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Muscle force loss and soreness subsequent to maximal eccentric contractions depend on the amount of fascicle strain *in vivo*

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Abstract

Aim: Defining the origins of muscle injury has important rehabilitation and exercise applications. However, current knowledge of muscle damage mechanics in human remains unclear *in vivo*. This study aimed to determine the relationships between muscle–tendon unit mechanics during maximal eccentric contractions and the extent of subsequent functional impairments induced by muscle damage.

Methods: The length of the muscle–tendon unit, fascicles and tendinous tissues was continuously measured on the gastrocnemius medialis using ultrasonography, in time with torque, during 10 sets of 30 maximal eccentric contractions of plantar flexors at $45°s^{-1}$, in seventeen participants.

Results: Muscle–tendon unit, fascicles and tendinous tissues were stretched up to 4.44 ± 0.33 cm, 2.31 ± 0.64 cm and 1.92 ± 0.61 cm respectively. Fascicle stretch length, lengthening amplitude and negative fascicle work beyond slack length were significantly correlated with the force decrease 48 h post-exercise ($r = 0.51$, 0.47 and 0.68, respectively; $P < 0.05$).

Conclusions: This study demonstrates that the strain applied to human muscle fibres during eccentric contractions strongly influences the magnitude of muscle damage *in vivo*. Achilles tendon compliance decreases the amount of strain, while architectural gear ratio may moderately contribute to attenuating muscle fascicle lengthening and hence muscle damage. Further studies are necessary to explore the impact of various types of task to fully understand the contribution of muscle–tendon interactions during active lengthening to muscle damage.

Keywords fascicle lengthening, muscle damage, shear wave elastography, ultrasonography.

Eccentric contractions are recognized as a major cause of cytoskeletal disruptions referred to as exercise-induced muscle damage (EIMD) (Clarkson & Hubal 2002, Guilhem *et al.* 2010). Although it is well documented that EIMD is associated with functional impairments such as force loss, delayed inflammation, oedema and soreness (Friden & Lieber 2001, Paulsen *et al.* 2012, Guilhem *et al.* 2013), our understanding of the mechanical processes involved in the eccentric contractions that govern the extent of subsequent

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muscle damage remains unclear *in vivo*. Current knowledge of muscle damage mechanics during eccentric contractions has been mainly inferred from animal and *in vitro* studies (Lieber *et al.* 1991, Lieber & Friden 1993, Butterfield 2010). Morgan (1990) proposed that active lengthening could quickly stretch sarcomeres and increase the tension in neighbouring myofibrils, leading to disruption of the weakest sarcomeres (Lieber *et al.* 2002). However, most of these *in vitro* studies imposed an electro-induced stretch (i.e. non-voluntary contraction), under a constant maximal level of activation (i.e. high muscle stimulation frequencies), to a small amount of fibres and beyond the physiological ranges of the fibre lengths (Brooks *et al.* 1995, Butterfield 2010). *In vivo*, due to the involvement of voluntary activation and elastic connective tissues, the behaviour of the muscle–tendon unit during eccentric contractions is more complex, and muscle damage processes cannot be solely extrapolated from studies that analyse single fibre disruptions (Butterfield 2010), challenging the transfer of animal studies to human situations.

In the last 15 years, the contribution of both fascicles and tendinous tissues to the behaviour of the total muscle–tendon unit has been widely analysed *in vivo* thanks to the advent of ultrasonography (Cronin & Lichtwark 2013). Interestingly, during the eccentric phase of human locomotion, several studies have shown no lengthening of the *gastrocnemius medialis* (GM) fascicles, which contract quasi-isometrically, whereas the muscle–tendon unit clearly increases in length (Fukunaga *et al.* 2001, Ishikawa *et al.* 2007, Lichtwark *et al.* 2007). Therefore, it may be considered that fascicle–tendon interactions reduce the magnitude and speed of stretch applied to the activated muscle fibres under natural conditions, when compared to isolated fibre studies (Butterfield 2010, Hoffman *et al.* 2014). Two main mechanisms may explain this reduction in fibre strain. First, the tendon acts like a shock absorber that dissipates energy and reduces fibre loads and elongation (Konow & Roberts 2015). Second, recent works have suggested that fascicle rotation (i.e. architectural gearing ratio, AGR) can minimize the stretch applied directly to fascicles during eccentric contractions (Azizi & Roberts 2014). Whether both mechanisms actually participate to protect pennate human muscles from EIMD remains a matter of debate.

In this context, although it is well known that the muscle force impairment after eccentric exercise differs greatly between subjects, this variability remains poorly understood (Chen 2006). Thus, we can speculate that the variability in fascicle lengthening due to variable tendon contributions and AGR could be involved in the variability in EIMD magnitude. To the best of our knowledge, only two studies have tested this hypothesis *in vivo* (Hoffman *et al.* 2014, Penailillo *et al.* 2015). During loaded (10% of body mass) backward walking on a 13% decline, Hoffman *et al.* (2014) observed an active lengthening of GM fascicles (amounting to 18% of optimal fascicle length on average). However, they did not report any correlation between the amount of fascicle lengthening during exercise and the subsequent decrease in electrically induced isometric torque due to muscle damage. In addition, Penailillo *et al.* (2015) did not impute the exercise-induced decrease in maximal voluntary isometric torque to the amplitude of the *vastus lateralis* fascicles lengthening observed during eccentric cycling. However, both studies analysed complex multijoint movements that involve high interindividual variability in muscle coordination, joint range of motion and contraction velocity, thus making it difficult to isolate the fascicle strain among the factors that could affect EIMD (Brooks *et al.* 1995, Butterfield 2010). More importantly, these two studies examined the effects of submaximal eccentric exercises that resulted in small muscle–tendon lengthening and non-significant or low force decrements (e.g. −7% 48 h after the exercise and −23% on average in Penailillo *et al.*). Hence, the link between the indexes of muscle damage and fascicle behaviour during maximal eccentric contractions that induce a higher level of force decrement and fascicle lengthening remained to be analysed (Kawakami *et al.* 2002, Ishikawa *et al.* 2003).

This study was designed to investigate (i) the behaviour of the GM (as a surrogate of plantar flexor muscles (Hauraix *et al.* 2013)) fascicles and muscle–tendon unit throughout repetitive maximal isokinetic eccentric and single-joint contractions of the plantar flexors and (ii) the potential relationships among fascicle lengthening during eccentric exercise, the subsequent decrease in muscle force and increase in soreness two days after exercise. An additional purpose was to analyse the potential influence of various parameters (i.e. AGR, mean torque during eccentric exercise, negative work) on these damage markers. We hypothesized that the amount of GM fascicle lengthening during maximal single-joint eccentric contractions is correlated with the decrease in muscle force and the increase in perceived soreness.

Materials and methods

Subjects

Nine males and eight females (age: 25.0 ± 3.7 years, height: 173.8 ± 9.2 cm and weight: 70.5 ± 13.8 kg) with no history of ankle disorder or injury participated in this study. All volunteers were informed regarding the nature, aims and risks associ-

ated with the experimental procedure before they gave their written consent to participate. This study was approved by the ethics committee of Paris 8 and the French Health and Safety Agency and conformed to the standards of the Declaration of Helsinki.

Experimental design

Participants performed a maximal isokinetic eccentric session of plantar flexors, which were chosen as a suitable muscle group for the study of muscle fascicle behaviour using ultrasonography (Cronin & Lichtwark 2013). Plantar flexor torque, joint angle, angular velocity, electromyographic (EMG) activity of the *gastrocnemius lateralis* (GL), muscle fascicle length and pennation angle of GM were continuously recorded during the eccentric exercise (Fig. 1). Two days before (Pre), as well as 5 min (Post) and 48 h (48 h) after the eccentric exercise, participants performed test sessions including the measurement of maximal voluntary isometric contraction (MVC) torque of plantar flexors, GL EMG activity, perceived delayed onset muscular soreness (DOMS) and muscle shear modulus.

Data processing during test sessions

Experimental set-up. Participants were lying prone (hip and knee fully extended) on a Con-Trex® isokinetic dynamometer (CMV AG, Dübendorf, Switzerland) with the right ankle fixed to the dynamometer's accessory with non-compliant straps to prevent the rise of the heel during contractions (Fig. 1). All mechanical signals (i.e. angular position, torque, velocity) were digitized by a 12-bit analog-to-digital converter (DT9804®; Data Translation, Marlboro, MA, USA) at 5000 Hz.

Soreness. Participants reported their DOMS on a visual analog scale with an ungraduated line from 'no pain' (0 mm) to 'intolerable pain' (100 mm), after the muscle insertions and the muscle belly of the GM, GM and *soleus* were palpated by the investigator.

Strength. After a standardized warm-up, the highest peak isometric plantar flexion torque was evaluated from three trials of 3-s duration isometric maximal voluntary contractions (MVC) performed at 0° (foot

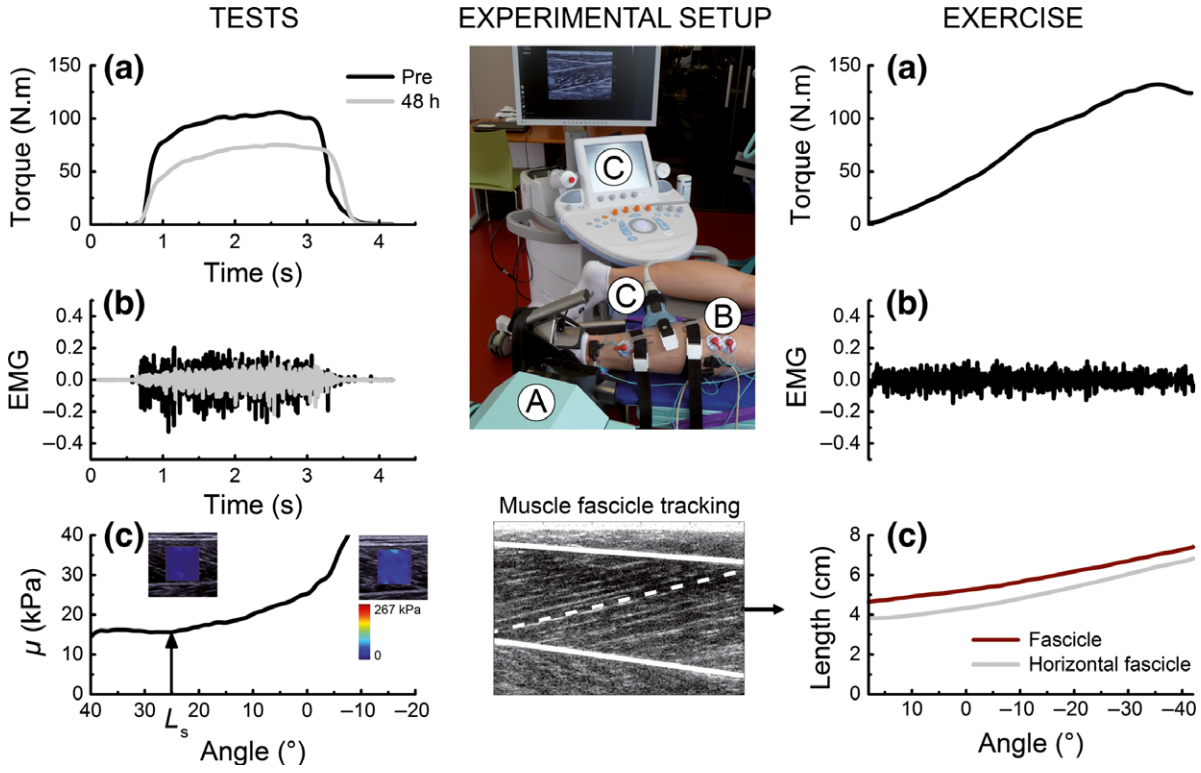

Figure 1 Experimental design. During tests sessions performed before (Pre), immediately (Post) and 48 h after eccentric exercise, plantar flexor torque (a) was measured during maximal isometric contractions. *Gastrocnemius medialis* slack length (L_s) was determined under EMG feedback from the relationship between the shear modulus (μ) and joint angle (c). During the eccentric exercise, plantar flexor torque (a), surface EMG activity of the GL (b) and the lengths of muscle fascicles (c) were continuously measured using ultrasonography. Each letter symbol corresponds to the site of measurements of the central picture (Experimental set-up) and the associated data plot on the left (Tests) and right (Exercise) panels.

perpendicular to the tibia) of ankle angle. The torque measured by the dynamometer was corrected with regard to inertia and the weight of the dynamometer attachment (i.e. without the foot being included) to obtain the external torque at the ankle joint. Mechanical signals were low-pass filtered (20-Hz second-order Butterworth filter). Participants were divided into two groups according to their force loss measured at 48 h (in % MVC at Pre), with a cut-off set at a −16% decrease in MVC torque. The group with low decrease in MVC torque (LOW) included eight participants (MVC torque decrease from 0 to −15.7%), while the group exhibiting high force decrements (HIGH) included nine participants (from −16.0% to −30.6%).

EMG. During MVC, surface EMG signals were recorded on GL using bipolar silver/silver chloride surface electrodes (interelectrode distance: 10 mm; Blue Sensor Q-00-S, Baltorpbakken, Denmark). Raw EMG signals were pre-amplified (Mazet Electronique Model®, Electronique du Mazet, Mazet Saint-Voy, France; impedance: 10 GΩ; CMRR: 100 dB; gain: 600; bandwidth: 6–500 Hz) and sampled through the same digital converter used for mechanical data.

Shear wave elastography. GM slack length (L_s) was determined using both B-mode ultrasound and shear wave elastography (Aixplorer scanner®, version 5.0; Supersonic Imagine, Aix-en-Provence, France, linear transducer array 4–15 MHz, Vermon, Tours, France) (Lacourpaille *et al.* 2012). Maps of GM muscle belly shear modulus were obtained at 1 Hz during passive ankle motion ($1°s^{-1}$) from 40° to −20° (after 5 cycles for conditioning). Online electromyographic (EMG) feedback of GL, *soleus* and *tibialis anterior* was provided to the examiner to ensure that there was no muscle activity during the cycles. The joint angle corresponding to the slack length (i.e. the muscle length beyond which the muscle begins to develop passive elastic force; Fig. 1c) was determined as the onset of shear modulus increase during the stretching (Lacourpaille *et al.* 2014). Additionally, the muscle shear modulus was measured at rest at 0° ankle angle to appraise the localized exercise-induced alterations in GM (Lacourpaille *et al.* 2014).

Eccentric exercise

Participants completed 10 sets of 30 maximal eccentric contractions of the plantar flexors (2-min rest between sets) at a constant angular velocity of $45°s^{-1}$ on the isokinetic dynamometer. Eccentric contractions of the plantar flexor muscles were set in eccentric mode, so as to be initiated once the participants started to exert a plantar flexor torque on the footplate. The return to the starting position was set in passive mode (no contraction) at $45°s^{-1}$. Before the exercise, three passive dorsiflexions were completed to determine the maximal dorsiflexed angle each participant was able to tolerate without pain. The starting position was set to perform eccentric contractions over a total range of motion of 60° ($18 \pm 5°$ in plantar flexion to $-42 \pm 5°$ in dorsiflexion on average).

Data processing during eccentric exercise

Mechanics. Mechanical signals were low-pass filtered as for the MVC test. Torque was normalized to the MVC torque measured at Pre for each participant and averaged from the angle corresponding to L_s to the angle corresponding to maximal dorsiflexion (torque beyond L_s).

EMG. EMG activity of GL was bandpass filtered (10- to 400-Hz, third-order Butterworth filter), consistently analysed with a 100-ms moving root mean square (RMS) window to produce a RMS envelope and normalized to the maximal EMG activity recorded during isometric MVC.

Ultrasound. The length of foot, shank and leg was measured to calculate the muscle–tendon unit (L_{MTU}) using the ankle angle signal and the anthropometric model proposed by Grieve *et al.* (1978). Ultrasonic raw data were acquired at 100 Hz on the GM muscle during 2 or 3 repetitions at the start, middle and end of each eccentric set. The probe was aligned vertically to the midline of the muscle so as to be in the same plane as the muscle fascicles, to obtain the longest possible fascicles and to minimize measurement error (Cronin & Lichtwark 2013). B-Mode images were then created by applying a conventional beam formation, that is, applying a time-delay operation to compensate for the travel time differences. The displacements of fascicles and aponeuroses were automatically tracked during each recorded contraction using the method proposed by Cronin *et al.* (2011) (Matlab®; The Mathworks, Nathick, MA, USA; Fig. 1c). When the fascicle was not fully visible, its length (L_F) was interpolated as the length of the straight line between the superficial and deep aponeurosis (Hauraix *et al.* 2013, Farcy *et al.* 2014). The angle between the fascicle and the deep aponeurosis corresponded to the pennation angle. The horizontal projection of the fascicle length (L_{FH}) was calculated as the L_F multiplied by the cosine of the pennation angle. Tendinous tissues length (L_{TT}) was considered

as the difference between L_{MTU} and L_{FH}. The lengthening velocity of each component corresponded to the first time derivatives of L_{MTU}, L_F, L_{FH} and L_{TT} respectively. The AGR was determined over each contraction from these parameters, based on the method proposed by Brainerd & Azizi (2005):

$$AGR = \frac{\Delta L_{FH}}{\Delta L_F} \quad (1)$$

The joint angle corresponding to the slack length previously measured was used to determine the slack length values of the different components. For that, we used the B-mode image corresponding to this specific joint angle obtained during the passive return of the foot during the eccentric exercise and the previously described tracking method. Then, L_{MTU}, L_F and L_{TT} changes were normalized to their respective slack lengths. Maximal lengthening and maximal lengthening velocity calculated from L_{MTU}, L_F, L_{FH} and L_{TT} were calculated. The product of maximal lengthening (normalized to L_s) and torque (normalized to MVC torque) was also calculated as an index of the mechanical work dissipated by muscle fascicles (Brooks *et al.* 1995, Azizi & Roberts 2014).

All the data continuously recorded during contractions were extracted and analysed using custom-written scripts designed using Origin 9.1 software (Origin®; OriginLab Corporation, Northampton, MA, USA) over the period of time corresponding to the eccentric contractions of the plantar flexor muscles, that is, from the starting (18° plantar flexion) to the ending (−42° dorsiflexion) ankle position. Plantar flexor muscles were relaxed during the return to the starting position (passive mode), and this return phase was therefore not considered in the data analysis. A linear interpolation technique was used to construct the curve as a function of joint angle every 1° (i.e. 61 regularly spaced angles) for the 300 repetitions. This relationship was averaged to obtain a value for the overall exercise, for the first and the tenth set.

Statistical analysis

Analyses were performed with Statistica® (StatSoft, Tulsa, OK, USA). The normality of the data was tested using a Shapiro–Wilk's test. All data being normally distributed, separate one-way (time effect) analyses of variance (ANOVAs) with repeated measures were applied to determine the effect of eccentric exercise on MVC torque, DOMS and GM shear modulus values. Two-way ANOVAs (set × angle) with repeated measures were applied to determine the changes in torque, GL EMG activity and GM fascicle length throughout the eccentric exercise. Two-way ANOVAs (component × angle) with repeated measures were applied to determine the differences in the raw length changes and lengthening velocity between muscle–tendon unit, fascicle, horizontal fascicle and tendinous tissues during eccentric contractions. When the sphericity assumption in repeated-measures ANOVAs was violated (Mauchly's test), a Geisser–Greenhouse correction was used. Post hoc tests were performed by means of Newman–Keuls procedures for comparison between time points. An unpaired t-test was first performed on each of the muscle–tendon unit behaviour parameters measured during the exercise to test the differences between the two groups of subjects distributed according to the decrease in MVC torque (LOW and HIGH). Then, linear correlations (Pearson r) were performed between decrease in MVC torque, DOMS at 48 h (post-measurement being potentially affected by muscle fatigue) and all the muscle–tendon unit behaviour parameters measured during the exercise, AGR, mean torque and mean torque produced beyond L_s during the exercise. For all tests, the significance level was set at $P < 0.05$. The data are presented as mean ± SD.

Results

Exercise-induced muscle damage

MVC torque significantly decreased at Post (−43.3 ± 15.2%; $P < 0.001$) and at 48 h (−15.6 ± 8.3%; $P = 0.03$). While DOMS did not change at Post ($P = 0.3$), it significantly increased to 44 ± 28 mm at 48 h ($P < 0.001$; Table 1). ANOVA showed a time effect on GM shear modulus values ($P = 0.03$), with a significant 28 ± 49% increase at Post ($P = 0.03$) and no persistent difference at 48 h ($P = 0.95$).

Changes in muscle–tendon mechanics throughout eccentric exercise

During the eccentric exercise, average torque increased from 11.8 ± 5.3 N.m at the starting position to 99.8 ± 35.3 N.m at −33 ± 8.5° ($P < 0.0001$) and remained elevated (89.7 ± 43.8 N.m) at maximal dorsiflexion. Average torque decreased by 31 ± 18% from set 1 to set 10 as reflected by a set effect ($P < 0.0001$). We observed a set × angle interaction ($P < 0.0001$), showing that peak torque occurred to a more extended position, and decreased from 89.4 ± 2.9% to 65.5 ± 2.9% of MVC torque from set 1 to set 10 (Fig. 2a).

EMG activity was influenced by ankle angle ($P = 0.009$), with a progressive decrease from 16° in plantar flexion to −42° in dorsiflexion (0.65 ± 0.21 to 0.29 ± 0.22; $P < 0.0001$; Fig. 2d). Inversely to torque, a lack of set effect ($P = 0.74$) showed that GL

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Table 1 Effect of eccentric exercise on DOMS, MVC torque and muscle shear modulus 48 h post-exercise

Parameter	Pre	Post	48 h
DOMS (mm)	3 ± 4	9 ± 16	44 ± 28*
MVC torque (N.m)	145.4 ± 35.0	80.7 ± 29.7*	123.4 ± 35.9*
Gastrocnemius medialis shear modulus (kPa)	27.9 ± 9.9	35.2 ± 16.0*	27.1 ± 5.0

*Significant difference with Pre ($P < 0.05$).

EMG activity remained stable throughout the eccentric exercise.

The muscle–tendon unit was stretched from 40.5 ± 3.8 cm to 45.0 ± 4.1 cm. Both GM fascicles and tendinous tissues length significantly increased during the eccentric contraction (angle effect, $P < 0.0001$), with a maximal lengthening of 2.31 ± 0.64 cm (55.1 ± 15.5% L_s) and 1.99 ± 0.61 cm respectively ($P < 0.0001$; Fig. 2). The two-way ANOVA (set × angle) showed no main effect of set on muscle–tendon unit, muscle fascicle and tendinous tissue length ($P > 0.05$). However, eccentric exercise significantly altered the relationship between torque and fascicle length, with a shift of the fascicle length where peak torque is produced from 3.85 ± 0.75 cm in set 1 to 4.72 ± 0.88 cm in set 10 ($P = 0.004$; Fig. 2b).

The contribution of tendinous tissues to the muscle–tendon lengthening was significantly higher than fascicles from 18° to −20°, while the opposite was found from −36° to −42° (Fig. 2e; $P < 0.05$). Muscle fascicles and tendinous tissues were lengthened at an average velocity of 1.64 ± 0.47 cm s^{-1} (0.41 ± 0.12 L_s s^{-1}) and 1.44 ± 0.49 cm s^{-1}, with a peak velocity of 2.29 ± 0.74 cm s^{-1} (1.00 ± 0.30 L_s s^{-1}) and 4.26 ± 1.43 cm s^{-1} respectively (Fig. 2f). ANOVAs demonstrated a component × angle interaction for the lengthening velocity of the different components of the muscle–tendon unit ($P < 0.0001$). Tendinous tissues were stretched faster than muscle fascicles in the horizontal direction at the beginning of the contraction (from 18° to 9°), while muscle fascicles were stretched faster in the horizontal direction than tendinous tissues in the last part of the contraction (from −21° to −42°; $P < 0.05$; Fig. 2f). GM muscle operated with an average AGR of 1.09 ± 0.04.

Relationship between muscle mechanics during eccentric exercise and muscle damage

During eccentric exercise, we did not find any differences in the parameters of MTU behaviour between LOW and HIGH groups ($P = 0.21$–0.96). When compared to the LOW group, the HIGH group showed higher maximal fascicle stretch length (1.24 ± 0.14 vs. 1.47 ± 0.21 L_s, respectively; $P = 0.02$), higher fascicle lengthening amplitude expressed as raw (1.96 ± 0.58 vs. 2.63 ± 0.57 cm, respectively; $P = 0.02$) and normalized data (0.47 ± 0.15 vs. 0.63 ± 0.12 L_s, respectively; $P = 0.02$), lower maximal length of tendinous tissues (1.08 ± 0.04 vs. 1.05 ± 0.01 L_s, respectively; $P = 0.04$) and lower maximal lengthening of tendinous tissues (0.06 ± 0.02 vs. 0.04 ± 0.01 L_s, respectively; $P = 0.02$).

When considering the whole sample, no significant correlation was observed between muscle–tendon unit or tendinous tissues behaviour parameters during eccentric contractions and the decrease in MVC torque at 48 h (Table 2). Inversely, normalized values (/L_s) of maximal fascicle stretch length and lengthening amplitude were significantly correlated with the decrease in MVC torque ($r = 0.51$ and 0.47, respectively; Fig. 3a and Table 2). Maximal fascicle length was also correlated with DOMS at 48 h ($r = 0.59$; $P = 0.01$; Fig. 3b), with a persistent trend when maximal fascicle length was normalized to L_s ($P = 0.06$). Moreover, the mean normalized torque (/MVC) produced multiplied by fascicle lengthening beyond L_s (i.e. fascicle braking work; (Azizi & Roberts 2014)) was strongly related to MVC torque decrease at 48 h ($r = 0.68$; Fig. 3c). In addition, we observed a trend of the mean fascicle lengthening velocity and maximal tendinous tissues lengthening during eccentric exercise being correlated with the decrease in MVC torque at 48 h (Table 2).

Discussion

The present study described the muscle–tendon behaviour during a single-joint and maximal eccentric exercise that induces muscle damage in plantar flexors. In accordance with our hypothesis, the maximal lengthening amplitude was correlated with the decrease in MVC torque, while maximal fascicle stretch length was correlated with DOMS 48 h after the eccentric exercise. Furthermore, negative work performed by the muscle fascicle was even more related to the MVC torque decrease at 48 h. These findings strongly suggest that the magnitude of the stretch applied to active muscle fascicles combined with high levels of force produced at extreme lengths

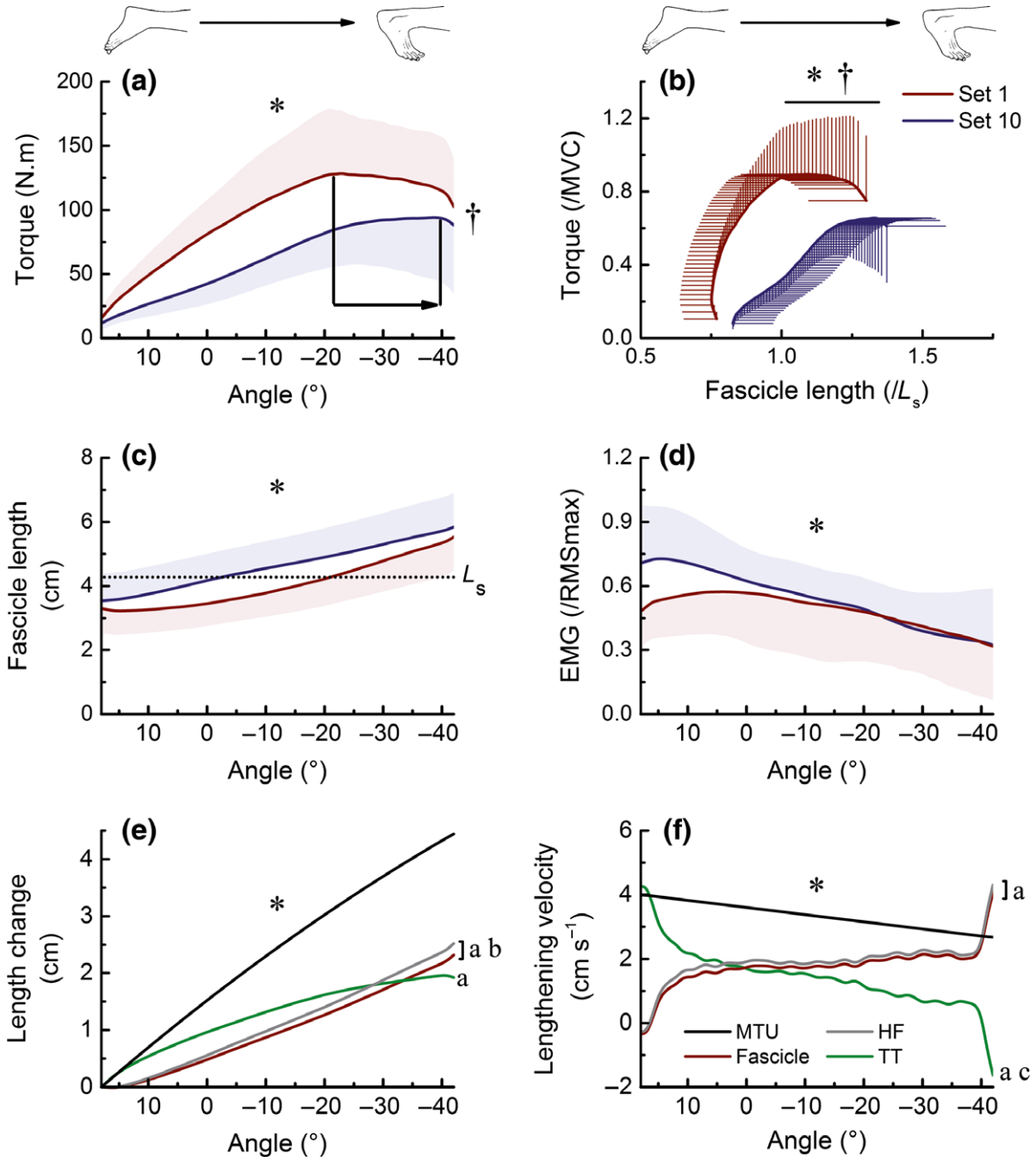

Figure 2 Muscle–tendon unit behaviour throughout eccentric contractions. Mean ± SD torque (a), muscle fascicle length (c) and muscle EMG activity (d) during the set 1 (in red) and set 10 (in blue) of eccentric contractions of the plantar flexors. Panel b displays the average curves (mean ± SD for x- and y-axis) of the relationship between torque and muscle fascicle length. The solid line corresponds to peak torque angle and the dashed line corresponds to the slack length (L_s). Muscle–tendon unit (MTU), muscle fascicles, horizontal fascicles (FH) and tendinous tissues (TT) length changes (e) and lengthening velocity (f) patterns are displayed as mean for clarity purposes. *, significant effect of joint angle. †, significant difference between set 1 and 10. a, significant difference with muscle–tendon unit. b, significant difference with tendinous tissues. c, significant difference with muscle fascicles. ($P < 0.05$).

during maximal eccentric contractions influences the extent of subsequent functional impairments induced by muscle damage.

Exercise-induced muscle damage. A significant relation has been demonstrated between the exercise-induced myofibrillar disruptions and the subsequent

Table 2 Correlations between parameters obtained during eccentric exercise and MVC torque decrease 48 h post-exercise

	Raw		Normalized	
Parameter	Mean ± SD	Pearson' r	Mean ± SD	Pearson' r
Functional parameters				
DOMS	4.4 ± 2.8	0.28	–	–
Mean torque (N.m; %MVC)	69.2 ± 18.3	0.02	0.49 ± 0.10	0.06
Max torque (N.m; %MVC)	107.9 ± 37.0	0.05	0.75 ± 0.20	0.03
Torque beyond L_s (N.m; %MVC)	88.6 ± 28.4	0.04	0.63 ± 0.20	0.01
Muscle–tendon unit mechanical properties				
Shear modulus at 0° (kPa)	18.8 ± 34.0	−0.27		
Maximal tendon lengthening (cm; L_s)	2.06 ± 0.49	−0.20	0.06 ± 0.02	0.14
Muscle–tendon unit behaviour during eccentric contractions				
Maximal length (cm; L_s)	45.0 ± 4.1	−0.32	1.10 ± 0.02	−0.30
Maximal lengthening (cm; L_s)	4.44 ± 0.33	−0.37	0.11 ± 0.01	−0.40
Mean velocity (cm s^{-1}; L_s s^{-1})	3.33 ± 0.25	−0.32	0.08 ± 0.00	−0.26
Maximal velocity (cm s^{-1}; L_s s^{-1})	3.99 ± 0.29	−0.04	0.10 ± 0.00	−0.09
Muscle fascicles behaviour during eccentric contractions				
Maximal length (cm; L_s)	5.80 ± 1.06	−0.32	1.36 ± 0.21	−0.51*
Maximal lengthening (cm; L_s)	2.32 ± 0.65	−0.37	0.55 ± 0.16	−0.47*
Mean velocity (cm s^{-1}; L_s s^{-1})	1.74 ± 0.49	−0.37	0.41 ± 0.12	−0.43†
Maximal velocity (cm s^{-1}; L_s s^{-1})	4.18 ± 1.18	−0.30	1.00 ± 0.30	−0.23
Gear ratio (%)	1.09 ± 0.04	−0.08	–	–
Tendinous tissues behaviour during eccentric contractions				
Maximal length (cm; L_s)	39.63 ± 3.98	−0.01	1.04 ± 0.03	0.31
Maximal lengthening (L_s; cm)	1.92 ± 0.64	0.39	0.05 ± 0.02	0.42†
Mean velocity (cm s^{-1}; L_s s^{-1})	38.91 ± 3.87	−0.05	1.02 ± 0.02	0.19
Maximal velocity (cm s^{-1}; L_s s^{-1})	39.63 ± 3.98	−0.01	1.04 ± 0.03	0.31

*Significant correlations ($P < 0.05$).
†Correlation trend ($P < 0.10$).

reduction in force-generating capacity (Raastad *et al.* 2010). Therefore, the present 15.6% decrease in peak isometric torque, together with the significant level of DOMS (44 mm), observed 48 h post-exercise can be considered as representative markers of the functional consequences of muscle damage (Paulsen *et al.* 2012). Furthermore, the shifts in the torque–angle and torque–fascicle length relationships to longer muscle lengths (+11°, Fig. 2a–b) are also classically identified as a symptom of muscle damage (Nosaka & Sakamoto 2001, Penailillo *et al.* 2015). One could notice that the occurrence of fatigue during the protocol may have induced a lower activation level and hence contributed to the shift in the torque–angle relationship observed in the last set (Butterfield & Herzog 2005). In this context, it is important to observe that the pattern of GL EMG activity during the contraction did not exhibit any breakdown suggesting that plantar flexor muscles were consistently activated over the entire range of motion and throughout the eccentric exercise protocol (i.e. from the set 1 to set 10, Fig. 2d). The application of the twitch interpolation technique would be necessary to be certain that voluntary drive was maximal during the exercise. At this stage, we cannot fully exclude the coexistence of damage and fatigue throughout the present eccentric exercise. At least, the present shifts could indirectly reflect an increase in muscle compliance due to progressive overextension and disruption of some sarcomeres induced by repetitive eccentric contractions (Proske & Morgan 2001). Because strength loss observed in the early moments after the eccentric exercise could also potentially include metabolic muscle fatigue unrelated to muscle damage, MVC torque was measured at 48 h (Prasartwuth *et al.* 2005). However, the damage-related shift in optimum length for active tension can affect the change in force production. The exploration of the electro-stimulated length–tension curve could contribute to confirm the impact of muscle fascicle lengthening on muscle damage extent characterized *in vivo* (Hoffman *et al.* 2014).

In the present study, GM muscle was considered as a surrogate of the behaviour of the plantar flexor muscles (Hauraix *et al.* 2013). Although there is currently no method validated to quantify the amount of damage in each muscle of a group, a recent study suggests that early increases in muscle shear modulus after exercise could reflect the perturbation of calcium

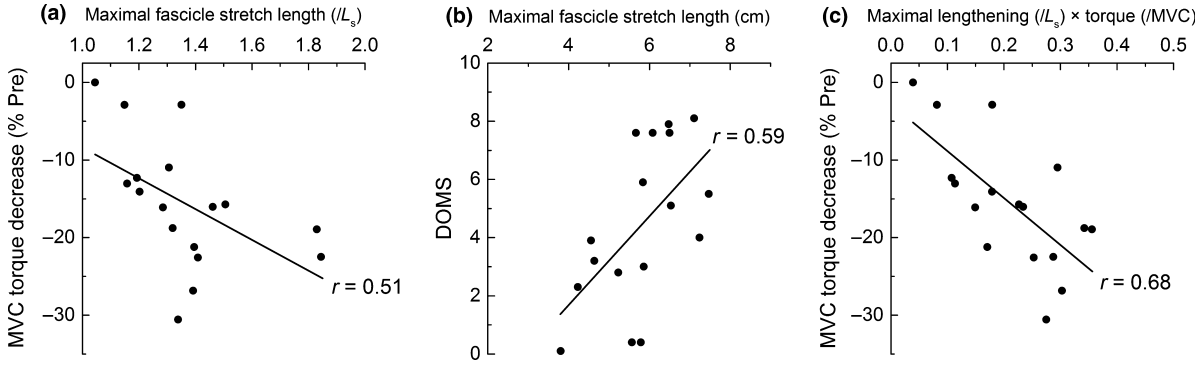

Figure 3 Correlations between muscle fascicles behaviour and muscle damage. Significant correlations were found (a) between maximal muscle fascicle stretch length (/L_s) and MVC torque decrease at 48 h (% Pre), (b) between maximal muscle fascicle stretch length (cm) and delayed onset muscle soreness (DOMS) and (c) between maximal fascicle lengthening (/L_s) multiplied by external torque (/MVC) and MVC torque decrease (% Pre).

homeostasis induced by cytoskeletal alterations (Lacourpaille *et al.* 2014). In addition, recent pilot analyses led by our group tend to show that this increase in shear modulus is strongly related to the MVC torque decrease 48 h post-exercise. Although the direct relation between the increase in shear modulus and muscle damage remains to be clearly established, the 28% increase in the GM shear modulus reported in the present study immediately after the eccentric exercise suggests that this muscle was noticeably damaged by our eccentric protocol.

Muscle–tendon unit behaviour during eccentric contractions. In line with previous data obtained during eccentric contractions of the plantar flexors (Wakahara *et al.* 2009), our results demonstrate that the fascicles contribute to more than half of muscle–tendon unit elongation during maximal single-joint eccentric contractions. The contribution of tendinous tissues to maximal muscle–tendon unit lengthening (43%) was also similar to those reported at a various range of eccentric velocities (45–50%) (Chino *et al.* 2008). Interestingly, Chino *et al.* (2008) showed a high interindividual variability in tendinous tissue behaviour, thus strengthening the relevance of further exploration of muscle–tendon unit behaviour during eccentric contractions.

According to the torque–length relationship in eccentric condition (Fig. 2b), the large amplitude towards dorsiflexion fixed in the present study resulted in a high muscle–tendon loading, as reflected by the important lengthening amplitude of tendinous tissues (i.e. 1.92 cm). However, after being stretched early during the contraction, tendinous tissues then remained at a quasi-constant length during the late part of the contraction (Fig. 2e). The additional lengthening of muscle–tendon unit in this last part is thus mainly supported by a large active stretch of muscle fascicles up to 2.31 cm. Given that force is not increasing at the end of the contraction (Fig. 2a), the tendon is not longer stretched and thus fascicles have to withstand the lengthening.

The present single-joint eccentric task stretched GM fascicles (up to 2.3 cm) on average to a greater extent than backward downhill walking, which causes a fascicle stretch of ~1.1 cm (Hoffman *et al.* 2014). In this previous study, the fascicle stretch induced a 23% decrease in electro-induced maximal isometric torque 2 h after exercise; however, the potential impact of fatigue due to prolonged exercise may have affected the force measured at 2 h and no data were provided regarding the strength loss at 48 h post-exercise (i.e. the most common index to identify EIMD) (Friden & Lieber 2001, Clarkson & Hubal 2002, Paulsen *et al.* 2012). Moreover, backward walking probably induced a fascicle lengthening mainly over the ascending limb of the force–length curve (Hoffman *et al.* 2014). The sarcomere non-uniformity hypothesis states that unstable sarcomeres rapidly elongate during active stretch at long muscle lengths, resulting in sarcomere disruption and damage predominantly on the descending limb of the muscle's length–tension curve (Morgan 1990). Nonetheless, in his review, Butterfield (2010) points out that this so-called popping sarcomere hypothesis was established on isolated fibres, raising the persistent necessity to further investigate how muscle fibres behave on the descending limb *in vivo*.

The present protocol was therefore designed to elicit maximal eccentric contractions (i.e. similar to strength training workout) and to stretch the fascicles over the descending limb of the length–tension curve (Fig. 2b) in physiological joint amplitudes. The amount of fascicle lengthening induced by eccentric contractions (1.36 L_s on average) was therefore higher than values reported for submaximal eccentric exercises (1.18 optimal length during backward walking in

Hoffman *et al.* 2014). While remaining realistic and within the physiological ranges on average, the maximal fascicle length reached 1.8 L_s for two individuals in the present study. These high values could originate from the normalization by the slack length, which is classically shorter than the optimal fascicle length (i.e. L_0, Hoffman *et al.* 2014). Alternatively, it must be acknowledged that such result seems to be due to particularly short slack length values for these individuals and then could reflect the specificity or potential limitations of the slack length measurement methodology. Our results showed the presence of significant exercise-induced muscle damage when muscle fascicles operate at long muscle lengths *in vivo*. On a different joint, Penailillo *et al.* (2015) observed that the *vastus lateralis* fascicles are lengthened by 8.0 cm on average during submaximal eccentric cycling (at 192 W) that induced a non-significant decline (−6.4%) in maximal voluntary isometric torque 2 days after. However, compared to the present eccentric bout performed maximally, the submaximal cycling intensity generated lower muscle activity levels and a lower resistance of muscle fascicles to active stretch. This can explain the high fascicle lengthening associated with a lack of significant muscle force impairment in their study.

Relationship between muscle–tendon unit behaviour and indexes of subsequent muscle damage. Our findings show a significant interindividual variability in both muscle–tendon unit and muscle fascicle behaviours during a standardized single-joint task, as it has been reported during the eccentric phase of stretch-shortening cycle tasks (Fukunaga *et al.* 2001, Ishikawa *et al.* 2007, Lichtwark *et al.* 2007) or during pure concentric maximal contractions (Hauraix *et al.* 2013). Maximal length and maximal lengthening amplitude of muscle–tendon unit induced by the present eccentric exercise were not significantly correlated with the maximal isometric torque decrease 48 h after exercise. However, in accordance with our hypothesis, the maximal length and the maximal lengthening amplitude of fascicle (normalized to the slack length) were (i) significantly higher for the participants that exhibit the largest decrease in MVC torque at 48 h and (ii) significantly related to the decrease in maximal isometric torque 48 h after the eccentric protocol (respectively, $r = 0.51$ and 0.44; Fig. 3a, Table 2). In addition, a significant correlation was observed between maximal fascicle length reached during eccentric contractions and DOMS evaluated 48 h after exercise ($r = 0.59$; Fig. 3b). Taken together, these results demonstrate for the first time *in vivo* that functional impairments due to exercise-induced muscle damage are linked to the lengthening amplitude applied to the muscle fascicles, rather than the global muscle–tendon unit length changes.

Importantly, the significant correlations previously mentioned were found only for values of maximal fascicle lengthening expressed as a function of the slack length. Indeed, it means that different amounts of muscle damage can occur in response to the same absolute fascicle length changes due to differences in individual fascicle properties. Therefore, the most important factor seems to be the amplitude of the relative stretch depending on its length at rest and especially the stretch induced beyond the fascicle slack length. This finding emphasizes that muscle injury may result from a strain-induced lengthening which may exceed the mechanical limits of the tissue (Proske & Morgan 2001, Butterfield 2010).

In addition, some authors have suggested that torque levels produced during eccentric contractions could also contribute to the magnitude of muscle damage in elbow flexor muscles (Nosaka & Sakamoto 2001). In the present study, despite the significant interindividual variability in the torque produced during the standardized maximal eccentric protocol, no direct correlation was observed between the MVC torque decrease at 48 h and the relative torque produced beyond the slack length (Table 2). Considering that both fascicle lengthening and the torque level could concomitantly influence the amount of muscle damage, the best correlation with the torque decrease at 48 h was obtained for the average torque produced beyond the slack length multiplied by the maximal fascicle lengthening ($r = 0.68$; Fig. 3c). This result provides a tangible evidence that the mechanical work dissipated by the sarcomere during the eccentric contraction can be considered as a relevant predictor of muscle damage or at least of its negative consequence on muscle function (Brooks *et al.* 1995).

Protective factors. The present study demonstrates the crucial role of the interactions between contractile and tendinous tissues in exercise-induced muscle damage and highlights both the fundamental influence of individual muscle–tendon elastic properties and architectural features due to pennation. Among the processes that can modulate the impact of mechanical strain on muscle fibres, the AGR operating in pennate muscles such as the GM has been suggested to reduce the stretch applied to muscle fibres during eccentric contractions (Brainerd & Azizi 2005, Azizi & Roberts 2014). Our findings confirm the greater length changes in muscle's line of action than in fascicle's (Fig. 2e), with an actual AGR of 1.09, similar to previous values obtained during eccentric contractions (on average ~1.0 to 1.2 in Shin *et al.* (2009)). However, correlation analyses did not reveal a significant link between the actual AGR

and subsequent muscle damage magnitude. This process thus appears to be a limited interindividual determinant of exercise-induced muscle damage. Nonetheless, it should be kept in mind that dynamic tasks involve both longitudinal and transverse deformation of aponeuroses and 3D rotation of muscle fascicles during contraction (Cronin & Lichtwark 2013, Herbert *et al.* 2015). To date, no 3D methods have currently been validated to measure fascicle motion during contraction. Therefore, these processes may have slightly influenced the results of the present study, and this should be explored in the future.

When visually inspecting the changes in fascicle length throughout the maximal eccentric contractions (Fig. 2e), it appeared that muscle fascicles were lengthened more rapidly at the end of the movement. Therefore, our results tend to show that for compliant tendon, muscle fascicles contract at shorter lengths at the beginning of the contraction and hence are actively lengthened to a lesser extent, the muscle–tendon unit lengthening being mainly taken by the tendinous tissues. At the end of the contraction, while torque levels were high, tendon became stiffer and most of the muscle–tendon lengthening took place in the muscle fascicles, which were still active as reflected by the EMG activity detected at the end of the contraction (Fig. 2d). These findings support three main conclusions: (i) tendinous tissues can act as a mechanical buffer to attenuate the subsequent stretch applied to the muscle fibres during eccentric contractions and hence potentially the amount of EIMD, (ii) muscle–tendon interactions are strongly influenced by the individual mechanical properties of the subject as well as the type and intensity of eccentric task itself (Hicks *et al.* 2013), and (iii) the last part of eccentric contractions (i.e. at long muscle–tendon lengths with high loads) likely explains most of the muscle damage reported in the present study. One could notice that the present study did not manipulate muscle–tendon properties to evaluate their influence on muscle damage. Further investigations including separate groups of individuals exhibiting clear different ranges of tendon stiffness and AGR values could more directly investigate this hypothesis.

To summarize, the results of the present study emphasize the crucial influence of the muscle fibre stretch amplitude combined with high levels of force produced at extreme lengths on the amplitude of changes in damage indexes (i.e. force loss, soreness) subsequent to eccentric contractions in humans. They also highlight the roles of muscle–tendon interactions and the pennation angle dynamics in the modulation of the stress directly applied to the muscle fibres *in vivo*. Thus, further studies are needed to explore the impact of various types of more complex task that impose different mechanical stress (e.g. sprint running, counter-movement jump, drop jump) on muscle damage extent. Considering the potential influence of morphological and architectural characteristics of muscle fibres (pennation angle, fibre length, fibre type proportion), the present results should also be confirmed on muscles more prone to muscle damage than GM (Jamurtas *et al.* 2005, Hedayatpour & Falla 2012). Such research would help to fully understand the influence of muscle–tendon interactions and their intrinsic properties in the muscle response on extreme lengthening and subsequent eccentric-induced damage.

Conflict of interest

No conflict of interest, financial or otherwise, is declared by the authors.

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