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ORIGINAL ARTICLE

Changes in electrical pain threshold of fascia and muscle after initial and secondary bouts of elbow flexor eccentric exercise

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Abstract
Purpose This study investigated changes in electrical pain threshold (EPT) after repeated eccentric exercise bouts to test the hypothesis that fascia would become more sensitive than muscle when greater delayed onset muscle soreness (DOMS) is induced.
Methods Ten young men performed two eccentric exercise bouts (ECC1, ECC2) consisting of ten sets of six maximal isokinetic eccentric contractions of the elbow flexors with the same arm separated by 4 weeks. Maximal voluntary isometric contraction torque, range of motion, muscle soreness assessed by a visual analogue scale (VAS) and pressure pain threshold (PPT) were measured before, immediately after and 1–5 days after exercise. EPT was assessed in the biceps brachii fascia (BBF), biceps brachii muscle, and brachialis fascia (BF) 1 day before, immediately after, and 1, 2 and 4 days after exercise.
Results All measures showed smaller changes ($P < 0.05$) after ECC2 than ECC1. EPT decreased after both bouts and the largest decreases were evident at 2 days post-exercise ($P < 0.05$). The decreases in EPT after ECC1 were greater ($P < 0.05$) for both BBF (Baseline: 1.45 ± 0.23 mA, 2 days post-exercise: 0.13 ± 0.11 mA) and BF (1.64 ± 0.29 mA, 0.26 ± 0.2 mA) than muscle (1.56 ± 0.29 mA, 0.69 ± 0.33 mA). Changes in EPT were correlated with the changes in PPT ($r = 0.63$–0.87, $P \leq 0.05$) but not with VAS ($r = -0.01$ to 0.50).
Conclusion These results show that fascia becomes more sensitive than muscle to electrical stimulation after the initial eccentric exercise, suggesting that damage inflammation to fascia than muscle fibres is more associated with DOMS.

Keywords Repeated bout effect · Delayed onset muscle soreness · Muscle damage · Pressure pain threshold · Visual analogue scale

Abbreviations

ANOVA	Analysis of variance
BBF	Biceps brachii fascia
BF	Brachialis fascia
CV	Coefficient of variation
DOMS	Delayed onset muscle soreness
ECC	Eccentric exercise
EPT	Electrical pain threshold
MVC	Maximal voluntary contraction
PPT	Pressure pain threshold
ROM	Range of motion
SEMs	Standard error of measurements
VAS	Visual analogue scale

Introduction

After performing unaccustomed exercise, people experience muscle pain starting several hours post-exercise and lasting for several days (Armstrong 1984; Cheung et al. 2003), which is referred to as delayed onset muscle soreness (DOMS). DOMS is characterised as muscular

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mechanical hyperalgesia (Murase et al. 2010), and pain is felt only when exercised muscles are moved, stretched or palpated. After performing the same or a similar exercise within several weeks, the magnitude of DOMS is substantially reduced or no DOMS is developed, which is a typical indication of the repeated bout effect (McHugh 2003; Howatson et al. 2007). It has been documented that damage to contractile proteins, intermediate filaments, and/or connective tissue surrounding muscle fibres, and the subsequent inflammatory responses are responsible for DOMS (MacIntyre et al. 1995; Cheung et al. 2003; Howatson and van Someren 2008). However, the mechanisms underpinning DOMS are still not fully understood.

Some studies have documented that connective tissue damage and inflammation are more responsible for DOMS than muscle fibre damage and inflammation (Crameri et al. 2007; Paulsen et al. 2010; Raastad et al. 2010). For example, Paulsen et al. (2010) reported no association between DOMS and inflammation of muscle fibres after 300 eccentric contractions of the quadriceps femoris, and noted that damage and remodelling of the extracellular matrix (ECM) than muscle fibres were related to DOMS. Crameri et al. (2007) compared muscle damage between 210 maximal eccentric contractions with electrical muscle stimulation (EMS) and 210 voluntary maximal eccentric contractions (VOL) of the knee extensors, and found that the magnitude of DOMS and increased staining of tenascin C were similar between EMS and VOL, but muscle fibres' morphological damage was evident only after EMS. Malm et al. (2004) reported that DOMS was related to a greater increase in the inflammatory markers such as T cells (CD3), granulocytes (CD11b) and leukaemia inhibitory factor (LIF) in the epimysium, but not in the skeletal muscle fibres after 45 min of downhill running. These results suggest that DOMS is attributed to increased sensitivity of connective tissue surrounding muscle fibres (endomysium), muscle bundles (perimysium) or fascia (epimysium) to mechanical stimuli, such as contraction, stretch, palpation and pressure.

It is known that skeletal muscles contain four types of afferent fibres: group I (Aα), II (Aβ), III (Aδ) and IV (C), and the free nerve endings of the latter two fibres (III and IV) mainly respond to noxious stimuli such as mechanical pressure, heat, cold and algesic substances (Millan 1999). Some researchers suggested that group I and II afferents were also involved in DOMS in addition to group III and IV afferents (Weerakkody et al. 2003; Proske 2005). Taguchi et al. (2005) demonstrated that only mechanical sensitivity of muscle thin-fibre sensory receptors (III and IV) was increased 2 days after eccentric contractions of extensor digitorum longus (EDL) muscle of rats. It should be noted that these free nerve endings (nociceptors) are located along the walls of arteries and mostly in the surrounding connective tissue (Stacey 1969; Mense 1993). It appears that connective tissue such as fascia, which contains a high density of nociceptors (Andres et al. 1985; Mense and Simons 2001), is responsible for muscle pain. In fact, Gibson et al. (2009) showed that fascia, rather than muscle tissue, in tibialis anterior muscle became more sensitive to hypertonic saline injection when DOMS was elicited.

Itoh et al. (2004) introduced an intramuscular electrical pain threshold (EPT) technique to assess pain threshold of the skin, fascia and muscle separately. They reported that EPT was significantly lower in the fascia compared with the muscle and skin of the forearm 2 days after eccentric exercise of the middle finger, and suggested that the sensitised nociceptors at the fascia level were responsible for DOMS. However, no previous studies have applied this technique to the elbow flexors and investigated changes in EPT in relation to the magnitude of muscle damage, which is largely different between the initial and secondary bouts of the same exercise. It has been well documented that the level of DOMS assessed by a visual analogue scale (VAS) or a pressure pain threshold (PPT) is significantly attenuated after the second eccentric exercise that is performed within several weeks after the first bout (Chen et al. 2007; Lau et al. 2013; Muthalib et al. 2012). However, it is not known whether this is also the case for the DOMS assessed by EPT.

Thus, the purposes of the present study are (1) to establish the reliability of EPT assessment applied for biceps brachii; (2) to investigate changes in EPT at biceps brachii fascia (BBF), muscle (biceps brachii) and brachialis fascia (BF) after the first and second bouts of maximal eccentric elbow flexion exercise separated by 4 weeks; and (3) to examine the relationship between EPT and other pain measures such as VAS to quantify the magnitude of pain and PPT to assess the sensitivity of muscle to pressure. It was hypothesised that (1) EPT would reliability provide region-specific pain sensitivity; (2) EPT would decrease more at the fascia regions than the muscle after the first than the second exercise bout; and (3) the magnitude of change in EPT would be significantly correlated with the magnitude of changes in VAS and PPT.

Methods

Subjects

This study was approved by the Institutional Human Research Ethics Committee and complied with the Declaration of Helsinki. Ten young men (22–28 years, average age: 24 years) with no current or previous upper arm injuries, who were not suffering from any upper arm pain and who had not performed resistance training of the upper

limbs for at least 6 months prior to the present study, were recruited for this study. The number of subjects was determined by a sample size estimation using the data of a previous study (Chen et al. 2007) that reported the repeated bout effect of maximal eccentric exercise of the elbow flexors. Based on α level of 0.05, a power $(1-\beta)$ of 0.80 and an expected 20 % difference in maximal voluntary contraction (MVC) torque recovery at 3 days after maximal eccentric elbow flexor exercise between the first and second bouts, at least ten subjects were deemed necessary. Their mean (±SD) body mass, height and MVC torque were 69.7 ± 14.3 kg, 170.1 ± 8.6 cm, and 50.6 ± 8.1 Nm, respectively. All subjects provided informed written consent, and a medical questionnaire was completed before participation in the study. Subjects were requested not to change their lifestyle and dietary habits, not to take any anti-inflammatory drugs or nutritional supplements, and not to perform unaccustomed exercise during the experimental period.

Eccentric exercise

All subjects performed two exercise bouts separated by 4 weeks, consisting of ten sets of six maximal isokinetic (60° s^{-1}) eccentric elbow flexor contractions on an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York, USA) using their non-dominant arm. Each subject was seated on the dynamometer seat with the shoulder joint being secured at 45° flexion, with the elbow being aligned with the axis of rotation of the lever arm of the dynamometer that was attached to the subject's wrist in a supinated position. For each eccentric contraction, the elbow joint was forcibly extended from a flexed (60°) to a fully extended position (0°) in 1 s at an angular velocity of 60° s^{-1}, while each subject was verbally encouraged to generate maximal force at the flexed position and to maximally resist against the elbow-extending action for the full range of motion. The smaller range of motion (60º) used in the present study when compared with that (90–130º) of previous studies (Jamurtas et al. 2005; Muthalib et al. 2012; Lau et al. 2013) was to induce greater DOMS to biceps brachii muscle, since a previous study reported that eccentric contractions at long muscle lengths induced greater damage and DOMS to bicep brachii (Nosaka et al. 2005). After each eccentric contraction, the isokinetic dynamometer was programmed to return the arm to the flexed position at a velocity of 6° s^{-1}, which provided a 10-s rest between contractions. The rest period between sets was 3 min. Torque signals were recorded via a data acquisition system (Powerlab with a Chart 7 software, ADinstrument, Bella Vista, Australia) at a sampling rate of 200 Hz, and real-time visual feedback of torque was displayed on a computer monitor.

Muscle damage markers

Indirect markers of muscle damage including maximal voluntary isometric contraction (MVC) torque, range of motion of the elbow joint (ROM), muscle soreness assessed by a visual analogue scale (VAS) and pressure pain threshold (PPT) were measured before, immediately after and 1–5 days after exercise.

MVC torque

Elbow flexion MVC torque was measured using the isokinetic dynamometer with the same positioning of the subject as that for the eccentric exercise described above. Each subject performed two 3-s maximal voluntary isometric contractions at an elbow joint angle of 90° with a 30-s rest between contractions. Measurements were taken twice and the peak torque of the two contractions was used as the MVC torque (Lau and Nosaka 2011; Lau et al. 2013).

ROM

A plastic goniometer was used to measure extended (EANG) and flexed elbow joint angles (FANG). The EANG was determined when subjects attempted to fully extend the elbow joint while standing and hanging the arm by their side, and the FANG was determined when subjects attempted to fully flex the elbow joint to touch the shoulder of the same side with the palm (Lau and Nosaka 2011). A semi-permanent ink pen was used to mark the lateral epicondyle of the humerus, the acromion process and the mid-point of the styloid process of the ulna and radius. Measurements were taken twice for each joint angle and the mean value of the two measurements was used to calculate the ROM by subtracting FANG from EANG (Lau and Nosaka 2011).

VAS

The level of muscle soreness was assessed using a 100-mm VAS in which '0' indicated no pain and '100' represented extreme pain (Lau and Nosaka 2011; Lau et al. 2013). Each subject was asked to mark the level of perceived soreness on the VAS, when he was lying on a massage table with his arm was placed on his side, and the elbow flexors were palpated by the investigator. The investigator placed his index and middle fingers over the subject's mid-belly of the biceps brachii at 9 cm above the elbow crease and applied pressure and palpated with the tips of the finger toward the deeper tissues for approximately 3 s. The pressure applied to the site was kept as constant as possible between days and among subjects, and the measurements were taken by the same investigator throughout the study.

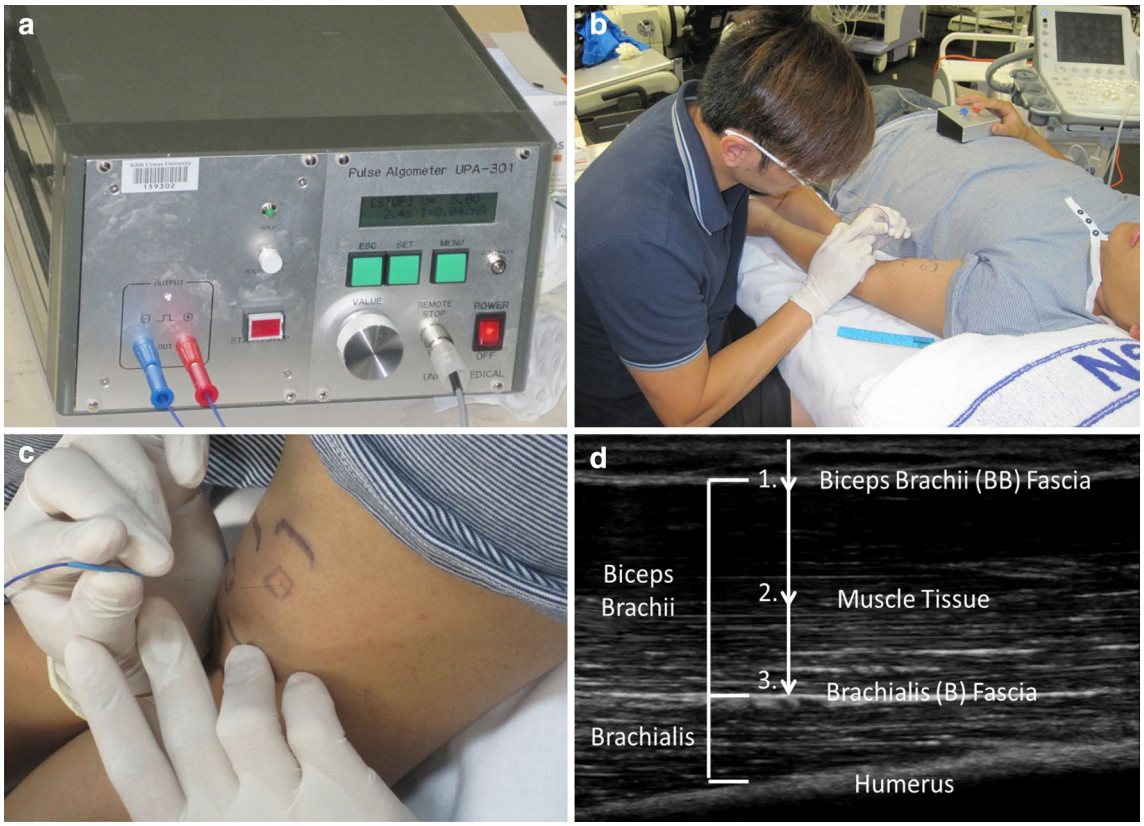

Fig. 1 A pulse algometer used in the study (**a**) and the measurement protocol for electrical pain threshold (EPT) using the pulse algometer with a stainless steel needle electrode and terminate current controller (**b**). A needle electrode was inserted in the mid-belly of the biceps brachii (**c**), and EPT at biceps brachii fascia, muscle tissue (biceps brachii, between the two fascias) and brachialis fascia as shown in the B-mode ultrasound image (**d**) was assessed

PPT

Pressure pain threshold was measured using an electronic algometer (Somedic AB, Hörby, Sweden). The probe head of the algometer (area of 1.0 cm^2) was placed perpendicular to the mid-belly of the biceps brachii at 9 cm above the elbow crease (the same site as the VAS measure) and force was gradually applied at a rate of 50 kPa s^{-1} until the subject reported the first feeling of noticeable pain of the muscle. The value (kPa) corresponding to the force applied to elicit pain was recorded. A 10-s interval was provided between measurements, and the average of the two measures was used for further analysis (Lau et al. 2013).

Electrical pain threshold

Electrical pain threshold of biceps brachii fascia (BBF), muscle (biceps brachii in between BBF and BF) and brachialis fascia (BF) that separated the biceps brachii and brachialis were measured separately by a pulse algometer (UPA-301, Unique Medical Co Ltd, Tokyo, Japan) while the subject lied supine on a massage table and relaxed his arm in a supinated forearm position (Fig. 1a, b). The frequency of the pulse algometer was adjusted to 40 Hz before each measurement. A stainless steel needle electrode insulated with acrylic resin (180 μm in diameter, Toyo Medical Institute, Osaka, Japan) was inserted into the mid-belly of biceps brachii muscle (approximately 9 cm above the elbow crease; Fig. 1c), and the BBF, muscle and BF pain thresholds were assessed (Fig. 1d). The location of the needle was confirmed using real-time B-mode ultrasound system (Hitachi Aloka F75 Co, Ltd., Tokyo, Japan) at a frame rate of 47 Hz with a 10-MHz electronic linear transducer (5 cm, UST-567) before each measurement (Fig. 1d). The pain threshold was determined for BBF followed by muscle, then BF. When the needle was inserted into BBF, the intensity of the current was increased from zero at a constant rate (0.05 mA s^{-1}) and the subject indicated the feeling of pain by pressing a button on the controller that records the stimulus current at that pain level. The pain threshold was automatically displayed on the digital display of the algometer in the units of mA. The intensity of the current stimulus was reset (back to zero), and the second stimulation was given with a 30-s interval between measurements.

Following this measurement, the needle was progressively inserted into the muscle with the needle location being confirmed by monitoring the remaining needle length, from which the depth was confirmed, and EPT of muscle (in between biceps fascia and brachialis fascia), and subsequently BF were measured twice for each as described for BBF. The average of the two measures for each region was used for further analysis. Because of the invasive nature of the EPT measures, the measurements were not performed on 3 and 5 days post-exercise.

Statistical analysis

Intra-class correlation (r), coefficient of variation (CV) and standard error of measurements (SEMs) were used to determine the test–retest reliability of the MVC torque, ROM, VAS and PPT measurements on two different days (1 day prior to and immediately before the eccentric exercise). The test–retest reliability of EPT measurements was also assessed on two different days (1 day prior to and immediately before the eccentric exercise) and on the same day separated by 1 h at 1 day prior to the eccentric exercise and at 2 days post-exercise when muscle soreness was peaked using intra-class correlation (r), CV and SEMs. Two-way repeated measures ANOVA was used to compare the first (ECC1) and second (ECC2) bouts for the changes in the muscle damage markers (MVC, ROM, VAS and PPT) over time (before, immediately after, 1–5 days after exercise). Changes in EPT over time (1 day prior to, immediately before and after, 1, 2 and 4 days after exercise) were also compared between ECC1 and ECC2 by two-way repeated measures ANOVA. Two-way repeated measures ANOVA was also used to compare the changes in the EPT between regions (biceps brachii fascia, muscle and brachialis fascia) for each bout separately. When the ANOVA showed significant interaction or time effects, a Tukey's post hoc test was used for multiple comparisons. Pearson's product moment correlation coefficients were computed between the changes in EPT and VAS, EPT and PPT, and VAS and PPT. Statistical significance was set at $P < 0.05$, and all data were presented as mean ± standard deviation (SD).

Results

Reliability of MVC torque, ROM, VAS, PPT and EPT measurement

The intra-class correlation (r) values were 0.91 for MVC torque, 0.98 for ROM, 0.98 for VAS and 0.92 for PPT, and CV for each measure was 5.9, 0.5, 2.2 and 3.3 %, respectively.

Table 1 The test–retest reliability of the EPT measurements indicated by intra-class correlation (r), coefficient of variation (CV), and standard error of measurement (SEM) for two different days for the baseline measures (1 day and immediately before exercise), two different time points separated by 1 h at 1 day before exercise, and the two time points separated by 1 h at 2 days after eccentric exercise (post-Ex)

Time	Region	r	CV (%)	SEM (mA)
Baseline, between-days	BBF	0.96	4.3	0.05
	M	0.98	2.7	0.04
	BF	0.99	2.7	0.03
Baseline, within-day (1 h)	BBF	0.94	5.0	0.06
	M	0.96	4.6	0.06
	BF	0.99	1.4	0.02
2 days post-Ex, within-day (1 h)	BBF	0.97	3.2	0.03
	M	0.93	5.5	0.05
	BF	0.98	2.1	0.02

The test–retest reliability of the EPT measures is shown in Table 1. The intra-class correlation (r) ranged from 0.96–0.99 for two different days for the baseline measures, 0.94–0.99 for two time points separated by 1 h at 1 day before exercise, and 0.93–0.98 for the two time points separated by 1 h at 2 days after eccentric exercise. CV ranged 2.7–4.3 % for two different days for the baseline measures, 1.3–4.6 % for two different time points separated by 1 h at 1 day before exercise, and 2.1–5.5 % for the two time points separated by 1 h at 2 days post-exercise. SEMs ranged from 0.03 to 0.05 mA for two different days for the baseline measures, 0.02–0.06 mA for two different time points separated by 1 h at 1 day before exercise, and 0.02–0.05 mA for the two time points separated by 1 h at 2 days after eccentric exercise.

Eccentric exercise

No significant differences in the changes in peak torque ($F = 0.57$, $P = 0.38$) and total work ($F = 0.45$, $P = 0.92$) over the ten sets of six eccentric contractions were evident between the first and second bouts.

Muscle damage markers

There were no significant differences in the pre-exercise values between bouts; baseline MVC torque was 50.6 ± 8.1 Nm for ECC1 and 49.5 ± 7.9 Nm for ECC2, ROM was 140.5° ± 4.5° for ECC1 and 140.1° ± 4.7° for ECC2, VAS was 0 cm (no pain) for both bouts, and PPT was 418.6 ± 45.6 kPa for ECC1 and 425.9 ± 45.6 kPa for ECC2. MVC torque decreased significantly ($P < 0.05$) immediately after exercise by approximately 60 % for both bouts, but recovered significantly faster ($F = 25.12$, $P = 0.01$) after ECC2 when compared with ECC1 (Fig. 2a). ROM

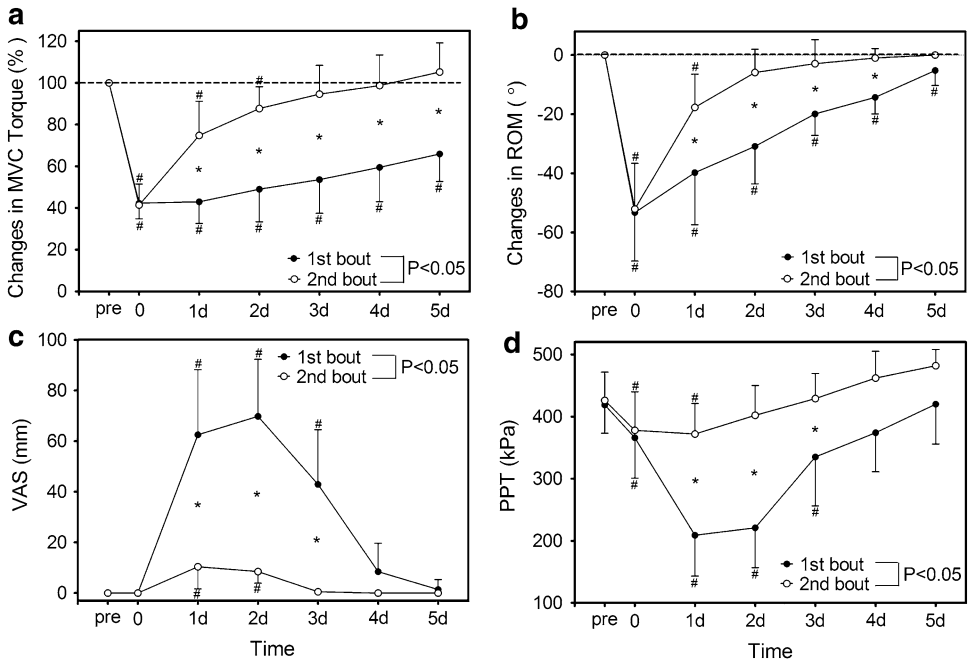

Fig. 2 Changes in maximal voluntary isometric contraction torque (**a**), range of motion (**b**), muscle soreness using a visual analogue scale (**c**), and pressure pain threshold (**d**) before (pre), immediately after (0), and 1–5 days after the first and second eccentric exercise bouts. A significant ($P < 0.05$) interaction effect is shown for all variables. *Asterisk* indicates a significant ($P < 0.05$) difference between bouts. *Hash* indicates a significant different from pre-exercise value

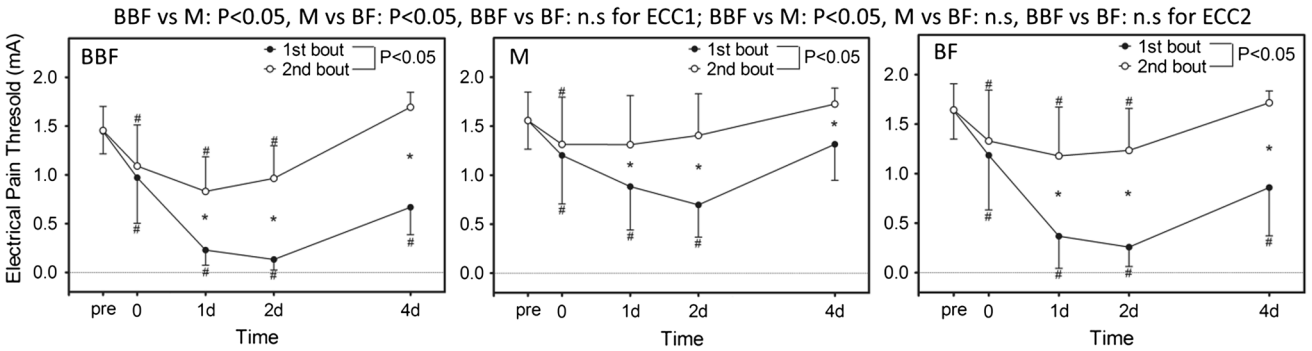

Fig. 3 Changes in electrical pain threshold at biceps brachii fascia (BBF), biceps brachii muscle (M) and brachialis fascia (BF) before (pre), immediately after (0), and 1, 2 and 4 days after the first and second eccentric exercise bouts. A significant ($P < 0.05$) interaction effect is shown for all locations. *Asterisk* indicates a significant ($P < 0.05$) difference between bouts. A significant ($P < 0.05$) difference was found between biceps brachii fascia (BBF) and muscle (M); brachialis fascia (BF) and muscle (M); however, no significant difference was found between BBF and BF. *Hash* indicates a significant ($P < 0.05$) difference from pre-exercise value

also decreased similarly between bouts immediately after exercise, with recovery being significantly ($F = 14.36$, $P = 0.02$) faster after ECC2 than ECC1 (Fig. 2b). VAS for muscle soreness increased significantly after both bouts; however, the magnitude of muscle soreness was significantly less ($F = 24.09$, $P = 0.01$) after ECC2 than ECC1 (Fig. 2c). PPT decreased significantly after both bouts, but the magnitude of the decrease was significantly smaller ($F = 20.15$, $P = 0.01$) after ECC2 than ECC1 (Fig. 2d).

EPT

Figure 3 shows changes in EPT of BBF, muscle and BF after ECC1 and ECC2. EPT decreased significantly ($P < 0.05$) after both bouts; however, the changes were greater ($F = 8.92$–28.43, $P = 0.01$) after ECC1 than ECC2 for the three regions. EPT decreased ($P < 0.05$) immediately after exercise and decreased further at 1–2 days after exercise for BBF (86–92 %), muscle (45–57 %) and BF (80–86 %), and remained significantly below baseline at 4 days post-ECC1. After ECC2, EPT decreased ($P < 0.05$) immediately after exercise, but did not show further large decreases and returned to the baseline at 4 days after exercise. When comparing the three regions, the magnitude of the decrease was significantly greater ($F = 5.07$–5.19, $P = 0.02$) for both BBF and BF (54–92 %) than muscle (16–57 %) at 1, 2 and 4 days post-ECC1, without a significant difference between BBF and BF. After ECC2,

the magnitude of the decrease was significantly greater ($F = 5.03$, $P = 0.02$) for BBF than muscle, but no significant difference was found between BF and muscle ($F = 2.36$, $P = 0.07$).

Correlation between EPT and VAS, EPT and PPT, and VAS and PPT

The correlations between EPT and VAS, EPT and PPT, and VAS and PPT measures are shown in Table 2. No significant correlation was found between the changes in EPT and VAS for all regions ($r = -0.01$ to 0.50) at 1, 2 and 4 days post-exercise. A significant correlation (0.63–0.87, $P < 0.05$) was found between the changes in EPT of BBF and PPT, and EPT of BF and PPT at 1 and 2 days post-exercise; however, no significant correlation was found at 4 days post-exercise. When combining all time points (1, 2 and 4 days), or taking the average between 1 and 2 days post-exercise, the results were basically the same as those using each time point such that EPT was significantly correlated with PPT ($r = 0.54$–0.82), but not with the VAS ($r = 0.36$–0.49). Furthermore, no significant correlation was found between the changes in VAS and PPT at any time points.

Table 2 Correlations between percent changes in EPT and VAS, and EPT and PPT for three regions (*BBF* biceps brachii fascia, *M* muscle, and *BF* brachialis fascia) at 1, 2 and 4 days after the first bout of eccentric exercise. Correlation between percent changes in VAS and PPT at 9 cm above the elbow crease at 1, 2 and 4 days after the first eccentric exercise of the elbow flexors is also shown

	Time	Region	*r*
EPT and VAS	1 day	BBF	−0.39
		M	−0.01
		BF	−0.42
	2 days	BBF	−0.19
		M	−0.13
		BF	−0.51
	4 days	BBF	−0.35
		M	−0.04
		BF	−0.27
EPT and PPT	1 day	BBF	0.87*
		M	0.02
		BF	0.74*
	2 days	BBF	0.64*
		M	0.05
		BF	0.63*
	4 days	BBF	0.36
		M	0.14
		BF	0.33
VAS and PPT	1 day	9 cm	−0.21
	2 days	9 cm	−0.34
	4 days	9 cm	−0.04

Asterisk indicates a significant ($P < 0.05$) correlation

Discussion

The present study tested the hypotheses that (1) EPT assessment would be reliable to access muscle pain sensitivity in normal condition and also when DOMS existed; (2) fascia would become more sensitive to electrical stimulation (i.e. more painful) than muscle after eccentric exercise, and the change would be greater after the first than second eccentric exercise bout; and (3) EPT would be significantly correlated with VAS and PPT. The indirect markers of muscle damage (MVC, ROM, VAS and PPT) indicated that the magnitude of muscle damage was less and the recovery was faster after the second than first eccentric exercise bout (Fig. 2), indicating a typical repeated bout effect. The magnitude of decrease in EPT was greater for biceps brachii fascia and brachialis fascia when compared with biceps brachii muscle (between the two fascias) after the first and second eccentric exercise bouts, and was smaller for the second bout than the first bout (Fig. 3). These results, therefore, supported the first two hypotheses. It should be noted that the changes in EPT at the fascia were significantly correlated with the changes in PPT, but a statistically significant correlation was not observed between the changes in EPT and VAS. This did not support the third hypothesis and suggested that the pain threshold assessed by EPT was different from the magnitude of pain expressed by VAS.

In the present study, EPT was not assessed at the same time points as those of VAS and PPT, because it was possible that the invasiveness of the measurement could damage muscle fibres and connective tissue, affecting pain sensation. The depth of the needle insertion was confirmed using B-mode ultrasonography before the measurements, thus we are confident that the tip of the needle was located precisely at the target for each measurement. However, it might be that the needle inserted into the "muscle" affected a mix of muscle fibres and the connective tissues surrounding the muscle fibres (endomysium) and fascicles (perimysium). Since group III and IV afferents are predominantly located in the perimysium surrounding larger or smaller muscle fibre bundles (Von Düring and Andres 1990), the EPT of the "muscle" affected these nociceptors. This may explain the significant decreases in "muscle" on EPT measurements after eccentric exercise (Fig. 3). Thus, it is important to note that the exact location of the needle in "muscle" is not known in the EPT assessment.

The test–retest reliability of EPT measurements between days and within days was high. However, it should be noted that a large inter-individual variability in EPT was evident.

To the best of our knowledge, this was the first study to investigate the changes in EPT at fascia and muscle of biceps brachii and brachialis. It is interesting that there was no significant difference in EPT between fascia and muscle before exercise as it was assumed that fascia would be more sensitive than muscle at baseline. The rationale for this hypothesis was based on the fact that nociceptors are considered to be more numerous in fascia than muscle (Stacey 1969; Mense and Simons 2001). It is possible that the muscle thin-fibre sensory receptors are activated when damage and/or inflammation are induced. This might explain why the difference in EPT between fascia and muscle was only evident after exercise. Murase et al. (2013) have shown elegantly that bradykinin triggers up-regulation of nerve growth factor (NGF) in EDL muscle of rat after eccentric contractions, which sensitises nociceptors and up-regulation of glial cell line-derived neurotrophic factor (GDNF) through cyclooxygenase 2 (COX-2) activation is essential to the muscular hyperalgesia after eccentric exercise. Ota et al. (2013) showed that transient receptor potential (TRP) ion channels were involved in DOMS using a mouse model, and found that TRPV1 was involved in up-regulation of NGF and GDNF, and TRPV4 was also involved in NGF and possibly GDNF up-regulation. It is interesting to investigate further how TRPV1 and TRPV4 are activated by eccentric contractions.

Itoh et al. (2004) measured EPT at the skin, fascia and muscle at 2 and 7 days after eccentric exercise of the middle (3rd) finger, and reported that EPT was 0.39–0.82 mA lower in the fascia compared with muscle and skin 2 days post-exercise, and suggested that the sensitised nociceptors at the fascia were responsible for DOMS. The results of the present study also showed that both biceps brachii and brachialis fascia became more sensitive to electrical stimulation-induced pain than the biceps brachii muscle (Fig. 3). This finding is consistent with previous studies showing that fascia (Gibson et al. 2009) and other connective tissues such as tendon/tendon–bone junction (Gibson et al. 2006) were more sensitive to hypertonic saline injection compared with muscle belly following eccentric contractions. Gibson et al. (2006) investigated the pain threshold sensitivity at the tendon, tendon–bone junction and muscle belly sites of the tibialis anterior muscle after three sets of ten eccentric dorsiflexor contractions, and reported that both the tibialis anterior tendon and tendon–bone junction became more sensitive to hypertonic saline injection compared with muscle tissue when assessed by visual analogue scale (VAS) and pressure pain threshold (PPT). In their subsequent study, Gibson et al. (2009) examined fascia and deep muscle sensitivities by hypertonic saline injection in tibialis anterior following three sets of ten eccentric contractions and found that fascia rather than muscle tissue was more sensitive to these saline injections at 2 days post-exercise when DOMS was prevalent. They suggested that the higher pain sensitivity found in the fascia reflected fascial/epimysium receptor sensitisation, and concluded that fascia rather than muscle tissue was important in DOMS-associated sensitisation.

It has been documented that damage and inflammation to connective tissue surrounding muscle fibres are responsible for DOMS (Cheung et al. 2003; Howatson et al. 2007). Paulsen et al. (2010) found a negative correlation between DOMS and leukocyte accumulation in inflamed muscle fibres after 300 eccentric quadriceps femoris contractions and concluded that damage and remodelling of the extracellular matrix (ECM) were associated with DOMS. Raastad et al. (2010) showed that tenascin C and N-terminal propeptide of procollagen type III increased staining in the endomysium of the exercised muscle after performance of the same exercise and concluded that the ECM was affected. Crameri et al. (2007) compared muscle damage between 210 maximal eccentric contractions with electrical muscle stimulation (EMS) and 210 voluntary maximal eccentric contractions of the knee extensors, and found similar increases in the staining of tenascin C after EMS-induced and voluntary contractions, although muscle fibre damage was evident only after EMS. Thus, it is possible that that damage and inflammation occurred in the biceps brachii and brachialis fascia during and/or after the eccentric contractions in the present study. However, further studies are necessary to explicitly examine the histological changes in fascia after eccentric exercise.

It has been documented that the free nerve endings (nociceptors) are located along the walls of arteries and mainly in the surrounding connective tissue (Stacey 1969; Mense 1993), and the density of nociceptors is different between connective tissue and muscle fibres (Andres et al. 1985; Mense and Simons 2001). Mense and Simons (2001) reported that the innervation density of nociceptors in the connective tissue surrounding the calcaneal tendon of the cat was approximately five times higher than in the gastrocnemius–soleus muscle but no difference was found in innervation density throughout normal muscle tissue. Tesarz et al. (2011) examined the density and distribution of nerve fibres in rats as well as human thoracolumbar fascia using immunohistological techniques, and reported that muscle fascia was densely innervated with PGP9.5-positive non-peptidergic nerve fibre endings and encapsulated mechanoreceptors. Deising et al. (2012) reported that the nociceptors in the fascia were sensitised and activated following nerve growth factor (NGF) injection to erector spinae at lumbar level (L4–L5), and suggested that the nociceptors in the fascia were particularly prone to sensitisation and this might contribute to acute or chronic muscle pain. Thus, it seems possible that damage to the connective tissues following eccentric contractions results in the activation of more nociceptors (increasing

peripheral sensitisation) in the fascia, releasing sensitised noxious chemical substances through the axon reflex (neurogenic inflammation) in the damaged region and, therefore, enhancing temporal summation of nociceptive input (increasing central sensitisation) to the spinal cord at the dorsal horn, in turn increasing the pain response to electrical stimuli at the fascia and inducing DOMS.

The time courses of changes in the VAS, PPT and EPT were different following eccentric exercise, such that muscle soreness assessed by VAS peaked 2 days post-exercise but the reduction of PPT was greatest at 1 day post-exercise, and both measures (VAS and PPT) returned to the baseline by 4 days post-exercise. However, the reduction in EPT was greatest at 2 days post-exercise in the fascia and remained below the baseline at 4 days after exercise. This discrepancy in the time course changes between EPT and VAS/PPT suggests that the events underlying the changes in EPT and VAS/PPT may not be the same. It has been reported that mild DOMS can occur with little or no changes in PPT (Umbel et al. 2009). Since myofibrillar remodelling has been observed at 7–8 days after eccentric exercise (Yu et al. 2004), it is plausible that the changes in EPT reflect remodelling processes of muscle fibres and their surrounding tissue rather than DOMS per se. No significant correlations between EPT and VAS, and between PPT and VAS suggest that the magnitude of pain is different from the threshold of pain. Lau et al. (2013) reported no significant correlation between VAS and PPT, and have stated that PPT measures the minimum pressure to induce pain, while VAS is a supra-threshold test to assess the magnitude of pain in response to a standardised stimuli (e.g. palpation, stretch). It should be noted that PPT and EPT are based on pain thresholds despite the stimulation method being different (pressure vs. intramuscular electrical stimulation). This could explain the significant correlation between the two.

In the present study, the first eccentric exercise bout resulted in severe muscle damage, since MVC torque was still 40 % lower than the baseline at 4 days post-exercise, and the magnitude of DOMS assessed by VAS was also high (>60/100 mm). This type of maximal eccentric exercise protocol has been demonstrated to lead to severe muscle fibre damage indicated by large increases in CK activity and/or myoglobin concentration in the blood and muscle fibre necrosis (Lauritzen et al. 2009; Paulsen et al. 2009). It is also likely that extracellular matrix disruption is induced after this type of exercise (Stauber et al. 1990). Therefore, the question arises as to how representative the findings of the present study are to eccentric exercise in general. The present study showed that the changes in EPT were small after the second bout of eccentric exercise, resulting in less damage than the first bout. Thus, it is assumed that if muscle damage is minor, changes in EPT are also small even for other muscles and other exercise protocols. Further studies are necessary to investigate this, and whether how sensitive EPT is in comparison to VAS or PPT to assess DOMS.

In conclusion, the present results showed that the magnitude of EPT decreased after eccentric exercise, but the decrease was greater after the first than the second exercise bout. The magnitude of decrease in EPT was greater for biceps brachii and brachialis fascia than muscle. Changes in EPT were correlated with the changes in PPT but not the VAS assessments. These results suggest that DOMS is more closely associated with the increased sensitivity of fascia than muscle.

Conflict of interest The authors declare that we have no conflict of interest.

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