effect (p=0.017). In particular, the EXP group increased by 26% the PA° variable while the CTRL one experienced a decrease of -3%. At the same time, FL showed no time, group or interaction effect (Table 4.3).

MVC_R showed a time effect but no group or interaction effect (p=<0.0001) (Table 5.3, Figure 1.3). However, it is interesting to notice that the percentage difference between the beginning and conclusion of the training protocol was significantly higher only in the EXP group (+32% p=0.036). Nevertheless, MVC_L showed time effect (p=0.001) but no group effect and an increase of +12% and +12% for the EXP and CTRL group respectively (Table 5.3).

Explosive Power of lower limb showed no group, time, or interaction effect in the three conditions tested. However, the EXP_DL and EXP_R increased both in EXP (+9% and +6% respectively) and CTRL groups (+5% and +7% respectively). On the other hand, EXP_L increased in the EXP group by +4%, while the CTRL group showed a tendency to decrease (-3%) (Table 4).

The SPPB test showed time effect ($p=0.009$) with an increase of 5% and 3% in the EXP and CTRL group respectively, but no group or interaction effect was found (Table 5.3).

When evaluating the performance in the 6MWT a strong time effect was found ($p<0.0001$), with an increase of 10% in the EXP group and 5% in the CTRL group. However, no group or interaction effect were found. Similarly, there was time effect in the TUG results but no other specific effects were found (Table 5.3).

The analysis of the balance performance evaluated by the COP_Area and COP_Length revealed no specific time, group or interaction effect (Table 5.3).

When analyzing the neuromuscular characteristics of the explosive muscle contractions in the first and last training sessions, no time, group or interaction effect were found. On the other hand, the RTD_0-50ms revealed both time ($p=0.003$) and group ($p=0.0007$) effect, with a higher value at the beginning of and after the training for the EXP group (+116% and +96% respectively) (Table 6.3). However, it is interesting to report that RTD_0-50ms decreased by 39% after the intervention training in the EXP group only (Figure 2.3). At the same time RTD_0-100ms showed the same trend, with time and group effect (but no interaction effect) ($p=0.001$ and $p=0.034$ respectively). In fact, RTD_0-100ms was higher at the beginning and after the training intervention in the EXP group (+86% and +106% respectively) (Figure 2.3; Table 6.3). The RTD_0-200ms revealed group ($p=0.008$) but no time or interaction effect. RTD_0-200ms was higher at the beginning and after the training intervention in the EXP group (52% and +68% respectively) (Figure 2.3; Table 6.3).

3.7 Discussion

In the present study we demonstrated that 6 weeks of explosive resistance training performed in older adults elicited: *i)* a modification in the morphological muscle characteristics of vastus lateralis muscle (increase PA and MT); *ii)* an increase in MVC of the right and left limbs; *iii)* an improved performance in all physical capacities; *iv)* an increased muscle output and *v)* decreased neuromuscular explosive characteristics.

The new training intervention proposed utilized an NMES conditioning contraction to produce a post-activation performance enhancement. Usually, applying this type of complex training is effective to develop both strength and power. However, previous studies demonstrated that there is no further improvement of complex training as compared to traditional resistance training (Mihalik et al. 2008; MacDonald et al. 2012). This might explain why no marked differences were found between the two training modalities proposed herein. Also, it is important to remember that elderlies are less responsive to PAP effect, probably because of changes in the excitation-contraction coupling mechanisms and atrophy-related factors (Hortobágyi et al. 1995; Hamada et al. 2000). However, it is important to state that after resistance training an increase in FL could be expected as mean of positive muscle adaptations. In the current study, no difference was depicted for the FL variable while PA increased only in the EXP group (Davis et al. 2020). Result in PA and MT parameters, along with a higher percentage increase in the MVC_R, might be indicators of a partial improve efficacy for the conditioning contraction protocol (EXP group) over the classic one (CTRL group). At the same time, adaptations in pennation angle usually facilitate increases in the isometric force rather than muscle volume by itself. It was demonstrated that 8 weeks of NMES-based training in older adults produced an increase in CSA of vastus lateralis and positive morphological adaptations (Jandova et al. 2020). Moreover, it is also important to remember that muscle morphological adaptations to strength training might start even later in time, and that the first adaptations may occur at the neural level only (Del Vecchio et al. 2019). In addition, an increase in the MVC_L was experienced by both groups. This result can indicate that both modalities were effective in producing a cross-education effect. This phenomenon has been studied as a result of both voluntary and electrically induced strength training. It is possible that the fatigue exercise was the main determinant of the cross-education effect. When central fatigue is experienced, the Central Nervous System is unable to send an optimal drive to motoneurons (Taylor and Gandevia 2008). Therefore, adaptations to the fatiguing exercise may occur at the central level and influence not only strength but also the synaptic connectivity within specific neural circuits which contribute to the ability of force generation (Lee and Carroll 2007).

Moreover, it is curious that no differences in the lower limb explosive power were found in either group after the training period. It is possible that the selective reduction in type II muscle fibers that is predominant in elderly population might be responsible for this result (Deschenes 2004). In fact, reduced strength and power is one of the main outcomes during aging. However, it has been observed that explosive-focused exercises, such as ballistic training, are safe and effective in increasing the CSA of type II muscle fibers (Zaras et al. 2013). At the same time, the specific type of resistance training might have impacted explosive power of lower limbs performed during a dynamic task. In fact, isometric resistance training can produce an improvement in force production but not velocity, with the latter being a key determinant for muscle power during dynamic task (Oranchuk et al. 2019). Unfortunately, in this particular population, 6 weeks of explosive training were not enough to show improvements in explosiveness of lower limbs.

Physical capacities significantly increased after the training period, but no differences were found between groups. In particular, an increase in the SPPB is a good indicator for general physical capacities in older adults. In fact, SPPB is associated with the risk of falls in elderly (Lauretani et al. 2019). By assessing daily life activities, the test is a good indicator for lower limb strength, walking speed and balance (Guralnik et al. 1994). Similarly, the TUG test values improved in both groups. The test can give important insights about the risk of falls in elderly because it evaluates balance, gait speed, and functional mobility (Beauchet et al. 2011). Previously, the TUG was reported to improve in populations with knee osteoarthritis after 8 weeks of strength training for lower limbs (Zhang et al. 2013). However, because in the present study the contraction performed during the training was isometric, it is possible that mobility and functional balance were equally affected along with strength improvement. Similar results can apply to the 6MWT, because no specific effect on the type of the intervention has been found. In fact, the test evaluates the aerobic capacity of the subjects and is particularly useful to assess functional capacity in cardiovascular diseases (Swisher and Yeater 2000). Also, the test predicts lower-limb function. Therefore, an increase in this parameter can also reflect an increase in leg function and the improved capacity to perform daily tasks efforts (Bautmans et al. 2004). However, because the two training modalities proposed in the present study differed only for the additional contraction (i.e. NMES conditioning contraction), the increased mechanical work could have been inadequate to produce a significant difference in the 6MWT for the EXP group only.

As mentioned for the SPPB test, balance is a key skill in elderly which prevent the risk of falls and is also the main determinant of independent daily living (Sibley et al. 2015; Tinetti and Kumar

2010). However, balance performance recorded during unilateral stance on a force plate was not affected by neither of the two training interventions. One possible reason for this result is the position of the body during both training interventions. A seated position might prevent proprioceptive adaptations of the foot and ankle joint receptors that are needed to maintain balance (Ducic et al. 2004; Kavounoudias et al. 1998). Moreover, the strength improvement was specific to the knee extensors muscle group, and plantarflexors were not affected by the training period. In fact, it was demonstrated that balance performance can actually be improved by a NMES-based training intervention when focused on plantarflexor muscles in elderly (Mignardot et al. 2015). Similarly, a resistance training or multi exercise is not effective in improving postural control which is a specific task (Low et al. 2017).

Finally, interesting results came from analyzing contractile characteristics of explosive contractions. In fact, RTD in the early phase of contractions (i.e. 0-50ms and 0-100ms) was higher at the beginning of the training intervention in the EXP than in the CTRL group. This effect might be the result of an enhanced potentiation performed by the electrical stimulus on type II muscle fibers and spinal circuitry (Grange et al. 1993). We previously demonstrated that a NMES conditioning contraction positively influenced an explosive contractions of knee extensors to a higher degree than a voluntary conditioning contraction on healthy adults (refer to Project 2, Chapter 2). In the present population of healthy elderlies, at the beginning of the training intervention the stimulated conditioning stimulus in the EXP group produced an increase in explosive characteristics of the voluntary contraction that ranged between +34% (RTD_0-200ms) and +54% (RTD_0-50ms) than the CTRL group. This increase is even higher than the one experienced in healthy adults, even though elderlies have a marked reduction in the number of type II fibers (Deschenes 2004). At the same time, it was previously reported during in-vivo studies that the lack of muscle fibers power in elderlies can be explained by a selective reduction in CSA of type II fibers (Sundberg et al. 2018). We can hypothesize that, before the training intervention, the combination of a stimulated conditioning contraction and explosive voluntary contraction was more advantageous than a simple voluntary explosive contraction because the NMES stimulus might recruit spinal circuities within the spinal cord or muscle portions with a higher number of type II fibers (Collins et al. 2001; Sweeney et al. 1993). However, a reduction in explosive characteristics calculated in the early phase of muscle contraction was reported after the training period only for the EXP group, but with values that were still higher than the CTRL one. It is possible that the reduction experienced was caused by an increased neuromuscular fatigue as a consequence of the training period (D'Emanuele et al. 2021). This result is even more curious when we consider the force increase after the end of the training period. In fact, when a higher level of force is produced, an increase in RTD is also expected (Bellumori et al. 2011). Nonetheless, it is interesting to report that even at the end of the training period RTD was always higher in the EXP group than the CTRL one.

3.8 Conclusion

In conclusion, 6 weeks of an explosive-focused resistance training is effective in increasing muscle force of knee extensors, physical capacities and muscle architecture in elderlies. Even though no differences were found in these parameters between groups, when the training paradigm is performed with a NMES-elicited conditioning contraction, explosive contractile characteristics of knee extensors are significantly enhanced.

Therefore, when the focus of the training intervention is the improved contractile characteristics of an explosive muscle contraction, an NMES conditioning contraction protocol should be preferred compared to a classical training modality. Further studies should be performed to evaluate the efficacy of longer period in both training protocols.

Table 1.3: Anthropometric and morphological characteristics of EXP and CTRL groups at baseline

	EXP (n=10)	CTRL (n=9)	p value
Age (year)	68.5 ± 2.8	66.4 ± 1.7	0.072
Stature (m)	1.66 ± 0.97	1.66 ± 0.82	0.958
Weight (kg)	67.9 ± 12.8	65.1 ± 13.2	0.641
BMI	24.4 ± 2.5	23.3 ± 3.0	0.458
CSA VL (cm^2)	19.89 ± 5.26	20.26 ± 6.67	0.895
CSA RF (cm^2)	8.80 ± 7.59	7.23 ± 2.49	0.563
MT (cm)	1.42 ± 0.25	1.62 ± 0.34	0.162
PA (°)	10.52 ± 2.06	12.15 ± 1.70	0.080
FL (cm)	7.40 ± 1.13	7.81 ± 1.19	0.453

Values are mean ± standard deviation.

BMI: Body Mass Index; CSA_VL: Cross Sectional Area of vastus lateralis muscle; CSA_RF: Cross Sectional Area of rectus femoris muscle; MT: Muscle Thickness of vastus lateralis; PA: Pennation Angle of vastus lateralis; FL: Fascicle Length of vastus lateralis

Significance is set at $p<0.05$

Table 2.3: Maximal Voluntary Contraction, Explosive Power, Neuromuscular characteristics Physical Performance Test and Balance test results of EXP and CTRL groups at baseline.

	EXP (n=10)	CTRL (n=9)	p value
MVC_R (Nm)	163 ± 54	189 ± 54	0.337
MVC_L (Nm)	160 ± 63	166 ± 36	0.798
EXP_DL (W*kg-1)	20.80 ± 7.28	21.40 ± 6.83	0.856
EXP_R (W*kg-1)	11.70 ± 3.40	11.58 ± 3.32	0.939
EXP_L (W*kg-1)	12.12 ± 3.32	12.33 ± 3.45	0.895
SPPB	11.2 ± 0.9	11.6 ± 0.7	0.366
TUG (sec)	6.84 ± 1.18	5.99 ± 0.54	0.063
6MWT (m)	580 ± 76	595 ± 51	0.625
COP_Area (cm)	1336 ± 596	1145 ± 308	0.400
COP_Length (cm)	3093 ± 378	3209 ± 209	0.429

Values are mean ± standard deviation.

MVC_R: Maximal Voluntary Contraction of knee extensors on right leg; MVC_L: Maximal Voluntary Contraction of knee extensors on left leg; EXP_DL: Explosive Power of lower limb performed with both legs; EXP_R: Explosive Power of lower limb performed with the right leg; EXP_L: Explosive Power of lower limb performed with both legs; SPPB: Short Physical Performance Battery Test; TUG: Time Up and Go; 6MWT: 6 Minutes Walking Test; COP_Area: Center of Pressure Area; COP_Length: Center of Pressure total length.

Significance is set at $p<0.05$

Table 3.3: Neuromuscular characteristics results of EXP and CTRL groups at baseline.

	EXP (n=10)	CTRL (n=9)	p value
TTI	428 ± 142	456 ± 164	0.704
Peak Torque_EC	126 ± 42	141 ± 42	0.448
Mean Torque_EC	91 ± 31	97 ± 32	0.718
RTD_0-50ms (Nm*s-1)	41 ± 16	19 ± 11	0.003*
RTD_0-100ms (Nm*s-1)	89 ± 28	48 ± 32	0.008*
RTD_0-200ms (Nm*s-1)	155 ± 47	102 ± 56	0.038*

Values are mean ± standard deviation.

TTI_EC: Torque Time Integral of the Explosive contractions; Peak Torque_EC: Peak Torque of the Explosive contractions; Mean Torque_EC: Mean Torque of the Explosive contractions; RTD_0-50ms: Rate of Torque Development in the 0-50 ms time window; RTD_0-100ms: Rate of Torque Development in the 0-100 ms time window; RTD_0-200ms: Rate of Torque Development in the 0-200 ms time windows;

* Significant difference by Unpaired t-test

Table 4.3 Effects of 6 weeks training periods in EXP and CTRL group on morphological characteristics

	EXP (n=10)		CTRL (n=9)		p value		
	PRE	POST	PRE	POST	G	T	GxT
CSA VL (cm^2)	19.89 ± 5.26	20.43 ± 6.12	20.26 ± 6.67	20.37 ± 6.54	0.956	0.404	0.589
CSA RF (cm^2)	8.80 ± 7.59	7.33 ± 1.61	7.23 ± 2.49	6.88 ± 2.39	0.973	0.789	0.345
MT (cm)	1.42 ± 0.25	1.67 ± 0.35	1.62 ± 0.34	1.60 ± 0.30	0.625	**0.030**	**0.015**
PA (°)	10.52 ± 2.06	13.23 ± 3.54	12.15 ± 1.70	11.75 ± 2.19	0.949	0.056	**0.017**
FL (cm)	7.40 ± 1.13	7.09 ± 1.11	7.81 ± 1.19	7.49 ± 0.97	0.282	0.388	0.990

Values are mean ± standard deviation.

CSA_VL: Cross Sectional Area of vastus lateralis muscle; CSA_RF: Cross Sectional Area of rectus femoris muscle; MT: Muscle Thickness of vastus lateralis; PA: Pennation Angle of vastus lateralis; FL: Fascicle Length of vastus lateralis

Two-Way repeated measure ANOVA: effects group (G), time (T) and interaction group x time (GxT)

Significance is set at $p<0.05$

Table 5.3: Effects of 6 weeks training periods in EXP and CTRL group on Maximal Voluntary Contraction, Explosive Power, Neuromuscular characteristics Physical Performance Test and Balance test results

	EXP (n=10)		CTRL (n=9)		p value		
	PRE	POST	PRE	POST	G	T	GxT
MVC_R (Nm)	163 ± 54	213 ± 66	189 ± 54	229 ± 72	0.472	**<0.0001**	0.412
MVC_L (Nm)	160 ± 63	180 ± 58	166 ± 36	187 ± 46	0.778	**0.001**	0.925
EXP_DL (W*kg-1)	20.80 ± 7.28	22.64 ± 6.62	21.40 ± 6.83	22.47 ± 7.94	0.948	0.086	0.634
EXP_R (W*kg-1)	11.70 ± 3.40	12.37 ± 3.66	11.58 ± 3.32	12.35 ± 3.80	0.966	0.086	0.899
EXP_L (W*kg-1)	12.12 ± 3.32	12.64 ± 3.41	12.33 ± 3.45	12.01 ± 3.82	0.894	0.764	0.218
SPPB	11.2 ± 0.9	11.07 ± 0.7	11.6 ± 0.7	11.09 ± 0.3	0.361	**0.009**	0.565
TUG (sec)	6.84 ± 1.18	6.15 ± 0.78	5.99 ± 0.54	5.78 ± 0.60	0.099	**0.008**	0.117
6MWT (m)	580 ± 76	635 ± 77	595 ± 51	624 ± 43	0.947	**<0.0001**	0.102
COP_Area (cm)	1336 ± 596	1199 ± 421	1145 ± 308	1666 ± 1189	0.603	0.367	0.138
COP_Length (cm)	3093 ± 378	3152 ± 547	3209 ± 209	3331 ± 384	0.314	0.454	0.798

Values are mean ± standard deviation.

MVC_R: Maximal Voluntary Contraction of knee extensors on right leg; MVC_L: Maximal Voluntary Contraction of knee extensors on left leg; EXP_DL: Explosive Power of lower limb performed with both legs; EXP_R: Explosive Power of lower limb performed with the right leg; EXP_L: Explosive Power of lower limb performed with both legs; SPPB: Short Physical Performance Battery Test; TUG: Time Up and Go; 6MWT: 6 Minutes Walking Test; COP_Area: Center of Pressure Area; COP_Length: Center of Pressure total length.

Two-Way repeated measure ANOVA: effects group (G), time (T) and interaction group x time (GxT)

Significance is set at $p<0.05$

Table 6.3: Effects of 6 weeks training periods in EXP and CTRL group on Neuromuscular characteristics results

	EXP (n=10)		CTRL (n=9)		p value		
	PRE	POST	PRE	POST	G	T	GxT
TTI	428 ± 142	478 ± 142	456 ± 164	491 ± 157	0.771	**0.007**	0.618
Peak Torque	126 ± 42	147 ± 41	141 ± 42	164 ± 54	0.436	**<0.0001**	0.801
Mean Torque	91 ± 31	101 ± 31	97 ± 32	108 ± 35	0.680	**0.002**	0.780
RTD_0-50ms (Nm*s-1)	41 ± 16	25 ± 11	19 ± 11	13 ± 5	**0.001**	**0.003**	0.148
RTD_0-100ms (Nm*s-1)	89 ± 28	69 ± 30	48 ± 32	34 ± 14	**0.001**	**0.034**	0.706
RTD_0-200ms (Nm*s-1)	155 ± 47	148 ± 61	102 ± 56	88 ± 19	**0.008**	0.395	0.797

Values are mean ± standard deviation.

TTI_EC: Torque Time Integral of the Explosive contractions; Peak Torque_EC: Peak Torque of the Explosive contractions; Mean Torque_EC: Mean Torque of the Explosive contractions; RTD_0-50ms: Rate of Torque Development in the 0-50 ms time window; RTD_0-100ms: Rate of Torque Development in the 0-100 ms time window; RTD_0-200ms: Rate of Torque Development in the 0-200 ms time windows;

Two-Way repeated measure ANOVA: effects group (G), time (T) and interaction group x time (GxT)

Significance is set at $p<0.05$

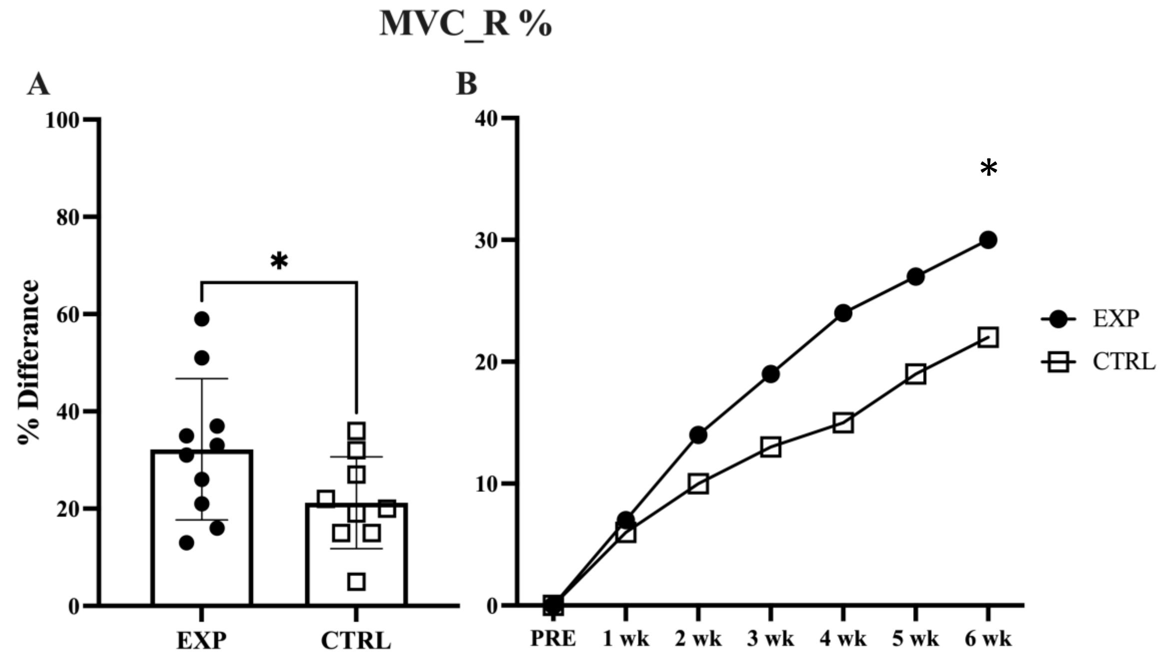

Figure 1.3: Percentage differences in Maximal Voluntary Contraction or the right leg in the EXP and CTRL group. **A**) Percentage Difference between the beginning and end of the training protocol. **B**) Percentage Difference at the beginning and at each week of the training protocol.

* Significant difference by Unpaired t-test

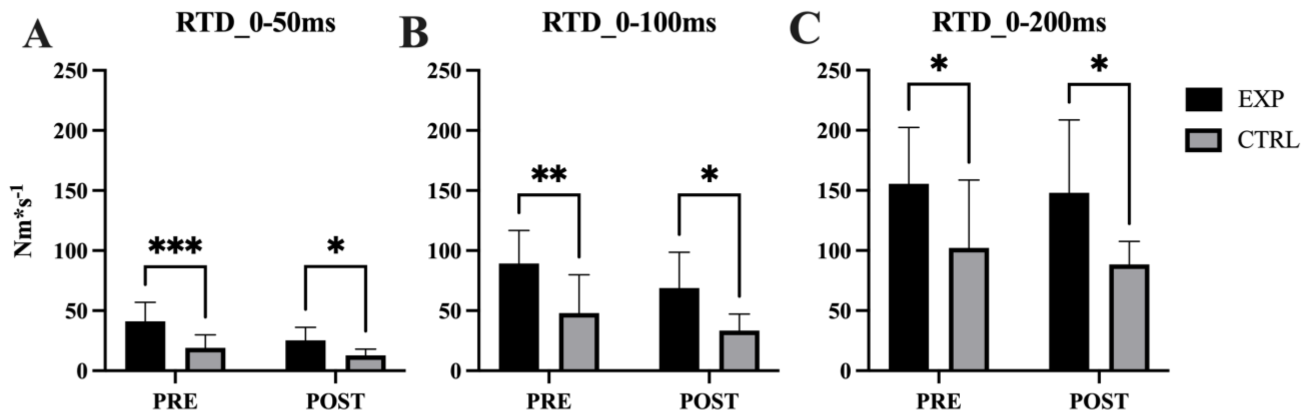

Figure 2.3: Rate of Torque development in the 0-50ms (RTD_0-50ms) (**A**), 0-100 m (RTD_0-100ms) (**B**) and 0-200ms (RTD_0-200ms) (**C**) time window in the EXP and CTRL group before (PRE) and after (POST) the training interventions.

* Significant difference by Two-Way repeated measure ANOVA

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ORIGINAL ARTICLE
EPIDEMIOLOGY AND CLINICAL MEDICINE

Physical capacities and leisure activities are related with cognitive functions in older adults

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ABSTRACT

BACKGROUND: This study aimed to evaluate the relationship between physical activity habits, physical performance and cognitive capacity in older adults' population of Italy and Slovenia.

METHODS: Anthropometric characteristics and body composition bioelectrical impedance analysis were evaluated in 892 older adults (60-80 y). Aerobic capacity was measured using the 2-km walk test and handgrip and flexibility tests were performed. Physical activity habits and cognitive functions were evaluated by the Global-Physical-Activity-Questionnaires (GPAQ) and by Montreal-Cognitive-Assessment (MoCA) questionnaires, respectively.

RESULTS: GPAQ scores were associated with lower BMI ($r=-0.096$; $P=0.005$), lower percentage of fat-mass ($r=-0.138$; $P=0.001$), better results in the 2-km walk test ($r=-0.175$; $P=0.001$) and a higher percentage of fat-free mass ($r=0.138$; $P=0.001$). We also evaluated that a higher MoCA Score correlates with age ($r=-0.208$; $P=0.001$), 2-km walk test ($r=-0.166$; $P=0.001$), waist-hip ratio ($r=-0.200$; $P=0.001$), resting heart-rate ($r=-0.087$; $P=0.025$) and heart-rate at the end of 2-km walk test ($r=0.189$; $P=0.001$).

CONCLUSIONS: Older adults with a higher level of daily physical activity showed reduction in fat-mass and BMI, and higher aerobic fitness; these characteristics have a protection effect on cognitive function.

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KEY WORDS: Aging; Exercise; Cognition; Habits.

In Europe, aging of population is settled into a well-defined trend of low birth rate and higher life expectancy. In 2016, 29.6% of the entire European population were older than 65 years, and between 2007 and 2017 in Italy and Slovenia people older than 65 years grown by 2.2% and 3%, respectively.[1] Data from 2016 reported that people over 65 were the 22% of the whole population in Italy and the 19% in Slovenia, but these numbers are expected to increase by 2070 reaching the 33% and 28% in Italy and Slovenia respectively.[1]

During aging, the body presents numerous modifications including changes in body composition, increase in fat mass, decrease in lean mass, and consequently sarcopenia, reduction in bone density, but also greater risks of Alzheimer's disease (AD) and different types of dementia.[2-5] Among these, decreased muscle mass in women,[6] decreased strength level and sarcopenia[7, 8] were found to be correlated with an increased limitation in performing daily activities, increased risk of hospitalization and nursing home admission, and premature death.[9, 10] It is worth mentioning that increasing sedentary behaviors as a consequence of limitation in daily activities or hospitalization, have been shown to negatively impact the general health status, as well as cognitive function.[11, 12] In fact, physical activity is associated with lower risk for cognitive decline and improved cognitive function via different mechanisms like neurogenesis, angiogenesis and synaptic plasticity and can also reduce the age-related neural inflammation.[13, 14] More specifically, cardiovascular fitness is positively correlated to hippocampal volume, which plays a crucial role in cognitive processing and memory function in women and episodic memory in men.[15] Noteworthy, combining different types of trainings (*i.e.* aerobic, strength and coordination) may have a higher efficacy on driving positive brain changes and, consequently, cognitive function.[16]

Current literature includes a wide variety of studies evaluating physical fitness or physical habits and cognitive function in aging population. However, we believe that it is necessary to evaluate anthropometric measurements and multiple aspects of physical fitness, including strength, flexibility and aerobic capacity, and the connection with physical daily habits and cognitive function on a large representative population of Caucasian free-living older adults.

For this reason, in this study we performed a wide battery of tests within a large cohort of older people living in northeast Italy and Slovenia. We collected anthropometric measurements and assessed body composition, physical fitness level, daily physical activity habits and cognitive performance. We assessed physical daily habits and cognitive performance via the Global-Physical-Activity-Questionnaires (GPAQ) and Montreal-Cognitive-Assessment (MoCA) questionnaires respectively. MoCA questionnaire is widely used as a rapid screening tool to assess cognitive function in elderly population and has been validated in the Italian and Slovenian population.[17, 18] We hypothesized that older adults who were more active (*i.e.* demonstrating a higher level of daily physical activity) would show better anthropometric characteristics, higher level of physical fitness and improved cognitive function compared to less active older adults.

Materials and methods

Ethical approval

The present study, PANGeA mass measurements study, was conducted according to the standards set by the latest revision of the Declaration of Helsinki and was approved by the National Ethical Committee of the Slovenian Ministry of Health on April 17, 2012, under the acronym IR-aging 1200. The purposes and objectives of this study were carefully explained to the subjects and written informed consent was obtained from all of them.

Subjects

Subjects were contacted via local newspapers both in Italy and Slovenia. The first inclusion criteria to participate in the PANGeA mass measurements study, which was reported in the newspaper, was having between 60 and 80 years of age. A total of 924 subjects from the North-East of Italy and Central and West Slovenia were first enrolled in the study and participated in the measurements. Prior to the beginning of the study, all subjects completed a multidomain questionnaire. The subjects reported, among other information, socio-demographic data, information on health status and drug therapy, and completed a self-reported questionnaire on the usual physical activity (GPAQ).[19] Subjects who were unable to walk independently and continuously for a distance of 2 km and/or subjects who had a severe cognitive decline defined as a MoCA Score less than 10 points (after correction for age and schooling) were excluded from the former analysis (N.=14). Moreover, subjects with acute illnesses or with a history of recent hospitalization (<6 months), diabetic subjects in insulin therapy or with drugs other than metformin were also excluded (N.=18). After the evaluation of inclusion criteria, the analysis was performed on a total of 892 free-living older adults (362 males and 530 females, age: 60-80 years).

Study protocol

Data were collected in six sport centers from Udine, Trieste, and Ferrara (Italy) as well as Koper, Ljubljana, and Kranj (Slovenia) in the summer of 2012. Subjects visited the sport center only once, the entire testing day took place in the same building and lasted approximately 2 hours for each subject. Priority was given to the accuracy of the measurements that were taken in a short period of time (three days for each center) and by the same sports scientists highly trained in running the physical test. At each center, cognitive tests (GPAQ and MoCA questionnaires) were administered individually and in a comfortable room by the same native scientists through the whole study period. Subjects were verbally instructed on how to complete the test by native speaker scientist. Physical capacity tests were administered in a gym set up to include multiple stations, one for each test.

Measurements

Anthropometric characteristics and body composition

Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board. Body mass index (BMI) was calculated as BM (kg) · stature^{-2} (m). Waist and hip circumference (m) were measured using a tape to the nearest 0.1 centimeter and for each site, the average of three measurements was taken. The ratio between waist and hip circumference (WHR) was also calculated. Body composition was measured by using bioelectrical impedance analysis with a tetra-polar impedance-meter (BIA101, Akern, Florence, Italy), according to accepted method.[20] Body composition (fat-free mass [FFM] and fat mass [FM]) was obtained from the software provided by the manufacturer, which contains predictive equations and specific reference values for the pediatric, adult and geriatric population.

Physical capacities and physical daily habits

Physical capacities were assessed using 3 physical fitness tests suitable for the age group. A representative spectrum of the target group's physical abilities was thus obtained.

Aerobic capacity

The 2-km walk test (UKK Test)[21] was used to evaluate aerobic capacity. The UKK Test was developed by the Urho Kaleva Kekkonen Institute for Health Promotion Research (UKK Institute), and serves as a measure of relative fitness, endurance and cardiorespiratory capacity. The test consists in completing the distance of two kilometers walking at the maximal steady velocity. The time to perform the test is expressed in minutes and is used to calculate the UKK Fitness Index (UKK-FI), considering age (yr), sex, Body Mass Index (BMI, kg/m^2) and the heart rate (HR) at the end of the test. The UKK-FI can be calculated as follow:

$$\text{Men: FI} = 420 - [(11.6 \times \text{min}) + (0.56 \times \text{HR}) + (2.6 \times \text{BMI}) + (0.2 \times \text{age})]$$

$$\text{Women: FI} = 304 - [(8.5 \times \text{min}) + (0.32 \times \text{HR}) + (1.1 \times \text{BMI}) + (0.4 \times \text{age})]$$

The equation results are the FI and can be inserted into 5 class domains that characterized the fitness level of the person.[21] The FI classes are divided as follow: <70; 70-89; 90-110; 111-130; <130; where <70 is considerably below the average and >130 is considerably above the average. Moreover, resting heart rate (HR_{pre}) and heart rate at the end of the UKK Test (HR_{post}) were measured using a heart rate monitor band (Polar, Kempele, Finland).

Handgrip strength

Strength was evaluated by the hand-grip test using a hand dynamometer (Jamar, Patterson Medical, Sutton-in-Ashfield, UK). The hand-grip test is considered a valid measure to evaluate strength in older adults.[22] The subject performed the test in a seating position with the elbow flexed at 90 degrees and positioned on the side, but not against, of the body. The hand was positioned firmly on the dynamometer with the thumb pointing up. The average of three trials measured in Newton was considered for further analysis.

Flexibility

The flexibility of lower back and hamstring muscles was measured by the Sit and Reach Test. The test is considered a valid measure of flexibility in a population of older adults.[23, 24] Each subject performed three trials, separated by at least 30 sec of rest, after a brief warm up. The test was performed using a box (Cartwright Fitness, Chester, UK) and the subject was asked to seat with both knees fully extended and pressed against the floor, with feet straight out. They were then asked to reach their toes or beyond. The maximum distance between the three measurements expressed in cm was then used for the analysis.

Physical daily habits

GPAQ[19, 25] was used to evaluate physical daily habits of each subject and was conducted in a face-to-face interview fashion. It consists of 16 questions divided in three domains and a sedentary behavior. The three domains are the following: 1) activity at work; 2) travel to and from places; and 3) recreational activities. GPAQ analysis uses the Metabolic Equivalents (METs) and assigns a total of 4 METs and 8 METs respectively for moderate and vigorous activity per time spent in the specific activity. Finally, after correcting the answers as reported in the GPAQ Guidelines, each question was inserted in the following formula:

$$\text{Total Physical Activity MET-minutes}\cdot\text{Week}^{-1} = [(P2*P3*8)+(P5*P6*4)+(P8*P9*4)+(P11*P12*8)+(P14*P15*4)]$$

In this formula P(n) is the answer to each question in the questionnaire.

World Health Organization recommendations give a cutoff value for total physical activity MET-minutes·Week^{-1} of 600 or 150 minutes of moderate to vigorous physical activity to be achieved in order to be considered healthy active.[26-28]

Cognitive performance

To assess the cognitive performance, we used the MoCA as it is a rapid validated screening test to detect mild cognitive impairment.[18, 29-31] From the raw data we then calculated the MoCA value applying the correction for age and total years of education as suggested by previous authors.[30] The test consists of one page with multiple questions divided in the following domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Ten minutes are necessary to complete the test and the maximal score achievable is 30 points. A score of 26 or above is considered normal.

Statistical analysis

All anthropometric characteristics, physical capacities and cognitive functions are described using mean and standard deviation (SD) and median and interquartile range (IQR) values. The normality of the distribution was checked with the Kolmogorov-Smirnov Test and it was found that all variables were non-normally distributed.

The comparisons between male and female were carried out using the Wilcoxon-Mann Whitney Test; Spearman correlation coefficients were used to assess the strength and direction of association and multicollinearity between independent variables. Then, Generalized Linear Multivariate Analysis was performed to evaluate the impact of some variables on cognitive functions. All statistical analyses were performed by SAS, Release 9.4 (SAS Institute, Cary, NC, USA), with a significance set at $P<0.05$.

Results

Anthropometric characteristics and body composition

When considering the whole population, mean age was 67.0±5.1 (years), BM 73.7±13.4 (kg), stature 1.65±0.09 (m) and BMI 27.0±3.9 (kg·m^{-2}). Also, as described in Table I, males were older than females (+1.5%, P=0.004) and had higher body mass, stature and BMI (+23.3%, +7.5% and +3.6%, respectively, P<0.001) (Table I). At the same time, males showed higher waist circumference, WHR and FFM (+8.2%, +9.3% and +27.4%, respectively, P<0.001) than females. However, males showed lower FM in absolute (-5.1%, P=0.011) and relative (-27.1%, P<0.001) values than females (Table I).

Physical capacities and physical daily habits

Males required less time to complete the 2-km walk test (-5.6%, P<0.001), had lower HR_{pre} and HR_{post} test (-6.0 and -5.3%, respectively, P<0.001) and UKK_FI (-18.1%, P<0.001) than females, results are described in Table II.

Males showed (Table II) higher handgrip strength (+37.3%, P<0.001) and lower flexibility (-31.4%, P<0.001) than females. Finally, males spent more time in physical daily activities (+10.1%, P<0.05) than females (Table II).

Cognitive performance

The cognitive performance, evaluated by the MoCA test, showed (Table II) that males had a higher education level (+3.9%, P<0.05), but lower values of MoCA test expressed in absolute (-4.0%, P<0.001) and adjusted (-4.3%, P<0.001) score, than females.

Factors of PA habits and cognitive abilities

Physical daily habits were directly related with relative FFM (r= 0.138, P=0.001) and UKK_FI (r=0.104,

TABLE I.—*Anthropometric characteristics and body composition of the subjects.*

	Male (N.=362)		Female (N.=530)		P
Age (y)	67.6±5.3	66 (8)	66.6±4.9	66 (7)	0.004
Body mass (kg)	82.2±11.2	81.5 (14.5)	67.9±11.5	66 (15.1)	0.001
Stature (m)	1.73±0.06	1.73 (9)	1.60±0.06	1.6 (7.4)	0.001
BMI (kg·m^{-2})	27.6±3.4	27.2 (4.4)	26.6±4.1	26 (5)	0.001
Waist circumference (m)	0.98±0.10	0.98 (0.13)	0.90±0.11	0.89 (0.15)	0.001
Hip circumference (m)	1.01±0.07	1.01 (10)	1.02±0.09	1.02 (0.11)	0.054
Waist to hip ratio	0.97±0.06	1 (0.1)	0.88±0.07	1 (0.1)	0.001
FFM (kg)	56.9±6.2	56.9 (8.3)	41.3±4.7	40.7 (6.5)	0.001
FM (kg)	25.3±6.8	24.5 (8.7)	26.6±8.4	25.3 (9.3)	0.011
FM (%)	30.3±4.9	30.5 (6.4)	38.5±6.2	38.2 (7.2)	0.001

All values are mean±SD and median (IQR).
BMI: Body Mass Index; FFM: fat-free mass; FM: fat mass.

TABLE II.—*Physical capacities and cognitive functions of the subjects.*

	Male (N.=362)		Female (N.=530)		P
2-km walk test (min)	19.6±2.3	19.4 (3.1)	20.7±2.3	20.5 (3.1)	0.001
HR_{pre} 2-km walk test (bpm)	70.3±11.8	69 (17.4)	74.5±11.3	74.5 (16)	0.001
HR_{post} 2-km walk test (bpm)	110.7±20.8	111 (27)	116.6±19.2	117 (28)	0.001
UKK Fitness Index (u.a.)	74.7±26.2	73.1 (37.1)	88.2±20.9	90.3 (27.7)	0.001
Hand-grip force (N)	45.6±7.9	46 (11)	28.6±5.5	28.7 (7)	0.001
Sit & Reach test (cm)	33.4±10.8	34 (15.2)	43.9±9.3	44.3 (13)	0.001
GPAQ (MET·min^{-1}·week^{-1})	5151±3760	4200 (4320)	4630±3374	3840 (3670)	0.047
Education (year)	12.7±3.5	13 (3)	12.2±3.7	13 (5.3)	0.035
MOCA test (u.a.)	24.7±2.9	25 (4)	25.7±2.9	26 (4)	0.001
MoCA test correct (u.a.)	23.3±2.7	23.6 (3.9)	24.3±2.5	24.6 (3.4)	0.001

All values are mean±SD and median (IQR).
HR: heart rate; GPAQ: Global Physical Activity Questionnaire; MoCA: Montreal Cognitive Assessment.

P=0.007), but inversely correlated with BMI (r=-0.096, P=0.005), relative (r=-0.138, P=0.001) and absolute FM (r=-0.114, P=0.001), and time spent in 2-km walk test (r=-0.175, P=0.001).

MoCA results were directly related with HR_{post} 2-km walk test (r=0.189, P=0.001), UKK_FI (r=0.149, P=0.001) and Education (r=0.343, P=0.001) but, inversely related with age (r=-0.208, P=0.001), waist circumference (r=-0.080, P=0.024), WHR (r=-0.200, P=0.001), HR_{pre} 2-km walk test (r=-0.087, P=0.025), time spent in 2-km walk test (r=-0.166, P=0.001).

Considering the adjusted MoCA for age and total year of education, HR_{post} 2-km walk test (r=0.130, P=0.001) and UKK_FI (r=0.112, P=0.005) were directly related with adjusted MoCA results. Conversely, WHR (r=-0.167, P=0.001) and HR_{pre} 2-km walk test (r=-0.086, P=0.028) were inversely related with results from the adjusted MoCA questionnaire.

In a multivariate analysis described in Table III, IV, the variables included into the equations provided a significant independent contribution to the model (P<0.001) for MoCA and adjusted MoCA test. When sex, age, time spent in 2-km walk test, WHR, HR_{pre} 2 km test and HR_{post} 2 km test were entered in the model, R^2 was 0.117 for MoCA test (Table III). It is important to highlight that we interpreted the dichotomic variable "sex" as male. In other words, for the purpose of the analysis, being male is positively correlated with the MoCA Score. In fact, when inserted in a multiple regression analysis, being male and younger, performing the 2-km walk test with a better time, having lower WHR, HR_{pre} 2-km walk test and higher HR_{post} 2-km walk test correlate with the result of MoCA Score.

TABLE III.—*Multiple linear regression analysis of MoCA.*

Parameter	Estimate	Standard Error	t value	Pr>|t|
Intercept	36.20	2.60	13.93	<0.0001
Sex	0.75	0.29	2.55	0.011
Age	-0.09	0.02	-3.87	0.001
2-km walk test	-0.15	0.05	-2.94	0.003
Waist to hip ratio	-3.93	1.79	-2.20	0.028
HR_{pre} 2-km walk test	-0.03	0.01	-2.72	0.007
HR_{post} 2-km walk test	0.02	0.01	3.36	<0.001
Root MSE	2.763			
Adj-R^2	0.117			
P	0.001			

TABLE IV.—*Multiple linear regression analysis of adjusted MoCA.*

Parameter	Estimate	Standard Error	t value	Pr>|t|
Intercept	28.75	1.81	15.89	<0.0001
Waist to hip ratio	-5.58	1.36	-4.10	<0.0001
HR_{pre} 2-km walk test	-0.02	0.01	-2.52	0.012
HR_{post} 2-km walk test	0.02	0.01	3.24	0.001
Root MSE	2.599			
Adj-R^2	0.047			
P	0.001			

At the same time, when WHR, HR_{pre} 2 km test and HR_{post} 2 km test were entered in the model, R^2 was 0.047 for adjusted MoCA test (Table IV). When the adjusted MoCA Score was inserted in the multiple regression analysis, having lower WHR, lower HR_{pre} 2-km walk test and higher HR_{post} 2-km walk test showed a significant correlation.

Discussion

The main results of this study indicated that older adults who are more active in their daily life showed better anthropometric characteristics and physical capacities, and better results in cognitive function.

As we hypothesized, we found a positive correlation between the GPAQ Score and anthropometric characteristics, body composition and physical capacities. In fact, it was shown that general physical activity, or even a certain period of exercise training, can positively impact body composition by decreasing BMI and fat-mass while increasing fat-free mass.[32-34] At the same time, as shown in the present study, being generally more active is also affecting physical fitness as evaluated by the decrease time to perform the 2-km walk test and the increased UKK_FI which is in line with previous findings and recommendations.[35, 36]

In addition, as previously reported, gait speed, anthropometric characteristics, and other evaluations of fitness, which may reflect a good level of physical activity, were found to be inversely correlated with cognitive impairment or dementia.[37, 38] These studies have identified physical activity as a potent lifestyle factor that plays a critical role in predicting rates of cognitive decline[39, 40] and the subsequent development of age-related neurodegenerative diseases like AD.[41, 42] It is worth to notice that, in the present study, the MoCA Score was highly correlated with the measures of physical fitness and more specifically with the time to complete 2 km, the heart rate and the FI, as observed previously.[5] In particular, decrease in brain volume and grey matter is a continuum during aging and associates with AD and dementia, but physical activity and higher cardiorespiratory fitness can lower this decline.[43] In addition, other physical activities like walking, can have a protective effect on AD, dementia and may also indirectly modify other potential risk factors (*i.e.* hypertension and obesity).[44, 45] Interestingly, during exercise the local blood flow to the brain increases as a direct response of the activity performed and such an increase indicates an enhanced neural activation.[46] Therefore, cerebral blood flow regulation can be considered of key importance when explaining the positive effects of exercise on cognitive function.[47, 48]

Other than aerobic activities, it was also demonstrated that a 6-month training period, including strength and flexibility, is enough to maintain cognitive function and general brain atrophy in people with mild cognitive impairment.[49] In fact, maintaining a higher muscle mass and lower body FM is associated with better cognitive performance.[50] However, in the present study, the MoCA Score was not directly correlated with strength or flexibility and this was also true when the adjusted value of MoCA was considered in the correlation analysis. In fact, when accounting for age and education year, the MoCA Score solely correlated with the WHR, HR and the FI. The reason for this may be found in the relative higher contribution of cardiorespiratory fitness rather than other physical factors in preserving cognitive function.

It was previously found that neurogenic factors like insulin-like growth factor-1 (IGF-1), brain-derived neurotrophic factor and vascular endothelial growth factor are of key importance in mediating structural and functional changes particularly in the hippocampus region when triggered by an exercise stimulus.[51, 52] In older adults, these neurogenic factors are related with a reduced atrophy in hippocampal volume, vascularization and therefore cognitive decline but positive changes are linked to exercise.[53, 54] As reported by the multiple regressions, herein the MoCA Score strongly correlates with WHR and HR measures. It is possible that the higher cardiovascular fitness exhibited in the present population could be linked to increased blood flow supply to the prefrontal and hippocampus region that protects from cognitive decline. Even though a direct correlation between the MoCA and the GPAQ questionnaire was not appreciable, a more active daily habits involving aerobic activities may be responsible for the correlation between WHR and MoCA. In fact, engaging in aerobic activities can improve the WHR along with the reduction in fat mass.[55]

Limitations of the study

Even though the present study was conducted on a relatively large population and on a wide variety of tests, it is important to take in consideration some limitations. In particular, we excluded from the former analysis subjects who scored less than 10 points in the MoCA test. This could be considered a confounding factor when evaluating extreme lower level of cognitive function. Moreover, we did not consider the time to perform the MoCA test which could give a deeper insight relative to the cognitive function in elderly population. At the same time, other domains of cognitive function (*i.e.* visual-constructional or memory) that are markedly declined during aging,[56] should be taken in consideration for further implications on cognitive function.

Conclusions

In conclusion, we collected data from a large population of older adults in Italy and Slovenia and demonstrated that, a higher level of physical activity habits can influence body composition by reducing FM, BMI and increasing FFM and the general aerobic fitness. Furthermore, it is also shown not only that these anthropometric measurements have a protection effect on cognitive impairment, but this can also be linked with daily physical activity. Finally, increased physical activity can directly improve cognitive performance but the opposite may also be true; that is, a high cognitive performance could lead to improved physical activity and better body composition. In an aging population with increasing incidence of dementia and cognitive impairment, physical activity intervention and strategies adapted for older adults are recommended to slow age-related decline and reduce disease-related cognitive impairment.

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Authors' contributions.—Federica Gonnelli: writing - original draft, visualization, data curation, formal analysis, validation; Nicola Giovanelli, Mirco Floreani, Andrea D'Amuri, Gloria Brombo, Edoardo Dalla Nora, Gianni Biolo, Filippo G. di Girolamo, Roberta Situlin: investigation, validation; Giulia Bravo: data curation, formal analysis, validation; Maria Parpinel: conceptualization, validation; Rado Pišot, Boštjan Šimunič, Saša Pišot: methodology conceptualization, validation; Angelina Passaro: conceptualization, methodology validation; Stefano Lazzer: project administration, supervision, visualization, methodology funding acquisition, conceptualization validation. All authors read and approved the final version of the manuscript.

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Chapter 4

4.1 Collaborations and supplementary projects

In this chapter, the parallel projects that composed the PhD period will be presented. In particular, the PhD candidate worked in two areas: Spinal Cord Injuries (SCIs) and Obesity.

The first theme was conducted throughout the whole period of the PhD during which in person and distance collaboration was conducted with the University of Louisville (Louisville, KY, USA) and with Professors Enrico Rejc and Yuri Gerasimenko. The second project was conducted with the local group of Exercise Physiology under the supervision of Professor Stefano Lazzer.

In particular, the focus of the collaboration with the University of Louisville core was the recovery of functional mobility (i.e. standing) in patients with chronic complete spinal cord injury. To recover independent standing in paralyzed subjects the use of the combination of Epidural Stimulation (ES) and locomotor-based training was used. The EMG activity of lower limb muscles was analyzed along with functional motor outcome of independent standing.

Lastly, the focus was shifted towards the analysis of locomotor training (i.e. stepping) with the use of Transcutaneous Spinal Cord Stimulation. In particular, we focused on analyzing coordination patterns, EMG activity of lower limb and trunk muscles before, during and after a specific locomotor training on a treadmill.

The first project resulted in a paper published on Scientific Reports Journal, while from the second project, an original article is currently under writing process.

The second collaboration focused on the analysis of the changes in the energy cost of locomotion during walking (Cw) related to the changes in body mass (BM, kg) and body composition in adolescents with obesity after a 9-moths multidisciplinary inpatient weight-reduction program consisting of lifestyle education, moderate energy restriction, and regular physical activity. This project resulted in an original article which was published on the Journal of Applied Physiology, Nutrition, and Metabolism.

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Neurophysiological markers predicting recovery of standing in humans with chronic motor complete spinal cord injury

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The appropriate selection of individual-specific spinal cord epidural stimulation (scES) parameters is crucial to re-enable independent standing with self-assistance for balance in individuals with chronic, motor complete spinal cord injury, which is a key achievement toward the recovery of functional mobility. To date, there are no available algorithms that contribute to the selection of scES parameters for facilitating standing in this population. Here, we introduce a novel framework for EMG data processing that implements spectral analysis by continuous wavelet transform and machine learning methods for characterizing epidural stimulation-promoted EMG activity resulting in independent standing. Analysis of standing data collected from eleven motor complete research participants revealed that independent standing was promoted by EMG activity characterized by lower median frequency, lower variability of median frequency, lower variability of activation pattern, lower variability of instantaneous maximum power, and higher total power. Additionally, the high classification accuracy of assisted and independent standing allowed the development of a prediction algorithm that can provide feedback on the effectiveness of muscle-specific activation for standing promoted by the tested scES parameters. This framework can support researchers and clinicians during the process of selection of epidural stimulation parameters for standing motor rehabilitation.

Individuals with motor complete spinal cord injury (SCI) are unable to stand, walk, or move their lower limbs voluntarily; this condition drastically affects their quality of life and implies severe limitations for functional recovery[1,2]. In the last years, there has been increasing evidence that the combination of lumbosacral spinal cord epidural stimulation (scES) and activity-based training that includes standing and stepping practice can promote the recovery of standing, walking and volitional leg movements in chronic, clinically motor complete[3–7] and incomplete[8] SCI individuals. To date, the prevailing view is that scES modulates the excitability of lumbosacral spinal circuitry by recruiting afferent fibers carrying somatosensory information[9–12]. This excitability modulation, in turn, can enable the spinal circuitry to generate appropriate muscle activation patterns in response to sensory information[5,13], and can also allow residual functionally silent descending input to modulate standing and stepping activation patterns[3,4,7,14].

The ability to stand with independent lower limb extension is a key achievement toward the recovery of functional mobility, and was consistently observed in all three motor complete SCI individuals that subsequently recovered over ground stepping and walking[3,7]. We showed that the appropriate selection of individual-specific scES parameters is crucial to promote standing with independent lower limb extension in this population[13]. The guidelines proposed for selecting a sub-set of electrode configurations to be tested for facilitating standing include adjusting cathodes (active electrodes) positon in order to target primarily extensor muscle groups according to

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the individualized map of motor pools activation[13]. Also, the use of multiple interleaving programs represents an important advantage compared to the use of a single program, as it allows to access different locations of the spinal circuitry with different intensities. However, to date there are no available algorithms or procedures that suggest the exact set of parameters to be applied for facilitating standing using tonic scES. In addition, the characteristics of muscle activation patterns leading to independent standing remain poorly understood. We have observed that EMG patterns that alternate bursts and negligible activation (i.e. similar to a rhythmic pattern) always result in poor standing ability[6]. On the other hand, overall continuous (i.e. non-rhythmic) co-activation of several lower limb muscles can promote standing with independent lower limb extension[6,7], but can also be observed during assisted standing. Understanding the characteristics of muscle activation patterns leading to independent standing can be of great importance for developing machine learning models capable of contributing to the selection of appropriate scES parameters.

Here, we introduce a novel framework for EMG data processing that implements spectral analysis and machine learning methods for characterizing EMG activity resulting in independent or assisted standing, and for identifying which of the tested sets of stimulation parameters promote muscle activation more effective for standing. We initially determined which spectral analysis method is more effective for identifying frequency-domain EMG features that characterize independent standing promoted by scES in humans with clinically motor complete SCI. We then integrated EMG frequency- and time-domain features in the computational model and tested its ability to accurately classify independent and assisted standing. Also, the physiological characteristics of EMG activity resulting in assisted and independent standing were defined. Finally, we applied the proposed framework on EMG datasets collected while research participants were testing different scES stimulation parameters for standing in order to rank the effectiveness of the muscle activation generated.

Results

Standing motor patterns with and without scES. Research participants required external assistance for lower limb extension when scES was not provided (Supplemental Video 1). Limited EMG activity was generally observed in response to the assisted sit-to-stand transition, and negligible EMG was recorded during standing with external assistance for hips and knees extension (assisted standing; Fig. 1a). When scES parameters for standing were applied, little activity and no movement was directly induced in sitting (Fig. 1b). On the other hand, without any change in stimulation parameters, sensory information related to the sit-to-stand transition and loading of the legs resulted in the generation of motor patterns with different characteristics (Fig. 1b,c; Supplemental Video 1 and 2). We have consistently observed that standing with independence of hip and knee extension (independent standing) is enabled by overall continuous (i.e. non-rhythmic) EMG activity of primary lower limb muscles crossing the hip, knee and ankle joints, with the exception of iliopsoas (Fig. 1b). However, continuous EMG patterns in these muscles can also be insufficient for achieving independent standing (Fig. 1c, top). On the other hand, the alternation between EMG bursts and negligible activity (i.e. similar to a rhythmic pattern) in lower limb muscles always resulted in assisted standing (Fig. 1c, bottom).

Time- and frequency-domain EMG features can accurately classify assisted versus independent standing. Two time-domain EMG variables (total power and pattern variability) were initially included in the proposed data processing framework aimed at classifying assisted and independent standing. This approach led to a classification accuracy for assisted and independent standing equal to 83.7% when all investigated muscles were considered for analysis. To improve this classification accuracy, we explored the inclusion of frequency-domain EMG features in the computational model. An initial step was devoted to the selection of an effective analysis method for EMG activity promoted by scES. When exemplary EMG signals recorded during assisted and independent standing were considered for analysis (Fig. 2a), Fast Fourier Transform (FFT) and, to a less extent, Short-Time Fourier Transform (STFT) primarily highlighted the content of frequencies related to epidural stimulation frequency (25 Hz) and its harmonics (Fig. 2b). On the other hand, Continuous Wavelet Transform (CWT) showed relevant frequency content that was not related to scES frequency. Also, the power of EMG signal collected during independent standing tended to be shifted toward lower frequency bins compared to that recorded during assisted standing. We then applied these three signal analysis methods on all EMG data collected during assisted and independent standing events from the 11 research participants considered in this study (Supplemental Table 1).

After normalization, dimension reduction and logarithmically transforming the EMG spectral feature values, the first three dimensions of standing data points (blue: independent standing; red: assisted standing) derived from the tested spectral analysis methods were plotted in Fig. 3a. It can be noted that the three analysis methods result in different distributions of the data points, and that CWT tends to present a clearer visual discrimination between assisted and independent standing data points. These feature values were subsequently used as input for K-nearest neighbor (KNN) classification. As expected from the exemplary data analysis and from the data points presented in Fig. 3a, we observed that CWT-derived features promoted the highest classification accuracy for assisted versus independent standing compared to STFT- and particularly FFT-derived features (Fig. 3b).

Hence, CWT-derived data were integrated with time-domain EMG features (EMG total power and pattern variability), resulting in a classification accuracy for assisted versus independent standing ranging from 92.2% to 97.5%, depending on the considered muscle(s) (Fig. 3c). This classification accuracy is higher and more consistent across examined muscles compared to when either frequency- or particularly time-domain EMG features alone were considered (Fig. 3b,c). Based on the results reported in this section, CWT-derived data were also considered for further analysis aimed at describing the physiological characteristics of muscle activation during standing with scES.

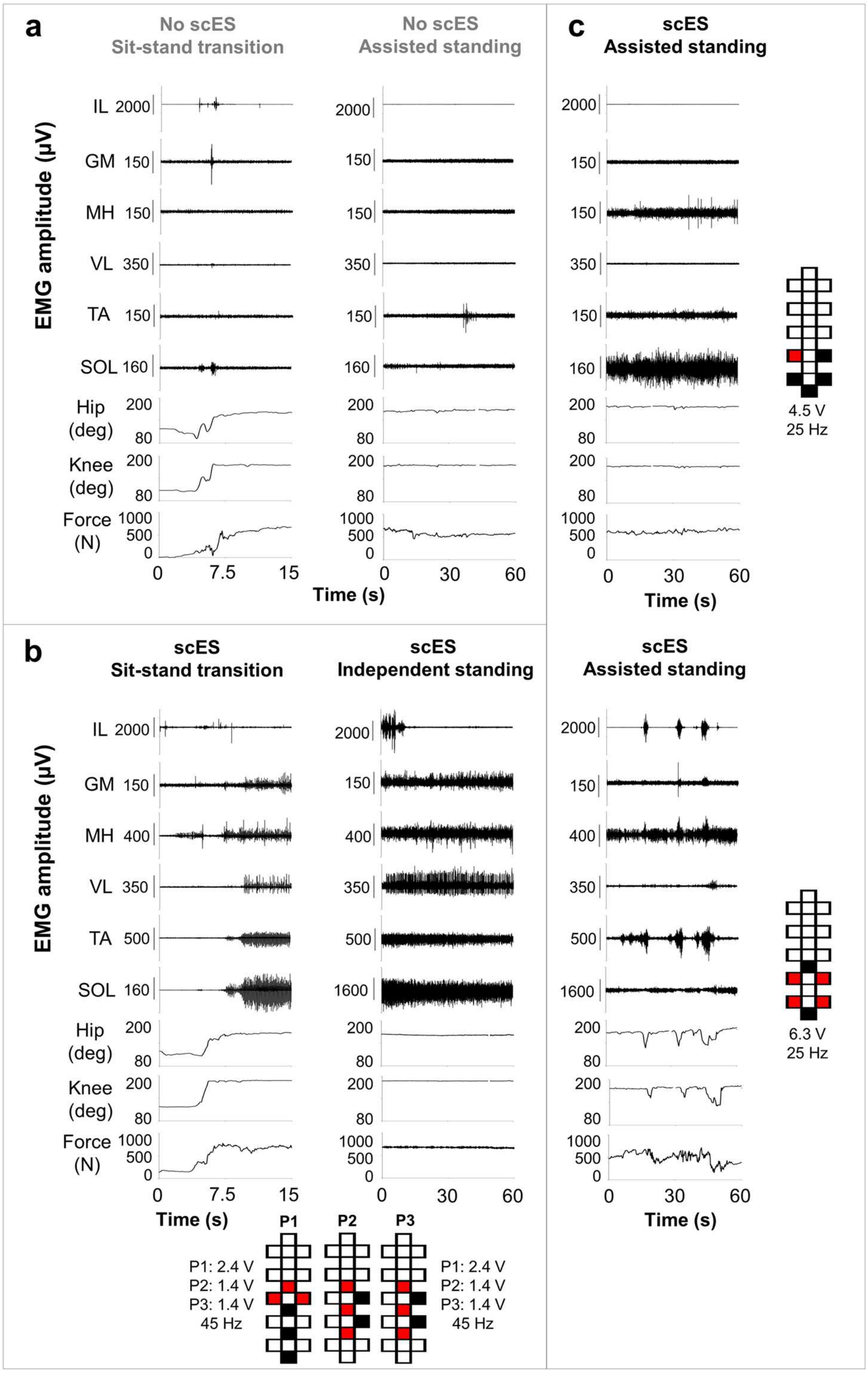

Figure 1. EMG, lower limb joint angles and ground reaction forces during sit-to-stand transition and during standing. Electromyography (EMG), hip and knee joint angle, and ground reaction forces recorded from research participant A59 during: (**a**) sit-to-stand transition and standing with external assistance for hips and knees extension (assisted standing) without spinal cord epidural stimulation (scES); (**b**) sit-to-stand transition and independent standing using scES; the participant held the hands of a trainer for balance control; **(c)** assisted standing with scES resulting from an overall continuous activation pattern (top) and from an EMG pattern characterized by the alternation of EMG bursts and little activation (bottom). Stimulation amplitude, frequency and electrode configuration (cathodes in black, anodes in red, and inactive in white) are reported for each standing condition. In (**b**), the participant was stimulated with 3 programs delivered sequentially at 15 Hz, resulting in an ongoing 45 Hz stimulation frequency. EMG was recorded from the following muscles of the right lower limb: IL, iliopsoas; GL, gluteus maximus; MH, medial hamstring; VL, vastus lateralis; TA, tibialis anterior; SOL, soleus.

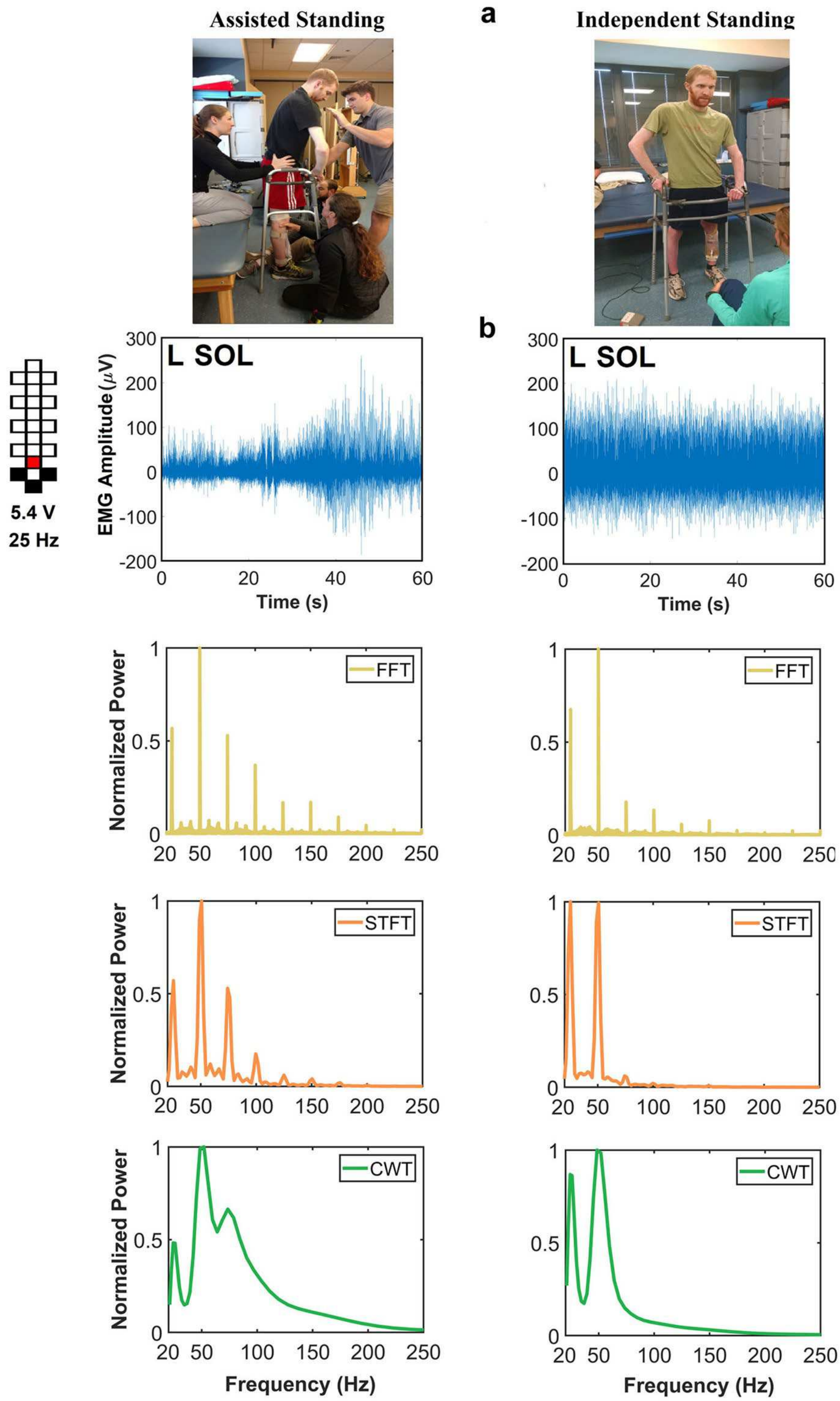

Figure 2. Spectral power density of EMG collected during standing. (**a**) Exemplary images of assisted standing (left) and independent standing (right). (**b**) EMG activity recorded from the left soleus (L SOL) of research participant A45 during assisted standing (left) and independent standing (right) with epidural stimulation, and related spectral power density generated by Fast Fourier Transform (FFT), Short-Time Fourier Transform (STFT) and Continuous Wavelet Transform (CWT). Stimulation amplitude, frequency and electrode configuration (cathodes in black, anodes in red, and inactive in white) are reported.

Physiological characteristics of muscle activation resulting in assisted or independent standing. Higher values of EMG pattern variability calculated from EMG linear envelope can characterize the muscle activation pattern consisting in the alternation between EMG bursts and lower activity (Fig. 4a; pattern

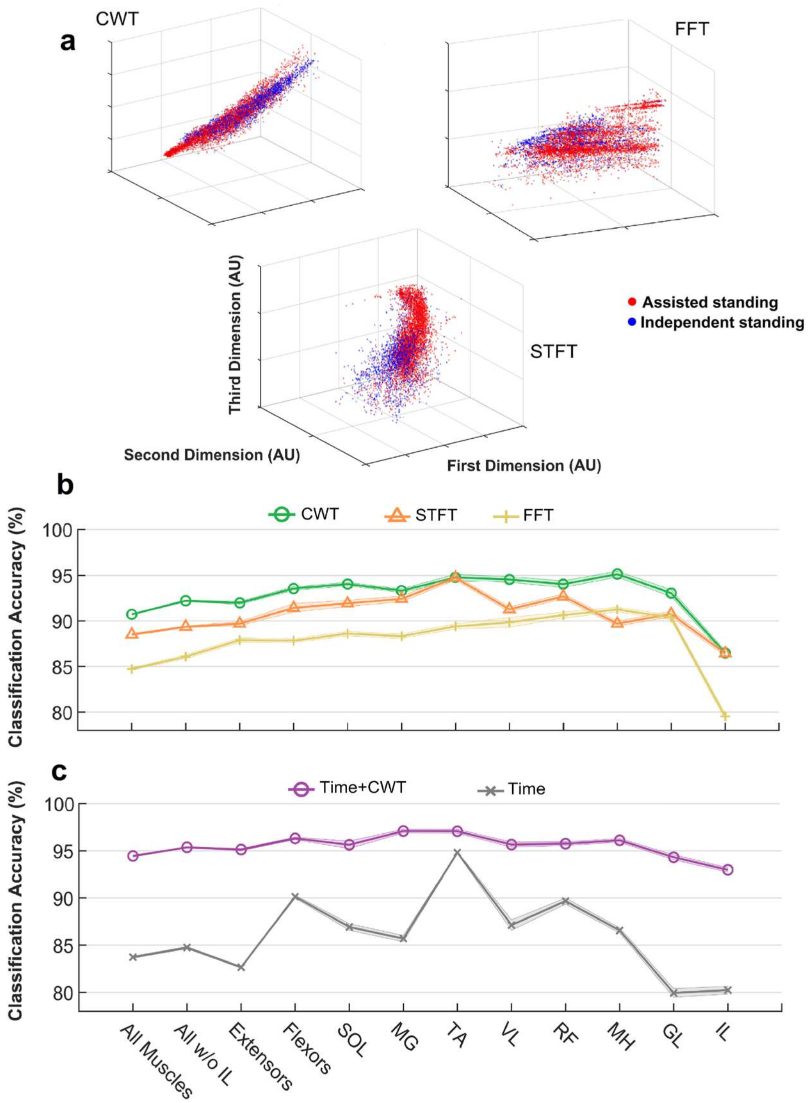

Figure 3. EMG-based classification of assisted vs independent standing. (**a**) First three dimensions of EMG standing data points (blue: independent standing, n = 2032 (127 standing events * 16 muscles); red: assisted standing, n = 4984 (316 standing events * 16 muscles, excluding 72 data points because of technical issues during EMG recordings) after normalization, dimension reduction and logarithmically transforming the spectral feature vectors extracted from Continuous Wavelet Transform (CWT), Short-Time Fourier Transform (STFT) and Fast Fourier Transform (FFT). Independent standing: independent lower limb extension and self-assistance for balance; assisted standing: external assistance for hip and knee extension and self-assistance for balance. K-nearest neighbor classification accuracy of the standing condition (assisted or independent standing) provided by feature vectors extracted from CWT, STFT and FFT **(b)**, and by time-domain features only or by the integration of time-domain features and CTW-extracted features (**c**), when considering all investigated muscles (left and right soleus (SOL), medial gastrocnemius (MG), vastus lateralis (VL), rectus femoris (RF), gluteus maximus (GL), tibialis anterior (TA), medial hamstring (MH), iliopsoas (IL); all muscles except left and right IL; primary extensor muscles (left and right SOL, MG, VL, RF, GL); primary flexor muscles (left and right TA and MH); and each investigated pair of muscles separately (left and right SOL, MG, TA, VL, RF, MH, GL, IL).

variability = 0.68), which results in poor, assisted standing. On the other hand, this feature does not discriminate between overall continuous EMG patterns resulting in assisted standing (Fig. 4b; pattern variability = 0.23) or independent standing (Fig. 4c; pattern variability = 0.22). CWT can provide additional information based on instantaneous EMG time- and frequency-domain features. For example, in case of the alternation between EMG bursts and limited activation, the maximum power variability is also relevant (Fig. 4a, Wavelet Scalogram; EMG maximum power variability = 1.35) compared to the condition of assisted standing with continuous EMG pattern (Fig. 4b; EMG maximum power variability = 0.35).

It is worth noting that differences in the CWT pattern can be observed also between the two similar continuous raw EMG activity recorded from the same individual during assisted and independent standing (Fig. 4b,c, respectively). In particular, assisted standing (Fig. 4b) tended to present greater EMG maximum power variability

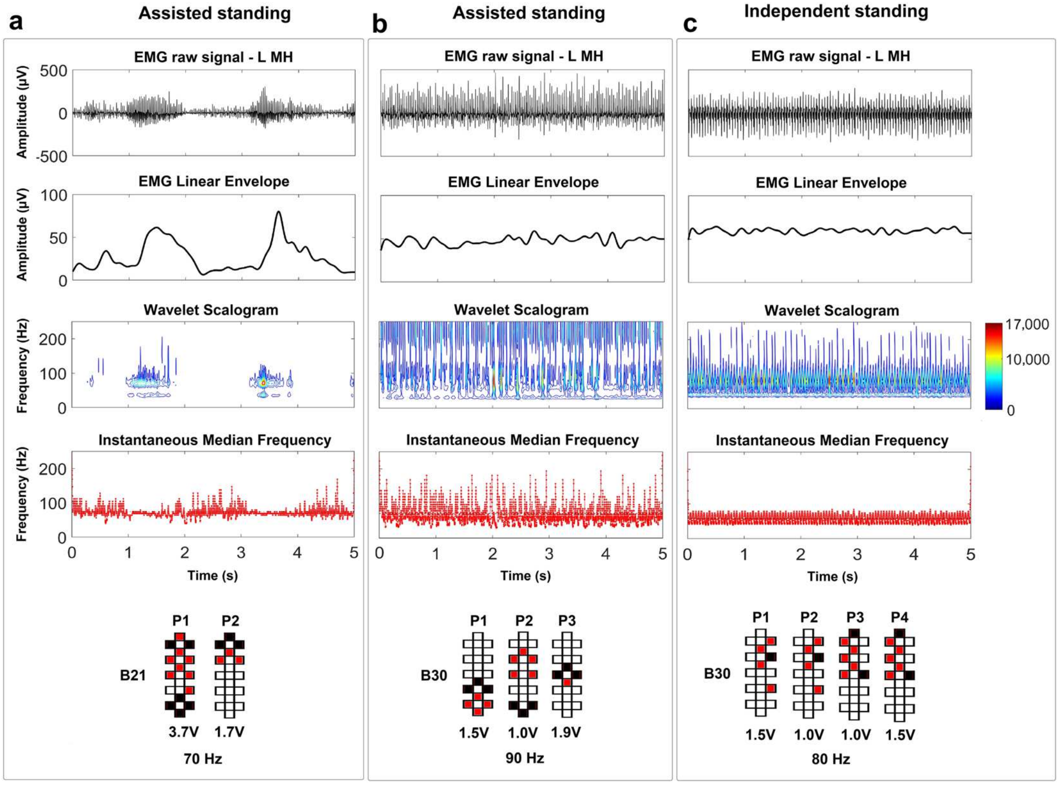

Figure 4. EMG-time and –frequency features characterizing exemplary standing activation patterns. EMG activity recorded from the left medial hamstrings (L MH) during standing with self-assistance for balance and external assistance for hip and knee extension characterized by (**a**) EMG bursts or by (**b**) continuous EMG activity, as well as (**c**) during independent standing with self-assistance for balance. For each standing condition, the EMG linear envelope, time-frequency power distribution of the signal (wavelet scalogram), and instantaneous median frequency are calculated from the plotted raw EMG. The wavelet scalogram is presented as contour plots, the power values of which are represented as colormaps, with the color scale showing the range of power values. Stimulation amplitude, frequency and electrode configuration (cathodes in black, anodes in red, and inactive in white) are reported. EMG activity reported in Panel A was collected from research participant B21. EMG activity reported in Panels B and C was collected from research participant B30.

(0.48), higher median frequency (70 Hz) and greater variability of median frequency (median frequency standard deviation = 32 Hz) compared to EMG activity that resulted in independent standing (0.35, 59 Hz, and 20 Hz, respectively; Fig. 4c). Interestingly, the same trend characterizing the differences in EMG features between assisted and independent standing can be also observed when the same exact stimulation parameters are applied (at different time points) to the same individual (Supplemental Fig. 1). In fact, even in this exemplary data, assisted standing tended to present greater EMG pattern variability (0.52), maximum power variability (1.51), median frequency (109 Hz) and median frequency standard deviation (49 Hz) as compared to standing with independent knees extension (0.23, 0.65, 81 Hz, and 23 Hz, respectively).

Paired comparisons (n = 8) show that standing with independent knees extension was promoted by significantly higher EMG total power, lower pattern variability, lower maximum power variability, lower median frequency standard deviation (SD), and lower median frequency as compared to assisted standing (Fig. 5). These differences were more pronounced when all investigated muscles and primary extensor muscles were considered as compared to primary flexor muscles. It is also worth noting that the average stimulation frequency was similar between the two conditions (47 ± 24 Hz for assisted standing and 51 ± 27 Hz for independent knees extension; $p = 0.844$).

We then performed a similar comparison including the 5 individuals who achieved assisted standing, standing with external assistance at the hips and independent knees extension, and independent standing (Supplemental Fig. 2). In summary, no substantial differences were observed between standing conditions with hips assisted or hips independent while the knees achieved independent extension. On the other hand, these two standing conditions demonstrating independent knees extension were characterized by higher EMG total power, lower pattern variability, lower median frequency variability, and lower median frequency compared to standing with hips and knees assisted, showing the same trend already reported in Fig. 5. Also, the average stimulation frequency was similar across these three standing conditions (58 ± 24 in assisted standing; 61 ± 29 Hz in hips assisted and knees independent; 62 ± 33 Hz in independent standing; $p = 0.182$).

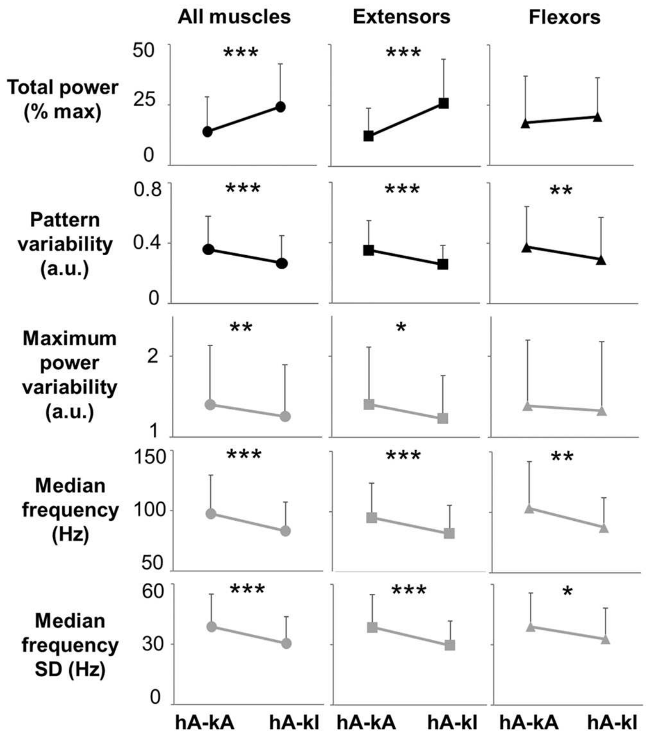

Figure 5. Quantification of EMG time- and frequency-domain features collected during standing with knees assisted or standing with independent knees extension. Representative time- and frequency-domain EMG features collected during standing with external assistance for hips and knees extension (hA-kA), and during standing with hips assisted and independent knees extension (hA-kI). EMG features values were averaged among research participants (n = 8) and among all investigated muscles (left and right soleus, medial gastrocnemius, vastus lateralis, rectus femoris, gluteus maximus, tibialis anterior and medial hamstring; total n = 114), among primary extensor muscles (left and right soleus, medial gastrocnemius, vastus lateralis, rectus femoris, gluteus maximus; total n = 80), or among primary flexor muscles (left and right tibialis anterior and medial hamstring; total n = 32). Values are expressed as mean ± standard deviation (SD). Differences were tested by Wilcoxon test. *p < 0.05; **p < 0.01; ***p < 0.001.

We then assessed standing conditions during which one lower limb (i.e. left side) achieved independent extension while the contralateral lower limb (i.e. right side) required external assistance. Similarly to the previous findings, higher EMG total power, lower pattern variability, lower maximum power variability, lower median frequency SD, and lower median frequency were detected from the limb achieving independent extension (Fig. 6). This trend showed more consistent statistical significance when all investigated muscles were pooled together for analysis.

It is worth noting that the higher median frequency and median frequency SD values observed during assisted standing can be attributed, at least partially, to the sharper peak shape of the spinal cord evoked responses generated (Fig. 7), which results in relevant increments of the instantaneous median frequency. Conversely, the smoother peaks of spinal cord evoked responses detected during independent standing contain more power at lower frequencies and result in a smaller instantaneous median frequency modulation.

Ranking the effectiveness of EMG activity for standing. The high classification accuracy for assisted versus independent standing provided by our EMG-based framework (Fig. 3) led us to develop a further computational step (prediction algorithm) aimed at ranking the effectiveness of muscle activation patterns generated for standing. We initially trained muscle-specific KNN models based on three different standing data sets related to different external assistance for standing. Classification accuracy was high (95.3% on average) for the KNN model trained with assisted and independent standing data set (Supplemental Fig. 3), while lower accuracy was observed for the other two models (Supplemental Table 2).

We then fed the prediction algorithm with a total of 48 assisted standing events performed by 6 individuals while different stimulation parameters were tested to search for optimal stand-scES parameters (Supplemental Fig. 3). The prediction algorithm correctly labeled as "assisted" (i.e. score between 0 and 0.5) 95.8% of the standing events considered (Supplemental Table 3). More importantly, its ranking scores varied substantially among stimulation parameters applied and investigated muscles. For example, participant A68 tested 9 different sets of scES parameters during the monitored standing session, obtaining average prediction scores ranging between 0.14 and 0.49 (Fig. 8a; Supplemental Table 3). In particular, during the standing attempt characterized by the lower score, only R IL and TA muscles showed EMG activity characteristics closer to independent standing. On the other hand, the standing attempt with the higher score was characterized by independent standing-like EMG

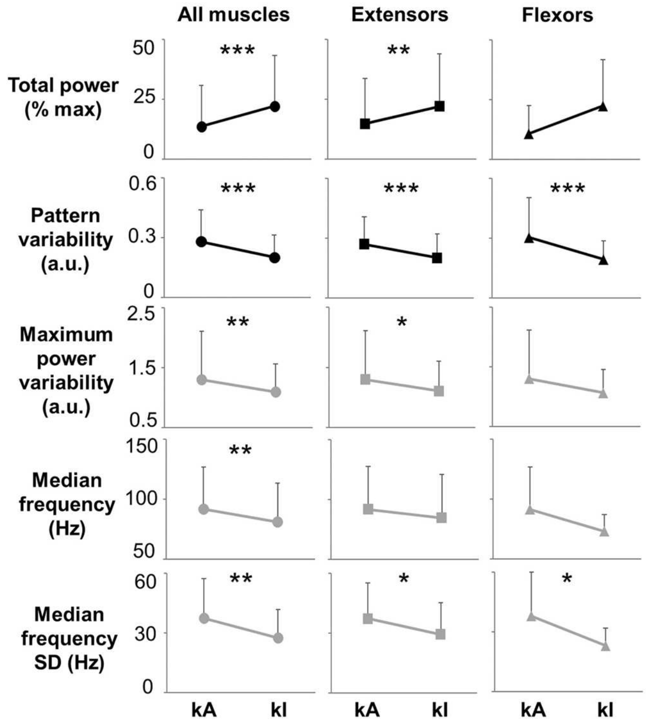

Figure 6. Quantification of EMG-time and –frequency domain features collected during standing with one lower limb assisted for knee extension. Representative time- and frequency-domain EMG features collected during standing when one lower limb achieved independent extension (kI) while the contralateral limb required external assistance for knee extension (kA). EMG features values were averaged among research participants (n = 7) and among different muscle groups of the assisted lower limb (kA) or the independent lower limb (kI). In particular, all investigated muscles (soleus, medial gastrocnemius, vastus lateralis, rectus femoris, gluteus maximus, tibialis anterior and medial hamstring; total n = 49), primary extensor muscles (left and right soleus, medial gastrocnemius, vastus lateralis, rectus femoris, gluteus maximus; total n = 35), or primary flexor muscles (left and right tibialis anterior and medial hamstring; total n = 20) were considered for analysis. Values are expressed as mean ± standard deviation (SD). Differences were tested by Wilcoxon test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

characteristics of several muscles (i.e. posterior thigh muscles and anterior muscles of the left lower limb). Also, EMG activity score of bilateral plantar flexor muscles was low in both standing conditions.

We then exemplified that the proposed prediction algorithm can be used also for ranking the effectiveness of EMG activity collected during standing with different amount of external assistance. For instance, it correctly labeled two standing events as "independent", and suggested that independent standing can be achieved even when the activation characteristics of few muscles are ranked as "assisted" (Fig. 8b). Also, when the algorithm is trained with the proper data set (data collected during standing with one lower limb assisted and the contralateral one generating independent extension, and during independent standing), it can rank the effectiveness of EMG activity generated by the lower limb assisted for knee extension (i.e. right side) while the other leg maintained independent extension (left side) (Fig. 8c).

Discussion

In this study, we developed a novel data processing framework for EMG activity promoted by spinal cord epidural stimulation during standing in individuals with severe SCI. This approach allowed us to uncover physiological characteristics of neuromuscular activation resulting in independent standing with self-assistance for balance. Additionally, we showed that, for each investigated muscle, the proposed machine learning algorithm can rank the effectiveness of EMG activity generated for standing. We discuss the implications of these findings in the context of mechanisms of motor pattern generation, and for the support this framework can provide during the selection of scES parameters, suggesting that it may contribute to facilitate the clinical translation of scES for standing motor rehabilitation.

Frequency-domain EMG features have been widely considered to study central motor control strategies during voluntary muscle activation[15–19], and the more recent development of technology for decomposing surface EMG signals has resulted in further insights on this topic[20–22]. On the other hand, EMG spectral features have been substantially neglected when the generation of activation patterns is promoted by scES. Gerasimenko and colleagues proposed a qualitative interpretation of spectral analysis (by FFT) performed on EMG signals collected from flexor and extensor muscles during stepping with scES[23]. In particular, they suggested that the dominant spectral peaks related to the stimulation frequency and its harmonics observed during the extension phase in extensor muscles reflected a predominance of monosynaptic-evoked responses. Conversely, the lack of

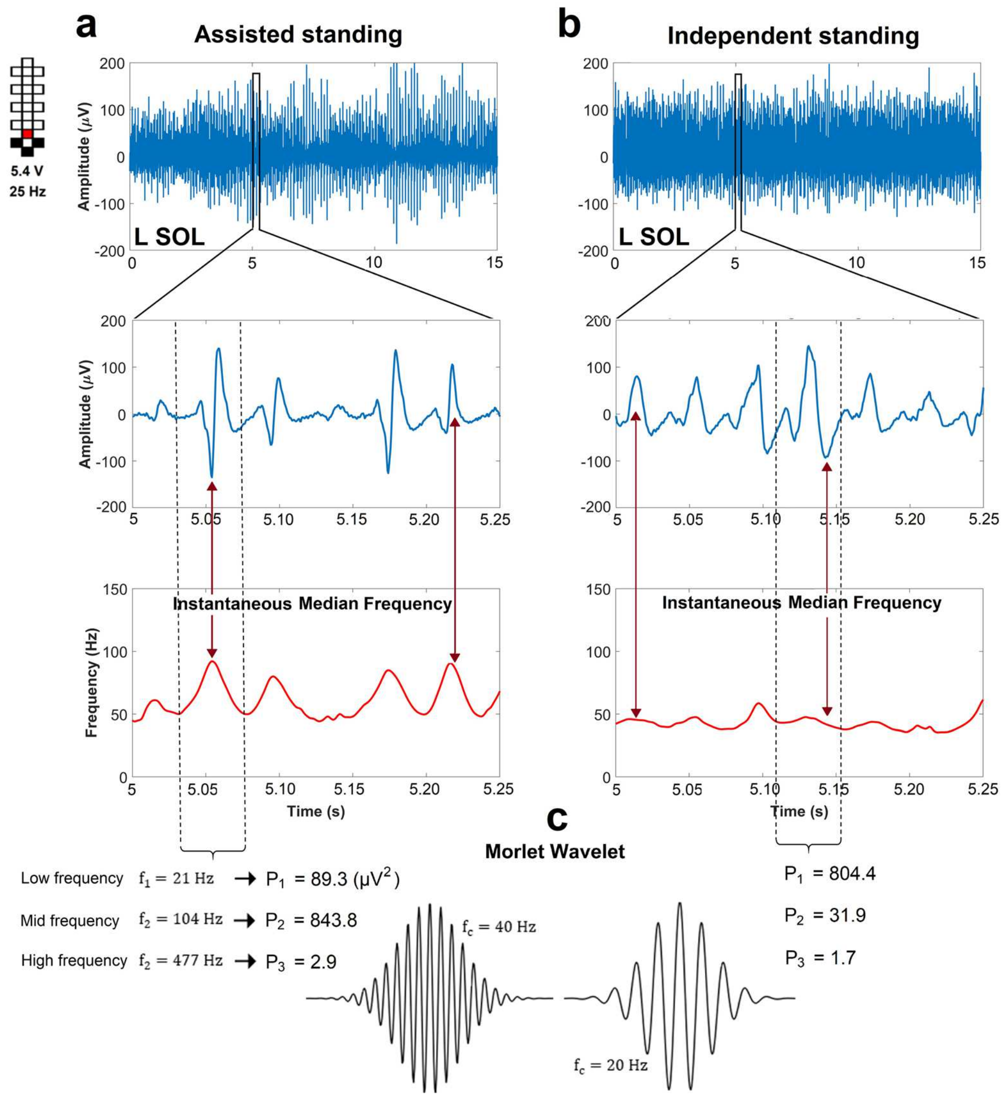

Figure 7. Representative spinal cord evoked responses collected during assisted and independent standing. EMG activity collected from the left soleus (L SOL) muscle of research participant A45 (**a**) during standing with external assistance for hips and knees extension and (**b**) during standing with independent lower limb extension. Spinal cord evoked responses and instantaneous median frequency calculated by continuous wavelet transform are reported for EMG activity included in the windows entered in the top panels. (**c**) Exemplary Morlet wavelet signals with high (40 Hz, left) and low (20 Hz, right) central frequency (f_c). The signal power (P, μV^2) at low (21 Hz), mid (104 Hz) and high (477 Hz) frequency bins is calculated for the spinal cord evoked responses highlighted by the dashed lines. Signal power collected during assisted standing is more concentrated at the mid frequency bin, while signal power collected during independent standing is more concentrated at the low frequency bin. Stimulation amplitude, frequency and electrode configuration (cathodes in black, anodes in red, and inactive in white) are reported for the two standing conditions.

consistent dominant peaks detected from the tibialis anterior muscle during the flexion phase of the gait cycle was interpreted as a predominance of polysynaptic-evoked responses. It is plausible that the marked dominant FFT spectral peaks related to the epidural stimulation frequency (i.e. Fig. 2a; Gerasimenko *et al.*[23]) have been often interpreted as features without relevant physiological meaning, thus discouraging further efforts aimed at quantifying scES-promoted EMG spectral parameters. Our approach was initially focused on understanding which spectral analysis method is more effective for identifying frequency-domain EMG features that characterize standing promoted by scES. This is important because, for example, FFT presents some intrinsic limitations such as poor time resolution, assuming the stationarity of EMG signal, and being unable to localize frequency content of the signal in the time domain, which may result in insufficient representation of the frequency content of scES-promoted muscle activation. Our results suggest that CWT is a spectral analysis method that can provide relevant frequency content not related to scES frequency (Fig. 2) as well as features resulting in the most accurate classification of assisted and independent standing (Fig. 3). This may be due to its high time and frequency resolution by decomposing the signal using numerous multi-resolution wavelets[24,25], which leads to an accurate

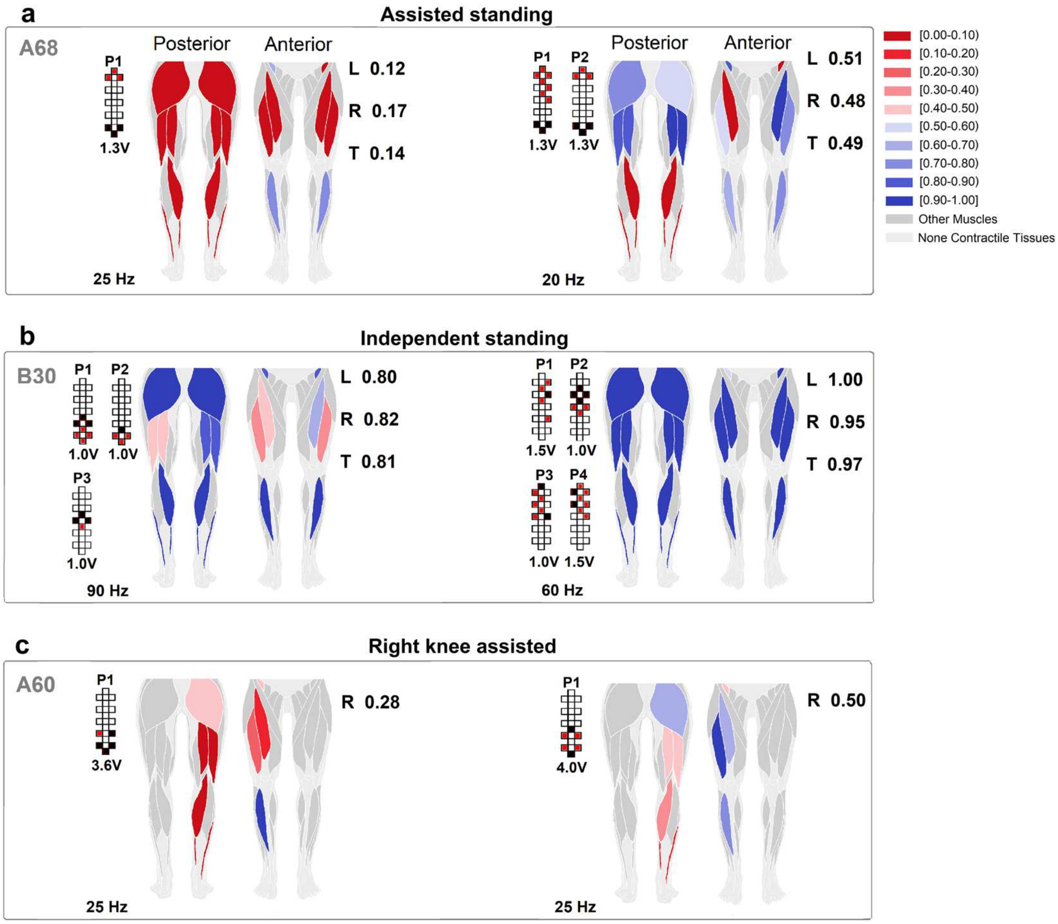

Figure 8. Colormap representing the effectiveness of standing muscle activation. The effectiveness of standing muscle activation is ranked by the prediction algorithm (see Methods). For each investigated muscle represented in the anatomical schematics, shades of red color rank activations labeled as assisted standing, while shades of blue color rank activations labeled as independent standing. Exemplary effects of different epidural stimulation parameters on muscle activation ranking during: (**a**) standing with external assistance for hips and knees extension; (**b**) standing with independent lower limbs extension; (**c**) standing with one lower limb assisted (right side) while the contralateral limb achieved independent extension. Average ranking score for the investigated muscles of the left (L) and right (R) lower limb, as well as for all muscles pooled together (total, T) are reported. Research participants' identification, stimulation amplitude, frequency and electrode configurations (cathodes in black, anodes in red, and inactive in white) are also reported.

characterization of the short time component within non-stationary signals[26]. Conversely, the resolution of STFT in time and frequency domain depends on the selected window size: longer window size increases the frequency resolution but impairs time resolution, which is not ideal for non-stationary signals like EMG[27].

To date, little is known about the characteristics of scES-promoted muscle activation resulting in the recovery of independent standing with self-assistance for balance after clinically motor complete SCI. We previously observed that the alternation between EMG bursts and negligible EMG activity (i.e. Fig. 1c, bottom) results in poor standing pattern and the need of external assistance[6]. Conversely, overall continuous (i.e. non-rhythmic) co-activation of several lower limb muscles was demonstrated when motor complete SCI individuals were able to maintain independent lower limb extension using spinal cord stimulation[6,7,13,28,29]. In this study, we identified additional EMG features that can discriminate the effectiveness of EMG activity for standing beyond the mere variability of the EMG pattern over time. In particular, independent standing events were promoted by EMG activity characterized by lower median frequency, lower variability of median frequency, lower variability of instantaneous maximum power as well as higher total power as compared to assisted standing (Figs 4–6; Supplemental Fig. 1). It is worth noting that the frequency-domain features can differentiate assisted and independent standing when the raw EMG signals are both overall continuous and demonstrate similar amplitude (Figs 4 and 7), and also when the same exact stimulation parameters are applied to the same individual resulting in different standing ability (Fig. 6; Supplemental Fig. 1). We then observed that the higher median frequency and higher variability of median frequency detected within overall continuous activation patterns during assisted standing reflect, at least partially, the sharper peaks of evoked responses, which carry more power at higher frequencies (Fig. 7). On the other hand, the smoother peaks of evoked responses detected during independent standing do not induce relevant increments in instantaneous median frequency. Partial desynchronization of motor units and/or greater involvement of polysynaptic responses, among others, may explain the smoother peaks of evoked responses detected during independent standing. Further studies involving the application of multi-channel surface EMG and dedicated signal processing may be useful for assessing the concurrent activity of many different motor units and investigating their firing pattern[30]. A limit of the present study is that it was not design to investigate how different stimulation parameters and activity-based training contributed to the activation pattern characteristics resulting in assisted or independent standing. Another limitation of the proposed framework is that it does not discriminate sufficiently between standing conditions with hips assisted and hips independent while the knees maintain independent extension (Supplemental Fig. 2; Supplemental Table 2). This may be due, at least partially, to the fact that trunk muscles contributing to hip joint control were not considered for analysis because of the presence of scES artifacts.

Presently, the prevailing view is that scES facilitates motor pattern generation by recruiting primarily large myelinated fibers associated with somatosensory information, and particularly with proprioceptive and cutaneous feedback circuits, at their entry into the spinal cord as well as along the longitudinal portions of the fiber trajectories, altering the excitability of lumbosacral spinal circuits[3–5,9–12,31–35]. This more functional excitability state, in turn, enables the spinal circuitry to use somatosensory information and residual supraspinal input as sources of control for generating motor patterns appropriate for standing and stepping. It is also important to consider that stimulation parameters play a crucial role in determining extent and proportion of the modulation of sensory-motor pathways impacted by scES[36]. For example, previous studies proposed that different stimulation frequencies may access different inhibitory and/or excitatory pathways within the spinal circuitry[37], and that higher stimulation frequencies may promote a progressive integration of additional afferent inputs through the greater involvement of interneurons[13,38,39]. While we attempt to select higher stimulation frequencies to promote the integration of afferent and residual supraspinal input through the greater involvement of interneurons and to promote more physiological (i.e. non-pulsatile) muscle contraction, this approach may also result in "bursting" EMG patterns not effective for standing (Figs 1c and 4a). This is conceivably due to the fact that these higher frequencies are applied in combination with stimulation sites and amplitudes that result in the reconfiguration of the interneuronal network (via presynaptic and synaptic mechanisms) to favor the generation of "bursting, locomotor-like" EMG patterns[37]. Because of the important role of stimulation frequency in contributing to the characteristics of activation pattern generated, an effort was devoted to understand whether the differences in EMG features observed in the present study between assisted and independent standing, and particularly the frequency-domain features, were associated with the application of different scES frequencies. Interestingly, the average stimulation frequencies delivered during assisted and independent standing were very similar (see description of Fig. 5 and Supplemental Fig. 2 in Results). Moreover, consistent differences in EMG features were also observed between the one lower limb achieving independent extension and the contralateral lower limb requiring external assistance, while the same spinal cord stimulation was applied (Fig. 6). Taken together, these findings further suggest that the characteristics of muscle activation result from the complex interaction among the stimulation parameters applied, the somatosensory information as well as any residual supraspinal input integrated by the spinal circuitry[3,8,14], and the characteristics of its extensive, individual-specific reorganization after SCI[40,41].

The integration of novel CWT-derived features with EMG total power and pattern variability enabled the proposed machine learning (KNN) algorithm to accurately classify assisted and independent standing (Fig. 3). It is interesting to note that the higher classification accuracy was achieved when considering medial gastrocnemius (96.3%) and tibialis anterior (97.5%), two primary ankle muscles. This may be due to the fact that plantar flexor muscles play a significant role in controlling and stabilizing the body during bipedal quiet standing[42,43], and that their net mechanical output is also influenced by their antagonist (TA). On the other hand, the lower classification accuracy (92.2%) resulted from the analysis of EMG collected from iliopsoas, a non-antigravity muscle that was monitored via fine-wire electrodes, which presented a greater intrinsic placement variability compared to the surface electrodes used for all other muscles. However, we took advantage of this overall high classification accuracy to develop a prediction algorithm capable of ranking the activation effectiveness of the investigated muscles for standing (Fig. 8; Supplemental Table 3). This approach results in a quasi-real time feedback on the effectiveness of scES-promoted muscle activation for standing, which can support researchers and clinicians during the process of selection of stimulation parameters. For example, the data reported in Fig. 8a suggests that left and right plantar flexors presented poor activation with both sets of stimulation parameters, being one of the possible factors limiting the achievement of independent standing. While the present framework does not propose the specific stimulation parameters adjustment for optimizing muscle activation, it can substantially improve the application of guidelines previously suggested for this task[13]. For instance, information on the individualized map of motor pools activation[44,45] may be retrieved and used to determine the electrode field of an additional interleaving program aimed at targeting primarily the location of the spinal circuitry related to plantar flexors. Then, a much smaller cohort of cathode-anode combinations as well as amplitude and frequency values can be tested, thus increasing the probability of achieving an improved activation pattern in a reduced amount of time. This is of particular interest considering that over 40 million different combinations of electrode configurations are potentially available when using a 16-electrode array, and that minor adjustments in the electrode configuration may or may not affect significantly standing motor pattern[13]. The second important contribution of the proposed framework is that it can suggest which of the tested set of stimulation parameters promotes activation patterns more effective for standing. This can be relevant when different sets of parameters result in the same need of external assistance (i.e. total score of Fig. 8a,c; Supplemental Table 3), and the decision on which parameters to apply for stand training should be made.

In conclusion, we have demonstrated that the proposed data analysis framework can characterize time- and frequency-domain EMG features resulting in the recovery of independent standing with self-assistance for

ID	Age (yrs)	Sex	Duration of Injury (Yrs)	Neurological Level	AIS Grade	AIS Score						Anal sensation	Anal contraction	Intervention
						Sensory (T10 - S5, core out of 24)				Motor (lower extremity)				
						L LT	L PP	R LT	R PP	L	R			
B13	33	M	4.2	C7	B	10	10	10	8	0	0	Yes	No	Motor #1
B07	24	M	3.4	T2	B	15	11	18	10	0	0	Yes	No	Motor #1
A45	24	M	2.2	T4	A	0	0	0	0	0	0	No	No	Motor #1
A53	27	M	2.3	T4	A	0	0	0	0	0	0	No	No	Motor #1
B23	32	M	3.3	C5	B	8	0	10	0	0	0	Yes	No	Motor #2
A59	26	M	2.5	T4	A	0	0	0	0	0	0	No	No	Motor #2
B30	22	F	3.3	T1	B	17	5	17	9	0	0	Yes	No	Motor #2
A60	23	M	3.1	T4	A	0	0	0	0	0	0	No	No	Motor #2
A68	35	M	3.8	C4	A	0	0	0	0	0	0	No	No	Cardiovascular
A41	24	M	7.2	C4	A	0	0	0	0	0	0	No	No	Cardiovascular
B21	31	M	7.0	C4	B	1	1	0	0	0	0	Yes	No	Cardiovascular

Table 1. Clinical characteristics of the research participants. Sensory score was designated by light-touch (LT) and pinprick (PP) of the left (L) and right (R) lower limb, below the level of injury. Neurological level: neurological level of the lesion; AIS: American Spinal Injury Association (ASIA) Impairment Scale. Each research participant was enrolled in an interventional study focused on either the facilitation of standing and stepping (Motor #1 and Motor #2) or the recovery of cardiovascular function (Cardiovascular).

balance in individuals with motor complete SCI using spinal cord epidural stimulation. This allowed us to develop a machine learning algorithm capable of ranking the effectiveness of muscle-specific activation for standing, which may facilitate the process of selection of stimulation parameters for standing motor rehabilitation. Future studies should be aimed at investigating the effects of stimulation parameters modulation on the EMG features related to standing ability. Also, the application of a similar framework on EMG activity collected during stepping with epidural stimulation may provide novel insights on mechanisms of motor pattern generation and selection of epidural stimulation parameters.

Methods

Participants. Eleven individuals with chronic, clinically motor complete or sensory and motor complete SCI individuals are included in this study (Table 1). The research participants signed an informed consent for lumbosacral spinal cord epidural stimulator implantation, stimulation, activity-based training and physiological monitoring studies, which were conducted according to the standards set by the Declaration of Helsinki, and were approved by the University of Louisville Institutional Review Board (ClinicalTrials.gov identifiers NCT02037620 and NCT02339233). Prior to epidural stimulator implantation, the International Standards for Neurological Classification of Spinal Cord Injury[46] was used for classifying the injury using the ASIA (American Spinal Injury Association) Impairment Scale (AIS). The research participants were implanted with a spinal cord epidural stimulation unit over 8 years (2009 to 2017), and were enrolled into interventional studies focused on either the facilitation of standing and stepping or the recovery of cardiovascular function. The research participants and other persons appearing in figures and supplemental videos included in this paper also gave written informed consent and granted full permission for their image to be used in publication online.

Surgical implantation of electrode array and stimulator. The epidural spinal cord stimulation unit (Medtronic, RestoreAdvanced) and the 16-electrode array (Medtronic, 5-6-5 Specify) were surgically implanted in the eleven research participants. The electrode array was positioned over the midline of the exposed dura, in correspondence of spinal segments L1-S1/S2 (Supplemental Fig. 3)[5,47]. EMG recordings from leg muscles were obtained intraoperatively during spinal stimulation at 2 Hz using midline, left and right electrode pairs in order to localize the optimal placement of the array. The wire leads were then internalized and tunneled subcutaneously to the abdomen and connected to the neurostimulator.

Experimental procedures. Experimental sessions devoted to the assessment of motor patterns generated during standing were performed over ground in a full bodyweight bearing condition, using a custom-designed standing apparatus. This standing apparatus is comprised of horizontal bars anterior and lateral to the individual that were used for upper extremity support and balance assistance as needed. Mirrors were placed in front of the participants and laterally to them, in order to provide visual feedback on their body position. Four individuals (B23, A59, B30, A60) performed standing also using a walker that was fixed to a wider aluminum frame base, a regular walker, or holding the hands of a trainer (hand-hold). Research participants always self-assisted balance control using their upper limbs during the standing events considered in this study.

scES was applied while the participant was seated. The sit to stand transition was performed with the research participants using their upper limbs to partially pull themselves into a standing position, and trainers positioned at the pelvis and knees manually assisting as needed the transition. If needed, research participants with higher level of injury and limited upper limb function were also assisted by trainers at the axillary triangle during the sit to stand transition.

When a stable standing position was achieved, if the knees or hips flexed beyond the normal standing posture, external assistance was provided at the knees distal to the patella to promote extension, and at the hips below the iliac crest to promote hip extension and anterior tilt. In particular, external facilitation was provided either manually by a trainer or by elastic cords, which were attached between the two vertical bars of the standing apparatus.

Selection of scES parameters for standing. The subset of scES parameters tested to facilitate standing were selected following dedicated guidelines[13], which are based on the literature as well as on previous assessments performed on the same research participants in supine position. Stimulation site and electrodes configuration have important implications for both topographical and functional organization of the activation pattern facilitated by scES. For example, we defined electrode fields that were more focused on the caudal portion of the electrode array to increase the excitability of distal muscles' motoneuron pools, or selected electrode fields that were more extended toward the rostral portion of the array to increase the excitability of proximal muscles' motoneuron pools[44]. Additionally, we initially positioned cathodes (active electrode) caudally, and more caudally than anodes, as this was shown to possibly promote better motor patterns characteristic of standing behavior in clinically motor complete SCI individuals while lying supine and standing[13,37]. Also, in case of activation differences between left and right lower limb, active electrodes were unbalanced between lateral columns of the electrode array, as the lateral placement of the epidural stimulation electrodes with respect to the spinal cord midline was shown to promote motor responses in muscles ipsilateral to the stimulation[9]. Furthermore, we adjusted cathodes position in order to target primarily extensors muscle groups according to the individualized map of motor pools activation. This was determined during previous assessments of muscle activation responses to different localized, two-electrode configurations using 2 Hz stimulation frequency, with the research participants in supine position (similarly to what has been previously reported[44]).

Epidural stimulation was initially delivered at a near-motor threshold stimulation amplitude that did not elicit directly lower limb movements in sitting, as the goal was to allow sensory information (and possibly residual descending input) to modulate the motor pattern. Stimulation amplitude and frequency were then synergistically modulated during standing in order to identify the higher stimulation frequency that elicited a continuous (non-rhythmic) EMG pattern effective to bear body weight, because higher stimulation frequencies may favor the integration of afferent and residual supraspinal input through the greater involvement of interneurons[38,39] and result in a more physiological (i.e. non-pulsatile) muscle contraction. These guidelines were also applied to multiple interleaving programs (i.e. Figs 1b and 4), which allow the access of different locations of the spinal circuitry with different stimulation intensities and frequencies. Each research participant underwent one or two experimental sessions aimed at selecting appropriate scES parameters for standing prior to the beginning of stand training. Stimulation parameters were also adjusted throughout stand training. In particular, dedicated sessions were performed approximately every 2–4 weeks to monitor motor behavior and lower limb EMG activity while testing different stimulation parameters to contribute to their selection.

Activity-based interventions. The standing experimental sessions considered for the present study were always performed using scES, and were carried out after scES implantation and prior to any training as well as after the different interventions defined for each of the three study groups, which are briefly described here below. All activity-based training protocols were always performed with scES optimized for the task that was practiced.

Motor #1 (Described by Rejc and colleagues[6]). Research participants underwent 81 ± 1 sessions of full weight-bearing stand training (1 hour of standing, five sessions per week). Stand training was performed using the custom-designed standing apparatus previously described. Participants were encouraged to stand for as long as possible throughout the training session, with the goal of standing for 60 min with the least amount of assistance. Seated resting periods occurred when requested by the individuals. Following the completion of stand training and respective experimental sessions, the research participants performed 81 ± 2 sessions of step training with body weight support (Innoventor, St. Louis, MO) on a treadmill (1 hour, five sessions per week). Body weight support, stepping speed and bouts duration were adapted to each individual to obtain appropriate stepping kinematics. Following a stepping bout, participants were encouraged to maintain standing. The research participants were also encouraged to practiced voluntary trunk and lower extremity movements with scES 5 days a week (1 hour per session).

Motor #2 (Described by Angeli and colleagues[3]). The initial portion of the training protocol (81 ± 6 sessions) consisted of one 1-hour training session per day for five days a week, and the trained motor task (standing or stepping) was alternated every session. During the second portion of the training protocol (79 ± 6 sessions), one supplementary training session was added every two weeks to the weekly schedule, to result in two training sessions per day. The research participants were encouraged to volitionally contribute to the motor pattern generation during training. Research participants were also encouraged to practice voluntary trunk and lower extremity movements with scES 5 days a week (1 hour per session).

Cardiovascular This study protocol included three different interventions, which were performed in sequential order and were cumulative. Research participants presenting with persistent low resting blood pressure initially completed 83 ± 3 two-hour sessions of daily scES aimed at increasing systolic blood pressure within 105 to 120 mm Hg, as reported by Harkema and colleagues[48]. In addition to this task, they subsequently performed approximately 80 training sessions of voluntary trunk and lower extremity movements practice (5 days a week, 1 hour per session). Following the completion of voluntary movements training, research participants also included stand training in their daily activities. In particular, research participants completed 83 ± 1 stand training sessions (5 days a week, 1 hour of standing per session).

Data acquisition. EMG, ground reaction forces and kinematics data were recorded at 2000 Hz using a custom-written acquisition software (National Instruments, Austin, TX). EMG activity of right (R) and left

(L) gluteus maximus (GL), medial hamstring (MH), rectus femoris (RF), vastus lateralis (VL), tibialis anterior (TA), medial gastrocnemius (MG) and soleus (SOL) was recorded by means of bipolar surface electrodes with fixed inter-electrode distance[5]. Bilateral EMG from the iliopsoas (IL) was recorded with fine-wire electrodes. Two surface electrodes were placed symmetrically lateral to the electrode array incision site over the paraspinal muscles in order to record the stimulation artefacts, which were used as indicators of the stimulation onset (time points when the stimulus pulses were applied). Lower limb joint angles were acquired using a high-speed optical motion capture system (Motion Analysis, Santa Rosa, CA). Ground reaction forces were collected using a high-resolution pressure sensing mat (HR mat system, TEKSCAN, Boston, MA) or force platforms (Kistler Holding AG, Winterthur, Switzerland).

Data analysis. Each standing event considered for analysis was characterized by consistent external assistance and stimulation parameters for a duration ranging between 40 and 70 seconds; the initial and final 5 seconds of each event were not considered for analysis. Each event was labeled as follow, based on whether hips and knees needed external assistance for maintaining standing or achieved independent extension: hips and knees assisted (assisted standing); hips assisted and knees independent; hips and knees independent (independent standing); one knee assisted and the contralateral knee independent.

The EMG processing framework consisted of several steps including spectral analysis, time- and frequency-domain features extraction, dimension reduction, classification and prediction, which are described here below.

EMG time domain features. The EMG pattern variability was assessed by calculating the coefficient of variation (standard deviation / mean) of the EMG linear envelope obtained by filtering the rectified EMG signal through a low-pass digital filter (cutoff frequency: 4 Hz)[6].

The EMG total power was calculated using the following equation:

$$P = \frac{1}{T}\int_0^T |x(t)|^2 dt \tag{1}$$

where x(t) is the recorded EMG signal and T is the length of the signal.

For each examined muscle, the total power was then normalized by the maximum value detected within each participant.

Spectral analysis. In this study, we initially applied three signal analysis methods to the scES-promoted EMG activity, with the goal of identifying the analysis method that better differentiates conditions of assisted standing and independent standing based on the spectral information provided. Fast Fourier Transform (FFT) is one of the most commonly used methods for spectral analysis of EMG signals[49]. It is characterized by high frequency resolution and poor time resolution, and cannot localize the frequency content of the signal in the time domain. Short-Time Fourier Transform (STFT) was designed to increase the time resolution of FFT by selecting a fixed-size window moving across the EMG signal[50]. Finally, Continuous Wavelet Transform (CWT) has been designed to effectively localize the frequency content of non-stationary signals in both time and frequency domains by using size adjustable wavelets, which do not compromise time or frequency resolutions[51–53].

Frequency domain features. Power spectral density (PSD) of FFT, STFT spectrogram ($s(t, f)$ and CWT scalogram ($p(f, t)$) (using Morlet wavelet, $\psi_{f,\,t}(\tau)$) was calculated as reported in Eqs 2 to 4, respectively.

$$\begin{aligned} FFT(f) &= \int x(\tau)\exp(-j2\pi f\tau)d\tau, \\ PSD(f) &= |FFT(f)|^2 \end{aligned} \tag{2}$$

$$\begin{aligned} STFT(t,\ f) &= \int w^*(\tau - t)x(\tau)\exp(-j2\pi f\tau)d\tau, \\ \text{STFT Spectrogram:}\ \ s(t,\ f) &= |STFT(t,\ f)|^2 \end{aligned} \tag{3}$$

$$\begin{aligned} CWT(f,\ t) &= \int x(\tau)\psi^*_{f,\,t}(\tau)d\tau \\ \text{Morlet wavelet: } \psi_{f,t}(\tau) &= \frac{1}{\sqrt{f_0/f}}\psi(\tau - t/(f_0/f)) \\ \text{Wavelet scalogram:}\ \ p(f,\ t) &= |CWT(f,\ t)|^2 \end{aligned} \tag{4}$$

where f_0 is the sampling frequency (2 kHz).

The STFT window size was selected at 0.3 seconds to increase the time resolution of FFT while minimally compromising the frequency resolution.

Mean frequency, median frequency, dominant frequency, and maximum power are the physiologically relevant features that were extracted from FFT output.

As for STFT and CWT, instantaneous values of mean frequency (IMNF), median frequency (IMDF), dominant frequency ($F_{max}(t)$) and maximum power ($P_{max}(t)$) were initially calculated (Eqs 5–8), and their average and standard deviation (SD) were considered as features for further analysis. In particular, EMG maximum power variability was assessed by calculating its coefficient of variation (SD/mean).

$$IMNF(t) = \frac{\sum_{j=1}^{M} f_j p(f_j,\ t)}{\sum_{j=1}^{M} p(f_j,\ t)} \tag{5}$$

$$\sum_{j=1}^{IMDF(t)} p\left(f_j,\ t\right) = \sum_{j=IMDF(t)}^{M} p(f_j,\ t) \tag{6}$$

$$F_{max}(t) = argmax_f(p(f,\ t)) \tag{7}$$

$$P_{max}(t) = max_f(p(f,\ t)) \tag{8}$$

where M is the number of frequency bins.

Classification. All the calculated EMG feature values (predictors) were normalized to their maximum to remove the effects of their units in the classification step. The non-negative matrix factorization (NNMF) algorithm was applied to the normalized measurements for dimensionality reduction[54] and the output values were logarithmically transformed in order to stabilize the variance[55]. We then performed preliminary analysis to determine which classification method resulted in the highest accuracy for classifying conditions of assisted standing versus independent standing based on the EMG features herein considered. In particular, K-nearest neighbor (KNN)[56] performed better than Naïve Bayes[57], binary Support Vector Machine[58], and ensemble decision trees[59]; therefore, KNN was the classification method applied in the present study.

The KNN classifier includes several parameters that need to be adjusted in order to achieve its best classification performance. These parameters include number of neighbors, distance measures, distance weights and standardization (centering and scaling the predictors). In order to find the optimized parameters for the classifier, the Bayesian optimization algorithm was used[60]. The objective function for the optimization is log (1 + Cross Validation Loss). The Cross Validation Loss is the ratio of misclassified observations during the cross validation step. The classification accuracy is calculated using 10-fold cross validation method and calculated as a percentage value of 1- Cross Validation Loss[57]. The KNN classifier with the parameter optimization algorithm and the cross validation step were iterated 10 times and the average accuracy values and the 95% confidence intervals are reported.

Prediction. All calculated EMG feature vectors (time- and CWT-derived features) that we included in the classification step were then used as a training dataset for the prediction part of the framework. A trained model is defined as a model that has captured the patterns in the training dataset. Based on these learnt patterns, the trained model can predict the class label (i.e. assisted or independent standing) for new observations that were not included in the training dataset. For this part of the study, we developed KNN models that are trained for each investigated muscle pair (i.e. left and right soleus) on three data sets related to the following different external assistance for standing: (*i*) hips and knees assisted *vs* hips and knees independent; (*ii*) one knee assisted *vs* hips and knees independent; (*iii*) hips assisted and knees independent *vs* hips and knees independent. The models related to the first data set were then used to predict the class labels for the prediction dataset, which includes assisted standing events collected from 6 research participants during experimental sessions aimed at testing the effectiveness of different scES parameters for standing. We also exemplified the application of models related to the second data set. The output of the prediction step is a score value ranging from 0 and 1, which is the posterior probability $P(C|X_{new})$ of "independent standing" class C given a new observation X_{new} (Eq. 9).

$$P(C|X_{new}) = \frac{\sum_{i=1}^{K} W(X_i) 1_{X_i=C}}{\sum_{i=1}^{k} W(X_i)} \tag{9}$$

Where K is the number of nearest neighbors to X_{new}, X_i is the ith nearest neighbor, $W(X_i)$ is the weight of X_i which is the distance value from X_{new} and normalized based on the class prior probability, i.e. the frequency of the number of observations in one class in the training dataset. The $1_{X_i=C}$ function returns 1 if observation X_i belongs to class C and 0 otherwise[61].

For each muscle, score values equal or less than 0.5 assign the given observation to the "assisted standing" class label, while values greater than 0.5 assign the observation to the "independent standing" class label. The number of neighbors for the prediction task is set to $K = 5$; this keeps the classification accuracy high for all muscle pairs and allows comparison of the prediction scores between KNN models. All EMG analysis steps are performed using MATLAB R2017b software and its Statistics and Machine Learning Toolbox.

Statistical analysis. Statistical analysis was performed using GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego, California, USA). A p value < 0.05 was considered statistically significant. The distribution of quantitative EMG variables was tested for normality using the Kolmogorov–Smirnov test, and the parametric or non-parametric tests reported below were applied accordingly. The effect of external assistance for standing on the EMG features considered (total power, pattern variability, maximum power variability, median frequency, median frequency SD) was tested on all muscles investigated with surface EMG pooled together (left and right SOL, MG, TA, MH, VL, RF, GL), on primary extensor muscles (left and right SOL, MG, VL, RF, GL), and on primary flexor muscles (TA, MH). Additionally, we tested whether the stimulation frequency applied was significantly different among standing conditions with different amount of external assistance. In particular, paired comparisons between standing conditions of hips assisted – knees assisted and hips assisted – knees independent (subjects number = 8) were performed by Wilcoxon test. Also, comparisons among standing with hips assisted – knees assisted, hips assisted – knees independent, and hips independent – knees independent (subjects number = 5) were performed by either Repeated Measures Anova (and following multiple comparisons by Bonferroni's post hoc test) or by Friedman Test (and following multiple comparisons by Dunn's post hoc test). Finally, when one lower limb (i.e. left side) achieved independent extension while the contralateral limb (i.e. right side) required external assistance for knee extension, paired comparisons (subjects number = 7) between the assisted and independent side were performed by Wilcoxon test.

Data Availability

Data that support the findings and software developed for the data analysis will be made available through material transfer agreement upon request.

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Author Contributions

All Authors contributed to study design and writing the manuscript; E.R. and S.M. contributed to conception of the study; C.A., E.R. and S.H. contributed to data collection; E.R., S.M., F.G. and A.E. contributed to data analysis; E.R., F.G., S.M. and S.H. contributed to interpretation of the results; S.M., E.R. and F.G. designed figures.

Additional Information

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Energy cost of walking and body composition changes during a 9-month multidisciplinary weight reduction program and 4-month follow-up in adolescents with obesity

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Running Head: Energy cost of walking in adolescents with obesity

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Abstract

The purpose of the present study was to investigate changes in the energy cost of locomotion during walking (Cw) related to the changes in body mass (BM, kg) and body composition in adolescents with obesity. Twenty-six (12 boys and 14 girls) obese adolescents (mean: BMI, 33.6±3.7 kg/m^2; 42.7±4.5 % fat mass) followed a 9-month multidisciplinary inpatient weight-reduction program consisting of lifestyle education, moderate energy restriction, and regular physical activity in a specialized institution. At baseline (M0), by the end of the 9-month program (M9) and after 4-months follow-up (M13), $V'O_2$ and $V'CO_2$ of standardized activity program were assessed by whole-body indirect calorimetry over 24 hours, and body composition was assessed by DXA. At M9, adolescents showed a 18% reduction in BM ($p<0.001$), 40% in total FM; while FFM (kg) remained stable in boys but decreased by ~6% in girls ($p=0.001$). Similarly, the mean Cw decreased by 20% ($p<0.001$). At M13, BM, FM and Cw were slightly higher compared than at M9. In conclusion, moderate energy restriction and regular moderate physical activities improved walking economy, improved exercise tolerance and induced beneficial changes in body composition of adolescents with obesity.

Novelty bullets

- Reduction of FM in the trunk region, and consequently reducing the work carried out by respiratory muscles, contribute to reduce Cw in adolescents with obesity.
- A lower cost of walking can be effective in improving exercise tolerance and quality of life in obese adolescents.

Keywords: Cost of walking; Trunk fat mass; Body composition; adolescents; obesity; physical activity

Introduction

Obesity affects all aspects of childhood life increasing the risk of comorbidities (i.e. cardiovascular disease or diabetes) and mortality (Ortega et al. 2016). Furthermore, adolescent obesity is rising in more industrialized countries (Lim et al. 2012; Ng et al. 2014), where daily habits favour increased food-intake altogether with a reduction of physical activity level. Walking is a convenient form of daily physical activity and is recommended for weight management, but the increased cost of locomotion during walking (Cw, *i.e.* the amount of energy spent above resting to transport 1 kg body mass (BM) over 1 meter distance) observed in subjects with obesity, negatively affects the exercise tolerance due to premature fatigue (Peyrot et al. 2010). Several factors contribute to the increase in Cw in adolescents with obesity such as a greater mechanical work for walking (Peyrot et al. 2012; Peyrot et al. 2010), excess of BM (Molina-Garcia et al. 2019) or excessive adipose tissue around the rib cage and in the visceral cavity, that will increase the resistive load on lungs, while increasing the respiratory pressure, pulmonary volumes and therefore the work of breathing during exercise (Oppenheimer et al. 2014). Hence, an increase trunk mass not only will increase the resting oxygen consumption ($V'O_2$) (Bhammar and Babb 2020) but also the oxygen uptake of muscles involved in breathing, which reduces oxygen availability to the muscles involved during exercise (Alemayehu et al. 2018), reducing exercise tolerance.

However, in adolescents with obesity, a weight-reduction program including moderate intensity continuous training, changes in food and behavioural habits, has been found effective in improving walking economy (Delextrat et al. 2015; Peyrot et al. 2012), reducing the O_2 cost of breathing (Babb et al. 2011; Bhammar et al. 2016), fat mass (FM) in the trunk region while maintaining fat-free mass (FFM) (Lazzer et al. 2004). Optimising the work of diaphragm and abdominal muscles in patients with obesity, even simply by reducing the external load of excessive fat mass around the rib cage, can be extremely effective in improving ventilatory parameters, increasing exercise tolerance and breaking the vicious cycle of inactivity (LoMauro et al. 2016).

Therefore, the main objective of the present study was to evaluate the effects of a 9-month multidisciplinary weight-reduction program on the Cw of adolescents with obesity, walking at various speeds and slopes. We hypothesised that our intervention could lead to (a) a decrease in the Cw and (b) this decrease was related to reductions of BM and trunk FM.

Materials and Methods

Subjects

Twenty-six (12 boys and 14 girls) adolescents with severe obesity, were recruited from the Paediatrics Department of Clermont-Ferrand University Hospital**.** The inclusion criteria were: age between 12 and 16 years, BMI above the 99th percentile for gender and chronological age (Rolland-Cachera et al. 1991). However, adolescents who had previously participated in weight management programs, were not in good health (eg, diabetes, hypothyroidism…), and those taking medications regularly or use of any medication known to influence energy metabolism, were excluded. All subjects had a full medical history and physical examination, with the routine haematology and biochemistry screens and urine analysis. BM was stable during the previous last two months. None of the subjects had evidence of significant disease, non-insulin-dependent diabetes mellitus or other endocrine disease, and none were taking medications regularly or any medication known to influence energy metabolism. The data considered in the present study are part of a larger study but had never been taken into account previously. The scientific questions being addressed in previous publications are related to the effects of a 9-months weight reduction program in obese adolescents on energy expenditure, lipid oxidation and adipocyte hormones regulation, and on leisure physical activities and sedentary behaviours level in free-living conditions (Lazzer et al. 2004; Lazzer et al. 2005a; Lazzer et al. 2005b)

Study protocol

The study was approved by the University Ethical Committee on Human Research for Medical Sciences (AU # 361). The purpose and objective of the study were explained to each subject and his or her legal representative, and written informed consent was obtained before beginning the study. The adolescents spent 10 months, 5 days per week, in a specialized nursing institution, and weekend and four holiday weeks at home. Subjects followed a 9-month personalized weight-reduction program consisting of lifestyle education, physical activity, dietary and psychological follow up. At the end of the weight-reduction program the adolescents returned home. Four months later they spent one week in the specialized institution. Full testing sessions were conducted just before the beginning (month 0, M0), at completion of the 9-month weight-reduction period (month 9, M9), and four months later (month 13, M13). The testing session included assessment of anthropometric characteristics, body composition, energy expenditure by whole-body calorimetry and ventilatory parameters by spirometry. In addition, individual anthropometric indexes and physical capacities were evaluated monthly to adjust physical training and food allowances. The latter were specially adjusted during the last two months to stabilize BM.

Diet and nutritional education

During the 9-month weight reduction period, personalized diets were offered on the basis of the baseline basal metabolic rate (BMR) test and physical activity level for each adolescent. Energy supply was adjusted to be close to 1.3 times initial BMR, that is about 15-20 % less than the estimated daily energy expenditure. Diet composition was formulated according to the French recommended daily allowances (Martin 2001). During the weight-reduction period the adolescents had dietetics lessons including choice and cooking of foods, and they were instructed to maintain their food habits after the end the weight-reduction period.

Physical activity

During the 9-month weight-reduction period, the adolescents participated in an exercise-training program including two 40-min endurance and strength training sessions (preceded and followed by 5-7 min stretching) per week under heart rate monitoring and medical supervision. Intensity of endurance exercises (cycle ergometer, treadmill walking, stepper, and stationary rowing) was set at a HR corresponding to 55-60 % of the initial V'O_2max. Strength training was performed on universal gym equipment. Physical training intensity was adjusted monthly according to the results of the physical capacity tests. In addition, subjects had two hours of physical education lessons (PEL) at school, and two hours of aerobic leisure activities at the institution per week. The adolescents and their parents were also advised to practice leisure physical activities during the weekend and holidays.

Measurements

Anthropometric characteristics and body composition

BM was measured to the nearest 0.1 kg using a calibrated manual weighing scale (Seca 709, Les Mureaux, France). Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board. Circumferences at the waist and hip were measured in triplicate to the nearest 0.1 cm using a steel tape according to the atlas of Sempè et al. (1979). Total and regional body composition was assessed by dual X-ray absorptiometry (DXA) using Hologic QDR-4500 equipment and version 9.10 of total body scans software (Hologic Inc, Bedford, MA, USA). FFM was defined as the sum of non-bone lean tissues and bone mineral content. Hydration of the FFM was assumed to be constant (73.2 %). The ability to measure changes in body composition by DXA was showed by Tylavsky et al. (2003).

Physical capacities

The aerobic capacities of the subjects were assessed by means of maximal oxygen uptake tests performed under medical supervision during walking on a treadmill before the beginning (M0), at completion of the weight-reduction program (M9), and four months later (M13). The subjects achieved several successive 2.5 min steps at constant speed (between 4.5 and 5.5 km/h according to the subject physical capacities) and increasing slope by 3% steps until exhaustion. Heart rate was recorded continuously (Cardiovit CS6/12, Scheller AG, Baar, Switzerland). Oxygen dioxide production were measured during the last 30 seconds of each step using the Douglas bag method. Oxygen consumption and carbon dioxide production were determined using a Tissot spirometer and gas analyzers (CPX ID; Medical graphics, St Paul). The oxygen and carbon dioxide analysers were calibrated using standard gas mixtures before each test period. Blood samples for lactate concentration measurement ([La]) were obtained by micro-puncture of the ear lobe within the 3 minutes following the completion of the tests. [La] were measured with lactate analyser (Analox LM5). The criteria for reaching maximal $V'O_2$ were: a respiratory exchange ratio (RER)> 1.1, and a maximal heart rate close to the theoretical maximum [220 – age (y)].

Energy cost of locomotion during walking

The Cw was measured by indirect calorimetry using two comfortable open-circuit whole-body calorimeter (Lazzer et al. 2004). Measurements were obtained three times: before the beginning (M0), at completion of the weight-reduction program (M9), and four months later (M13), as previously described by Lazzer et al. (2004). Briefly, the adolescents spent 36 hours in the whole-body calorimeters, one evening and one night for adaptation and 24 hours for measurement. They followed the same standardized activity program composed of five main periods: 1) sleeping, 2) sedentary activities (watching television, video games, listening to music, board games, school work, 10.5 hours), 3) miscellaneous activities (washing and dressing, making the bed and tidying the room, 1 hour), 4) meals (breakfast, lunch, snack and dinner, 2 hours), 5) six 20-min-exercises of walking at six different speeds (2 hours). Before the beginning of the weight-reduction program subjects walked at their own speed on the treadmill and the slope was altered to obtain different intensities of exercises.

Gas exchanges were computed from outlet air flow, differences in gas concentrations between air entering and leaving the calorimeter, atmospheric pressure, chamber air temperature and hygrometry, after correction for the drift of the gas analysers and the response time of the whole system. The gas analysers were calibrated twice a day using the same gas mixture during the whole study. In addition, the validity of gas exchange measurements was checked gravimetrically by injecting gas (N_2 and CO_2) into the chambers. HR was measured by telemetry (Life scope 6, Nikon Kohden, Tokyo, Japan)(Lim, #79) and recorded continuously during the stay in the calorimeters.

The Cw ($J \cdot kg^{-1} \cdot m^{-1}$) was calculated by dividing net V'O_2 by speed and BM. V'O_2 is obtained by subtracting pre-exercise rest V'O_2 from gross V'O_2, and converted to joules according to the formula given by Garby and Astrup (1987), which accounts for the RER (dependent variation of O_2 energy equivalence). In addition, RER was monitored to ensure that it remained under the specific threshold of 1.0. All these precautions were required to indicate that the metabolism was essentially oxidative.

Spirometry

Before the beginning of the weight-reduction program (M0), at completion (M9), and four months later (M13), the adolescents performed standard spirometry tests (forced vital capacity, FVC; forced expiratory volume in 1 sec, FEV1; $FEV1 \cdot FVC^{-1}$ ratio) by utilizing a metabolic cart (MedGraphics CPX/D, Medical Graphics Corp., USA). Pulmonary function testing was performed according to the guidelines of the American Thoracic Society (Miller 2005). The predicted values were based on Hankinson et al. (1999).

Statistical analysis

Statistical analyses were performed using GRAPH PAD PRISM software, version 8.0.1, 2020 (GraphPad Software, Inc. - San Diego, CA, USA). The data are presented as mean and standard deviation (SD). Differences between the periods (M0-M9 and M9-M13) are presented as mean differences, 95% confidence intervals (CI) and effect size (ES). ES was calculated using Cohen's d ($0 < d < 0.20$, small; $0.20 < d < 0.50$ medium; $d > 0.50$, large) (Cohen 1988). Significance was set at $P < 0.05$. Normality of data set was tested with a Shapiro wilk test. Bivariate associations were determined by Pearson's or Spearman's correlation coefficients. A General Linear Mixed Model repeated measures was used to determine the effects of the period (M0, M9 and M13), gender and their interaction (P x G) on body composition, metabolic and spirometry parameters. Sphericity has been assessed using Mauchly's test. Greenhouse-Geisser estimate correction was used in case of sphericity assumption violation. Significant main effects (P, G) or interactions (P x G) were further analysed by the Tukey post hoc test. The relationships between the different factors were investigated using Pearson product–moment correlation coefficient.

Results

Effects of the weight-reduction program (M9-M0)

Anthropometric characteristics and body composition

At M0, mean age, BM, stature, and BMI were not significantly different between boys and girls (Tab. 1). Waist circumference was also not significantly different between boys and girls, but boys had lower hip circumferences (-8%, p=0.003) and higher waist/hip ratio (+7%, p<0.001, Tab. 1).

During the 9-month weight-reduction program, both boys and girls reduced their body mass, BMI, waist and hip circumferences, and waist/hip ratio by mean ~18%, ~21%, ~15%, ~11 and 5%, respectively (p <0.001), without P x G interaction (Tab. 1).

At baseline, total FFM (kg) and total FM (%) were not significantly different between boys and girls, while FM (kg) was higher in girls (+12%, p=0.003, Tab. 2) than in boys. Furthermore, boys had similar FFM (kg) but significantly lower FM (kg) than girls in arms, legs and trunk districts (-6%, p=0.007; -11%, p=0.011; and -15%, p=0.002; respectively, Tab. 2).

During the 9-month weight-reduction period, total FFM (kg) remained stable in boys, while it decreased by ~6% in girls (p=0.001); total FM (kg) decreased by ~50% (p<0.001) in boys and ~30% in girls (p<0.001) (Tab. 2). FFM (kg) in arms and legs did not change significantly in boys and girls; while trunk FFM (kg) decreased in girls (by ~7%, p=0.023) but not in boys. On the other hand, FM (kg) decreased in boys by ~45% in arms, ~44% in legs and ~59% in the trunk (p <0.001) and in girls by ~26% in arms, ~27% in legs and ~35% in trunk (p <0.001, Tab. 2).

Physical capacities

$V'O_2max$, $V'O_2max \cdot FFM^{-1}$, HR_{max} and [La] max were not significantly different between boys and girls (Table 1).

During the 9-month weight-reduction program $V'O_2max$, $V'O_2max \cdot FFM^{-1}$ and HR_{max} did not change significantly. Finally, [La] max decreased significantly only in boys (by mean ~31%, p=0.035, Tab. 1).

Spirometry

At M0, FVC, FEV1 and $FEV1 \cdot FVC^{-1}$ were not significantly different between sexes (Tab. 3).

During the 9-month weight-reduction program FVC and FEV1 increased by ~7% (p=0.009) and ~9% (p=0.014), respectively, in boys but not in girls; while $FEV1 \cdot FVC^{-1}$ did not change significantly. FVC, FEV1 and $FEV1 \cdot FVC^{-1}$ were not significantly different from the standard reference values at M0 and M9, both in boys and girls (Tab. 3).

In addition, changes in FVC (ΔFVC, L) were inversely related to changes in TrunkFM (Δ TrunkFM, kg) at M9-M0 (R^2: 0.375, p: 0.0015; Fig. 1 A) and directly related to changes in ΔV'O_2 max during the period M9-M0 (R^2: 0.208, p: 0.0250; Fig. 2 A).

Metabolic parameters measured in the whole-body calorimeters

At M0, V'O_2, HR and Cw were not significantly different between boys and girls (Fig. 3).

At M9, V'O_2 and HR were significantly lower at all slopes by 20% and 16% on average, respectively ($p<0.001$, Fig. 3 B, E). As well, the Cw decreased by ~20% ($p <0.001$, Fig. 3 H) at all slopes. In addition, changes in Cw were not significantly related to changes in BM (ΔBM, kg) or TrunkFM (ΔTrunkFM, kg) at M9-M0.

Changes after the end of the weight-reduction program (M13-M9)

Anthropometric characteristics and body composition

At M13 the body mass of boys was ~7% (p=0.020) higher than at M9 while their BMI, waist and hip circumferences, and waist/hip ratio were not significantly altered; whereas none of the above-mentioned parameters changed significantly in girls. It is worth considering that, even though the parameters recorded were worse at M13 than at M9, they were much better than at M0 for both groups ($p < 0.05$, Tab. 1).

At M13 total FFM, arms FFM and legs FFM were ~5% (p=0.006), ~8% (p=0.009) and ~5% (p=0.006), respectively, higher than at M9 in boys; whereas trunk FFM was not significantly altered (Tab. 2). By contrast the above parameters did not show any significant changes in girls (Tab. 2).

Finally, total FM (kg), arms FM (kg), legs FM (kg) and trunk FM (kg) did not change significantly both in boys and girls and remained 36%, 35%, 34% and 41%, respectively, lower than at M0 ($p<0.05$, Tab. 2).

Physical capacities

$V'O_2max$, $V'O_2max \cdot FFM^{-1}$, HR max and [La] max were not significantly different at M13 and M9 both in boys and girls. [La] max remained significantly lower than at M0 in boys and girls (by mean ~32%, p=0.014, Tab. 1)

Spirometry

FVC was ~3% higher at M13 than at M9 (p=0.002, Tab. 3) in boys but not FEV1 and the $FEV1 \cdot FVC^{-1}$ ratio. In girls, FEV, FEV1 and $FEV1 \cdot FVC^{-1}$ ratio did not change significantly (Tab. 3). Finally, FVC and FEV1 were ~10% and ~13% higher, respectively, at M13 than at M0 ($p<0.001$, Tab. 3) in boys; whereas they were not significantly different in girls (Tab. 3).

In addition, changes in FVC (ΔFVC) were inversely related to changes in TrunkFM (Δ TrunkFM, kg) at M13-M9 (R^2: 0.280, p: 0.0078; Fig. 1 B) and M13-M0 (R^2: 0.656, p: 0.0001; Fig. 1 C) and directly related to changes in $\Delta V'O_2$ max at M13-M9 (R^2: 0.208, p: 0.0248; Fig. 2 B) but not at M13-M0 (Fig. 2 C).

Metabolic parameters measured in the whole-body calorimeters

$V'O_2$ was significantly higher at M13 than at M9 for all speeds at 2% and 4% slopes ($p<0.001$) while it was not significantly altered at 0% and 6% slope (Fig. 3 C). HR did not change at any speeds and slopes (Fig. 3 F). As well, Cw did not change at any speeds and slopes (Fig. 3 I). In addition, changes in Cw were not related to changes in BM (ΔBM, kg) and TrunkFM (ΔTrunkFM, kg) at M13-M9.

Discussion

The present study shows that a 9-month multidisciplinary weight-reduction program including physical training, regular physical activity and moderate energy restriction, induced in adolescents with severe obesity: 1) a significant reduction of Cw, 2) considerable reductions of BM and FM, particularly in the trunk region, and 3) no adverse effect on FFM in boys, and a slight but significant FFM loss in girls.

Our intervention was effective in reducing Cw, $V'O_2$ and HR during walking at different speeds and slopes, which suggested an improved walking economy and exercise tolerance (Alemayehu et al. 2018; Peyrot et al. ; Peyrot et al. 2010). However, our hypothesis was rejected as no correlation was found between changes in Cw and changes in BM or trunk FM. Cw was ≃20% lower at M9 than at M0. Only few studies reported a significant decrease in Cw of this magnitude (i.e. ranging from 9% to 22%) after a weight reduction program (Delextrat et al. 2015; Peyrot et al. 2012; Peyrot et al. 2010). The first factor accounting for the decreased in Cw in adolescents with obesity after weight loss, was the decrease in BM (Lazzer et al. 2004; Peyrot et al. 2010). However, recent studies showed that, when BM was normalized for internal work (i.e., work required to move the limbs with respect to the centre of mass, (Cavagna and Kaneko 1977; Willems et al. 1995), BM did not affect the total mechanical work and was not directly responsible for the higher Cw during walking in adults suffering from obesity (Menéndez et al. 2020). As well, in agreement with previous studies, which considered physical activity and diet as the main components of a weight reduction program, maintaining FFM (Stiegler and Cunliffe 2006) and improving physical capacities (Delextrat et al. 2015), due to physical training, contribute to the reduction of the Cw.

Therefore, respiratory and mechanical parameters were suggested to play an important role in the reduction of Cw (Hunter et al. 2008; Menéndez et al. 2020; Oliveira et al. 2020; Peyrot et al. 2012). Particularly, at the end of the 9 months weight reduction program, we found improvements of the spirometry parameters with an increase in FVC, although the values of FVC were within the range of normality (Kochli et al. 2019; Oliveira et al. 2020). In addition, the reduction of trunk FM was positively related to the improvement in FVC, which reflects the total compliance of both lung and chest wall (Forno et al. 2018). The extra weight or mass added to the chest wall, as in obesity condition, causes a compression of the thorax, thus decreasing respiratory compliance and lung volumes (Wang and Cerny 2004). As previously observed in healthy and lean subjects, a load on the trunk between 15-20% of body weight, as observed in obesity (Hong et al. 2008; Hudson et al. 2020), causes chest wall restriction with a concomitant increase in work of breathing and Cw (Faghy et al. 2016; Faghy and Brown 2014; Phillips et al. 2016). Therefore, previous studies showed that a 12-week diet and physical activity program, induced BM loss and reduction of trunk FM, which significantly enhanced breathing mechanics in men and women with obesity (Babb et al. 2011; Bhammar et al. 2016). Moreover, it was shown (Alemayehu et al. 2020; Salvadego et al. 2017) that a decrease in Cw was associated with a reduction of $V'O_2$ of respiratory muscles after 3 weeks of training of respiratory muscles, or acute respiratory muscle unloading. Potential mechanisms underlying the decrease of O_2 cost of breathing in adolescents with obesity are partially described by decreased FM and load on the trunk region (Babb et al. 2011; Milic-Emili et al. 2007), augmented efficiency of respiratory muscles thanks to an increase in both chest wall and compliance and lung compliance (Babb et al. 2011; Pelosi et al.), and decreased airway resistance (Babb et al. 2011). Reducing the work of the respiratory muscles by decrease in trunk FM, would not only interrupt or attenuate the metaboreflex, but would also positively affect “central” hemodynamics (Alemayehu et al. 2018; Vogiatzis et al. 2011) and enhance O_2 delivery to locomotor muscles, thereby reducing the O_2 cost of these locomotor muscles and further decreasing Cw (Dominelli et al. 2017; Hogan et al. 1999). Increasing O_2 availability to locomotor muscles may also delay or reduce the development of peripheral muscle fatigue, maintaining metabolic stability in these muscles and preventing the recruitment of additional less efficient muscle fibers (Babcock et al. 2002; Cleland et al. 2012; Dominelli et al. 2017). Another potential hypothesis may explain the Cw improvement in subjects with obesity as related to the impact of ectopic muscle lipid infiltration. Indeed, intramyocellular lipid is well recognized as a cause of metabolic disturbances (insulin resistance as well as anabolic resistance) (Beals et al. 2018; Guillet et al. 2009; Tardif et al. 2014) but it is also a possible factor of an impaired muscle contraction (Choi et al. 2016; Collins et al. 2018). So, a weight reduction program is supposed to improve insulin sensitivity, to reduce intramyocellular content, and to improve finally muscle structure and quality, which could contribute to this lower Cw.

It is worth mentioning that, in the present study, a significant reduction in BM and FM, and particularly in TrunkFM, was associated with a significant FFM loss in girls but not in boys.

The adolescents involved in the present study were in a growth phase: boys were in the peak of growth, while girls were in the final phase of growth. Indeed, the growth-related increment in FFM in boys during the 9 months weight-reduction program could have compensated for the FFM loss due to energy restriction, which eventually did not occur in girls (Lazzer et al. 2004).

Finally, it is important to consider that a reduction in TrunkFM could affect FVC and then also $V'O_2$ max. Nevertheless, because no changes were evaluated in $V'O_2$max after the weight-reduction program, it is possible to assume that lung volumes explained only partially the variance of the improvements of cardiorespiratory fitness in adolescents with obesity (Mendelson et al. 2016).

In conclusion, a weight-reduction program including regular physical activity and moderate energy restriction decreased BM and FM in adolescents with obesity, together with improvements of Cw and pulmonary parameters. Although the spirometry values of participants were in the normal range, we have shown that reducing FM in the trunk region, and consequently reducing the work carried out by respiratory muscles, may reduce possible respiratory limitations, thus contribute to reduce Cw. In addition, this strategy can be effective in improving exercise tolerance and quality of life of adolescents with obesity, considering that most activities of everyday life are linked to walking activities.

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Conflict of Interest

There are no real or potential conflicts of financial or personal interest with the financial sponsors of the scientific project.

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Table 1. Changes in physical characteristics and capacities of adolescents (Boys, B; Girls, G) before the beginning (M0), at completion of the weight-reduction program (M9), and four months later (M13).

		M0	M9	M13	ΔM9-M0		ΔM13-M9	
					diff (95%CI)	ES	diff (95%CI)	ES
Age	B	13.47 ± 1.27	14.23 ± 1.27	14.63 ± 1.27	0.75* (0.78 to 0.71)	0.59	0.40* (0.40 to 0.40)	0.31
(y)	G	14.87 ± 1.57	15.63 ± 1.57	16.03 ± 1.57	0.75* (0.78 to 0.71)	0.48	0.40* (0.40 to 0.40)	0.25
Body mass	B	89.8 ± 20.1	71.4 ± 14.4	76.2 ± 17.4	-18.4* (-12.9 to -23.9)	0.92	4.8* (-8.86 to -0.77)	0.33
(kg)	G	92.0 ± 14.7	76.1 ± 12.7	78.2 ± 13.3	-15.9* (-11.9 to -19.9)	1.08	2.1 (5.95 to -1.75)	0.17
Stature	B	1.63 ± 11.58	1.68 ± 11.29	1.70 ± 11.09	0.04* (0.05 to 0.03)	0.39	0.02* (0.02 to 0.01)	0.00
(m)	G	1.64 ± 10.64	1.65 ± 10.34	1.65 ± 10.41	0.02* (0.02-0.01)	0.15	0.00 (0.01 to 0.00)	0.00
BMI	B	33.1 ± 4.2	25.0 ± 3.4	26.2 ± 4.2	-8.07* (-6.7 to -9.5)	1.94	1.2 (2.4 to -0.1)	0.34
(kg m^{-2})	G	34.2 ± 3.4	27.7 ± 2.7	28.4 ± 3.0	-6.5* (-5.0 to -8.0)	1.91	0.7 (2.1 to -0.7)	0.27
Waist circum.	B	110 ± 12	90 ± 8	92 ± 11	-20* (-15 to -25)	1.70	3 (6 to -1)	0.32
(cm)	G	110 ± 11	96 ± 12	94 ± 10	-14* (-9 to -19)	1.23	-2 (3 to -7)	0.17
Hip circum.	B	104 ± 9	91 ± 7	93 ± 8	-13* (-11 to -16)	1.51	2 (5 to -1)	0.26
(cm)	G	113 ± 8 †	103 ± 10 †	103 ± 8 †	-10* (-8 to -15)	1.24	0 (3 to -4)	0.05
Waist hip^{-1}	B	1.05 ± 0.05	0.99 ± 0.05	0.99 ± 0.05	-0.06* (-0.04 to -0.09)	1.33	0.01 (0.01 to 0.00)	0.12
	G	0.97 ± 0.06 †	0.93 ± 0.04†	0.91 ± 0.05 †	-0.04* (-0.01 to -0.07)	0.65	-0.01 (0.00 to -0.05)	0.35
VO_2max	B	2.77 ± 0.69	2.86 ± 0.58	2.82 ± 0.46	0.09 (0.32 to -0.13)	0.03	-0.04 (0.21 to -0.29)	0.07
(l/min)	G	2.59 ± 0.40	2.47 ± 0.48	2.45 ± 0.44	0.12 (0.39 to -0.29)	0.37	-0.02 (0.20 to -0.25)	0.05
VO_2max FFM^{-1}	B	51.29 ± 2.87	53.71 ± 3.91	51.42 ± 7.63	2.42 (5.22 to -0.38)	0.84	-2.29 (1.70 to -6.27)	0.58
(ml kg^{-1} min^{-1})	G	49.24 ± 4.88	49.75 ± 4.60	49.10 ± 3.30	0.51 (4.28 to -3.27)	0.10	-0.65 (4.04 to -5.35)	0.14
HR max	B	197 ± 7	191 ± 11	188 ± 14	-6 (2 to -13)	0.76	-3 (1 to -8)	0.28
(bpm)	G	198 ± 11	194 ± 14	193 ± 11	-4 (0.34 to -8)	0.35	-1 (-4 to -6)	0.08
[La] max	B	6.95 ± 2.37	4.78 ± 1.34	4.21 ± 1.20	-2.17* (-0.28 to -4.07)	0.92	-0.57 (0.29 to -1.42)	0.42
(mmol l^{-1})	G	7.36 ± 2.14	6.31 ± 2.98	5.67 ± 2.61	-1.05 (1.43 to -3.53)	0.49	-0.64 (0.47 to -1.75)	0.22

Values are expressed as means and SD. *: significantly different, $P<0.05$; †: significantly different between genders, $P<0.05$; 95% CI: 95% confidence interval; ES: effect size.

VO_2max, maximal oxygen consumption; VO_2max FFM, maximal oxygen consumption normalized for fat-free mass; HR max, maximal heart rate; [La] max, maximal blood lactate concentration.

Table 2. Changes in body composition of adolescents (Boys, B; Girls, G) before the beginning (M0). at completion of the weight-reduction program (M9). and four months later (M13).

		M0			M9			M13			ΔM9-M0			ΔM13-M9		
		Mean	±	SD	Mean	±	SD	Mean	±	SD	diff	(95%CI)	ES	diff	(95%CI)	ES
Total body																
Fat-free mass	B	54.21	±	14.46	53.77	±	13.29	56.24	±	13.95	-0.44	(1.33 to -2.21)	0.03	2.47*	(4.35 to 0.60)	0.19
(kg)	G	53.67	±	8.62	50.46	±	7.76	49.88	±	8.71	-3,20*	(-1.57 to -4.83)	0.37	-0.58	(0.86 to -2.02)	0.07
Fat mass	B	35.61	±	6.41	17.62	±	6.40	19.96	±	7.93	-17.99*	(-13.36 to -22.62)	2.57	2.34	(5.65 to -0.38)	0.37
(kg)	G	40.56	±	7.84 †	28.09	±	7.29 †	28.32	±	7.27 †	-12.46*	(-10.01 to -14.91)	1.61	0.23	(3.32 to -2.88)	0.03
Fat mass	B	42	±	4	30	±	7	32	±	8	-12.11*	(-8.45 to -15.77)	3.38	2	(4.20 to -0.92)	0.27
(%)	G	43	±	4	34	±	7	33	±	7	-8.93*	(-6.08 to -11.78)	2.03	-1	(0.81 to -4.15)	0.14
Arms																
Fat-free mass	B	5.66	±	1.84	5.61	±	1.68	6.07	±	1.93	0,05	(0.22 to -0.32)	0.00	0.46*	(0.80 to 0.12)	0.27
(kg)	G	5.21	±	0.93	4.98	±	1.06	4.71	±	0.97	-0.23	(0.04 to -0.49)	0.25	-0.27	(0.01 to -0.55)	0.26
Fat mass	B	4.24	±	0.65	2.29	±	0.55	2.39	±	0.60	-1.96*	(-1.51 to -2.40)	3.16	0.10	(0.37 to -0.17)	0.18
(kg)	G	4.53	±	0.77 †	3.33	±	0.93 †	3.28	±	0.83 †	-1.19*	(-0.80 to -1.59)	2.94	-0.05	(0.37 to -0.47)	0.05
Legs																
Fat-free mass	B	18.40	±	5.09	18.56	±	4.71	19.52	±	4.76	0.16	(1.10 to -0.78)	0.03	0.96*	(1.61 to 0.30)	0.20
(kg)	G	18.92	±	3.59	17.88	±	4.09	17.22	±	3.58	-1.04*	(-0.10 to -1.96)	0.29	-0.66	(0.42 to -1.74)	0.16
Fat mass	B	13.87	±	2.59	7.79	±	1.73	8.61	±	2.06	-6.07*	(-4.29 to -7.85)	2.35	0.82	(1.76 to -0.12)	0.47
(kg)	G	15.61	±	3.19 †	11.30	±	3.36 †	10.98	±	2.31 †	-4.30*	(-3.34 to -5.27)	1.35	-0.32	(0.83 to -1.49)	0.10
Trunk																
Fat-free mass	B	26.23	±	7.35	25.72	±	6.57	26.57	±	6.90	-0.51	(0.45 to -1.48)	0.07	0.85	(1.98 to 0.27)	0.13
(kg)	G	25.77	±	4.03	23.92	±	2.85	24.39	±	4.03	-1.85*	(-0.35 to -3.36)	0.46	0.47	(2.00 to -1.06)	0.17
Fat mass	B	16.49	±	4.38	6.74	±	2.31	8.12	±	3.54	-9.75*	(-7.11 to -12.38)	2.22	1.37	(3.00 to -0.25)	0.59
(kg)	G	19.44	±	4.35 †	12.51	±	4.06 †	13.15	±	3.96 †	-6.93*	(-5.39 to 8.46)	1.59	0.64	(2.37 to -1.10)	0.16

Values are expressed as means and SD. *: significantly different, $P<0.05$; †: significantly different between genders, $P<0.05$; 95% CI: 95% confidence interval; ES: effect size.

Table 3. Changes in spirometry parameters of adolescents before the beginning (M0), at completion of the weight-reduction program (M9), and four months later (M13).

		M0	M9	M13	ΔM9-M0			ΔM13-M9		
		Mean ± SD	Mean ± SD	Mean ± SD	diff	(95%CI)	ES	diff	(95%CI)	ES
FVC (L)	B	3.67 ± 0.90	3.92 ± 1.05	4.05 ± 1.04	0.25*	(0.42 to 0.08)	0.28	0.13*	(0.25 to 0.01)	0.13
	G	3.68 ± 0.41	3.75 ± 0.46	3.84 ± 0.53	0.1	(0.17 to -0.03)	0.17	0.09	(0.27 to -0.10)	0.19
FVC (% p)	B	97.7 ± 12.6	99.3 ± 12.1	98.4 ± 12.7	1.6	(6.4 to -3.2)	0.13	-0.8	(6.8 to -3.5)	0.07
	G	108.6 ± 5.6	106.0 ± 9.9	105.3 ± 8.0	-2.6	(3.3 to -8.5)	0.46	-0.8	(4.75 to -6.25)	0.08
FEV1 (L)	B	3.25 ± 0.70	3.53 ± 0.89	3.68 ± 0.81	0.28*	(0.49 to 0.07)	0.41	0.15	(0.34 to -0.04)	0.17
	G	3.19 ± 0.37	3.28 ± 0.41	3.31 ± 0.45	0.1	(0.19 to - 0.03)	0.22	0.03	(0.16 to -0.09)	0.08
FEV1 (% p)	B	100.3 ± 11.8	103.4 ± 10.6	101.8 ± 10.7	3.2	(9.1 to -2.7)	0.27	-1.6	(4.6 to -7.7)	0.15
	G	106.0 ± 7.4	105.8 ± 10.2	104.1 ± 10.2	-0.2	(6.1 to -6.4)	0.02	-1.8	(3.0 to -6.5)	0.17
FEV1/FVC	B	0.89 ± 0.04	0.91 ± 0.04	0.92 ± 0.07	0	(0.03 to - 0.00)	0.35	0.01	(0.05 to -0.02)	0.28
	G	0.87 ± 0.05	0.87 ± 0.05	0.86 ± 0.06	0	(0.03 to -0.02)	0.11	-0.01	(0.00 to -0.03)	0.20

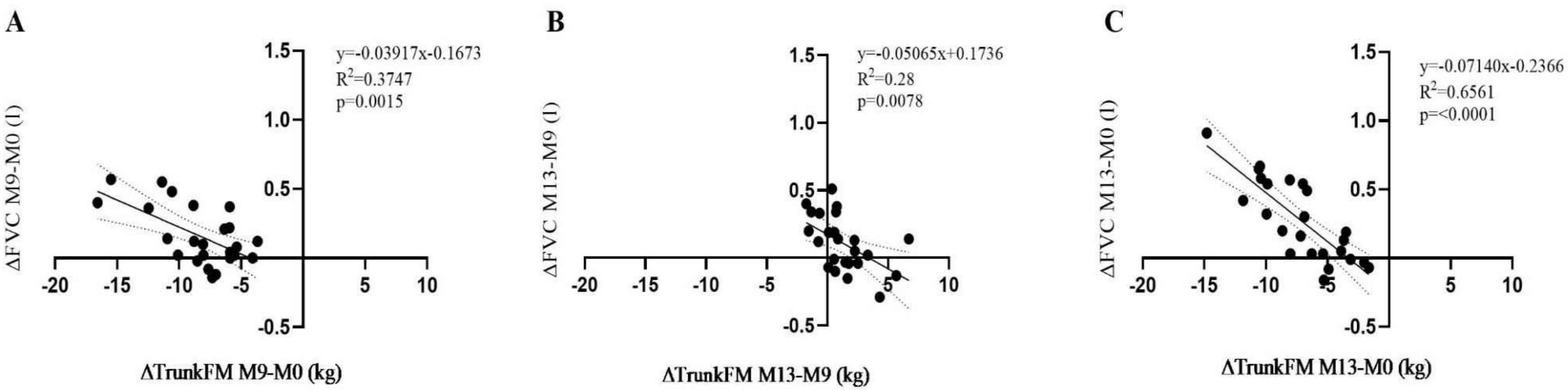
Values are expressed as means and SD. *: significantly different, $P<0.05$; 95% CI: 95% confidence interval; ES: effect size; B, boys; G, girls. FVC, forced vital capacity; FEV1, forced expiratory volume in 1 sec; (% p), values as percentage of reference (predicted) values.

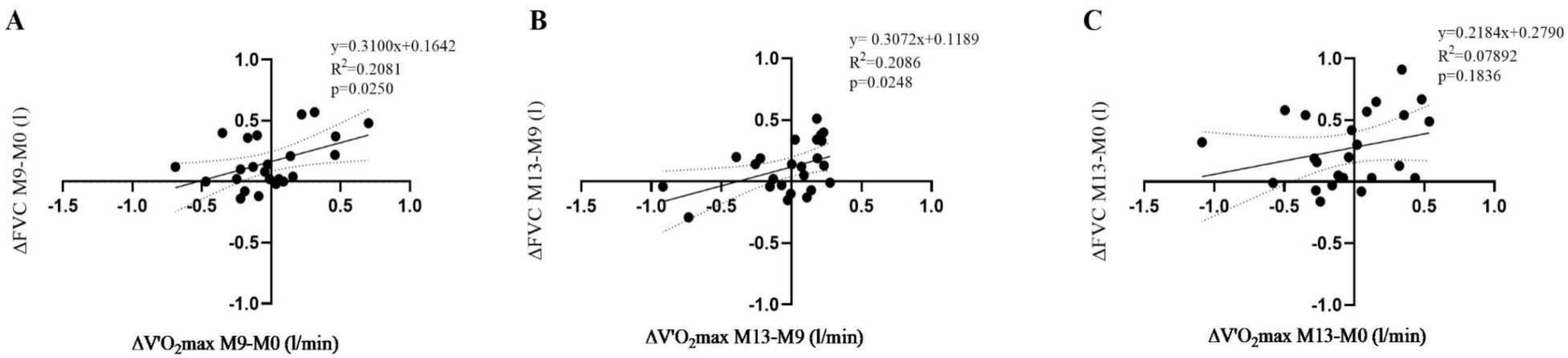
Figure Legends

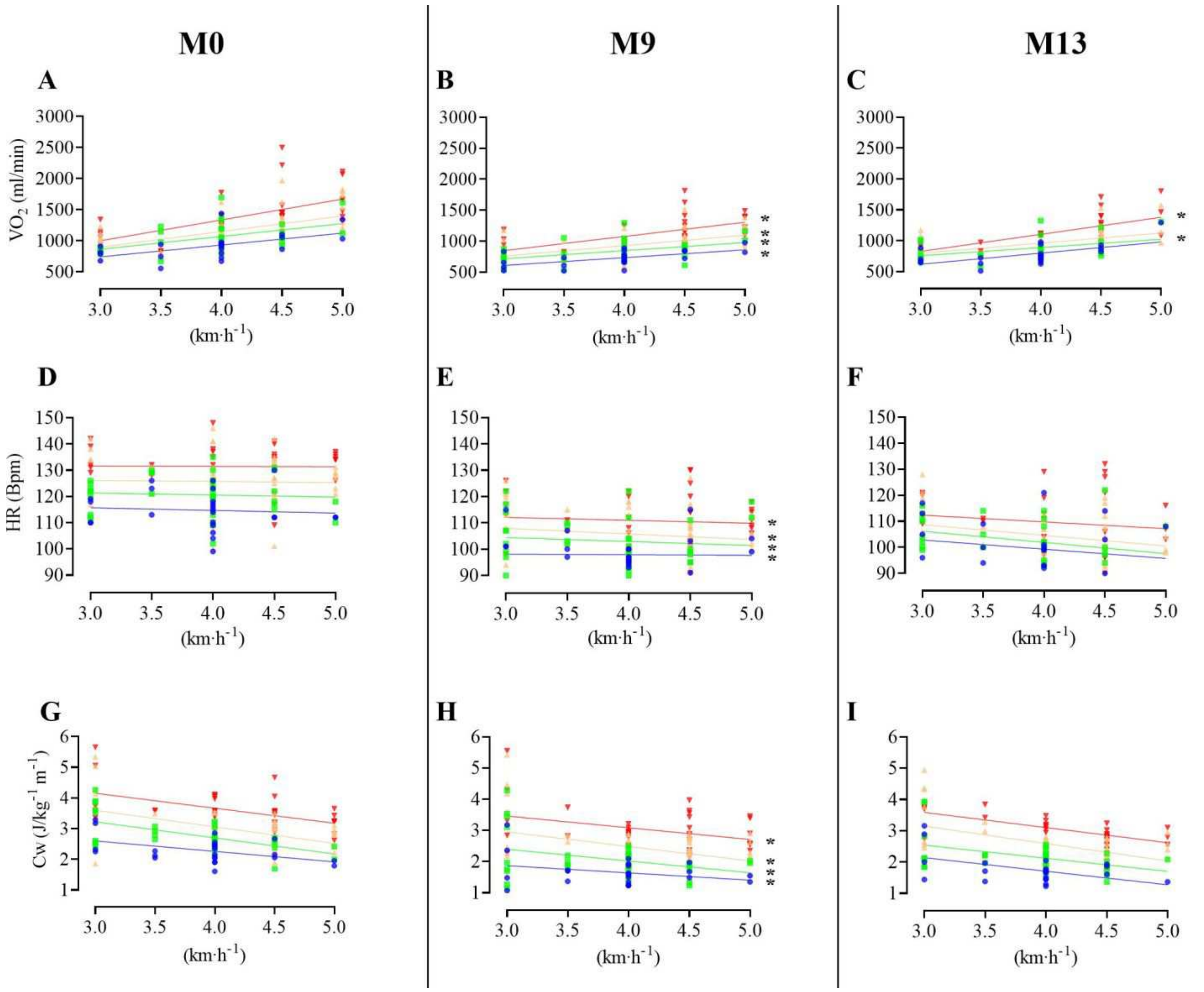
Figure 1. Linear regression between changes in FVC (ΔFVC) and Trunk Fat Mass (ΔTrunkFM, kg) at M9-M0 (panel A), M13-M9 (panel B) and M13-M0 (panel C).

Figure 2. Linear regression between changes in FVC (ΔFVC) and V'O_2max (ΔV'O_2max) at M9-M0 (panel A), M13-M9 (panel B) and M13-M0 (panel C).

Figure 3. Changes in oxygen consumption (V'O_2, mL min^{-1}; panels A, B and C), Heart Rate (HR, bpm; panels D, E and F) and energy Cost of walking (Cw, J kg^{-1} m^{-1}; panels G, H and I) as a function of speed (km h^{-1}) and slope (0 ---, 2 ---, 4 --- and 6 --- %) of adolescents. Before the beginning (M0; panels A, D and G) at completion of the weight-reduction program (M9; panels B, E and H), and four months later (M13; panels C, F and I).


A
y=-0.03917x-0.1673
R2=0.3747
p=0.0015
ΔFVC M9-M0 (l)
ΔTrunkFM M9-M0 (kg)
B
y=-0.05065x+0.1736
R2=0.28
p=0.0078
ΔFVC M13-M9 (l)
ΔTrunkFM M13-M9 (kg)
C
y=-0.07140x-0.2366
R2=0.6561
p=<0.0001
ΔFVC M13-M0 (l)
ΔTrunkFM M13-M0 (kg)


A
y=0.3100x+0.1642
R2=0.2081
p=0.0250
ΔFVC M9-M0 (l)
ΔV'O2max M9-M0 (l/min)
B
y= 0.3072x+0.1189
R2=0.2086
p=0.0248
ΔFVC M13-M9 (l)
ΔV'O2max M13-M9 (l/min)
C
y=0.2184x+0.2790
R2=0.07892
p=0.1836
ΔFVC M13-M0 (l)
ΔV'O2max M13-M0 (l/min)



*. significantly different from M0: p<0.05

Conclusion

In conclusion, during the three-year PhD course, we analyzed the different acute or chronic adaptations to the use of NMES technique.

In particular, we evaluated that NMES parameters can deeply influence the metabolism, muscle mechanical output and MUs recruitment pattern on knee extensors in healthy and spinal cord injured individuals.

Then, we evaluated the efficacy of using the NMES based conditioning contraction compared to a voluntary one on explosive characteristics of knee extensors in healthy adults. Therefore, we indicated applicative considerations on selecting the type of the conditioning stimulus to regulate muscle performance. These indications could be useful in the context of rehabilitations and to improve explosiveness in different task such as jumping.

Lastly, we observed and compared adaptations of two training modalities focusing on explosiveness of knee extensors in elderlies. We evaluated the similar effects of training using a NMES conditioning contraction protocol and a classic training modality (without a conditioning stimulus) on strength improvement, physical capacities, and muscle architecture. Interestingly, in elderlies NMES-elicited conditioning contraction enhanced neuromuscular explosive characteristics of a following explosive contractions to a higher degree than what was experienced in healthy adults.

Further studies would be necessary to evaluate the influence of a longer training period on the above-mentioned characteristics both in elderlies and in healthy adults or in the context of rehabilitation protocols.

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