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ORIGINAL ARTICLE

Muscle alterations induced by electrostimulation are lower at short *quadriceps femoris* length

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Abstract

Purpose This study aimed at determining through MRI investigations, force and soreness assessments whether the modulation of muscle length is a relevant strategy for minimising neuromuscular electrical stimulation (NMES)-induced muscle damage in young healthy participants.

Methods Comparison of 2 NMES sessions (40 isometric electrically-evoked contractions of the knee extensors) was randomly performed on 1 knee flexed at 50° (short muscle length) and the contralateral at 100° (long muscle length) in a single group of healthy participants. Indirect markers of muscle damage including changes in maximal voluntary isometric contraction (MVC) force, muscle volume and transverse relaxation time (T_2) were measured before, 2 days (D2), 4 days (D4) and 7 days (D7) after the NMES sessions in each limb of the ten participants.

Results Although stimulation intensity was similar during the NMES session on both limbs, significantly lower force production was recorded at long muscle length (peak at 30 ± 5% MVC force) as compared to short muscle length (peak at 61 ± 12% MVC force). In the following days, MVC force at long muscle length was decreased from D2 to D7, whereas no significant change occurred at short muscle length. Increases in muscle volume and T_2 were found at each time point in stimulated muscles at long muscle length, whereas no change was found at short muscle length.

Conclusion For the same stimulation intensity, NMES-induced isometric contractions generate higher knee extension force output and result in lower muscle tissues alterations that could be related to a lower intramuscular shear strain when exercise is performed at short muscle length.

Keywords MRI · Muscle damage · Acute exercise · Neuromuscular electrical stimulation · Muscle strength · Thigh

Abbreviations

ANOVA	Analysis of variance
D2	Day #2
D4	Day #4
D7	Day #7
MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction

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NMES	Neuromuscular electrical stimulation
POST	Immediately after the NMES session
PRE	Before the NMES session
RF	*rectus femoris*
SAR	*sartorius*
T_2	Transverse relaxation time
VAS	Visual analog scale
VI	*vastus intermedius*
VL	*vastus lateralis*
VM	*vastus medialis*

Introduction

Repetition of unaccustomed maximal voluntary eccentric or isometric contractions can lead to muscle damage (Armstrong 1984; Crameri et al. 2007; Nosaka et al. 2011). Structural alterations of muscle organization have been reported within days following the damaging exercise (Crameri et al. 2007; Mackey et al. 2011). These changes were accompanied by decreased maximal voluntary isometric contraction (MVC) force (Clarkson et al. 1992; Fouré et al. 2015b) and range of motion (Clarkson et al. 1992; Nosaka and Clarkson 1996), muscle swelling (Howell et al. 1993; Fouré et al. 2014), increased muscle stiffness (Lacourpaille et al. 2014, 2017; Xu et al. 2018), plasmatic muscle protein concentration (Clarkson et al. 1992; Nosaka and Clarkson 1996; Fouré et al. 2014) and delayed onset muscle soreness (Howell et al. 1993).

Interestingly, it has been suggested that the magnitude of muscle damage induced by unaccustomed exercises could be modulated by various factors. For instance, the amount of muscle damage has been found to be higher in upper limb muscles as compared to the lower limb ones (Jamurtas et al. 2005; Saka et al. 2009). Moreover, it has been demonstrated that muscle damages were larger during eccentric exercise performed at a long muscle length and for a large range of motion as compared to those resulting from eccentric exercise performed at short muscle length and small range of motion (Nosaka et al. 2005). Similarly, eccentric exercise involving high force generation and a high number of repetitions can cause large muscle fibre structural alterations (Lieber and Friden 1993; Paulsen et al. 2012). Using real-time ultrasound imaging, it has been reported that the amount of fascicle length changes during maximal voluntary eccentric contractions and then the level of negative work imposed by the exercise is highly involved in the subsequent MVC force loss (Guilhem et al. 2016; Doguet et al. 2019). Muscle damage has also been reported as a result of intense voluntary isometric contractions (> 60% MVC) performed at long muscle length (Allen et al. 2018) even if muscle force and length change were lower than what could be generated during maximal voluntary eccentric contractions.

Deleterious effects of isometric contractions have also been reported as a result of neuromuscular electrical stimulation (NMES) (Fouré et al. 2014, 2015a). To our knowledge, these changes have only been reported for contractions performed at long muscle length and at relatively low force levels (~ 30% MVC) (Aldayel et al. 2010; Fouré et al. 2015a) so that the damaging effects of isometric contractions performed at short muscle length have not been assessed. On the contrary to what occurs during voluntary contractions, the motor units' recruitment during NMES is synchronous, spatially fixed and involves fast and slow motor units at the same time (Maffiuletti 2010). Such an activation of fast muscle fibres even at relatively low levels of evoked force (Gregory and Bickel 2005) can induce early fatigue and generate muscle damage and extracellular matrix deadhesion (Mackey et al. 2008, 2011). Based on the "*popping sarcomere hypothesis*", fast muscle fibres have a shorter optimum length (Brockett et al. 2002; Allen et al. 2018) and are more prone to alterations (Morgan and Proske 2004). One can hypothesize that fibres' alterations could be alleviated during NMES-induced isometric contractions performed at short muscle length, considering a lower strain (i.e., the relative fascicle length change) reported to be a key factor in the force loss after maximal eccentric plantarflexions (Guilhem et al. 2016).

The aim of the present study was to assess the effects of muscle length on the occurrence and extent of NMES-induced muscle damage on the basis of multimodal MRI investigations, force and soreness assessments conducted over a 7-day post-exercise period.

Methods

Ten healthy participants (27 ± 4 years, 173 ± 10 cm, 69 ± 10 kg, four women) volunteered to participate in this study. None of them were engaged in any training or exercise programs. Participants were instructed to avoid any intensive and non-familiar physical activities throughout the duration of the protocol. They were fully informed about the nature and aims of the study and gave their informed written consent to participate. The study was approved by the Local Human Research Ethics Committee (*Sud Mediterranée V*, #2012-04 A00449-34) and conducted in conformity with the Declaration of Helsinki. Participants were asked to keep their diet habits and limit their alcohol consumption throughout the study period. They were instructed to avoid consuming caffeine and smoking before the experiments. Consumption of medication was also prohibited during the experimental protocol. All testing sessions were performed at the same time of day. The experiment included four sessions aiming at assessing muscle function and tissue integrity before (PRE), immediately after the NMES session

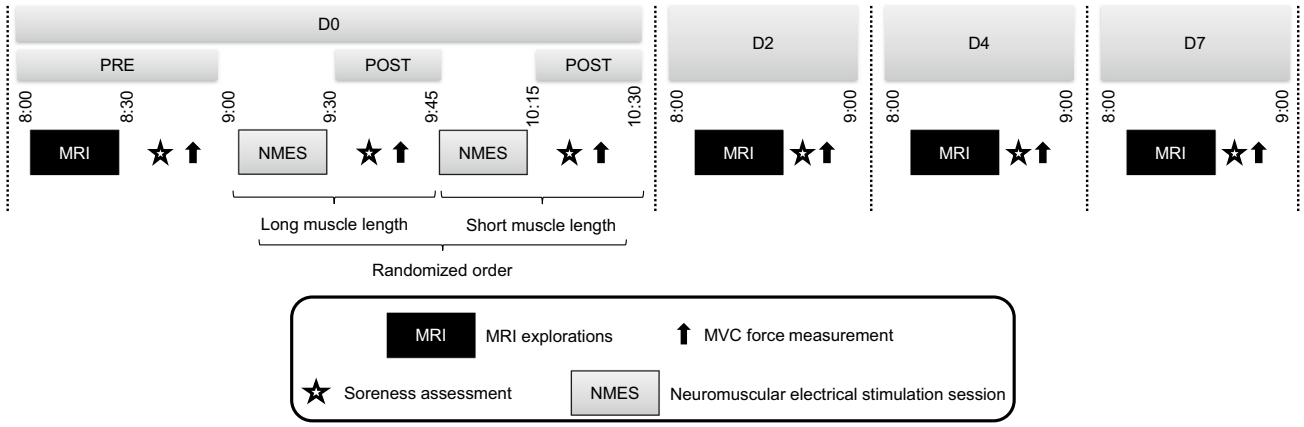

Fig. 1 Schematic representation of the experimental design

(POST) and in the following days (two [D2], four [D4] and seven [D7]—Fig. 1).

Participants were seated on a chair (Multi-Form', La Roque d'Anthéron, France) customized with a force sensor. Adjustable belts secured hip and ankle joints to hold the hip at ~ 90° (0° corresponding to the joint fully extended). Similar NMES sessions were randomly performed either with the knee flexed at 100° corresponding to a long muscle–tendon length (hereafter referred to as long muscle length) and with the contralateral knee flexed at 50° corresponding to a short muscle–tendon length (hereafter referred to as short muscle length). Link between knee joint angle and muscle fascicle length was performed on the basis of ultrasound imaging measurements on the *vastus lateralis* during eccentric contractions with a mean fascicle length of 85 mm and 100 mm at a knee flexion angle of 40° and 100°, respectively (Doguet et al. 2019). Considering that VL muscle slack length was assessed for a knee flexion angle of 24° [range 16°–30°] (Stubbs et al. 2018), we assume that the two testing positions were beyond the VL slack length (Stubbs et al. 2018; Xu et al. 2018). The two different NMES conditions of the present study correspond to knee joint flexion angles that can be used in a clinical context to minimise muscle atrophy in patients. Knee extensor muscles were stimulated using three electrodes placed over the thigh, a 5 × 10 cm rectangular electrode on the proximal part of the thigh (i.e., placed ~ 5 cm below the inguinal ligament) and two 5 × 5 cm square electrodes on the *vastus lateralis* (VL) and *vastus medialis* (VM) muscle bellies. Biphasic symmetric rectangular pulses were delivered at a frequency of 100 Hz with a pulse duration of 400 µs (40 contractions, 5 s on and 35 s off throughout the NMES session) using a portable battery-powered stimulator (Compex® Performance, DjoGlobal, France). Stimulation intensity was gradually increased during the 40 contractions to reach the highest tolerated (considering the pain threshold) level of electrically-evoked force for each participant as previously described (Fouré et al. 2014, 2015a). Stimulation intensities and electrically-evoked force outputs were recorded every five contractions during the entire series of each NMES session. Evoked force was normalized to MVC performed at the corresponding muscle length.

Each participant was seated on a chair with the knee flexed (i.e., at 100° for one knee and 50° for the other) and performed a 5-min warm-up including a set of sub-maximal knee extensions under isometric conditions. MVC was assessed on each side in the same position used for the NMES session. Participants were instructed to perform three unilateral MVC. The MVC trials were separated by a resting period of at least 30 s. MVC force was considered as the highest value recorded among the three trials.

The level of muscle soreness was assessed by the same experimenter (A.F.) using circular palpation (Lau et al. 2015) on the VL and the VM muscle bellies at rest (i.e., before MVC trials) and using a visual analog scale (VAS) with a 100 mm horizontal line with "no pain" on one end (0 mm) and "extremely painful" on the other (100 mm). An additional measure was performed immediately after MVC trials. Soreness was quantified as the mean value obtained from the three measures.

Participants were positioned supine with the limb of interest centred in a 1.5-T super-conducting magnet (MAGNETOM Avanto, Siemens AG, Healthcare Sector, Erlangen, Germany). A flexible surface six-channel coil (Siemens AG, Healthcare Sector, Erlangen, Germany) was placed around the thigh muscles. Muscle volume was determined from high-resolution T_1-weighted images (20 slices, field of view = 220 mm × 220 mm; matrix = 576 × 576; relaxation time = 549 ms; echo time = 13 ms; number of repetitions = 1; slice thickness = 6 mm; gap between slices = 6 mm, acquisition time = 5 min 18 s). T_2-weighted

images were acquired with a segmented (15 segments) echo planar imaging sequence with time echos = 15, 25, 35, 45 and 55 ms. Other acquisition parameters were as follows: field of view = 220 mm × 220 mm; matrix = 192 × 192; repetition time = 4800 ms; $N_{EX} = 1$; number of slices = 20; slice thickness = 6 mm; gap between slices = 6 mm, fat saturation; acquisition time = 5 min 10 s. The most distal slice was always acquired at approximately 20 mm (i.e., 5% of the thigh length measured for each participant) above the proximal border of the patella.

Regions of interest (i.e., thigh muscles including the VL, VM, *vastus intermedius* (VI), *rectus femoris* (RF), *sartorius* (SAR), *gracilis*, *adductor longus*, *adductor magnus*, *biceps femoris* short and heads, *semintendinosus*, *semimembranosus*) were manually delimited using FSLView (FMRIB, Oxford, USA). Muscles were delineated in every two slices. Corresponding masks on the remaining slices were generated using an interpolated process (Ogier et al. 2017). Using the truncated cone formula, muscle volume of each muscle was calculated by summing areas of all the slices, taking into account the slice thickness and the gaps between slices.

T_2 maps were generated using a linear fit on a pixel-by-pixel basis using the following equation:

$$\ln(S(TE)) = \ln(S_0) - (TE/T_2),$$

where S(TE) is the signal at time equal to echo time and S_0 is the equilibrium magnetization. Regions of interest initially drawn on T_1-weighted images were used to analyse T_2 maps and to determine a mean T_2 value for each muscle.

The reproducibility of measurements (*e.g.*, MVC force, volume, T_2) was reported in previous studies (Fouré et al. 2014, 2015a). The number of participants was determined on the basis of a statistical power calculation ($\alpha = 0.05$ and $1-\beta = 0.9$) and previous measurements (Fouré et al. 2014) with the aim of detecting a significant increase of 7% in the mean T_2 of the whole *quadriceps femoris*. Normality of the data distribution was initially investigated using Shapiro–Wilk test. Two-way repeated-measure ANOVA (time × muscle length) was used (Statistica, Statsoft, Tulsa, USA) to assess changes in parameters assessed during the NMES session (i.e., stimulation intensity and relative output force), MVC force, soreness, volume and T_2 in each muscle. Sphericity hypothesis was checked with a Mauchly's test and results were adjusted using the Greenhouse–Geisser correction if required. A Tukey's HSD post hoc analysis was used when appropriate. The level of significance was set at $P < 0.05$ and results are reported as mean ± standard deviation (SD).

Results

During the NMES session, stimulation intensity was increased similarly for both muscle length conditions and reached 59 ± 10 mA (Fig. 2a). The resulting force level was significantly larger for the short muscle length as compared to the long muscle length condition ($P < 0.01$, Fig. 2b). The mean relative force output recorded throughout the NMES sessions was 23 ± 7% MVC force at long muscle length and 45 ± 15% MVC force at short muscle length. The maximal electrically-evoked force reached 30 ± 5% MVC force and 61 ± 12% MVC force when NMES was performed at long and short muscle length, respectively.

The relative MVC force was significantly lower for the long length at D2, D4 and D7 compared to the short length ($P < 0.01$). For the long muscle length condition, MVC force decreased by -28 ± 7% immediately after the NMES session (from PRE: 408 ± 110 N to POST: 301 ± 85 N) and was still lower than the baseline value at D2 (−34 ± 10%, 276 ± 85 N), D4 (−27 ± 7%, 303 ± 89 N) and D7 (−21 ± 5%, 326 ± 93 N) (Fig. 2c). On the contrary, for the short muscle length condition, MVC force remained unchanged immediately after, at D2 and at D4 but significantly increased at D7 (+17 ± 13%, from PRE: 401 ± 148 N to D7: 453 ± 143 N, $P < 0.001$).

The amount of soreness at D2 was significantly higher at long length compared to the short length condition ($P < 0.01$). Muscle soreness significantly increased and reached its maximum at D2 for the long muscle length condition (30 ± 24 mm, Fig. 2d). No significant change in soreness was detected for the short muscle length condition ($P > 0.05$).

Muscle volume and T_2 were increased in muscles of both limbs after the NMES session (Table 1). A larger increase was found for the long muscle length condition (Fig. 3) especially for the two muscles located beneath the stimulation electrodes (i.e., VL and VM).

Regarding muscle volume, a statistical time effect was found at both short and long muscle lengths for SAR at D2, D4 and D7 ($P < 0.05$, Table 1). A significant time × muscle length interaction was found for VL and VM ($P < 0.01$ and $P < 0.05$, respectively) likely due to a significant muscle volume increase at long muscle length for VM (D2: + 5 ± 5%, D4: + 5 ± 5% and D7: + 3 ± 4%) and VL (D2: + 8 ± 4%, D4: + 12 ± 7% and D7: + 12 ± 9%).

A significant time effect was also found for T_2 changes at both short and long muscle length for SAR at D2, D4 and D7 ($P < 0.01$, Table 1). Additionally, time × muscle length interactions were identified for T_2 in the four muscles of the *quadriceps femoris* ($P < 0.01$). Overall, a significant T_2 increase was observed at long muscle length

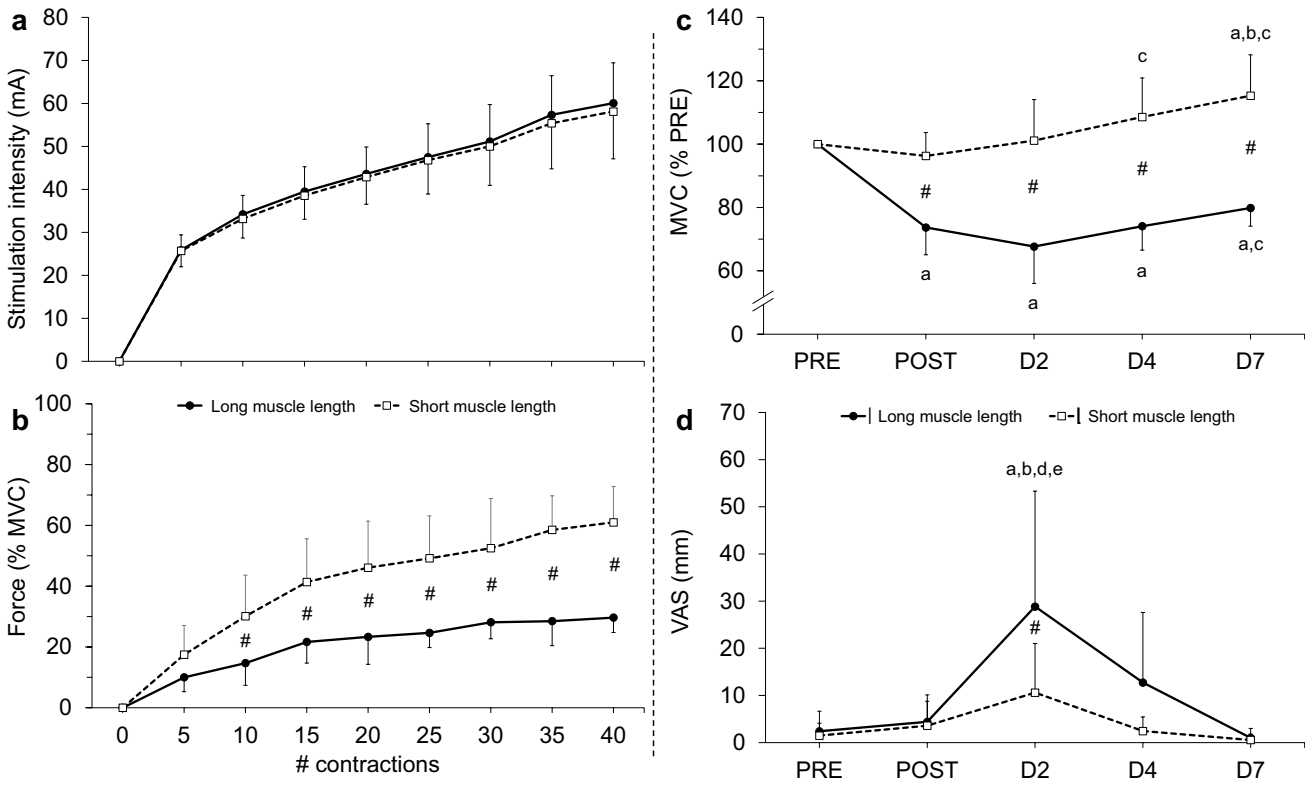

Fig. 2 **a** Time course of current stimulation intensity and **b** relative force production during the neuromuscular electrical stimulation (NMES) session performed with knee flexed at 100° (solid line: long muscle length) and knee flexed at 50° (dotted line: short muscle length). **c** Maximal voluntary contraction (MVC) force and **d** muscle soreness assessed from visual analog scale (VAS) score before (PRE), immediately after and 2, 4 and 7 days (POST, D2, D4 and D7, respectively) after the neuromuscular electrical stimulation (NMES) session performed with knee flexed at 100° (solid line: long muscle length) and knee flexed at 50° (dotted line: short muscle length). Error bars represent standard deviations. a: significantly different from PRE ($P < 0.05$), b: significantly different from POST ($P < 0.05$), c: significantly different from D2 ($P < 0.05$), d: significantly different from D4 ($P < 0.05$), e: significantly different from D7 ($P < 0.05$), #: significant difference between long and short muscle length conditions ($P < 0.05$)

for RF (D2: +5±5%, D4: +6±6% and D7: +5±5%), VI (D2: +3±3%, D4:+ 4±4% and D7: +3±3%), VM (D2: +6±4%, D4: +11±7% and D7: +11±7%) and VL (D2: +14±6%, D4: +27±14% and D7: +36±18%). In addition, a significant increase from D2 to D4 was observed for VM (+ 4±4%) and VL (+ 12±9%). For the other thigh muscles (i.e., not involved in knee extension), experimental conditions mostly had no effect on muscle volume and T_2 changes (see Supplementary Table).

Discussion

The main results of the present study demonstrated that the occurrence and extent of isometric NMES-induced muscle damage in knee extensors are lower at short muscle length. Long-lasting MVC force reduction and large muscle T_2 increase were recorded in *quadriceps femoris* muscles within days following the NMES session performed at long muscle length. These deleterious changes were not observed at short muscle length.

While the stimulation intensity was similar throughout the NMES sessions performed at both short and long muscle length, significantly lower force levels were produced by the knee extensors at long muscle length as compared to the short length condition. This reduced force production under similar stimulation conditions might be due to a lower involvement of both VL and VM muscles. A less-efficient force production by knee extensor muscles resulting from the force–length relationship might also been advocated. Accordingly, based on force–length relationships assessed in human cadavers, Herzog et al. demonstrated that the individual force produced by the *vastii* muscles was maximal for a knee angle near 50°–80° in isometric conditions (Herzog et al. 1990). It is noteworthy that the peak torque measured in vivo during knee extensors isometric MVC occurs around 70° of knee flexion as reported previously (Knapik et al. 1983; Marginson and Eston 2001; Lanza et al. 2019).

Table 1 MRI parameters of knee extensor muscles assessed before (PRE), 2 days (D2), 4 days (D4) and 7 days (D7) after the NMES session

Mean ± SD [95% CI]	Short muscle length				Long muscle length			
	PRE	D2	D4	D7	PRE	D2	D4	D7
Muscle volume (cm^3)								
VL	840 ± 114 [768–912]	870 ± 116 [796–943]	857 ± 119 [781–932]	861 ± 126 [782–941]	834 ± 119 [758–909]	905 ± 146[a] [813–997]	941 ± 166[a] [836–1045]	935 ± 170[a] [828–1043]
VM	736 ± 128 [655–818]	751 ± 134 [666–836]	745 ± 135 [660–831]	746 ± 134 [661–830]	736 ± 102 [672–801]	771 ± 115[a] [698–844]	777 ± 116[a] [704–850]	761 ± 113[a] [690–833]
VI	782 ± 151 [687–878]	793 ± 168 [687–899]	788 ± 168 [682–894]	790 ± 168 [684–897]	827 ± 138 [740–915]	840 ± 148 [746–933]	827 ± 149 [733–921]	825 ± 155 [727–922]
RF	238 ± 86 [184–292]	241 ± 83 [188–293]	240 ± 87 [185–295]	240 ± 82 [188–293]	258 ± 86 [204–313]	266 ± 87 [211–321]	266 ± 83 [213–318]	267 ± 85 [214–321]
SAR	161 ± 37 [137–184]	165 ± 37[a] [142–189]	168 ± 38[a] [145–192]	169 ± 40[a] [144–195]	166 ± 35 [144–188]	174 ± 38[a] [149–198]	175 ± 40[a] [150–200]	174 ± 41[a] [148–199]
T_2 (ms)								
VL	33.8 ± 1.1 [33.1–34.5]	34.9 ± 1.4 [34.1–35.8]	34.7 ± 1.1 [34.0–35.4]	34.3 ± 1.6 [33.3–35.4]	33.1 ± 0.9 [32.5–33.6]	37.6 ± 2.4[a] [36.1–39.1]	42.2 ± 5.2[a,b,#] [38.9–45.4]	45.0 ± 6.1[a,b,#] [41.1–48.8]
VM	33.3 ± 1.1 [32.6–34.0]	34.2 ± 0.9 [33.6–34.8]	33.8 ± 0.8 [33.3–34.3]	33.7 ± 1.2 [32.9–34.5]	33.5 ± 0.7 [33.0–33.9]	35.6 ± 1.4[a] [34.7–36.5]	37.2 ± 2.3[a,b,#] [35.7–38.6]	37.3 ± 2.3[a,b,#] [35.8–38.7]
VI	34.3 ± 1.1 [33.5–35.0]	34.6 ± 1.0 [34.0–35.2]	34.3 ± 0.6 [33.9–34.7]	34.2 ± 1.1 [33.5–34.8]	33.9 ± 0.9 [33.3–34.4]	34.8 ± 1.1[a] [34.1–35.6]	35.2 ± 1.6[a] [34.2–36.2]	35.0 ± 1.3[a] [34.2–35.8]
RF	32.6 ± 1.6 [31.5–33.6]	32.9 ± 1.2 [32.2–33.7]	32.6 ± 0.8 [32.0–33.1]	32.3 ± 0.9 [31.7–32.9]	33.0 ± 0.9 [32.5–33.6]	34.4 ± 1.7[a] [33.3–35.5]	34.9 ± 1.9[a,#] [33.7–36.1]	34.8 ± 1.9[a,#] [33.6–36.0]
SAR	32.8 ± 1.6 [31.8–33.9]	33.8 ± 1.6[a] [32.8–34.8]	35.9 ± 2.2[a,b] [34.5–37.3]	36.5 ± 2.8[a,b] [34.8–38.3]	32.9 ± 1.2 [32.1–33.7]	34.6 ± 1.5[a] [33.7–35.5]	35.9 ± 2.6[a,b] [34.2–37.5]	36.2 ± 3.4[a,b] [34.1–38.4]

VL: *vastus lateralis*; VM: *vastus medialis*; VI: *vastus intermedius*; RF: *rectus femoris*; SAR: *sartorius*. a: significantly different from PRE ($P < 0.05$), b: significantly different from D2 ($P < 0.05$), #: significant difference between long and short muscle length conditions ($P < 0.05$)

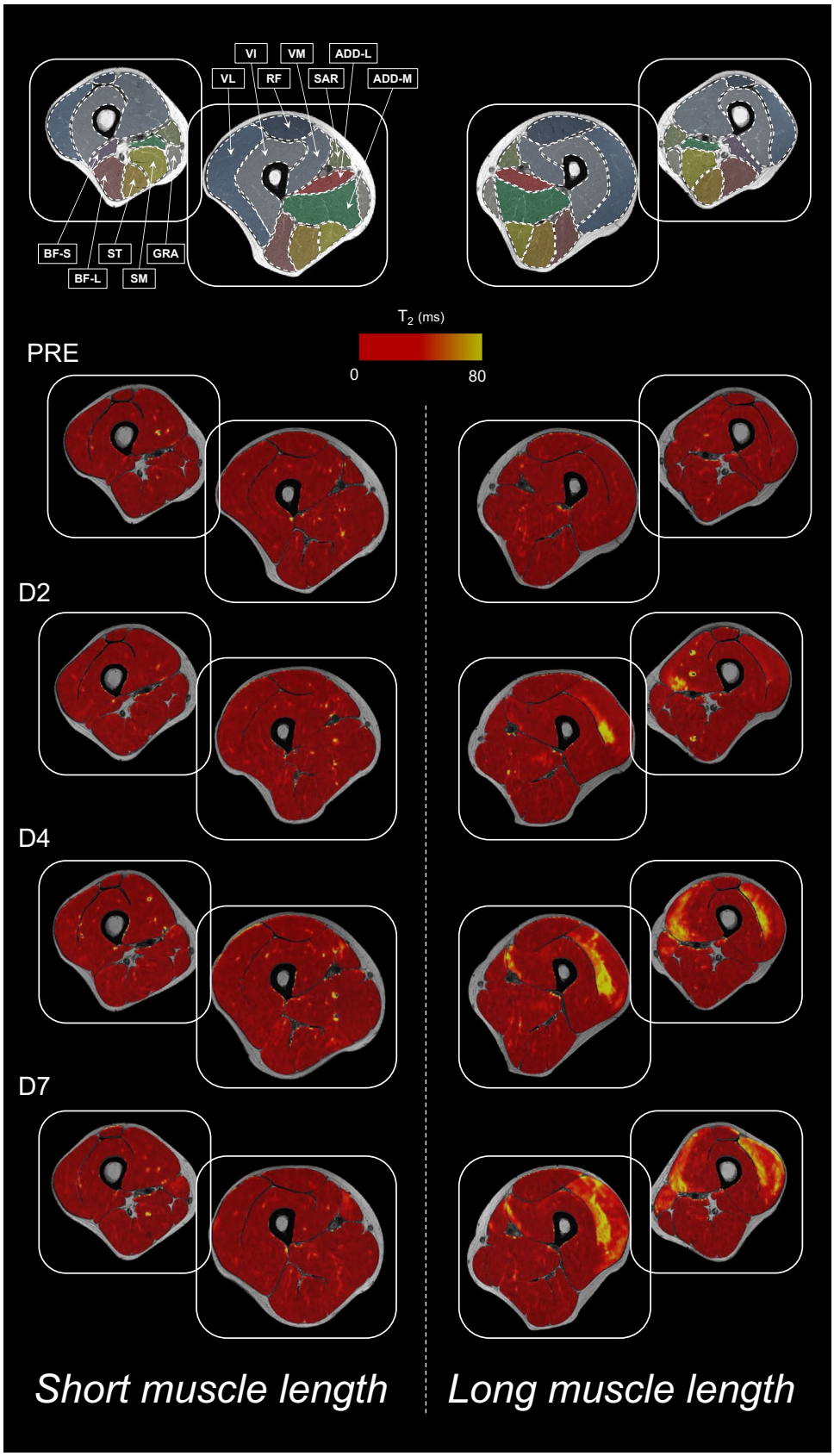

Fig. 3 Axial T_2 maps superimposed on T_1-weighted images (exposed in the upper part with muscle delineations for *ADD-L*: adductor longus, ADD-M: *adductor magnus*, BF-S: *biceps femoris* short head, BF-L: *biceps femoris* long head, GR: *gracilis*, RF: *rectus femoris*, SAR: *sartorius*, SM: *semimembranosus*, ST: *semintendinosus*, VI: *vastus intermedius*, VM: *vastus medialis*, VL: *vastus lateralis*) acquired on a representative participant with a comparison of the explorations performed before (PRE), 2 days (D2), 4 days (D4) and 7 days (D7) after the isometric NMES session at long and short muscle length during the NMES exercise. Changes in T_2 maps colour are due to muscle alterations resulting from the damaging exercise

Surprisingly and despite the twofold lower electrically-evoked force resulting from the NMES session performed at long muscle length, larger muscle alterations were recorded as illustrated by the large MVC force reduction, the increased muscle soreness and T_2 when compared to the short length condition. MVC force dropped after the long muscle length NMES session with a time-course similar to what has been previously described (Aldayel et al. 2010; Nosaka et al. 2011; Fouré et al. 2014). An additional long-lasting effect was measured at D7 ($-21 \pm 5\%$) illustrating an incomplete MVC force recovery resulting from the severe muscle damage (Paulsen et al. 2012). In contrast, a reduced MVC force or an increased muscle soreness was recorded neither immediately after nor within days following the short muscle length NMES session. Similarly, muscle volume and T_2 were significantly increased in the directly stimulated muscles during the long muscle length condition, whereas no change occurred for the short muscle length condition. These significant differences illustrated a muscle length dependence of NMES-induced damage in agreement with what has been recently reported 24 h after maximal voluntary isometric contractions (Allen et al. 2018). The muscle-length dependence of isometric NMES-induced damage could be related, at least in part, to the so-called *popping sarcomere hypothesis* (Morgan and Proske 2004). Accordingly, considering the sarcomere length-tension curve (Morgan 1990), Allen et al. (2018) suggested that the overstretching of some sarcomeres beyond the myofilaments overlap can account for the initiation of muscle damage. In addition, both muscle fascicle lengthening and muscle tension were involved in the amount of muscle damage following eccentric contractions (Guilhem et al. 2016). On that basis, muscles similarly stimulated at a long muscle length would be more sensitive to damage than those activated at a short muscle length. Considering that the motor unit recruitment associated with NMES is mainly superficial (Maffiuletti 2010), the localization of T_2 changes in the superficial part of the VM muscle within days after the exercise performed at long muscle length would support the assumption of active sarcomeres overstretch. However, in the VL, sarcomeres length might change a little over the range of knee angles used in the present study (Herbert et al. 2002). A mean sarcomere length change of about 0.4 µm, representing an increase of about 10% between the two knee flexion positions, has been recently measured in the VL using microendoscopy (Chen et al. 2016). Although the mean sarcomere length change was relatively minor, the tension capacity may have been altered. In addition, between the two knee flexion positions used in the present study, the length change of VL and VM muscle–tendon units has been of about 6–7% on the basis of measures performed on cadavers (Visser et al. 1990). Therefore, the resistance to the shortening sarcomeres during contraction could have been higher due to a potential shorter "toe region" of the passive structures in series within the muscle–tendon unit. However, a recent study demonstrated that muscle alterations were mainly located in less activated areas following the NMES exercise (Fouré et al. 2019). Therefore, the deeper localization of T_2 changes for the VL muscle would suggest that other mechanisms than the *popping sarcomere hypothesis* are involved.

The increased muscle T_2 in the deep part of the VL muscle might be associated with the alteration of costameres and/or passive components of muscle tissue. From a comparative analysis between muscle damage induced by voluntary and NMES eccentric contractions, Crameri et al. (2007) indicated that a significant disruption of cytoskeletal proteins (i.e., desmin) only occurred after the NMES contractions. In addition, ultrasound elastography measurements have recently illustrated that connective tissues can also be exposed to stretch at long muscle length (Freitas et al. 2019; Xu et al. 2018) contributing to increase the tension on contractile tissues and overstretch during contractions. The increased passive stress and strain on knee extensor muscles during passive stretches can be considered as facilitating conditions for a potential disruption of passive structures including connective tissues. Overall, intramuscular alterations illustrated in the present study by T_2 changes could result from damage of the muscle extracellular matrix (Mackey et al. 2008, 2011) and/or structural elements of muscle cells (Crameri et al. 2007; Mackey et al. 2008). Further investigations are warranted to determine the potential alteration of the latter structures in the deep and superficial parts of the VL and VM muscles in response to NMES-induced isometric muscle contractions at long length. One can also put forth that the inhomogeneous activation of the four muscles within the *quadriceps femoris* group might have disturbed the mechanical coupling between musculotendinous structures (Maas and Finni 2018). The relative displacement of two neighbourhood muscles (e.g., VL and VI) during the isometric NMES protocol could have created a stress between muscle fascias thereby contributing to an increased shear strain on intramuscular connective tissues and so more particularly at long muscle length. Additional investigations would be of interest to assess the behaviour of muscle fibres during the NMES-induced isometric contractions in both knee flexion positions and the potential subsequent changes in local mechanical properties using ultrasound imaging and/or elastography. That would help to understand the biomechanical conditions leading to damage occurring only at long muscle length. We did not measure fascicle length changes during the NMES exercise and this could be viewed as a limitation of the present study. It would have been of interest to assess the strain on active fascicles but also on passive structures such as series elastic components during the NMES-isometric contractions throughout the exercise. The impact of the fascicle/passive structures

strain at short and long muscle lengths on the extent of muscle T_2 increase and MVC force decrease could have been assessed as reported recently during voluntary eccentric contractions (Guilhem et al. 2016; Doguet et al. 2019).

Slight changes in muscle volume and T_2 were also detected for SAR and antagonist muscles independently of the length condition. This might be linked to potential contractions occurring as a result of reflexes, co-activation and/or to preserve knee and hip joints integrity during the NMES sessions. A significant T_2 increase was also measured in the RF muscle at D4 and D7 for the long muscle length condition. One can hypothesize that water diffusion could have occurred from the neighbourhood muscles (i.e., from VL and VM for which muscle volume increased due to the edema). Overall, our results further support that isometric NMES-induced contractions performed at long muscle length led to severe muscle alterations (Paulsen et al. 2012) as illustrated by large MVC force changes. In addition, the same NMES protocol performed at short muscle length prevented these deleterious changes and was linked to a paradoxical MVC force increase at D7 which would need to be further documented. The contribution of neuromuscular adaptations (e.g., increased intra- and inter-muscular coordination and/or a decreased contribution of knee flexors during knee extension MVC) to a single NMES-isometric contractions session leading to an increase in MVC force 7 days post-exercise observed in the present study cannot be ruled out.

During the NMES session in the present study, a high frequency stimulation protocol with a manual increase in stimulation intensity was used in order to induce muscle damage as described in previous studies (Fouré et al. 2014, 2015a). Similar stimulation intensities were used at both short and long muscle lengths, but a larger relative evoked force was recorded at short muscle length. That phenomenon could be, at least in part, related to a larger muscle volume involvement, a potential difference in VL and VM relative contribution to maximal isometric knee extension and/or a slight shift of stimulation electrodes location on the muscles already described as the *soft tissue artefact* (i.e., a skin sliding over the muscle with joint angle change). Regarding the latter potential issue, methodological cautions were taken to accurately stick the electrodes on the muscle belly of each muscle and so for each experimental position. Further investigations based on T_2 measurements to assess muscle activation and relative contribution at short and long muscle lengths could have been of interest. In addition, it could have been relevant to assess the MVC force length relationship considering that the peak of this relationship can be shifted following a damaging exercise leading to a potential change in MVC force assessed at a single knee angle (Gregory et al. 2007). Therefore, additional assessment of the MVC force–length relationship for the two experimental conditions reported in the present study are needed to clarify the potential influence of the shift of the optimal length for MVC following isometric NMES-induced muscle damage.

Moreover, neuromuscular alterations can occur following NMES-isometric contractions at long muscle length thereby inducing a decreased MVC force and muscle damage as reported previously (Fouré et al. 2014). In the latter study, a disturbance of excitation–contraction coupling was characterized from a prolonged low-frequency electrically-evoked force depression. This result is in accordance with the alteration of neuromuscular transmission after eccentric exercise-induced muscle damage (Hedayatpour and Falla 2012) and the disturbance in post-synaptic regulation of acetylcholine as a result of neuromuscular junction remodeling at the injured sites (Warren et al. 1999). In addition, a decreased voluntary activation has been reported (Fouré et al. 2014) which can be related to the corticospinal contribution in the decreased MVC force observed following damaging eccentric contractions (Doguet et al. 2019). Based on the T_2 changes reported in the present study, muscle alterations were highlighted at long muscle length in comparison to short muscle length condition. However, the contribution of neuromuscular adaptations after NMES-isometric contractions inducing muscle damage to the decreased MVC force at long muscle length should also be considered.

Considering that the electrically-evoked force levels remain the main determinant of NMES efficiency (Maffiuletti 2010) and that muscle hypertrophy can occur independently of the occurrence of muscle damage (Damas et al. 2018), we suggest that rehabilitation training programs including electrically-induced isometric contractions should be performed at short muscle length.

Conclusion

A muscle length dependence was clearly illustrated regarding damage resulting from NMES-induced isometric muscle contractions. The short muscle length condition was associated to a higher force production during the NMES protocol as compared to the long length protocol, whereas low muscle damage was only found in SAR. The use of NMES at short muscle length minimize the occurrence and extent of muscle damage (as compared to the longer one) and could contribute to the muscle remodelling despite the lack of muscle tissues alterations. Therefore, NMES performed at short muscle length appears of utmost interest in the field of rehabilitation medicine for both deconditioned patients and injured athletes.

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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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