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# AREAS OF MUSCLE TISSUE ALTERATION CAN DIFFER FROM ACTIVATED REGIONS DURING ELECTRICALLY-INDUCED ISOMETRIC CONTRACTIONS

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## Introduction

Neuromuscular electrical stimulation (NMES) has been shown to induce severe muscle damage under isometric conditions. Structural alterations [1] have been assessed in the muscles located beneath the stimulation electrodes. These findings have been supported by muscle transverse relaxation time ( $T_2$ ) mapping using magnetic resonance imaging (MRI)[2].

Although muscle activation is considered to be mainly superficial using NMES [3], MRI investigations have demonstrated a relative spatial heterogeneity regarding  $T_2$  changes [4]. Therefore, two main contributing causes could be linked to isometric NMES-induced muscle damage. A direct damage of weaker sarcomeres as suggested by the *popping sarcomere hypothesis* [5] in the activated muscle areas during the NMES exercise and/or an indirect damage of less activated muscle areas associated to intramuscular shear strain during the NMES exercise.

This study aimed at comparing the localization of activated and damaged muscle areas after a NMES session in order to distinguish these potential causes related to isometric NMES-induced muscle damage.

## Materials & Methods

**Population.** Thirteen subjects ( $26 \pm 3$  y,  $173 \pm 8$  cm,  $70 \pm 9$  kg, 4 women) volunteered to participate in this study. They were fully informed about the nature and the aim of the study and gave their informed written consent to participate in this study conducted in conformity with the Declaration of Helsinki

**Study design.** All testing sessions were performed at the same time of day before (PRE) and immediately after the damaging exercise (POST) and then seven (D7), fourteen (D14) and twenty-one (D21) days after the first exploration as detailed in Figure 1.

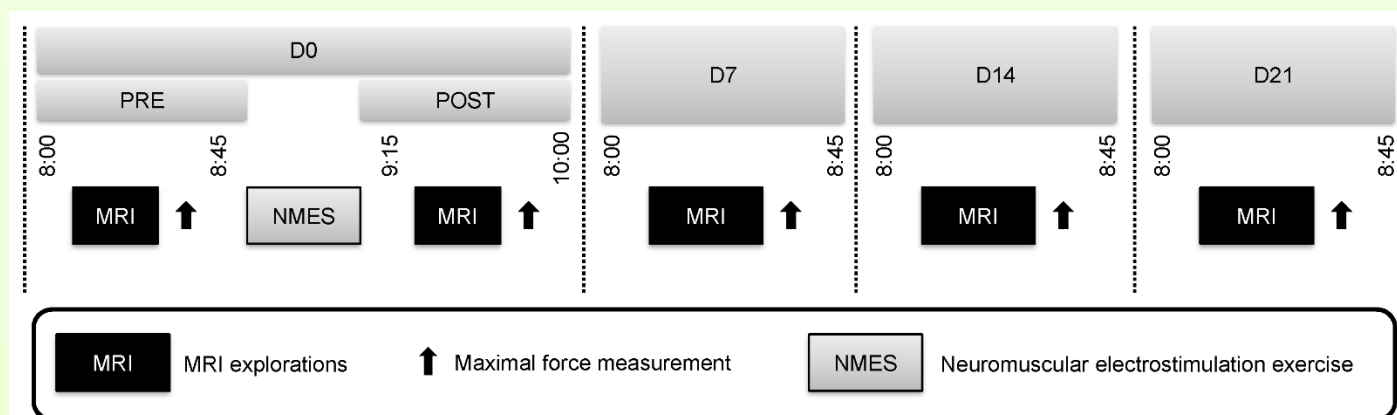


Figure 1: Schematic representation of the experimental design

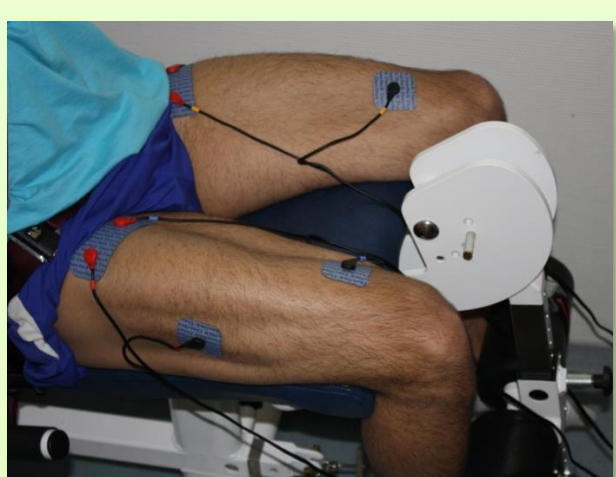


Figure 2: Subject position during the neuromuscular electrostimulation session

**Exercise.** During NMES exercise inducing muscle damage, subjects were positioned on *vastus lateralis* (VL) and *vastus medialis* (VM) as shown in Figure 2. NMES exercise consisted of 40 isometric contractions (5s on and 35s off) evoked with pulses delivered at 100Hz (pulse duration of 400 $\mu$ s). Intensity was increased until maximally tolerable level.

**MRI.** MR images were acquired at rest using a 1.5T scanner.  $T_1$ -weighted images were assessed for anatomical muscle delineations of the thigh.  $T_2$  maps were determined with a segmented spin-echo echo-planar imaging sequence with the following parameters: 20 slices, slice thickness = 6mm, slice spacing = 6mm, TR = 4800ms, TE<sub>s</sub> = 15/25/35/45/55ms.

**$T_2$  quantification in deep and superficial parts of the VM and VL muscles.** Two depth levels (*i.e.*, superficial and deep) were considered for VM and VL the most damaged muscles located beneath the stimulation electrodes. As previously described [6], a polar coordinate system (with the bone center used as the reference) was used to determine the thickness of damaged muscles at different angles and the frontiers between superficial and deep parts was then characterized in each slice [6]. Regarding muscle length, three levels were considered (*i.e.*, distal from slice #1 to #7, central from slice 8 to #13 and proximal from slice #14 to #20).  $T_2$  was then quantified for each region and at each time point

**Statistics.** Normality of the data distribution was initially investigated using Shapiro-Wilk test. One-way ANOVA (time) were used (Statistica, Statsoft, Tulsa, USA) to assess immediate (*i.e.*, PRE vs. POST) and remaining (*i.e.*, PRE vs. D7 to D21) changes in volume and  $T_2$  of each muscle. In addition, two-way ANOVAs (muscle depth \* time) were used to assess  $T_2$  differences between superficial and deep parts over time for both VL and VM and so for each muscle length level (*i.e.*, proximal, mid and distal). A Tukey's HSD post hoc analysis was performed when appropriate.

## Conclusion

On the basis of immediate and long lasting  $T_2$  changes recorded after an isometric NMES session, the present results clearly demonstrated that muscle areas activated during the contractions can be spatially different than the damaged muscle regions.

The discrepancies between activation areas and alteration regions in the *vastus lateralis* implies that tissues other than active skeletal muscle fibers were altered during unaccustomed NMES-induced isometric contractions.

## Results

Significant decrease in MVC was found at POST ( $-26 \pm 9\%$ ) and in delayed time at D7 ( $-20 \pm 6\%$ ) and D14 ( $-12 \pm 5\%$ ).

Although activation of muscle was statistically detected through  $T_2$  increase at POST in superficial parts of the two muscles located beneath the stimulation electrodes (*i.e.*, *vastus lateralis* and *vastus medialis*), alterations quantified in a delayed time from increased  $T_2$  were mainly located in the deep muscle region of the *vastus lateralis* ( $+57 \pm 24\%$ ) and superficial area of the *vastus medialis* ( $+24 \pm 16\%$ ) at D7 and were still observed in whole muscle at D21 (Table 1).

Table 1: Muscle  $T_2$  values in deep and superficial parts along the *vastus lateralis* and *vastus medialis* before (PRE), immediately after (POST) and seven days (D7), fourteen days (D14) and twenty-one days (D21) after NMES-induced muscle damage.

Mean $\pm$ SD (ms)	VL		VM		
	Sup	Deep	Sup	Deep	
PRE	Proximal	32.5 $\pm$ 1.2	32.5 $\pm$ 1.2	33.0 $\pm$ 1.0	32.4 $\pm$ 0.8
	Central	31.2 $\pm$ 0.8	31.9 $\pm$ 0.8	32.4 $\pm$ 0.6	32.3 $\pm$ 0.8
	Distal	32.4 $\pm$ 1.0	33.1 $\pm$ 1.0	33.6 $\pm$ 0.5	33.5 $\pm$ 0.8
	Whole muscle	32.2 $\pm$ 1.0	32.5 $\pm$ 1.0	33.3 $\pm$ 0.6	32.9 $\pm$ 0.7
POST	Proximal	38.2 $\pm$ 1.7 <sup>a,#</sup>	36.1 $\pm$ 1.4 <sup>a</sup>	34.5 $\pm$ 1.5 <sup>a</sup>	33.3 $\pm$ 1.0 <sup>a</sup>
	Central	37.0 $\pm$ 2.0 <sup>a</sup>	36.7 $\pm$ 1.7 <sup>a</sup>	35.6 $\pm$ 2.0 <sup>a,#</sup>	33.8 $\pm$ 1.6 <sup>a</sup>
	Distal	37.6 $\pm$ 2.1 <sup>a</sup>	38.4 $\pm$ 2.2 <sup>a</sup>	38.4 $\pm$ 2.1 <sup>a,#</sup>	36.1 $\pm$ 1.7 <sup>a</sup>
	Whole muscle	37.8 $\pm$ 1.7 <sup>a</sup>	36.6 $\pm$ 1.4 <sup>a</sup>	36.9 $\pm$ 1.9 <sup>a,#</sup>	34.9 $\pm$ 1.4 <sup>a</sup>
D7	Proximal	45.3 $\pm$ 8.0 <sup>a</sup>	51.2 $\pm$ 8.9 <sup>a</sup>	37.1 $\pm$ 3.6 <sup>a,#</sup>	33.5 $\pm$ 1.1
	Central	44.3 $\pm$ 7.1 <sup>a,#</sup>	52.8 $\pm$ 6.8 <sup>a</sup>	40.9 $\pm$ 5.7 <sup>a,#</sup>	34.7 $\pm$ 2.4
	Distal	44.3 $\pm$ 6.7 <sup>a</sup>	50.3 $\pm$ 10.0 <sup>a</sup>	42.7 $\pm$ 5.6 <sup>a,#</sup>	37.6 $\pm$ 3.8 <sup>a</sup>
	Whole muscle	44.6 $\pm$ 7.0 <sup>a,#</sup>	51.1 $\pm$ 7.8 <sup>a</sup>	41.1 $\pm$ 5.0 <sup>a,#</sup>	35.8 $\pm$ 2.6 <sup>a</sup>
D14	Proximal	37.7 $\pm$ 3.0 <sup>a,b</sup>	40.9 $\pm$ 3.7 <sup>a,b</sup>	35.7 $\pm$ 2.2 <sup>a</sup>	33.5 $\pm$ 1.4
	Central	36.9 $\pm$ 3.1 <sup>a,b</sup>	41.3 $\pm$ 3.4 <sup>a,b</sup>	36.9 $\pm$ 2.5 <sup>a,b</sup>	33.8 $\pm$ 1.5
	Distal	37.1 $\pm$ 3.3 <sup>a,b</sup>	39.4 $\pm$ 4.4 <sup>a,b</sup>	38.6 $\pm$ 2.0 <sup>a,b</sup>	36.0 $\pm$ 2.2
	Whole muscle	37.4 $\pm$ 2.8 <sup>a,b</sup>	40.7 $\pm$ 3.4 <sup>a,b</sup>	37.6 $\pm$ 1.9 <sup>a,b</sup>	34.8 $\pm$ 1.6
D21	Proximal	36.4 $\pm$ 2.5 <sup>a,b</sup>	38.4 $\pm$ 3.0 <sup>a,b</sup>	34.4 $\pm$ 1.3 <sup>b</sup>	32.9 $\pm$ 1.0
	Central	35.3 $\pm$ 2.4 <sup>b</sup>	38.6 $\pm$ 2.8 <sup>a,b</sup>	35.5 $\pm$ 2.4 <sup>a,b</sup>	33.1 $\pm$ 1.1
	Distal	35.6 $\pm$ 1.8 <sup>a,b</sup>	37.9 $\pm$ 3.1 <sup>a,b</sup>	37.1 $\pm$ 1.9 <sup>a,b</sup>	35.3 $\pm$ 1.7
	Whole muscle	36.0 $\pm$ 2.3 <sup>b</sup>	38.4 $\pm$ 2.8 <sup>a,b</sup>	36.2 $\pm$ 1.8 <sup>a,b</sup>	34.1 $\pm$ 1.2

VL: *vastus lateralis*; VM: *vastus medialis*. Sup: superficial part, Deep: deep part

a: significantly different from PRE ( $P < 0.05$ ), b: significantly different from D7 ( $P < 0.05$ ), #: significantly different from deep part ( $P < 0.05$ ).

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