



Mild to moderate damage in knee extensor muscles accumulates after two bouts of maximal eccentric contractions

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Abstract

Purpose This study aimed to determine whether mild to moderate muscle damage accumulates on the knee extensors after two bouts of maximal eccentric contractions performed over two consecutive days.

Methods Thirty participants performed an initial bout of maximal eccentric contractions of knee extensors during the first day of the protocol (ECC₁). Then, they were separated in two groups. The Experimental (EXP) group repeated the eccentric bout 24 h later (ECC₂) while the Control (CON) group did not. Indirect markers of muscle damage (i.e., strength loss, muscle soreness, and shear modulus) were measured to quantify the amount of muscle damage and its time course.

Results Two days after the initial eccentric session, participants from EXP had a higher strength deficit ($-14.5 \pm 10.6\%$) than CON ($-6.6 \pm 8.7\%$) ($P=0.017$, $d=0.9$). Although both groups exhibited an increase in knee extensors shear modulus after ECC₁, we found a significant increase in muscle shear modulus ($+13.3 \pm 22.7\%$; $P<0.01$; $d=0.5$) after ECC₂ for the EXP group, despite the presence of mild to moderate muscle damage (i.e., strength deficit about 16%).

Conclusion Although the markers of muscle damage used in the current study were indirect, they suggest that the repetition of two bouts of maximal eccentric contractions with 24 h apart induces additional muscle damage in the knee extensors in presence of mild to moderate muscle damage.

Keywords Exercise-induced muscle damage · DOMS · Shear modulus · Shear wave elastography · Lengthening contractions

Abbreviations

ANOVA	Analysis of variance
CON	Control group
D ₁ , D ₂ , D ₃ , D ₄ , D ₇	Day 1, Day 2, Day 3, Day 4, Day 7
ECC ₁ , ECC ₂	Eccentric training 1, Eccentric training number 2
EXP	Experimental group
MVC	Maximal voluntary contraction
RF	Rectus femoris
VM	Vastus medialis
VL	Vastus lateralis

Introduction

Most sport activities involve repeated eccentric contractions, which are a major cause of cytoskeletal disruptions, commonly referred as muscle damage (Armstrong 1984). Muscle damage triggers a cascade of symptoms such as long-lasting strength loss, muscle soreness, and inflammatory response (Paulsen et al. 2012). Although a single bout of eccentric exercise limits the magnitude of such symptoms from subsequent eccentric bouts [i.e., repeated bout effect; McHugh (2003)], it is a long-held belief that muscle damage may accumulate with the repetition of training stimulus over consecutive days (Kataoka et al. 2022; Mohr et al. 2016; Opar et al. 2012; Vila Pouca et al. 2021). In other words, additional muscle damage could be induced in presence of existing muscle damage. In professional sport, one aim of training periodization is to manage muscle damage and prevent potential accumulation within the week, at least close to competitive events. To do it, Lovell et al. (2018) proposed that eccentric-based lower limb injury prevention should be scheduled in the midst of the microcycle to avoid muscle

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damage accumulation and enhance the preparation of the upcoming game. Despite this belief and practices, the current literature still lacks robust experimental evidences to draw definitive conclusions.

Previous works have demonstrated that the repetition of soccer games (Mohr et al. 2016) and simulated soccer situations (Page et al. 2019) three times a week has been reported to induce greater muscle damage. Specifically, they reported that strength loss, muscle soreness and inflammatory response were greater after the second situation compared to the first. This suggests that there may be a potential accumulation of muscle damage related to repeated soccer situations. However, the absence of control group (i.e., a group that should have performed only the first soccer game/situation) limits the interpretation of these findings. Additionally, they used indirect markers of muscle damage that gradually change and peak few days after the first soccer game/situation (Clarkson et al. 1992; Mohr et al. 2016), when the second game or simulated situation was performed. Taken together, it is impossible to determine whether the time-course of the markers of muscle damage was altered due to the presence of additional damage and/or due to other mechanisms (e.g., muscle fatigue, hormonal response). An experimental design that i) considers the presence of a control group and ii) provides an early detection of muscle damage (i.e., immediately after the soccer situation) could provide a better understanding of muscle damage accumulation. A series of studies reported a strong linear relationship between changes in muscle shear modulus 30 min after exercise cessation, and strength loss recorded 48 h post exercise in both upper- (elbow flexors) and lower-limb muscles (knee extensors and hamstring), suggesting that this technique provide an indirect, early, and non-invasive estimation of the amount of damage (Chalchat et al. 2022; Goreau et al. 2022; Heales et al. 2018; Lacourpaille et al. 2017). Taking advantage of this technique, it is possible to estimate the magnitude of muscle damage induced by each eccentric bout.

The possibility of muscle damage accumulation has been studied in laboratory settings (Chen and Hsieh 2001; Nosaka and Newton 2002; Paddon-Jones et al. 2000). Strikingly, all of these studies did not reported any muscle damage accumulation when eccentric repeated bouts were performed during a mono-articular exercise (Chen 2003, 2006; Chen and Hsieh 2001; Paddon-Jones et al. 2000). Chen and Hsieh (2001) observed that the magnitude and time-course of markers of muscle damage were similar between the experimental group that daily performed repeated maximal isokinetic eccentric contractions over a period of seven consecutive days, and the control group that performed a unique eccentric bout on one day. The putative discrepancy of the results between sport vs. laboratory settings may be explained by the amount of muscle damage induced by the first eccentric bout. Mild exercised-induced muscle damage

was reported in sports settings [$< 10\%$ strength loss at 48 h post-exercise, Mohr et al. (2016); Page et al. (2019)], while works conducted in laboratory settings induced severe damage [$> 30\%$ strength loss at 48 h after exercise, Chen and Hsieh (2001); Nosaka and Newton (2002); Paddon Jones et al. (2000)]. In presence of moderate to severe muscle damage, it is likely that the central nervous system reduced the activation of fast-twitch motor units, which are more impacted by muscle damage, and consequently limits the generation of additional muscle damage to preserve participant's integrity (Chen 2003; Warren et al. 2000).

With these considerations in mind, this study aimed to determine whether mild to moderate muscle damage accumulates on the knee extensors after two bouts of maximal eccentric contractions performed over two consecutive days. The time-course of strength loss and muscle soreness were used as markers of the amount of muscle damage (Paulsen et al. 2012). The early changes in shear modulus were used as a marker of the induction of muscle damage for the superficial head of the knee extensors [i.e., vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF)] after each eccentric bout. We tested two hypotheses (i) an alteration in the time course of changes in maximal strength would be observable for participants who performed two eccentric sessions in two days, and (ii) an increase of muscle shear modulus after the second session would be observable, highlighting the creation of muscle damage, in presence of initial muscle damage.

Materials and methods

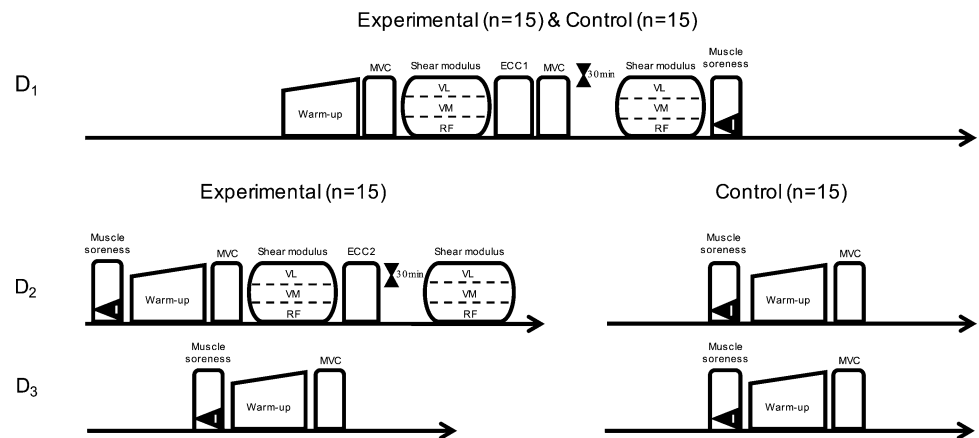
Participants

A total of 30 physically active (~ 2 h/week) volunteers participated in the study. They were separated in two equivalent maximal torque-matched groups, i.e., experimental group (EXP, $n = 15$; 6 females; age: 21 ± 2 years; height: 175.5 ± 9.6 cm; weight: 66.8 ± 10.2 kg) and control group (CON, $n = 15$; 6 females; age: 21 ± 1 years; height: 171.1 ± 11.7 cm; weight: 64.9 ± 11.1 kg). None of the participants had recent history of musculoskeletal injuries in the lower limbs. All participants were informed about the nature, aims and risks associated with the experimental procedures, before their written consent was collected. The experimental procedures were approved by the Ethics Committee (CPP IDF I, n°2018-A02675-50), and all procedures adhered to the Declaration of Helsinki.

Design

The first experimental session (D_1) was similar for participants from both EXP and CON groups (Fig. 1). First, we

Fig. 1 Experimental design of the study. *MVC* Maximal voluntary contractions, *VL* Vastus lateralis, *VM* Vastus medialis, *RF* Rectus femoris, *ECC1* First eccentric bout, *ECC2* Second eccentric bout (Experimental group). *D₁* Day of the first bout of eccentric contractions, *D₂* Day of the second bout of eccentric contractions (Experimental group). *D₃* 48 h after the first eccentric bout, and 24 h after the second eccentric bout (Experimental group)



assessed (i) maximal isometric voluntary contraction (MVC) and (ii) resting shear modulus. Immediately after these initial testing, participants performed an intense eccentric exercise (ECC₁) with the dominant leg, i.e., the leg used to kick a ball (n = 29 participants with the right leg and 1 participant with the left leg). It consisted in 30 to 75 repeated isokinetic maximal eccentric contractions of knee extensors until a strength loss of 20%. MVC was performed immediately after the last contraction. Resting shear modulus of superficial heads of the knee extensors (VL, VM, and RF) were measured 30 min after the final contraction (Lacourpaille et al. 2017). This first session ended by a muscle soreness measurement. The second session (D₂) was performed 24 h after D₁, and was similar to this first session for participants from the EXP group. They performed a second work-matched eccentric exercise (ECC₂) while the CON group only performed MVC and muscle soreness measurements. Note that electromyographic (EMG) signals of the VL, VM and RF were recorded during eccentric contractions of all sessions for another purpose.

Eccentric exercise

The eccentric exercise was performed on an isokinetic dynamometer (Con-Trex; CMV AG, Dübendorf, Switzerland) in a seated position (hip = 80°; 0° = lying supine). Participants were first asked to perform two sets of 15 maximal isokinetic eccentric contractions of the knee extensors. These two initial sets were separated by 2 min of passive recovery. Then, MVC was tested. If MVC loss was > 20%, the exercise was stopped. If not, participants performed another set of 15 maximal eccentric contractions, with an evaluation of MVC after each additional set, until they reach the target level of strength loss. Note that the eccentric exercise was definitely stopped after 5 bouts of eccentric contractions (i.e., 75 contractions). The threshold of 20% was chosen to induce mild to moderate muscle damage (Paulsen et al. 2012). The

eccentric contractions were performed with the dominant leg from 10° to 110° of knee flexion (0° corresponding to the full knee extension) at a constant angular velocity of 60°s⁻¹ (Lacourpaille et al. 2017). Participants were firmly attached to the dynamometer in a seated position. Between each eccentric contraction, the leg was passively returned at the initial position (i.e., 10° of knee flexion) by the dynamometer at a constant angular velocity of 30°s⁻¹. Participants were encouraged to produce the greatest torque possible during each contraction all along the range of motion. The amount of work during ECC₁ was measured using the dynamometer. During ECC₂, participants of EXP group performed the number of sets of 15 repetitions until they reached the total workload done during ECC₁. A visual feedback of torque signal was provided to the participants throughout the protocol. All mechanical signals (i.e. torque, angular position, angular velocity) provided by the dynamometer were recorded.

Indirect markers of muscle damage

Strength loss

MVC was measured using the isokinetic ergometer in isometric mode (knee flexion of 80°) and used as an indirect marker of the magnitude of damage (Paulsen et al. 2012). Before the eccentric bout, two 5-s MVC were performed (interspaced by 2-min of passive recovery). The trial with the highest peak torque was kept for further statistical analysis. If more than 10% of variation was found between the two trials, a third one was performed. MVC was also measured between each 15-contractions after the two first sets to determine whether the participants reached the threshold of 20% (i.e., to limit the magnitude of muscle damage). Further, MVC were assessed at D₂ and D₃ to quantify the magnitude of muscle damage. Note that MVC at D₂ was measured before the eccentric session for EXP.

Muscle soreness

Muscle soreness was evaluated using a visual analogue scale with a continuous line that represent « no pain » at one side (left) and « very high pain » at the other side (right). Participants were asked to move a mark on the line to indicate the soreness after self-palpation of self-the knee extensors and seated knee flexion/extension. A score between 0 to 10 was collected for further analysis.

Muscle shear modulus

Before and 30 min after the eccentric exercise, resting muscle shear modulus of VL, VM and RF muscles were measured at 110° of knee flexion (Chalchat et al. 2022; Heales et al. 2018; Lacourpaille et al. 2017) using an ultrasound scanner (Aixplorer version 12.4, Supersonic Imagine) coupled with a linear transducer array (2–10 MHz, SuperLinear 10–2; Vermon, France). Shear wave elastography mode was used to provide shear modulus in real time (1–1.2 Hz). The ultrasound probe was positioned within the direction of the fascicles for each muscle and perpendicular to the skin (Fig. 2) (Le Sant et al. 2015). We considered the position as correct when muscle fascicles were observable on the entire image without interruption. The location and orientation of the transducer was marked on the skin using waterproof marker to keep it constant between recordings (i.e., between *pre* and *post* eccentric measurements and between D_1 and D_2 for the EXP group). For each recording, participants were asked to remain as relaxed as possible. Two 10-s videos were recorded before and 30 min after the eccentric exercise for each muscle (Fig. 2). Each video was processed

on MATLAB software (The Mathworks, Nathicks, USA) with the ElastoGUI open software (<https://bio.tools/elastogui>). The region of interest was defined manually for each clip of every participant. Then, an average-value of shear modulus was obtained for every 10-s video clip for each muscle. Finally, a second average was performed between both clip of each muscle to save one value of shear modulus (Lacourpaille et al. 2012).

Statistical analysis

Statistical analyses were performed with Statistica software (StatSoft Inc., Tulsa, Oklahoma, USA). Normality of the data was tested by a Shapiro–Wilk test. Data are reported as mean \pm standard deviation. We performed independent Student's t-test to confirm that the age, weight, height, MVC, number of contractions and work at D_1 were not different between groups. Additionally, a dependent student's t-test was performed to confirm that the amount of work performed during ECC_2 was not different than ECC_1 for EXP. To determine whether the repetition of eccentric bouts alters the strength loss and muscle soreness, independent two-way repeated-measures analysis of variance (ANOVA) were performed [within subject factor: Time ($D_{1\text{PRE}}$: before ECC_1 ; $D_{1\text{POST}}$: immediately after ECC_1 , D_2 , D_3) and Group (EXP, CON)]. To determine whether the amount of muscle damage induced by ECC_1 was different between EXP and CON group at D_1 , we used an independent three-way ANOVA [within subject factors: Time ($D_{1\text{PRE}}$: before ECC_1 ; $D_{1\text{POST}}$: 30 min after ECC_1), Muscle (VL, VM and RF) and Group (EXP and CON)]. To determine whether the amount of muscle damage varied between sessions (D_1 and D_2) after

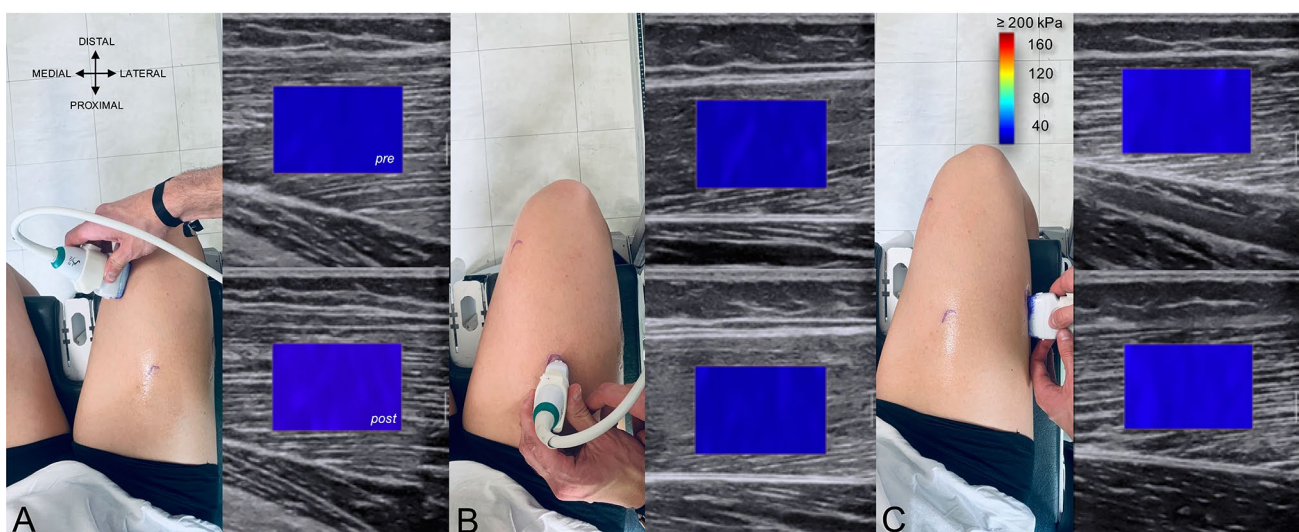


Fig. 2 Typical ultrasound images and elasticity maps of vastus medialis **A**, rectus femoris **B** and vastus lateralis **C**. The blue box represents the region of interest for shear modulus analysis. Partici-

pants were in a sitting position and positioned at 110° of knee flexion. *kPa* kiloPascal. *pre* muscle shear modulus measurements before eccentric. *post* muscle shear modulus measurements after eccentric

each eccentric exercise for EXP group, we used an independent three-way ANOVA on shear modulus [within subject factors: Time ($D_{1\text{ PRE}}$: before ECC_1 ; $D_{1\text{ POST}}$: 30 min after ECC_1 ; $D_{2\text{ PRE}}$: before ECC_2 ; $D_{2\text{ POST}}$: 30 min after ECC_2), Muscle (VL, VM and RF) and Session (ECC_1 , ECC_2)]. When appropriate, post hoc analyses were performed using Newman-Keuls test. Effects size were calculated using Cohen’s *d* (Cohen 1988) considering 0.2, 0.5 and 0.8 as a small, medium and large effect, respectively. The significance level was set to $P < 0.05$.

Results

Individual and exercise features

No differences between groups were found for the age, height, weight and knee extension strength (EXP = 268.5 ± 70.8 Nm; CON = 267.0 ± 68.5 Nm) (all *P* values > 0.25 ; all *d* values < 0.2). At D_1 , participants from EXP performed 3.8 ± 0.9 bouts of 15 maximal

eccentric contractions, and participants from CON performed 3.2 ± 1.4 bouts of 15 maximal eccentric contractions ($P = 0.1$; $d = 0.5$), corresponding to $-13\,919 \pm 4\,085$ Joules and $-10\,789 \pm 4\,683$ Joules ($P = 0.06$; $d = 0.7$) for EXP and CON, respectively. At D_2 , EXP group performed $-13\,835.7 \pm 4\,005.9$ Joules ($P = 0.5$; $d = 0.02$), corresponding to 4.5 ± 1.4 sets.

Strength loss

Results related to strength loss are displayed in Table 1. ANOVA revealed a significant effect of Time ($P < 0.01$) and a significant Time \times Group interaction ($P < 0.01$). As expected, strength loss was not different between EXP and CON at $D_{1\text{ POST}}$ ($P = 1.0$; $d = 0.1$) and D_2 , ($P = 0.96$; $d = 0.1$). Interestingly, a *large* significant difference in strength loss was found at D_3 between EXP (227.7 ± 58.6 Nm) and CON (248.2 ± 62.3 Nm) corresponding to $(-14.5 \pm 10.6\%)$ and $(-6.6 \pm 8.7\%)$ respectively ($P = 0.017$, $d = 0.8$) (Fig. 3).

Table 1 Indirect markers of exercise-induced muscle damage after a single (Control) or two (Experimental) eccentric bouts over two consecutive days for the knee extensors

Group	Day 1 _{pre}	Day 1 _{post}	Day 2	Day 3
Strength (% of Day 1 _{pre})				
Control	100	$78.8 \pm 7.3^*$	$83.5 \pm 6.9^*$	93.4 ± 8.8
Experimental	100	$77.8 \pm 5.6^*$	$83.9 \pm 6.9^*$	$86.1 \pm 10.1^{*\#}$
DOMS (a.u.)				
Control	0	1.6 ± 2.1	$3.7 \pm 2.7^*$	$3.2 \pm 2.6^*$
Experimental	0	$3.4 \pm 2.3^*$	$4.2 \pm 2.1^*$	$5.8 \pm 2.3^{*\#}$
Muscle	ECC_1		ECC_2	
	Day 1 _{pre}	Day 1 _{post}	Day 2 _{pre}	Day 2 _{post}
Shear modulus (kPa)				
Control				
RF	4.2 ± 1.5	5.9 ± 4.3 <i>$33.6 \pm 54.3\%$</i>		
VL	5.1 ± 0.8	5.5 ± 1.1 <i>$9.6 \pm 15.8\%$</i>		
VM	4.4 ± 1.1	4.6 ± 0.7 <i>$8.4 \pm 25.3\%$</i>		
Experimental				
RF	3.8 ± 0.9	4.7 ± 1.4 <i>$30.9 \pm 57.1\%$</i>	4.1 ± 1.3	4.9 ± 1.1 <i>$20.5 \pm 26.3\%$</i>
VL	5.3 ± 0.7	5.9 ± 1.6 <i>$10 \pm 28.1\%$</i>	5.4 ± 0.8	5.9 ± 1.1 <i>$9.7 \pm 16.5\%$</i>
VM	4.5 ± 0.5	4.9 ± 0.9 <i>$9.4 \pm 12.2\%$</i>	4.6 ± 0.6	5.0 ± 0.8 <i>$9.7 \pm 24.1\%$</i>

Values are presented as mean \pm SD. *: Significant difference when compared with the PRE. #: Significant difference when compared with the CON. *Italic* writing for shear modulus results are the changes, in percentage, between PRE and POST measurement performed the same day. *a.u* Arbitrary Unit, *kPa* kilopascal, *RF* Rectus femoris, *VL* Vastus lateralis, *VM* Vastus medialis, ECC_1 first eccentric bout, ECC_2 second eccentric bout

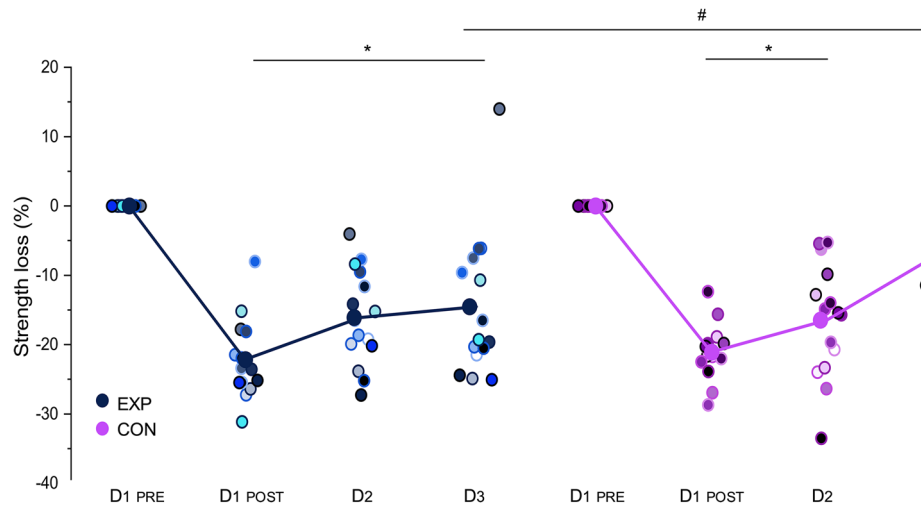


Fig. 3 Time course of strength loss for Experimental and Control groups. The first eccentric bout was performed at D₁ for both Experimental and Control groups. The second eccentric bout was performed at D₂ for the Experimental group. Note that only the strength loss before the second eccentric bout is depicted in the. D₁ PRE MVC measurements before the first bout of eccentric contractions. D₁ POST

MVC measurements immediately after the last contraction of the first bout of eccentric contractions. D₂ MVC measurements 24 h after the first eccentric bout. D₃ MVC measurements 48 h after the first eccentric bout, and 24 h after the second eccentric bout (Experimental group). *Significant difference when compared with the D₁ PRE. #Significant difference when compared with the CON

Muscle soreness

We found a significant effect of Time on muscle soreness ($P < 0.001$). We also observed a significant Time \times Group interaction ($P < 0.01$), with *large* difference in muscle soreness between EXP (5.8 ± 2.3) and CON (3.2 ± 2.6) at D₃ ($P = 0.02$; $d = 1.1$), while no difference at D₁ POST ($P = 0.35$; $d = 0.8$) and D₂ ($P = 0.60$; $d = 0.5$) (Table 1).

Muscle shear modulus

The ANOVA performed on ECC₁ revealed a Time \times Muscle interaction ($P = 0.02$) on muscle shear modulus. Regardless of group, ECC₁ induced a significant increase in RF- ($d = 0.8$ and $d = 0.5$, for EXP and CON, respectively; $P < 0.01$), but not in VL- ($d = 0.4$ and $d = 0.5$, for EXP and CON, respectively; $P = 0.16$) and VM-shear modulus ($d = 0.6$ and $d = 0.2$, for EXP and CON, respectively; $P = 0.22$) (Table 1). We reported no Time \times Group interaction ($P = 0.73$) nor Time \times Muscle \times Group interaction ($P = 0.40$). The second ANOVA performed on EXP revealed a significant effect of Time ($P < 0.001$) indicating that the shear modulus was increased ($+13.3 \pm 22.7\%$; $d = 0.5$). We did not find any significant Time \times Session interaction ($P = 0.68$) nor Time \times Muscle \times Session interaction ($P = 0.77$), indicating that the increase in muscle shear modulus was not different between ECC₁ and ECC₂ (Fig. 4). This suggests that the amount of muscle damage induced at ECC₁ and ECC₂ was similar.

Discussion

The present study provides the first experimental evidence in a laboratory setting that mild to moderate muscle damage of knee extensors accumulates after two bouts of maximal eccentric contractions performed over two consecutive days. This finding is supported by three results obtained from indirect markers of muscle damage. We found that strength loss and muscle soreness were higher at D₃ for participants from EXP, that have performed two bouts, compared to CON group, who performed one bout. These results were corroborated by the significant increase in shear modulus after the second bout of eccentric contractions in the participants from EXP, indicating that additional damage was induced by the second bout of eccentric contractions.

A prerequisite of this study was to induce mild to moderate muscle damage [i.e., $< 20\%$ of strength loss at 48 h after exercise, Paulsen et al. (2012)]. Participants performed 3.5 ± 1.0 sets of 15 maximal eccentric contractions of knee extensors to reach about a $\sim 20\%$ MVC decrease immediately after the final contraction of the first eccentric session (ECC₁), regardless of the group. This led to a $16.2 \pm 7.4\%$ decrease in MVC at D₂. The moderate peak muscle soreness score reported at D₂ for both EXP (4/10) and CON (3.5/10) confirmed the presence of mild to moderate muscle damage (Paulsen et al. 2012). For some individuals, however, muscle damage was considered as moderate to severe, with 20% (3/15) and 27% (4/15) of participants from EXP and CON groups, respectively, who exhibited a strength loss higher than 20% (range: 23.4–33.5%) at D₂. This was

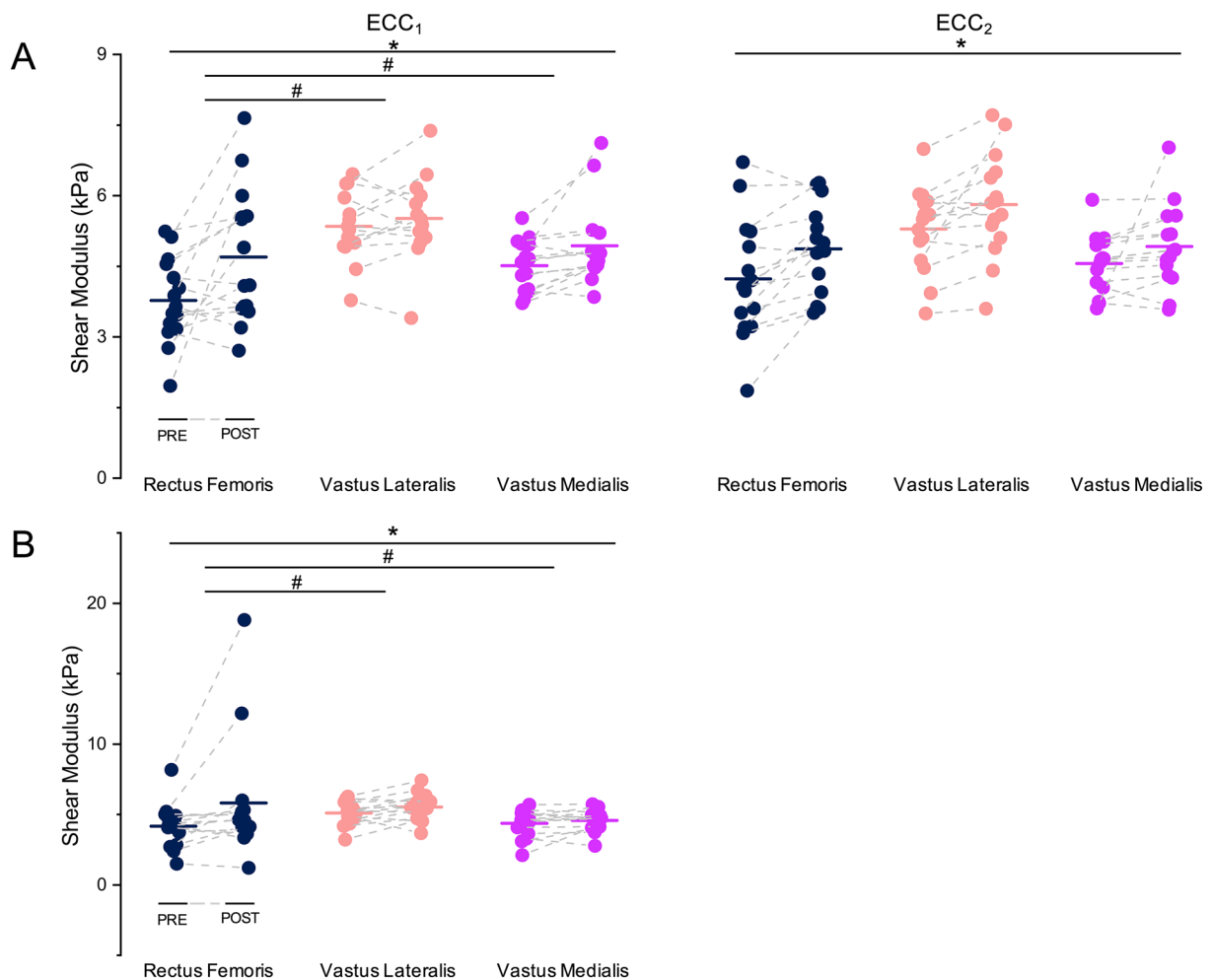


Fig. 4 Changes in shear modulus of the superficial heads of the knee extensors after the first eccentric bout (ECC₁) for experimental **A** and control **B** group and after the second eccentric bout (ECC₂) for

the experimental group **A**. *Significant effect of Time. #Significant Time \times Muscle interaction

corroborated by their high soreness peak score (6/10). This large inter-individual difference in the sensitivity to muscle damage is well known and linked to different parameters [i.e., training background, flexibility, age, genetics (Chen 2006; Hubal et al. 2007; Paulsen et al. 2012)]. Overall, the 20% threshold for strength loss immediately post-exercise used in the current study was an effective strategy to induce mild to moderate damage, as the proportion of individuals with moderate or severe muscle damage were limited compared with other studies that used a similar volume of maximal eccentric contractions [e.g., 40% of the participants in the low load protocol, Lacourpaille et al. (2017); 43%, Shoji et al. (2021)].

There is no study showing different patterns in the time course of strength loss when two specific conditions were compared, i.e., repetition of eccentric bouts over two consecutive days (EXP) vs. unique eccentric bout on one day (CON). In the current study, participants from EXP

exhibited a larger strength loss at D₃ ($-14.5 \pm 10.6\%$) compared to participants from CON ($-6.6 \pm 8.7\%$) ($P=0.017$; $d=0.8$). At the individual level, 8 out of 15 participants from EXP exhibited a lower MVC at D₃ compared with D₂, while it was only 2 out of 15 for participants from CON. The alteration of the time course of muscle soreness found for EXP group make us confident about the accumulation of muscle damage. As the strength loss was still significant at D₃ for both groups, we performed additional time measurements (four and seven days after the first eccentric bout, D₄ and D₇, respectively) for ten participants to describe its entire time course (EXP, $n=5$; CON, $n=5$). We found a *small* ($d=0.3$) larger strength loss at D₄ for EXP ($-8.5 \pm 9.2\%$) compared to CON ($-5.1 \pm 11.4\%$). MVC was returned to baseline at D₇ for both groups. Similarly, a *large* ($d=1.1$) difference was still present at D₄ between EXP (4.8 ± 3.2) and CON (2.0 ± 2.2) while muscle soreness returned to baseline at D₇ for both groups. The altered time course of strength in EXP

group is further reinforced by the changes in shear modulus after the second eccentric bout (i.e., in presence of initial muscle damage, see below for further details). A main hypothesis supports the discrepancy between our results and the other laboratory studies. Strength loss at 48 h post-ECC₁ (Fig. 3) was lower than that reported in other laboratory studies that was usually around 30% (Chen and Hsieh 2001; Nosaka and Newton 2002; Paddon-Jones et al. 2000). In absence of muscle damage, neuromuscular activity is attenuated during maximal eccentric contractions, as suggested by decreased level of central activation and spinal motor neuron excitability [for review see Aagard (2018)]. It is therefore reasonable to assume that the decrease in neuromuscular activity would be exacerbated in presence of moderate to large muscle damage to limit the recruitment of additional motor unit, and in turn, muscle tension. This protective adaptation would reduce the risk of developing an extensive homeostasis disturbance, or even a pathophysiological condition [e.g., rhabdomyolysis (Noakes 2005)]. It is reasonable to assume that this putative decreases in neuromuscular activity was limited in presence of mild to moderate muscle damage. Further electrophysiological investigations are needed to determine how the amplitude of muscle damage (i.e., mild to moderate versus moderate to severe) could impact the neuromuscular activity.

The early increase in shear modulus after a single bout of eccentric contractions is now consensual (Chalchat et al. 2022; Goreau et al. 2022; Green et al. 2012; Heales et al. 2018; Lacourpaille et al. 2017). The prevailing theory of changes in muscle shear modulus after eccentric contractions is linked to the increase of calcium concentration in the intramuscular area due to muscle fiber disruption (Whitehead et al. 2001). It has been shown that this increase in shear modulus was reflective of both cycle -dependent and -independent processes that generate a supplemental passive force (Lacourpaille et al. 2014), which can be attributable to resting cross-bridges and to the structural protein titin (Labeit et al. 2003; Linke and Krüger 2010), as they are sensitive to intramuscular calcium concentration (Herzog 2014; Whitehead et al. 2001). After ECC₁, we found that the increase in shear modulus of the superficial heads of the quadriceps muscles was in line with those reported in previous studies, regardless of the experimental group (+17.6% and +26.7% in this study and in Lacourpaille et al. (2017), respectively). Chalchat et al. (2022) recently demonstrated that the early increase in shear modulus reported after an initial bout of eccentric exercise was limited for both RF and VL after a second one, that was performed 14 days after the first exercise in presence of a repeated bout effect. In the current study, we did not find any difference in the increase in shear modulus between ECC₁ and ECC₂ ($P=0.68$) (Fig. 4). This may first be explained by the amount of work matched between the two bouts while the maximal strength was

reduced at the beginning of ECC₂. Second, the lower magnitude of muscle damage induced by the first eccentric bout in our study [i.e., $-16.2 \pm 7.4\%$ of strength deficit 24 h after the first session in the present study vs $\sim 20\%$ of strength deficit 48 h after the first eccentric bout in Chalchat et al. (2022)] is also a candidate. This increase in shear modulus after ECC₂, in presence of muscle damage is a major result of this study. At the individual level, this increase in shear modulus after ECC₂ was observed in 10 out of 15 of the participants while they exhibited MVC loss about $-16.1 \pm 6.9\%$ (i.e., from -27.2 to -4.0%). This confirms our hypothesis that additional muscle damage may occur in presence of existing mild to moderate muscle damage. It is important to note that a larger increase in shear modulus was observed for RF compared to VL and VM after ECC₁ (mean difference = 22% and 23% with VL and VM respectively) and ECC₂ (mean difference = 11% with both VL and VM). These findings align with previous shear wave elastography (Lacourpaille et al. 2017; Xu et al. 2019) and MRI (Maeo et al. 2018) based-studies, suggesting that RF is more sensitive to muscle damage compared to VM and VL. Although speculative, this finding is likely explained by its predominance of fast twitch fibers, more prone to muscle damage (Kulig et al. 2001) combined with a larger strain during seated eccentric knee extension compared to daily life multi-joints tasks (Prior et al. 2001).

In sport settings, this means that the repetition of intense/unaccustomed training sessions should be considered in training load management within the microcycle. This is in line with the recommendations from Lovell et al. (2018) suggesting that the eccentric lower limb injury prevention exercises should be scheduled early in the microcycle to avoid compromising preparation for the following game. Further investigations are needed to determine whether the accumulation of muscle damage plays a role in the development of muscle injury.

Conclusion

The current protocol induced mild to moderate damage on the knee extensors after one (CON) or two (EXP) work-matched bouts of maximal eccentric contractions performed one day apart. Both the concomitant alteration of the time course of the strength loss and muscle soreness, combined with an increase in shear modulus after the second bout of eccentric contractions provide evidence that EXP group exhibited an accumulation of muscle damage. Although the markers of muscle damage used in the current study were indirect, they provide evidence that the repetition of two bouts of maximal eccentric contractions with 24 h apart induced additional muscle damage in knee extensors in presence of mild to moderate muscle damage.

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Author contributions Lilian Lacourpaille conceived and designed research. Titouan Morin and Inès Boulaouche conducted the experiments and collected the data. Lilian Lacourpaille and Titouan Morin analyzed the data. Antoine Nordez, Marc Jubeau, Robin Souron, Lilian Lacourpaille and Titouan Morin drafted the manuscript. All authors provided critical appraisal to the manuscript and approved the final version.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest No potential conflict of interest was reported by the authors. The results of the study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

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