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Time-course effect of exercise-induced muscle damage on localized muscle mechanical properties assessed using elastography

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Abstract

Aim: Changes in muscle stiffness after exercise-induced muscle damage have been classically inferred from passive torque–angle curves. Elastographic techniques can be used to estimate the shear modulus of a localized muscular area. This study aimed to quantify the changes in shear elastic modulus in different regions of the elbow flexors after eccentric exercise and their relation to muscle length.

Methods: Shear elastic modulus and transverse relaxation time (T_2) were measured in the biceps brachii and brachialis muscles of sixteen participants, before, 1 h, 48 h and 21 days after three sets of ten maximal isokinetic eccentric contractions performed at 120° s^{-1} .

Results: The shear elastic modulus of the elbow flexors significantly increased 1 h (+46%; $P = 0.005$), with no significant change at 48 h and 21D, post-exercise. In contrast, T_2 was not modified at 1 h but significantly increased at 48 h (+15%; $P < 0.05$). The increase in shear elastic modulus was more pronounced at long muscle lengths and reached a similar extent in the different regions of the elbow flexors. The normalized hysteresis area of shear elastic modulus–length relationship for the biceps brachii increased 1 h post-exercise (31%) in comparison with the pre-exercise value (18%), but was not significantly altered after five stretching cycles ($P = 0.63$).

Conclusion: Our results show homogeneous changes in muscle shear elastic modulus within and between elbow flexors. The greater increase in shear elastic modulus observed at long muscle lengths suggests the putative involvement of both cross-bridges number and titin in the modifications of muscle shear elastic modulus after damaging exercise.

Keywords eccentric contractions, hysteresis area, oedema, passive tension, supersonic shear imaging.

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Eccentric muscle contraction imposes high mechanical strain on the muscle-tendon system leading to muscle damage (Clarkson & Hubal 2002, Guilhem *et al.* 2010). These cytoskeletal alterations typically result in impairment of neuromuscular function (Guilhem *et al.* 2013, Heroux & Gandevia 2013, Semmler *et al.*

2013) including a decrease in maximal strength and range of motion, combined with an increase in muscle stiffness and perceived soreness in the days following exercise (Howell *et al.* 1993, Friden & Lieber 2001). Passive torque is increased close to its peak value in the early moments after exercise, remaining elevated

for the next 4 days and restoring its initial value 1 week after exercise (Howell *et al.* 1993, Chleboun *et al.* 1998, Proske & Morgan 2001, Whitehead *et al.* 2001). However, because the passive joint torque results from a composite of various muscular (i.e. agonists and antagonists) and non-muscular structures, such as tendon, skin, articular structures and ligaments (Riemann *et al.* 2001, Herbert *et al.* 2011), this global measure does not provide a direct estimation of mechanical properties of individual muscles.

Elastographic techniques can be used to assess shear elastic modulus (i.e. elasticity) of a localized area of muscle at rest (Gennisson *et al.* 2010, Shinohara *et al.* 2010, Debernard *et al.* 2011), during isometric contraction (Dresner *et al.* 2001, Nordez & Hug 2010, Shinohara *et al.* 2010) and passive stretching (Maïsetti *et al.* 2012, Koo *et al.* 2013). Using magnetic resonance elastography, Green *et al.* (2012) reported an increase in the shear storage modulus for both *gastrocnemius medialis* and *soleus* after an eccentric exercise. Interestingly, the magnitude and time course of increase in the shear storage modulus differed between the muscles with a peak at 48 h (+21%) and 1 h (+9%) for *gastrocnemius medialis* and *soleus* respectively (Green *et al.* 2012). Three hypotheses could explain this result. First, as suggested by the authors, the protocol used (i.e. backward downward walking) and muscles anatomical features (i.e. mono- and bi-articular muscles) implied a greater stretching of *gastrocnemius medialis* than the *soleus*. Second, despite similar recruitment during the eccentric exercise, the activated muscles can be affected differently by lengthening contractions due to their specific architecture (i.e. fascicle length and pennation angle) and fibre typology (Kulig *et al.* 2001). Indeed, compared with *soleus*, *gastrocnemius* is composed by a higher percentage of type II muscle fibres which are more susceptible to damage (Friden & Lieber 1998, Takekura *et al.* 2001). Finally, muscles can be heterogeneously stretched during high-tension eccentric contractions, leading to spatial variability in muscle damage (Sbriccoli *et al.* 2001, Takekura *et al.* 2001). For example, *gastrocnemius* muscle injuries preferentially affect the myotendinous junction and the medial head (Weishaupt *et al.* 2001). In addition, the greatest tenderness and muscle swelling have been reported to occur within the distal region of the *biceps brachii* muscle after eccentric exercise (Cleak & Eston 1992). Taken these elements in mind, it is possible that exercise-induced muscle damage is not homogeneously distributed within and between agonist muscles leading to a spatial variability of the changes in muscle mechanical properties.

The rise in passive tension subsequent to muscle damage has been proposed to be due to the release of

Ca²⁺ caused by membrane damage accompanying sarcomere disruption during eccentric exercise (Morgan 1990), which triggers low-level contraction of the muscle (Morgan & Allen 1999). As muscle fibres sensitivity to Ca²⁺ increases as muscle is elongated (Stephenson & Wendt 1984), passive torque has been shown to be maximal at long muscle-tendon lengths (Whitehead *et al.* 2001, Hoang *et al.* 2007). Consequently, it is expected that changes in shear elastic modulus might differ according to muscle length.

This study was designed to quantify the time-course changes in muscle shear elastic modulus subsequent to exercise-induced muscle damage. Muscle shear elastic modulus was measured using shear wave elastography (Supersonic Shear Imaging; Lacourpaille *et al.* 2012) from different locations of two elbow flexors, *biceps brachii* (BB) and *brachialis* (BA), which have different anatomical properties (i.e. bi- vs. mono-articular muscle) and are known to be greatly affected by eccentric exercise (Jamurtas *et al.* 2005). These measures were performed at different locations and elbow joint angles to test any potential site and length dependencies of changes in shear elastic modulus. Oedema was assessed by measuring the transverse relaxation time (T_2) using magnetic resonance imaging (MRI). We hypothesized that (1) muscle damage would induce a significant increase in muscle shear elastic modulus that would be more pronounced at long muscle lengths; (2) this increase would occur in both BB and BA before the occurrence of oedema; and (3) BB and BA would be affected differently by the eccentric exercise, leading to different changes in muscle shear elastic modulus.

Methods

Participants

Sixteen healthy participants (11 males and five females; age: 23.6 ± 3.2 years; height: 1.8 ± 0.1 m; weight: 68.5 ± 8.5 kg) participated in this study. They were informed regarding the nature, aims and risks associated with the experimental procedures before their written consent was given. The study was conducted in accordance with Good Publishing Practice in Physiology (Persson & Henriksson 2011) and the Declaration of Helsinki (Schuklenk 2001).

Experimental design

The study was spread over 21 days. On the first day, participants performed an initial test session (PRE). An eccentric exercise was performed the following day. The same test session as PRE was repeated 1 h, 48 h and 21 days after the eccentric exercise (1H, 48H, and 21D respectively) at the same time of day.

Eccentric exercise

Participants were seated on a Con-Trex MJ isokinetic dynamometer (CMV AG, Dübendorf, Switzerland) so that the hip was flexed at 85° (0° = full hip extension). They completed three sets of ten maximal eccentric contractions of the elbow flexor muscles of the dominant arm (the one used to throw a ball) at 120° s⁻¹. The eccentric contractions were executed from a 60° to a 175° elbow angle (180° = full extension) with a shoulder abduction angle of 0° (i.e. arm along the body). Between each contraction, the arm was passively repositioned at 60° (at 30° s⁻¹). A passive recovery period of 1 min 30 s was carried out between each set to avoid short-term fatigue (Semmler *et al.* 2013). Participants were vigorously encouraged to produce the greatest torque during each contraction.

Test sessions (PRE, 1H, 48H, 21D)

All measurements were taken in the same order at each test session. During test sessions [except for delayed onset muscular soreness (DOMS) evaluation], participants were seated on the ergometer as in the eccentric exercise session. The arm was firmly strapped to the attachment dedicated to the elbow joint and positioned in the horizontal plane with the shoulder abducted at 90° (Lacourpaille *et al.* 2013), to suppress any effect of gravity on torque measurements.

Muscle soreness. Delayed onset muscular soreness was evaluated using an illustrated visual analogue scale numbered from 0 ('my muscles do not feel sore at all') to 10 ('my muscles feel so sore that I do not want to move them'), with a sliding pointer. Participants stood in an upright position, flexed their forearm slowly to a 90° angle and returned to the initial position. Then, they moved the sliding pointer along the scale to indicate the sensation of soreness in their arm.

Maximal voluntary contraction. After 10 concentric extensions and flexions performed at 120° s⁻¹ as a warm-up, maximal isometric elbow flexion torque was evaluated during 5 s duration isometric maximal voluntary contractions (MVC) performed at an elbow angle of 90°. A total of three trials were performed, and the trial with the highest isometric torque (MVC) was considered for further analysis. All mechanical signals provided by the dynamometer (i.e. joint angle, torque and velocity) were digitized by a 12-bit analog to digital converter (DT 9804, Data Translation, Marlboro, MA, USA).

MR imaging. As described in Guilhem *et al.* (2013), longitudinal T₂-weighted magnetic resonance images of

the damaged arm (Fig. 1a) were obtained using a dedicated low magnetic field (0.25 tesla) MRI system (ESA-OTE, Genoa, Italy). Participants laid in the supine position and placed their arm into an arm-dedicated optimized coil. A thermoformed splint was adjusted to the arm to maintain a constant elbow angle of 110° during each test session. Two plastic straps containing gel-like crosses were placed on the skin at the same location used for the acquisition of elastographic recordings. Due to the low magnetic field in the extremities of the coil, two sequences were performed. During the first sequence, the arm was placed with the proximal mark located in the centre of the coil, while the second sequence was centred on the distal mark. Both extremities of the coil were clearly labelled on the splint in order to ensure reliable positioning of the arm during the subsequent test sessions. The parameters of the magnetic resonance sequences were as follows: spin echo technique; repetition time/echo time = 3000 ms/20, 50, 85 ms; 256 × 256 matrix; two excitations; 250-mm field of view; 8 mm slice thickness; gap between slices: 27.1 mm; six slices per sequence. During a short scout sequence, the gel-like crosses were easily located and the 3rd slice was set in order to pass through the centre of the humerus bone and the gel-like cross. This slice in the medial location was considered for further analysis.

Elastographic measurements. An Aixplorer ultrasound scanner (version 5.0; Supersonic Imagine, Aix-en-Provence, France) coupled with a linear transducer array (4–15 MHz, SuperLinear 15-4; Vermon, Tours, France) was used in shear wave elastography mode (musculoskeletal preset) as previously described (Bercoff *et al.* 2004). Assuming a linear elastic behaviour, the muscle shear elastic modulus was obtained as follows (1):

$$\mu = \rho V_s^2 \quad (1)$$

where ρ is the muscle density (1000 kg m⁻³) and V_s is the shear wave speed.

The hypothesis of linear material is well accepted in muscle elastographic studies, for both transient elastography (Bercoff *et al.* 2004, Catheline *et al.* 2004) and magnetic resonance elastography (Dresner *et al.* 2001, Debernard *et al.* 2011). Maps of the shear elastic modulus (μ) were obtained at 1 Hz with a spatial resolution of 1 × 1 mm (Fig. 1b).

B-mode images were used to determine the orientation of BB muscle fibres during the PRE session. When several fascicles could be observed without interruption across the image, the probe orientation was traced on the skin. In addition, proximal and distal marks aligned with the fibres' orientation and separated by 9 cm were labelled on the skin in order to allow the accurate

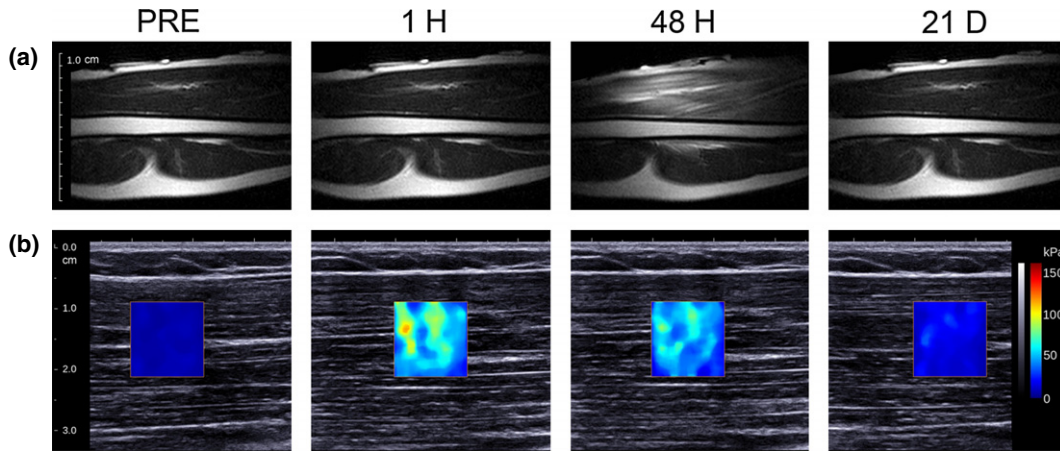


Figure 1 Typical example of T_2 (a) and shear elastic modulus (b) measurements for the *biceps brachii* before (PRE), 1 h, 48 h and 21 days (21D) post-exercise at an elbow angle of 110° . The white signal observable at 48 h (a) indicates the presence of fluid accumulation (oedema). The coloured region represents the shear elasticity map (b, scale on the right).

placement of two plastic straps containing gel-like crosses used for MRI measurements (described above). These marks were used to ensure that MRI and elastographic measurements were performed at the same locations. The shear elastic modulus was determined in BB (Fig. 1b) and BA muscles at 70° , 110° and 160° of elbow angle on the damaged arm and at 110° of elbow angle on the contralateral arm (not exercised). For each elbow joint angle, the shear elastic modulus of the BB was quantified from three locations (i.e. proximal, medial and distal) and from the medial part of the BA (Bouillard *et al.* 2012). During all of these static measurements, participants were asked to remain as relaxed as possible.

Then, the participant's elbow flexor muscles were passively stretched through five slow (4° s^{-1}) loading/unloading cycles from elbow joint angles of 70° to 170° . To study the changes in mechanical properties during the five cycles (Whitehead *et al.* 2001), the shear elastic modulus was measured only for the first and the fifth cycles. Immediately after the fifth cycle, the shear elastic modulus was assessed during a very slow (2° s^{-1}) loading cycle (i.e. elbow extension) over the same range of motion in order to determine the joint angle corresponding to the slack length (i.e. the muscle length corresponding to the length beyond which the muscle begins to develop passive elastic force). A slower velocity was chosen for this cycle in order to obtain a better resolution for the determination of slack length. In order to accustom the participants to the procedure and ensure they were as relaxed as possible during the passive stretching, a familiarization session of five cycles was performed before the start of the PRE session. The participants observed a 10-min rest period following the familiarization before performing five new loading/unloading

cycles that were used for further analysis. Online electromyographic (EMG) feedback of BB and *triceps brachii* was provided to the examiner to ensure that there was no muscle activity during the cycles. A microphone (MB Quart K800, frequency response: 40–18 000 Hz, sensitivity: 16.25 mV/Pa) was used to record the noise associated with the SSI impulse during each measurement (1 Hz) to synchronize elastographic measurements with elbow joint angle.

Surface EMG activity. Bipolar surface EMG signals of BB and *triceps brachii* muscles were recorded during loading/unloading cycles using bipolar silver/silver chloride surface electrodes (Blue Sensor Q-00-S, Medicotest, France). EMG electrodes were attached to the skin with an interelectrode distance of 10 mm, longitudinally with respect to the underlying muscle fibre arrangement. Wires and electrodes were well secured to the skin to avoid movement-induced artefacts. Raw EMG signals were pre-amplified (Mazet Electronique Model, Electronique du Mazet, Mazet Saint-Voy, France; input impedance: 10 G Ω ; common mode rejection ratio: 100 dB; gain: 600; bandwidth: 6–500 Hz) and sampled through the same digital converter used for mechanical data at 1000 Hz.

Data processing

The analysis of MRI data and the plotting of graphs were performed with custom-written scripts designed using ORIGIN 9.0 software (OriginLab Corporation, Northampton, MA, USA). The analysis of SSI data was performed using the Aixplorer scanner software for static measurements, while MATLAB 10.0 (The Mathworks, Natick, MA, USA) was used to analyse the passive cycles.

MRI data. T_2 relaxation time was determined in the BB and the BA muscles from the three same locations (i.e. proximal, medial and distal) as SSI measurements. The muscle region of interest (ROI) was determined using public domain image processing software (IMAGE J; National Institute of Health, Bethesda, MD, USA). Special care was taken to avoid inclusion of subcutaneous fat, fascia, blood vessels or bony anatomy in the ROI. Muscle T_2 was determined for each slice by fitting the relationship between the signal intensity on the ROI and the echo time, according to the following equation (2):

$$y = a \times \exp\left(\frac{x}{T_2}\right) \quad (2)$$

where y is signal intensity, x is echo time (ms), T_2 is relaxation time (ms) and a is magnitude. The same investigator blindly performed the imaging synchronization, scan and the T_2 calculation.

SSI data. For each static measure, shear elastic modulus values of BB and BA were averaged over the largest region (average 12-mm-circle ROI) from the ultrasound software scanner (Q-Box function). SSI recordings from the six passive stretching cycles (five loading/unloading cycles and one loading phase) were exported in 'mp4' format and sequenced into 'jpeg' images. Image processing converted the coloured map into shear elastic modulus values. The average value of shear elastic modulus over an approx. 12-mm-square region of BB was calculated for each image. After synchronization of elastographic measurements with elbow joint angle, shear elastic modulus values during the loading and unloading phases were interpolated every 5° throughout the whole range of motion (i.e. from 70° to 170°). Using these data, the area under the shear elastic modulus loading curve (E), the area under the shear elastic modulus unloading curve (ER) and the hysteresis area (ED) were calculated (Nordez *et al.* 2009). The normalized hysteresis area (DC) was calculated as follows (3):

$$DC = \frac{ED}{E} = \frac{E - ER}{E} \quad (3)$$

The elbow angle corresponding to the slack length of BB was visually determined from the loading phase of the sixth passive stretching (i.e. extension phase) as the first increase in shear elastic modulus (Lacourpaille *et al.* 2013).

Statistical analysis

Due to methodological constraints (in particular the use of the MRI scanner), the 16 participants could not complete all measurements. The total sample was thus shared so that each parameter was measured on 12 subjects. A total of eight participants performed all

the experiments (static elastographic measurements, loading/unloading cycles and MRI measurements): four participants performed both static elastographic and MRI measurements, and four participants performed only the loading/unloading cycles. All analyses were performed with STATISTICA version 7.1 (StatSoft, Tulsa, OK, USA). Data distributions consistently passed the Shapiro–Wilk normality test. Analyses of variance (ANOVA) for repeated measurements were used to test the effects of muscle damage on the different parameters. A one-way ANOVA (time effect) was performed on DOMS. A two-way ANOVA [time (PRE, 1H, 48H, 21D) × arm (damaged, contralateral)] was performed on MVC torque. A three-way ANOVA [time × angle (70°, 110°, 160°) × site (proximal, medial, distal, BA)] was performed on raw shear elastic modulus values. A two-way ANOVA [time × site (proximal, medial, distal, BA)] was performed on T_2 values. Two-way ANOVAs were performed to evaluate the effect of time (PRE, 1H, 48H, 21D) and cycle (first and fifth) on E and DC . A one-way ANOVA was performed to characterize the effect of time (PRE, 1H, 48H, 21D) on the angle corresponding to the slack length. 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 all tests, the significance level was set at $P < 0.05$. The data are presented as mean ± SD.

Results

Delayed soreness

A significant effect of time on muscle soreness was found ($P < 0.0001$). DOMS was significantly increased at 1 h (1.5/10; $P = 0.0002$) and peaked at 48 h post-exercise (3.8/10; $P < 0.0002$ vs. PRE and $P = 0.0001$ vs. 1H), while the 21D value was not significantly different from PRE ($P = 0.70$; Fig. 2a).

Muscle strength

A significant time × arm interaction was observed on MVC ($P < 0.0001$). MVC of the damaged arm was significantly reduced at 1 h ($-29 \pm 13\%$; $P < 0.001$) and 48 h ($-22 \pm 18\%$; $P < 0.001$) post-exercise, whereas no changes were observed in the contralateral arm. No significant change in MVC was found at 21 days when compared with the initial value (Fig. 2b).

T_2 relaxation time

Magnetic resonance imaging revealed mean T_2 values ranging from 29.7 ± 1.7 ms at PRE to 33.5 ± 7.7 ms

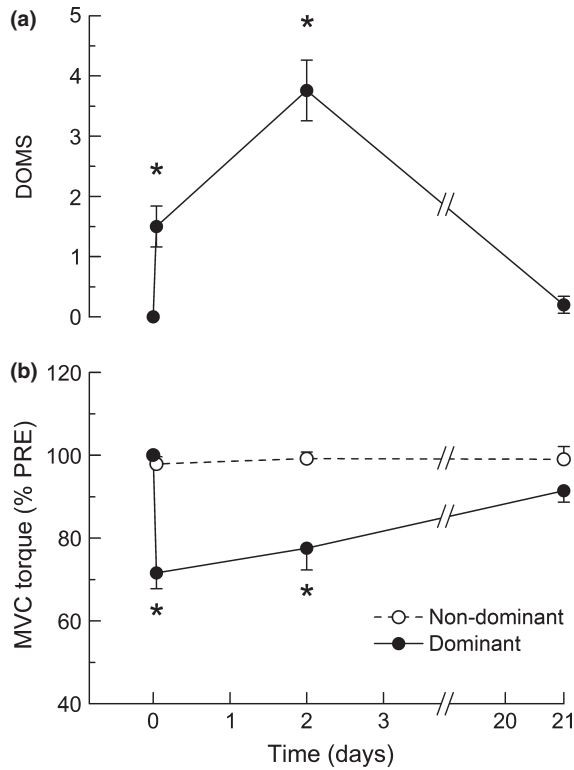


Figure 2 Delayed onset muscular soreness (DOMS) obtained from a visual analogue scale numbered before and 1 h, 48 h and 21 days after eccentric exercise (a). Changes in maximal voluntary isometric elbow flexor torque of dominant (damaged) and non-dominant arms performed during isometric maximal voluntary contractions at each time point (b). Values are presented as mean \pm SD. * $P < 0.05$: significant difference when compared with the PRE value.

at 48H for BB and from 30.9 ± 1.2 ms at PRE to 37.6 ± 7.4 ms at 48H for BA. The course of T_2 relaxation time of elbow flexor muscles is displayed in Figure 3. A significant main effect of time was found ($P = 0.01$). While T_2 level was not affected at 1H and 21D, it increased significantly in BB at 48H ($+16 \pm 32$, $+13 \pm 33$ and $+11 \pm 17\%$, for proximal, medial and distal region respectively) and in BA ($+22 \pm 23\%$; $P < 0.009$). T_2 values were not differently changed between within BB and between BB and BA after the eccentric exercise (time \times site interaction – $P = 0.16$).

Shear elastic modulus

Static measurements. Mean shear elastic modulus values measured for each muscle, each elbow angle and each location are depicted in Table 1. Figure 4 depicts the changes in shear elastic modulus measured in static conditions for the elbow flexor muscles. The statistical analysis revealed significant effects of time ($P < 0.0001$), angle ($P = 0.0001$) and site ($P = 0.03$) on shear elastic modulus. No significant time \times

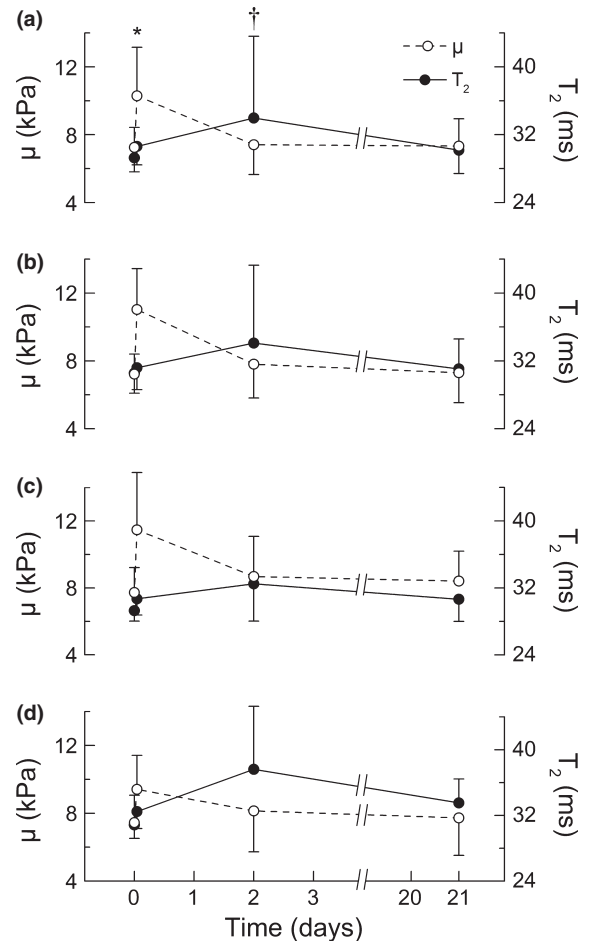


Figure 3 Dissociated time courses of shear elastic modulus (μ) and T_2 measurements assessed at an elbow angle of 110° for the elbow flexor muscles, before and 1 h, 48 h and 21 days after eccentric exercise. Values are represented as mean \pm SD. * $P < 0.05$: significant difference when compared with the PRE value for shear elastic modulus. † $P < 0.05$: significant difference when compared with the PRE value for T_2 .

angle \times site interaction was observed for the shear elastic modulus values ($P = 0.52$). The statistical analysis showed a significant time \times angle interaction ($P < 0.0001$). More precisely, a significant increase in shear elastic modulus of elbow flexor muscles (with no difference between the muscles) was found at 1H for each site of measurements (i.e. BB proximal, medial, distal and BA) and both 110° ($+42 \pm 28$, $+54 \pm 33$, $+48 \pm 30$ and $+43 \pm 36\%$ respectively; $P = 0.005$) and 160° ($+81 \pm 70$, $+78 \pm 55$, $+78 \pm 69$ and $+55 \pm 44\%$ respectively; $P = 0.0001$) of elbow joint angle. Shear elastic modulus was not altered when measured at 70° at all time points (P -values ranged from 0.75 to 0.95). At 160° , the shear elastic modulus was still significantly higher than PRE value at 48H (average across the sites: $+39 \pm 25\%$; $P = 0.001$)

Table 1 Raw values of shear elastic modulus of the elbow flexor muscles

Elbow angle	Muscle	Site	Shear elastic modulus (kPa)			
			PRE	1H	48H	21D
70°	Biceps brachii	Proximal	3.7 ± 0.7	4.0 ± 0.6	3.5 ± 0.5	3.7 ± 0.6
		Medial	3.9 ± 1.2	3.9 ± 0.5	3.7 ± 0.9	3.6 ± 0.6
		Distal	4.3 ± 1.2	4.4 ± 0.7	4.1 ± 0.7	4.0 ± 0.7
110°	Biceps brachii	Proximal	7.3 ± 1.2	10.3 ± 2.9*	7.4 ± 1.8	7.3 ± 1.6
		Medial	7.2 ± 1.2	11.0 ± 2.4*	7.8 ± 2.0	7.3 ± 1.8
		Distal	7.7 ± 1.5	11.5 ± 3.4*	8.7 ± 2.4	8.4 ± 1.8
160°	Biceps brachii	Proximal	17.3 ± 3.4	30.4 ± 10.7*	23.6 ± 6.4*	21.7 ± 4.1*
		Medial	16.8 ± 2.8	29.6 ± 10.3*	24.8 ± 8.0*	20.5 ± 4.4*
		Distal	19.0 ± 3.4	33.6 ± 12.9*	26.3 ± 6.0*	21.0 ± 4.9*
	Brachialis	Medial	19.9 ± 3.0	30.2 ± 7.0*	26.2 ± 7.1*	19.6 ± 3.7*

Shear elastic modulus measurements were obtained at three relaxed elbow joint angles (70°, 110° and 160°) using supersonic shear imaging, before (PRE) and 1 h, 48 h, and 21 days (21D) after eccentric exercise. Values are presented as mean ± SD.

* $P < 0.05$: significant difference when compared with the PRE value.

and 21D (average across the sites: $+14 \pm 19\%$; $P = 0.001$; Fig. 4).

Loading/unloading cycles. The changes in shear elastic modulus as a function of joint angle measured during passive stretching are presented in Figure 5. We observed a significant time \times cycle interaction on the area under the loading curve ($P = 0.0001$). More precisely, the area under the loading curve was significantly increased at 1H for both the first ($P = 0.0001$) and the fifth cycles ($P = 0.0001$) and at 48H for the first cycle only ($P = 0.0003$; Fig. 6a). In addition, a significant time \times cycle interaction was found for the normalized hysteresis area ($P = 0.01$). The normalized hysteresis area of the first cycle was significantly higher at 1H ($31 \pm 13\%$) than PRE ($18 \pm 9\%$; $P = 0.0005$) but was not significantly changed for the fifth cycle ($P = 0.63$; Fig. 6b). The elbow joint angle corresponding to the slack length of BB shear elastic modulus significantly decreased from PRE ($87.6 \pm 5.2^\circ$) to 1H ($83.2 \pm 4.9^\circ$; $P < 0.0001$; Fig. 6c).

Discussion

The aim of this study was to investigate the changes in mechanical properties of the main elbow flexor muscles (i.e. BB and BA) after exercise-induced muscle damage. The novelty of this work was to explore the putative relationship between the changes in muscle shear elastic modulus and both muscle length and damage location. The results showed that the muscle shear elastic modulus was significantly increased 1 h after the eccentric exercise (while oedema was absent).

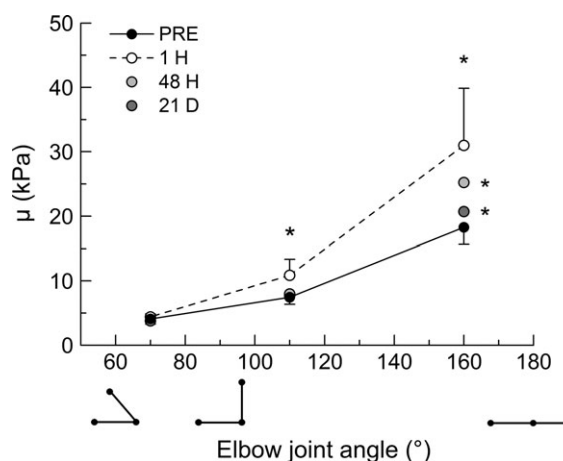


Figure 4 Raw values of shear elastic modulus of the elbow flexor muscles (μ ; proximal, medial, distal and *brachialis* values are pooled due to the lack of 'site' effect) obtained at three relaxed elbow joint angles (70°, 110° and 160°) using supersonic shear imaging, before (PRE) and 1 h, 48 h, and 21 days (21D) after eccentric exercise. Values are presented as mean ± SD. * $P < 0.05$: significant difference when compared with the PRE value.

This increase was higher and persisted longer (up to 21 days) at long (i.e. 160° of elbow joint angle) than at short muscle lengths. In addition, this increase reached a similar extent within the different locations in BB, and between BB and BA. Changes in the mechanical properties of the elbow flexors obtained during loading/unloading cycles contribute to the understanding of the potential mechanisms underlying the localized changes in muscle passive tension after eccentric exercise.

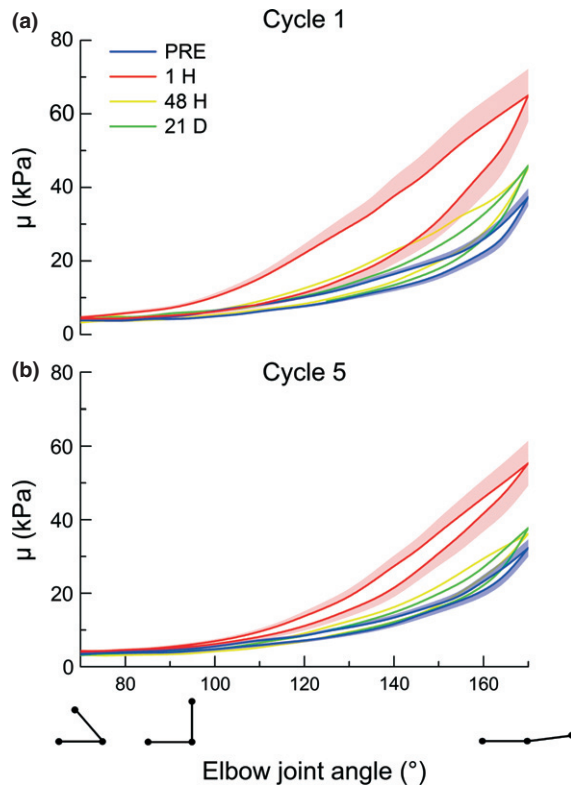


Figure 5 Shear elastic modulus (μ) of *biceps brachii* obtained for the first (a) and fifth (b) passive loading/unloading stretching cycles between elbow joint angles of 70° and 170° (180° = full extension). The changes in *biceps brachii* shear elastic modulus before (PRE) and 1 h, 48 h and 21 days (21D) after eccentric exercise are plotted in blue, red, yellow and green respectively. For the sake of clarity, SD is presented only for PRE and 1H.

Shear elastic modulus measurements showed pre-exercise values in accordance with previous data obtained with the same technique at rest [approx. 4.0 kPa at 70° or 90° of elbow angle (Gennisson *et al.* 2010, Lacourpaille *et al.* 2012)] and passive conditions [approx. 8.0 ± 0.9 kPa at 110° and 25.6 ± 6.4 kPa at 160° of elbow angle (Lacourpaille *et al.* 2013)]. In accordance with previous results obtained using magnetic resonance elastography (Green *et al.* 2012), our findings showed that muscle damage induced an increase (+46%; $P = 0.0005$) in the shear elastic modulus of the elbow flexors measured at 110° at 1 h and returned to initial value at 48 h post-exercise. These results differ from those reported in previous studies (Howell *et al.* 1993, Proske & Morgan 2001) where muscle stiffness was inferred from the passive torque–angle curve. These studies reported an increase in stiffness immediately after the exercise that remained elevated for the next 4 days (Howell *et al.* 1993, Proske & Morgan 2001). These discrepancies may be explained by the fact that

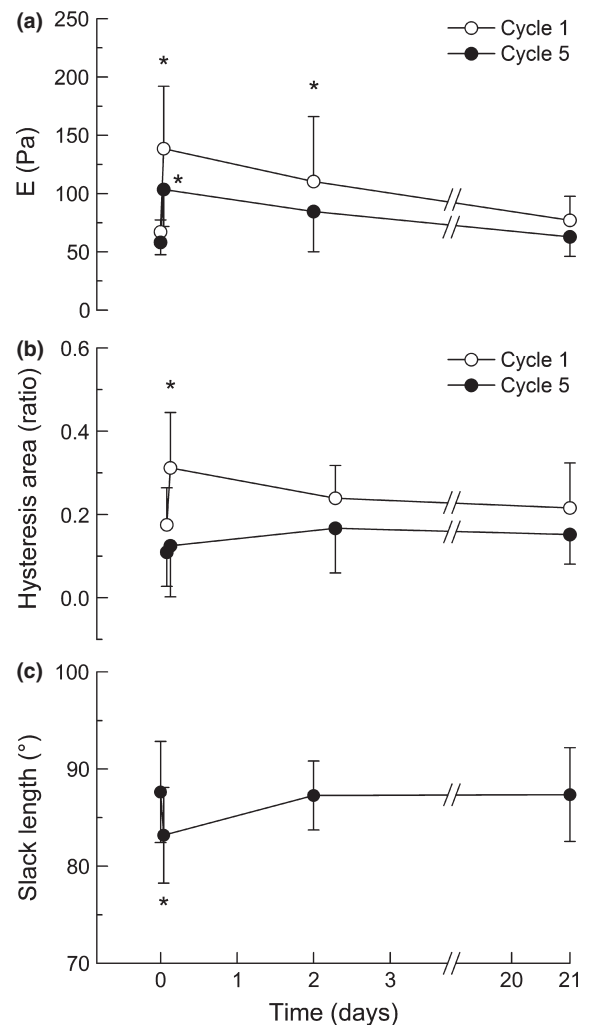


Figure 6 Time course of area under the loading curve (a), normalized hysteresis area (b) and slack length (c) of the *biceps brachii* before and after eccentric exercise. The area under the loading curve (E) and normalized hysteresis area are presented for the first (black circles) and the fifth (white circles) stretching cycles, before and 1 h, 48 h and 21 days after the exercise. Changes in the elbow joint angle corresponding to the *biceps brachii* slack length were determined during a sixth 2° s^{-1} passive stretching. Values are presented as mean \pm SD. * $P < 0.05$: significant difference when compared with the PRE value.

passive torque is influenced not only by muscular but also by non-muscular structures. Interestingly, we observed that the increase in shear elastic modulus preceded the occurrence of oedema (T_2 level), which peaked at 48 h. This confirms that the early changes in muscle shear elastic modulus could not be attributed to the delayed fluid accumulation occurring after eccentric exercise (Whitehead *et al.* 2001). Nonetheless, our findings do not exclude the possible contribution of swelling to the maintained elevation of global

passive torque, 24 h post-exercise onwards, as suggested by Whitehead *et al.* (2001).

As the T_2 values were similarly affected within BB (proximal, medial and distal regions) and between BB and BA 48 h after the exercise (Fig. 3), our hypothesis that these two elbow flexors would be affected differently by the exercise was not verified. Together with the similar increase in shear elastic modulus observed in the different regions of elbow flexors, the results suggest that the changes in shear elastic modulus are similar within and between synergistic muscles when oedema is homogeneously spread. While no significant differences were observed 1 h after eccentric exercise at 70°, the shear elastic modulus of elbow flexor muscles was increased by $46 \pm 25\%$ at 110° and by $72 \pm 50\%$ at 160° of elbow joint angle (Fig. 4). Hence, we showed that the length dependency of changes in muscle mechanical properties observed using global measurements (Howell *et al.* 1993, Chleboun *et al.* 1998, Whitehead *et al.* 2001, Hoang *et al.* 2007) is also observed at a muscle level in a similar fashion within and between synergistic muscles.

Green *et al.* (2012) reported a significant increase in shear storage modulus of *soleus* and *gastrocnemius medialis*, respectively, at 1 and 48 h after eccentric exercise, with measurements performed at a relative short muscle-tendon length (i.e. foot perpendicular to the leg). Considering that passive torque increase has been shown to be maximal at long muscle-tendon lengths (Whitehead *et al.* 2001, Hoang *et al.* 2007), one could expect that larger increases in shear elastic modulus would have been observed at longer muscle lengths. As discussed by the authors, the protocol used (i.e. backward downward walking), anatomical features and fibre composition may also have differently influenced the response of the activated muscles to eccentric exercise (Takekura *et al.* 2001, Weishaupt *et al.* 2001, Green *et al.* 2012).

Some methodological considerations relative to the SSI technique need to be discussed. First, due to the anisotropic nature of muscle, the shear elastic modulus depends on the angle between the muscle fibre and the probe direction (Gennisson *et al.* 2010). To account for this limitation, we used B-mode images to determine the fibres' orientation and trace marks on the skin to reliably locate the ultrasound transducer in respect of the fibres direction. As *biceps brachii* are fusiform, we assume that the fibre's orientation does not change during passive stretching. Consequently, it is unlikely that our results are affected by muscle anisotropy. Second, even if the measurements performed using SSI are in two dimensions and the size of the ROI is relatively small (approx. 1.45 cm^2), we argue that the mean value of shear elastic modulus over the map is representative

of muscle stress within the considered muscle region as demonstrated *in vivo* (Bouillard *et al.* 2012, Maïsetti *et al.* 2012) and *in vitro* (Koo *et al.* 2014). Also, a good reliability of muscle shear elastic modulus measurements (using SSI) has been reported at rest (Lacourpaille *et al.* 2012), during contraction (Bouillard *et al.* 2012) and passive stretching (Maïsetti *et al.* 2012). Therefore, it is unlikely that either the relative spatial selectivity or reliability of the SSI technique could affect the main conclusions of the present study.

Whitehead *et al.* (2001) showed that normalized hysteresis area was significantly increased immediately after eccentric exercise (+60%). The main hypothesis proposed to explain this finding is linked to an increase in the proportion of stable attached cross-bridges between myofilaments in muscle fibres due to an increase in intramuscular calcium concentration (Chen *et al.* 2007). However, such an increase in hysteresis has never been directly investigated *in vivo*, although it could be of great interest to better understand the putative origin of the changes in mechanical properties after muscle damage. Several studies focused on the potential elastic energy stored by the musculo-articular complex and the dissipation coefficient calculated from the torque-angle relationship (Magnusson 1998, Nordez *et al.* 2009). However, as the shape of torque-angle relationship is influenced by all the structures acting on the joint (Howell *et al.* 1993, Chleboun *et al.* 1998), individual muscle behaviour could not be directly inferred from these studies. As previous studies showed that the shear elastic modulus is linearly related to muscle tension during passive stretching (Maïsetti *et al.* 2012, Koo *et al.* 2013), the present study proposed a way to investigate force-length relationship of an individual muscle after a damaging exercise. We reported a significant increase in the area under the loading curve for the first cycle at 1 h ($+110 \pm 95\%$) and 48 h ($+35 \pm 37\%$) after the eccentric exercise. Interestingly, the significant increase in the normalized hysteresis area at 1 h after the eccentric exercise ($+13 \pm 13\%$) (Fig. 6b) reported herein was close to the 13% increase reported by Whitehead *et al.* (2001). These adaptations could originate from the perturbation of the intramuscular calcium homeostasis (Chen *et al.* 2007) that could trigger an augmentation of stable attached cross-bridges number (Whitehead *et al.* 2001), leading to an increase in both muscle shear elastic modulus and dissipative properties for the first cycle. Two additional results from the present study could corroborate the involvement of this mechanism in the modification of muscle shear elastic modulus. First, the sensitivity of muscle fibres to Ca^{2+} has been reported to increase as muscle is elongated (Stephenson & Wendt 1984, Balnave & Allen 1996, Claflin *et al.* 1998). Thus, the

higher increase in shear elastic modulus observed at long than at short muscle lengths might be due to the involvement of calcium-dependent physiological processes [e.g. cross-bridges number (Proske & Morgan 1999) or titin giant protein (Labeit *et al.* 2003)]. Second, the disturbance of calcium homeostasis due to damaged muscle membranes [for a review see (Morgan & Allen 1999)] induces a homogeneous increase in calcium levels within the muscle (Balnave *et al.* 1997). Such an alteration has been reported to be sufficient to trigger a low level of activation of muscle fibres (i.e. 'contracture clots'), responsible for an increase in muscle passive tension, particularly at long muscle lengths (Howell *et al.* 1993, Whitehead *et al.* 2001, Hoang *et al.* 2007). The absence of change in shear elastic modulus at short muscle lengths (70° of elbow extension) may be explained by the fact that these physiological consequences of Ca²⁺ homeostasis perturbation were marginal (because of the low Ca²⁺ sensitivity).

We observed that the area under the loading curve of the fifth cycle, quantified 1 h post-exercise, was still higher than the pre-exercise value (+85 ± 76%, Fig. 6a) without significant concomitant changes in the normalized hysteresis area (Fig. 6b). Conversely, Whitehead *et al.* (2001) found that the coefficient of dissipation measured during the fifth cycle was still higher than the initial value after an eccentric exercise. This difference can be explained by different experimental setups (e.g. 150 vs. 30 eccentric muscle contractions). In addition, we showed that the elbow joint angle corresponding to the slack length of the *biceps brachii* was significantly decreased 1 h after exercise (−6.4 ± 3°) and returned to pre-test values at 48 h and 21 days post-exercise (Fig. 6c). These findings are thus reflective of cycle-independent processes that generate a supplemental passive force, which cannot be attributable to resting cross-bridges. Indeed, it has been demonstrated that, in addition to collagen and cross-bridges attachment, the structural protein titin contributes to the passive stiffness of a muscle in a calcium-dependent way (Labeit *et al.* 2003, Linke & Kruger 2010). Given these elements and the persistence of increased passive shear elastic modulus after five loading/unloading cycles, our results support the role of a specific structural protein (i.e. titin) in the modulation of shear elastic modulus (Herzog 2014). Experimental evidence suggests that titin is able to bind to actin via its proline-glutamate-valine-lysine segment (PEVK segment) (Yamasaki *et al.* 2001, Labeit *et al.* 2003) in the presence of a high intramuscular calcium concentration (i.e. 1 h after eccentric exercise) (Chen *et al.* 2007). In this context, the titin molecule might be stretched to a greater degree when muscle is passively lengthened. Such a process might

result in both the increase in passive shear elastic modulus without a stretching-cycle effect (i.e. persistence of the increase in the area under the loading curve during the fifth cycle) and the reduction in the distance necessary to stretch the muscle elastic components generating an early rise in passive tension (i.e. slack length), 1 h after the eccentric exercise (Herzog 2014).

Unlike previous studies (Howell *et al.* 1993, Chleboun *et al.* 1998, Hoang *et al.* 2007), muscle shear elastic modulus was still higher 21 days post-exercise compared with initial value (Fig. 4). It is possible that this has not been observed previously due to the lack of sensitivity of the global measurements to assess small changes in the mechanical properties of muscle. This result could suggest the putative rapid adaptation of muscle mechanical properties after exercise-induced muscle damage, which is in agreement with the theory based on the reshuffling of cytoskeleton proteins partly involved in the repeated-bout effect (Nosaka *et al.* 2001, McHugh 2003). Nonetheless, given that 9% of the initial force remained to be recovered 21 days after the exercise (Fig. 2b), the higher passive tension observed at 21D might be still a reflection of persistent damage. Further investigations are required to better explain the origin of this long-term modification of the muscle mechanical properties and its potential role in the repeated-bout effect.

In conclusion, this study showed that the shear elastic modulus and the dissipative properties of the elbow flexor muscles were increased after a damaging exercise and that these changes occurred before fluid accumulation (oedema) in the damaged area. These modifications of muscle mechanical properties were more pronounced at long muscle lengths and reached a similar extent within and between elbow flexors. Our findings corroborated the uniform augmentation in resting calcium throughout the muscle, and the increased sensitivity of tension to this released calcium in skeletal muscle fibres upon stretch, suggested by global measurements. In addition, this study underlined the putative involvement of both cross-bridges number and titin in the changes in muscle passive tension after eccentric exercise.

Conflict of interest

No conflict of interests, financial or otherwise, are declared by the authors.

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