Exp Brain Res (2009) 194:299–308
DOI 10.1007/s00221-008-1699-8

RESEARCH ARTICLE

Increased pain from muscle fascia following eccentric exercise: animal and human findings

William Gibson · Lars Arendt-Nielsen · Toru Taguchi · Kazue Mizumura · Thomas Graven-Nielsen

Received: 20 November 2006 / Accepted: 23 December 2008 / Published online: 21 January 2009

Abstract Mechanisms and structures which are involved in eccentric exercise-induced delayed onset muscle soreness (DOMS) are not yet clarified. Tissue and site specificity may be important considerations in afferent sensitisation following eccentric exercise. This study investigated the nociceptive response to hypertonic sodium solution applied to fascial/epimysium tissue and mechanically sensitised sites in muscle by assessing (1) afferent recordings in animals and (2) psychophysical assessment in humans. Seventeen male rats underwent eccentric contraction of extensor digitorum longus muscle, while 11 rats served as an unexercised naïve group. Two days post-exercise, group IV afferent fibre activity was recorded in response to superfusion of hypertonic Krebs solution on the mechanically sensitised muscle/epimysium site. Mechanical sensitisation was confirmed with significant increases in afferent response and decreases in threshold to mechanical stimulation in the eccentrically exercised rats compared to naïve rats. There was no difference in afferent response magnitude to hypertonic Krebs solution between exercise and naïve groups. In the human study, 13 volunteers participated. After bilateral assessment of pressure pain thresholds (PPT) along the tibialis anterior muscles, eccentric exercise was performed to induce DOMS in m. tibialis anterior of one leg. Site of maximal mechanical sensitivity was identified 24 h later and injected with hypertonic saline at fascial and deep muscle levels. The corresponding site on the opposite unexercised leg served as a control. Fascial injection of the exercised muscle caused significantly higher pain intensity compared to all other injections. Response to deep muscle stimulation was not different between sides. This suggests that fascia rather than muscle tissue is important in DOMS associated sensitisation.

Keywords Experimental pain · Deep tissue · Hypertonic saline · Fascia · Eccentric exercise · DOMS

W. Gibson · L. Arendt-Nielsen · T. Graven-Nielsen (✉)
Laboratory for Experimental Pain Research,
Department of Health Science and Technology,
Center for Sensory-Motor Interaction (SMI), Aalborg University,
Fredrik Bajers Vej 7D-3, 9220 Aalborg, Denmark
e-mail: tgn@hst.aau.dk

W. Gibson
e-mail: w.gibson@curtin.edu.au

T. Taguchi · K. Mizumura
Department of Neuroscience,
Division of Stress Recognition and Response,
Research Institute of Environmental Medicine,
Nagoya University, Nagoya, Japan

W. Gibson · T. Graven-Nielsen
School of Physiotherapy, Curtin University of Technology,
Bentley, Perth, WA 6845, Australia

Introduction

Eccentric contraction of muscle tissue frequently causes delayed onset muscle soreness (DOMS) in humans (Slater et al. 2005; Bajaj et al. 2000; Prasartwuth et al. 2005; Gibson et al. 2006). Recently, increased group IV afferent fibre activity to mechanical muscle stimulation has been demonstrated 2 days after eccentric exercise in animals (Taguchi et al. 2005a, b). This appears to be the animal correlate of human DOMS.

Hypertonic saline is frequently used to excite nociceptors in deep tissues (Graven-Nielsen 2006; Bennell et al. 2004) and in particular, experimental pain in muscle tissue has been investigated extensively (Slater et al. 2003;

Graven-Nielsen et al. 1998; Hodges et al. 2003). However, fascial tissue response to hypertonic saline remains relatively unexplored (Kellgren 1938), although fascial tissue is known to be highly sensitive in human and animal investigations (Itoh and Kawakita 2002; Kawakita et al. 1991; Itoh et al. 2004). Previous studies examining response to intramuscular hypertonic saline injection during DOMS have shown no increase in pain intensity (Slater et al. 2005; Weerakkody et al. 2003; Gibson et al. 2006) compared with unexercised muscle. These studies have, however, not specifically been injecting the site with maximal DOMS effect or targeting the tissue causing the DOMS.

The response of muscle tissue to eccentric exercise particularly involves the cytoskeletal proteins desmin and actin (Yu and Thornell 2002; Yu et al. 2002; Lieber et al. 2002), as well as causing sarcomere disruption (Morgan and Proske 2004; Friden and Lieber 1998) with resulting sarcomerogenesis/myofibrillar remodelling (Yu and Thornell 2002; Butterfield and Herzog 2006). It is speculated that DOMS may be an event occurring in various tissues including epimysium/fascia rather than muscle tissue proper (Malm et al. 2004; Birtles et al. 2003). This is in line with facilitated flexor-withdrawal response to electrical stimulation found specifically at the level of the fascia in rabbit gastrocnemius muscle 48 h post-eccentric exercise (Itoh and Kawakita 2002). Markedly, increased sensitivity to electrical stimulation in fascial tissue has been shown in humans following eccentric exercise (Itoh et al. 2004).

The current study examined fascial and deep muscle tissue pain/afferent activity in response to hypertonic sodium solution applied to mechanically hyperalgesic sites post-eccentric exercise. It is acknowledged that eccentric exercise-induced mechanical hyperalgesia is not mediated purely peripherally but also involves centrally facilitated pain mechanisms (Bajaj et al. 2000; Weerakkody et al. 2001; Gibson et al. 2006; Barlas et al. 2000; Weerakkody et al. 2003). This could not be assessed in the animal study component of this paper; however, the human study may be reflective of the combined effect of peripheral and central sensitisation.

The following hypotheses were investigated: (1) hypertonic sodium solution applied to mechanically sensitised (eccentric exercise) receptive fields in rat muscle will cause greater group IV nociceptive afferent activity compared to unexercised muscle. (2) Hypertonic saline injection into fascial/epimysium tissue of maximally sensitive sites in DOMS affected muscle in humans will result in higher pain intensity, compared to fascial/epimysium tissue of an unexercised muscle and higher pain intensity, compared to muscle belly (deep) injection at the same site in the DOMS muscle.

Methods

Animal study

Twenty-eight male Sprague–Dawley rats (SLC Inc., Japan) weighing 335–440 g (10–13 weeks) were used. One group of animals ($n = 17$) received eccentric contractions 2 days before single fibre recording, and the other received no treatment ($n = 11$) and served as the naïve group. The animals were kept 1–3 per cage under a 12 h light/dark cycle in an air-conditioned room (22–24°C). Food and water were available ad libitum. All experimental procedures were approved by the Animal Care Committee, Nagoya University, and all animal experiments were done at Nagoya University.

Exercise protocol

The animals received eccentric contractions under anaesthesia with pentobarbital sodium (50 mg/kg, intra-peritoneally). Rectal temperature was kept in the physiological range (37–38°C) with a heating pad during the exercise period. The exercise protocol was the same as previously described by Taguchi et al. (2005a). Briefly, a pair of needle-electrodes, insulated except for the tips, were transcutaneously inserted near the common peroneal nerve that innervates the extensor digitorum longus (EDL) muscle. Correct location of the needles was assured by dorsiflexion of the ankle joint and extension of the toes upon electrical stimulation of the common peroneal nerve. Repetitive contraction of the EDL muscle was induced by electrical stimuli applied to the common peroneal nerve. Parameters of electrical stimulation were as follows: constant current intensity three times the twitch threshold, 50 Hz, 1 ms duration, and stimulus period of 1 s. The foot of the same side was plantar-flexed with a servomotor to stretch the EDL muscle in synchrony with electrical stimulation of the nerve over a 1 s period and then returned to the starting position over a 3 s period. This pattern was repeated every 4 s for a total of 500 repetitions. After the rats recovered from anaesthesia following exercise, they survived until single-fibre recording experiments were done.

Electrophysiology

The EDL muscle with the common peroneal nerve remaining attached was carefully excised under pentobarbital anaesthesia (50 mg/kg, i.p.), 2 days after eccentric-lengthening. The EDL muscle obtained from animals with no treatment served as the naïve group. One preparation was taken from each animal *except* for naïve animals in which muscle of *both* sides were used, and one nerve fibre was

recorded from each preparation. Animals were killed with an overdose of the anaesthetic after dissection of the preparation. The isolated preparation was placed in an organ bath, similar to the one used for the skin–nerve preparation reported by Reeh (1986), with the proximal and distal ends of the EDL muscle pinned in the test chamber. The preparation was maintained at 34.0 ± 0.5°C (pH 7.4) under superfusion with Krebs–Henseleit solution (Krebs solution), which contained (in mM) 110.9 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 $NaHCO_3$, and 20.0 glucose and the perfusate was continuously bubbled and equilibrated with a gas mixture of 95% O_2 and 5% CO_2. The common peroneal nerve was drawn through a hole to the recording chamber which was filled with paraffin-oil and single nerve fibre activities were obtained by dissection method. Action potentials were amplified, filtered, displayed on an oscilloscope (Fig. 1d) and continuously recorded on magnetic tapes (for off-line analysis). Recordings were analysed on a computer with an analogue–digital converter and a SPIKE/SPIDI software package (Forster C., University of Erlangen-Nurnberg, Germany). Muscle thin-fibre sensory receptors that fulfilled the following criteria were used in this study: (1) sensitive to mechanical stimulation from probing the EDL muscle with a glass rod. (2) No intensity-dependent increase in the discharge rate, while the muscle was stretched by a length of a few millimetres. (3) Conduction velocity slower than 2.0 m/s (i.e. group IV). The conduction velocity of the fibres was calculated from the distance and conduction latency between the recording and the stimulating electrodes (≤50 V, 100–500 μs duration), the latter being placed on the receptive field.

Spontaneous activity of a fibre was calculated during a 60 s period. A ramp-mechanical stimulus, linearly increasing from 0 to 196 mN in 10 s, was applied with a servo-controlled mechanical stimulator (manufactured by Aizawa S., Goto College of Medical Arts and Science, Tokyo, Japan, tip area 2.28 mm^2) to the most sensitive point of the identified receptive field. The mechanical threshold was defined as the intensity that induced a discharge that exceeded the mean frequency plus 2 standard deviations (SD) of the spontaneous discharges without stimulation. The mechanical response magnitude was defined as the total number of evoked discharges during the ramp-mechanical stimulus application period. Investigators were not blind as to muscle group (eccentrically exercised versus unexercised).

Hypertonic Krebs solution containing 734.1 mM Na was superfused (5 ml over 30 s) to the identified receptive fields through a 3 mm diameter tube, with the opening being placed in very close proximity to the identified receptive field (Taguchi et al. 2005a).

Human experiment

Thirteen subjects participated (6 females and 7 males). Mean age was 25 with a range of 22–34 years. All subjects were healthy and not suffering from any present or ongoing lower leg pain or musculoskeletal dysfunction. Subjects were given a detailed verbal explanation and signed an informed consent form. The study was approved by the local ethics committee (VN 2006/0004) and conducted according to the declaration of Helsinki. All human experiments were conducted in Aalborg, Denmark.

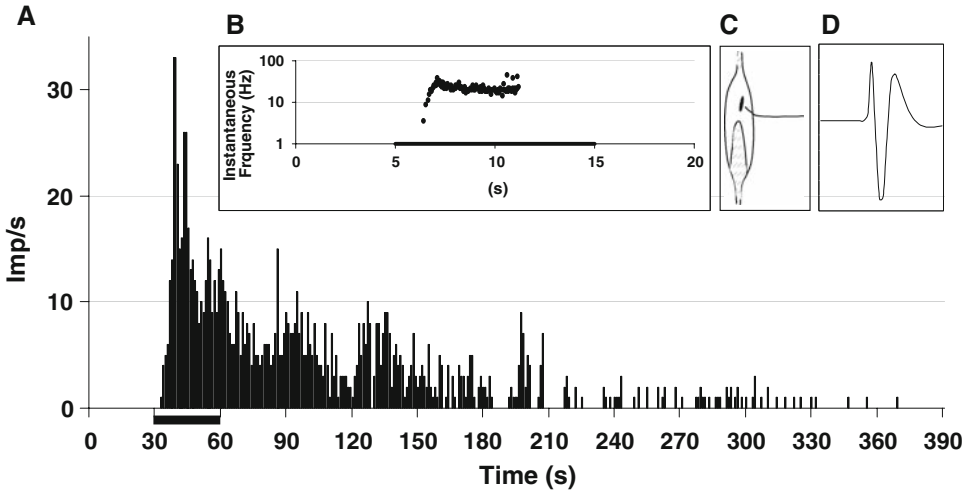

Fig. 1 **a** Response pattern of rat group IV afferent from eccentrically exercised muscle preparation to hypertonic Krebs solution (734 mM) is shown. Hypertonic Krebs solution was superfused to the identified receptive field for 30 s (shown with *thick bar* on the abscissa). **b** Mechanical response of this fibre is shown with an instantaneous *frequency plot*. A ramp shaped mechanical stimulation, linearly increasing from 0 to 196 mN in 10 s, was applied (*thick bar* on the abscissa). **c** Receptive field of this fibre (*solid*) was located on the front side on this EDL muscle. Upper tendon is toward proximal end. *Shading* shows the tendinous area. **d** Recorded spike form of the receptor

Experimental protocol

The experiment consisted of two experimental days separated by 24 h. DOMS was induced on day 1 with a period of eccentric exercise. Pressure pain thresholds (PPTs) along the muscle length (experimental and control muscles) were recorded on day 1 and then again on day 2. Maximum voluntary contraction (MVC) to dorsiflexion was assessed in the experimental muscle on day 1 pre- and post-eccentric exercise. Hypertonic saline injection was given to the maximally mechanically sensitive site on day 2 (identified by maximum PPT decreases from day 1) at both deep muscle and fascial depths in the experimental leg. The same injection procedure was repeated in the control leg at the anatomically corresponding site. Injection sequence was randomised as was the selection (right or left legs) of DOMS/control legs.

Pressure pain sensitivity and injection site selection

Pressure pain thresholds in both legs were measured prior to eccentric exercise on day 1 and prior to hypertonic saline injections on day 2. PPTs were assessed at eight sites in each tibialis anterior muscle with the subject in a half-supine position. Sites were spaced at 2 cm intervals beginning at the distal musculotendinous junction and covering the muscle belly length. Each PPT measure was repeated three times at all sites on each-day. Averages of these values were used for further analysis. PPT was defined to the subject as "the point at which the pressure sensation just becomes painful". Pressure pain thresholds were measured by a computer-controlled pressure algometer utilizing step motors (Aalborg University, Denmark) that controlled the probe in the horizontal plane. The pressure was delivered at a constant rate (0.6 kg/s, 58.4 kPa/s) by an actuator. The round, flat algometer rubber tip had an area of 1 cm^2. The software controlling the system randomised the assessment site order. It was ensured that the algometer tip approached the stimulation site perpendicular to the target tissue. For each subject, the average values of day 1 PPT assessment were compared with the average day 2 values and the site of greatest reduction indicated the site of maximal sensitivity. This area of the muscle (and the anatomically corresponding site of the control leg muscle) was then used as the injection site for the hypertonic saline.

Delayed onset muscle soreness (DOMS)

Prior to and immediately after the eccentric exercise period, the MVC to dorsiflexion was recorded with the use of a footplate/torque transducer (Aalborg University, Denmark). The subject was seated with the ankle secured to the footplate at 90°. The seat was positioned such that the thigh was horizontal to the floor. Each MVC was repeated three times with 1 min of rest intervals. The maximal recorded value of the three MVCs was used for further comparison. The method of DOMS-induction in tibialis anterior muscle has been described in a previous work (Gibson et al. 2006b). In short, the subject stood on a 13 cm-high metal platform placed approximately 45 cm from a wall. Subjects stood with the heel of the experimental leg on the edge of the platform with the mid and forefoot extending over the edge, resting on a metal footplate. The footplate was attached to the platform with a hinge, allowing free plantar and dorsiflexion. The palms of the subject's hands were placed flat on the wall at shoulder level for support only. Subjects lifted the non-experimental leg off the platform by flexing at the hip and knee, transferring to single leg stance on the experimental leg side. The subject then performed a slow plantar flexion of the foot and ankle (requiring controlled eccentric-lengthening of tibialis anterior) allowing the forefoot to descend until the footplate contacted the floor. At this point, the non-experimental leg was extended until weight bearing and used to assist in returning the subject to the initial starting position. Subjects repeated this process ten times per set. Efficacy of eccentric activity was assessed by the subject heel-walking and performing a sustained dorsiflexion. Typically, three sets of ten contractions separated by 20 s rest were required. Poor performance of these tasks on the experimental side (defined as reduced dorsiflexion range, decreased duration and tremor) was assumed to be due to eccentric contraction induced fatigue/muscle damage. Eccentric contraction induced fatigue was verified by repeat MVC testing of dorsiflexion. Subjects were given a modified Likert scale diary to complete daily for the 7 days following exercise with instructions to complete it in morning and evening.

Saline-induced deep tissue pain

Sterile hypertonic (5.8%, 1 M) saline infusion was delivered via a computer-controlled syringe pump (Asena, Alaris Medical Systems, UK) with a 10 ml disposable syringe. This was connected to an extension tube (IVAC G30303) which was in turn connected to a disposable needle (27G, 20 mm). The saline was infused as a 0.5 ml bolus over 20 s. The injections were made at the identified sites mentioned above. For deep injections the needle was passed through the fascia to a typical depth of approximately 1.5 cm within the muscle tissue. For fascial injections the needle was initially inserted just into the subcutaneous tissue. The subject then performed a small dorsiflexion of the ankle. No oscillatory movement of the needle confirmed that the needle had not yet pierced the fascial tissue and was not being perturbed by contractile tissue. The needle was then advanced in small

increments and the dorsiflexion movement repeated. The point at which the needle moved in an oscillatory-fashion in synchrony with the dorsiflexion was deemed as the point at which the needle tip had just pierced the fascia. The needle was held in this position and the computer-controlled infusion started. Upon cessation of the injection, correct needle placement was confirmed by the subject after performing a very-small dorsiflexion and observing the needle oscillation in synchrony with the movement. The four injections were randomised in such a way that each site/depth of injection was balanced in terms of the order of injection sequence. An interval of 15 min without pain was kept between successive injections.

Assessment of saline induced pain intensity and distribution

Intensity of induced pain was recorded continuously on a 10 cm-long electronic visual analogue scale (VAS). The bottom of the scale was marked 'no pain' and the top marked 'maximum pain'. Subjects rated their pain using the VAS, from onset to resolution and the data was automatically sampled every 2 s and recorded for 900 s. Peak pain scores, and area under VAS-time graph were extracted. Upon resolution of pain the subject were filled in a bodychart marking along all areas of pain. This was later digitized (ACECAD D9000+, Taiwan) and the pain areas were estimated. Referred pain was defined as pain isolated and distinct from the local pain caused by injection.

Statistical analysis

In the animal studies; data of mechanical threshold, mechanical response magnitude and response magnitude to hypertonic saline are presented as median with interquartile range (IQR), and analysed using a Mann–Whitney *U* test between groups. All other data are expressed as mean and standard errors of the mean (SE). Normality tests were passed and parametric repeated measures ANOVA with 'leg' and 'injection depth' as factors was used in the analysis of VAS data. Analysis of MVC, 'DOMS leg' PPT and 'control leg' PPT data was accomplished using repeated measures ANOVA with 'pre-exercise', 'post-exercise' and site (PPT data only) as factors. Newman Keuls (NK) test as post-hoc was used when appropriate. Pre-and post-comparisons of MVC and PPT data were made with post-exercise changes expressed as percentage of baseline (pre) measures. Likert scale DOMS results were analysed using Friedman ANOVA and non-parametric NK post-hoc test. A P value less than 0.05 were accepted as the significance level.

Results

Afferent recordings

There was no significant difference between the unexercised and exercised muscles in terms of conduction velocity of fibres selected for recording: 0.58 ± 0.08 m/s versus 0.75 ± 0.12 m/s (naïve vs. eccentric). There was no significant difference in spontaneous discharge activity between naïve and eccentric groups: 0.19 ± 0.08 imp/s versus 0.09 ± 0.04 imp/s (naïve vs. eccentric).

Almost all fibres which are examined showed an increased discharge rate with the application of hypertonic Krebs solution (naïve: 14/14, 100%; eccentric: 16/17, 94.1%). The fibre in the eccentrically exercised preparation that did not respond to a 30 s application of hypertonic Krebs solution responded with a latency of 66 s when the application period was prolonged to 2 min, suggesting that the receptor terminal was located deep in the muscle. There was a large variation of the response magnitude among fibres, and one example of large response to hypertonic Krebs solution is shown in Fig. 1a. There was no significant difference in afferent nerve response magnitude between unexercised and eccentrically exercised muscles during the application period of hypertonic Krebs solution (30 s) and the subsequent 60 s (Fig. 2). Receptive fields of recorded fibres were distributed widely on the muscle with a tendency that more receptive fields were located near the musculo–tendinous junction especially in the eccentric exercise group (Fig. 3).

Typical response to ramp-mechanical stimulus is shown in Fig. 1b. Response threshold to ramp-mechanical stimulation in the eccentrically exercised muscles was significantly lower than that of unexercised muscles (Fig. 4a). Additionally, response magnitude to the ramp-mechanical stimulation in the eccentric group was significantly higher than the unexercised group (Fig. 4b).

Delayed onset muscle soreness

Immediately post-eccentric exercise, all subjects showed a significant decrease in maximum voluntary contraction force to dorsiflexion with an average reductions of $25.2 \pm 2.3\%$ (ANOVA $F_{1,12} = 124$, $P < 0.001$, NK $P < 0.002$). All subjects were experienced DOMS at 24 h post-exercise. Likert scores for day 1 and day 2 post-exercise (3.4 and 2.4, respectively) were significantly higher than pre-exercise (0) and 3, 4, 5, 6 and 7 days post-exercise (1.3, 0.6, 0.25, 0 and 0, respectively; Friedman ANOVA $P < 0.001$, NK $P < 0.05$). Sites of maximal sensitivity post-eccentric exercises were concentrated mainly around the mid muscle belly area (61% of subjects) although there was considerable individual variation (Fig. 5). The average PPT

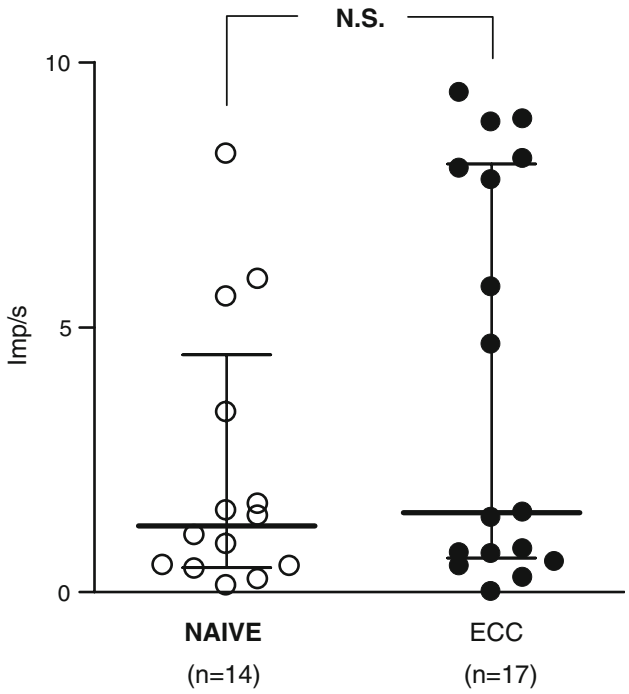

Fig. 2 Response magnitude to hypertonic saline. Median of the response magnitude (with IQR) given by mean afferent discharge rate during application period of hypertonic saline (30 s) + the subsequent 60 s. Naïve 1.25 imp/s (IQR; 0.46–4.48 imp/s) versus eccentric 1.50 imp/s (IQR; 0.65–8.08 imp/s), no significant difference, $P = 0.32$. *ECC* eccentric group, *NAÏVE* naïve group, *NS* not significant

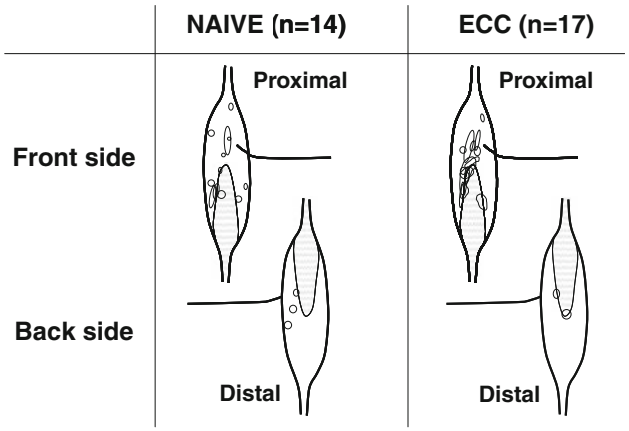

Fig. 3 Distribution of the receptive field of recorded receptors. *Front side* the side facing the tibialis anterior muscle, *back side* the opposite side. *Shading* shows the tendinous area. Each *spot* represents the receptive field of a group IV receptor, and its shape corresponds to the shape of the receptive field. *ECC* eccentric group, *NAÏVE* naïve group

compared to pre-eccentric exercise values at the most sensitive site was significantly decreased by, on an average $32 \pm 4\%$ (ANOVA $F_{1,12} = 74$, $P < 0.001$, NK $P < 0.002$). The opposite control leg site showed no significant reduction in PPT ($-7 \pm 6.4\%$). There was no significant difference in PPT values for the control leg between day 1 and day 2.

Hypertonic saline induced pain

Fascial-directed injection of the DOMS affected muscle caused significantly higher peak VAS scores and area under the VAS-time curve compared to all other injections (ANOVA $F_{1,12} > 4.7$, $P < 0.05$; NK $P < 0.006$; Fig. 6; Table 1). Fascial-directed injection of the control leg caused significantly higher peak VAS scores compared to deep injection of the control and DOMS leg (ANOVA $F_{1,12} = 5.3$, $P < 0.05$; NK $P < 0.05$; Fig. 6; Table 1). There was no significant difference in VAS parameters between experimental and control legs following deep muscle injection (Fig. 6; Table 1).

Injections caused referred pain to varying degrees. Deep muscle injection of the DOMS muscle caused the highest referred pain frequency (85%) followed by fascial-directed injection of the DOMS muscle (69%), fascial-directed injection of the unexercised muscle (62%), and deep injection of the unexercised muscle (54%). There was no significant difference in bodychart pain areas for each of the four injections.

Discussion

This study found unchanged C-fibre response magnitudes to hypertonic sodium solution superfused to receptive fields in rat EDL muscle that were demonstrably mechanically sensitised following a period of eccentric exercise (compared to unexercised muscle). The facilitated mechanical sensitivity of C-fibre receptors from exercised muscle confirms previous results (Taguchi et al. 2005a). In contrast, in humans it was found that fascial/epimysium targeted injection of hypertonic saline to the eccentrically exercised, DOMS affected muscle produced greater pain than injection into the deep muscle tissue of the same muscle site and greater pain than fascial/deep injections to the unexercised control muscle.

Mechanical hyperalgesia following eccentric exercise

Augmented mechanical sensitivity of group IV afferent fibres in rat, eccentrically exercised muscle tissue 2 days post-eccentric exercise, was found in this study. Further, spontaneous discharge in the eccentrically exercised muscle was not significantly increased compared to the unexercised muscle. This fits with human perception of DOMS. Typically, maximum mechanical sensitivity is apparent at 24–48 h post-exercise (Newham 1988; Whitehead et al. 2001) and at rest there is no spontaneous pain (Nie et al. 2005).

All human subjects reported DOMS 24 h post-exercise with significantly increased mechanical sensitivity. The

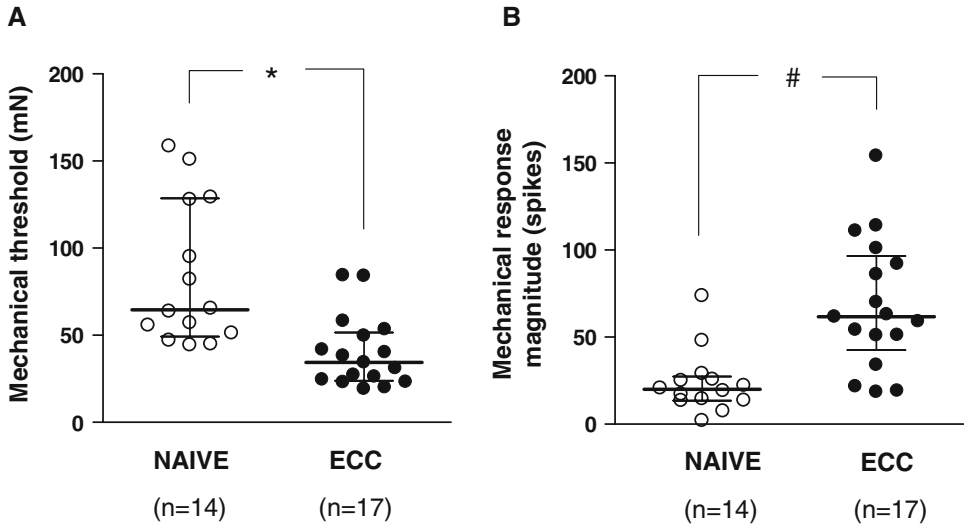

Fig. 4 Comparison between naïve (NAÏVE) and eccentric (ECC) rat EDL muscles of mechanical threshold and mechanical response magnitude to ramp-mechanical stimulation. **a** *: Mechanical threshold significantly lower in eccentric compared to naïve group. Eccentric 34.4 mN (IQR; 23.9–51.6 mN) versus naïve 64.6 mN (IQR; 49.2–128.6 mN), $P < 0.001$, non-parametric Mann–Whitney U test. **b** #: Mechanical response magnitude significantly higher in eccentric compared to naïve group. Eccentric 61.7 spikes (IQR; 42.5–96.5 spikes) versus naïve 20.0 spikes (IQR; 13.7–27.4 spikes), $P < 0.001$ non-parametric Mann–Whitney U test

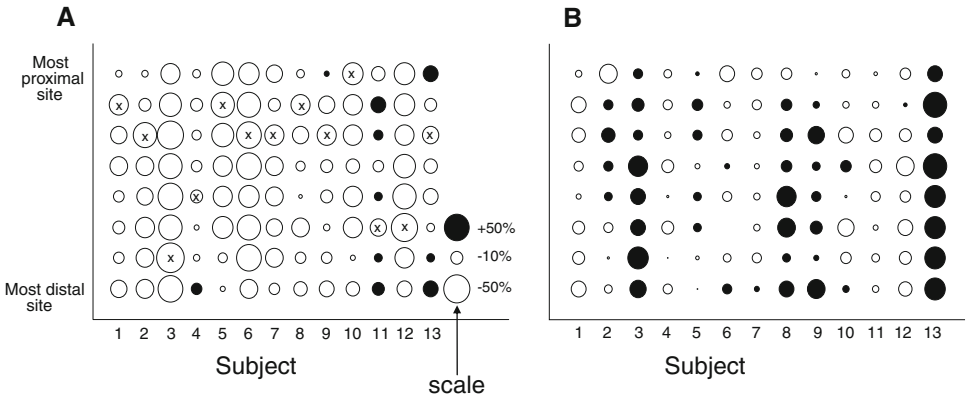

Fig. 5 Pressure pain thresholds (PPTs) day 2 (% of day 1). **a** Sites of maximal sensitivity along tibialis anterior muscle 24 h post-eccentric exercise in exercised (eccentric) leg. **b** PPTs in control leg. *Open circles* represent PPT reductions, *filled circles* represent PPT increases. *Size of circle* represents size of percentage change of PPT. × Highlights site of greatest reduction in PPT; i.e. the most sensitive site (only shown in the exercised leg)

location of sites of maximal mechanical sensitivity showed some variability between subjects. This is reflective of previous findings in DOMS, describing a non-uniform, variable pattern of increased sensitivity along the muscle (Andersen et al. 2005; Weerakkody et al. 2003). The unchanged PPT values for the unexercised control leg reinforce the argument that there was no 'spread' of central sensitisation meaning, this was a valid control site.

The mechanical stimulus applied in the human and animal studies obviously differed in size and application technique. This difference is acknowledged; however, due to the clearly demonstrated mechanical sensitivity increases in both eccentrically exercised animals and humans it is suggested that the method is sufficiently robust to allow further discussion regarding hypertonic sodium stimulation under these mechanically sensitised conditions.

Afferent nerve response magnitude to hypertonic saline

In the animal experiment, hypertonic sodium solution applied to sites on the eccentrically exercised muscle that were demonstrably mechanically sensitised did not induce a higher afferent fibre response magnitude compared to the same application to unexercised muscles. Because hypertonicity may shrink the muscle afferent receptor terminals directly and act as a mechanical stimulation, this unchanged

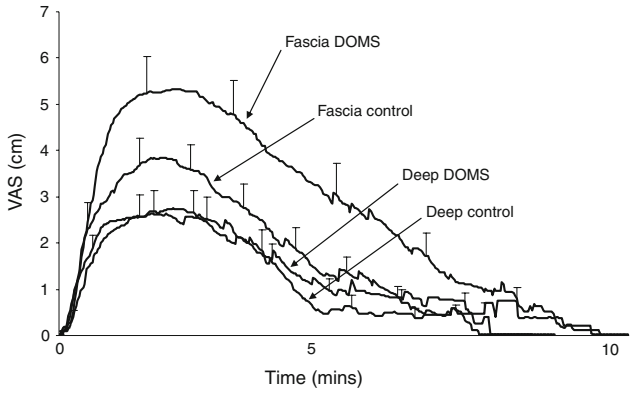

Fig. 6 Average (±SE) VAS profiles following hypertonic saline injection (0.5 ml, 5.8%) to the fascial and deep structures in the DOMS and control muscles

Table 1 Mean ± SE VAS parameters following hypertonic saline injection

Injection site and type	Area under Vas-time graph (cms)	Max VAS (cm)
Deep DOMS muscle	758 ± 163	2.97 ± 0.5
Fascial DOMS muscle	1,807 ± 325*	5.64 ± 0.82*
Deep control muscle	683 ± 164	2.85 ± 0.57
Fascial control muscle	1,057 ± 190	4.06 ± 0.6**
ANOVA *P* values	<0.05	<0.05

* Significantly different compared to all other injections, NK $P < 0.05$

** Significantly different compared to deep injections in DOMS and control leg, NK $P < 0.05$

response to hypertonic saline application to mechanically sensitised muscle receptive fields was unexpected. The stretch-inactivated channel (a sub-type of the transient receptor potential vanilloid receptor, TRPV1) expressed on small diameter sensory neurones is a likely source of afferent activity subsequent to hypertonic exposure (Schumacher et al. 2000). On the other hand, our preliminary experiment in visceral afferents has shown that hypertonicity itself has only a small effect, and sodium ions in hypertonic solution are important to excite sensory receptors (data not shown). Channels such as Nav2/NaG sodium channels that are expressed in the brain and the dorsal root ganglion and sense extracellular sodium ion concentration (Watanabe et al. 2000; Hiyama et al. 2002) might be involved. Therefore, mechanical sensitivity and sensitivity to high sodium concentrations could be differently modulated by an eccentric protocol.

Rat EDL does not have a thickened epimysium forming a distinct fascia (unlike human tibialis anterior) and the results from our EDL-peroneal nerve preparation are likely to reflect the response of afferents in proximity to the epimysium as well as in deeper tissue to the superfusion of the hypertonic sodium solution. The possibility remains that specifically fascial afferents might have displayed increased sensitivity to hypertonic sodium solution. It has been suggested that eccentric exercise-induced damage/adaptation can manifest at different depths/areas within the muscle (Butterfield and Herzog 2006). In addition, the musculo–tendinous junction has been reported to be more susceptible to eccentric exercise (Friden and Lieber 2001; Saxton and Donnelly 1996). It is possible in this study that group IV fibres in different locations were influenced to differing extents, and sensitisation to hypertonic sodium solution may have been detected if different receptive fields were compared. However, this would have been prohibitively time consuming. It has to be acknowledged that hypertonic sodium solution may cause afferent activity to similar levels, regardless of mechanical hypersensitivity and presumed afferent sensitisation post-eccentric protocol. It has been shown that hypertonic saline excites all group IV afferents (Hoheisel et al. 2005) and there may be an 'all or nothing' response, rendering it less effective as a measure of sensitisation in afferent activities. It must also be added, that the sensitisation mechanism may not be the same for all sensitivities of afferents (Taguchi et al. 2005a).

Hypertonic saline and superficial injections

This study significantly found higher pain levels from superficial injections targeting fascial/epimysium tissue compared to deep muscle tissue injections. This confirms previous human experimental pain findings in fascial tissue (Kellgren 1938). The injections were given as superficially as possible, literally as soon as muscle fascia was pierced. This leaves the method open to some speculation as to whether it was subcutaneous tissue (or superficial muscle tissue/epimysium) being infiltrated with saline. There are a number of reasons why this was not the case in this methodology. First, subcutaneous injections of 0.5 ml saline result in a 'button' of saline which is visible and palpable. This was not seen after any of the superficial muscle injections. Second, a small dorsiflexion upon completion of the infusion demonstrated needle oscillation in synchrony with the movement, confirming the needle had remained in contact with contractile tissue.

The significantly higher pain intensity following fascial injection of the DOMS affected muscle may be reflective of fascial/epimysium receptor sensitisation with possible excitation of these receptors subsequent to the injection procedure; and/or facilitated with central processing of their afferent activity. It has been previously demonstrated that a hypertonic saline bolus injected into a muscle tends to track longitudinally as opposed to being penetrative into the deeper aspects of the muscle (Graven-Nielsen et al. 1997). While it cannot be asserted in this study that the saline

bolus is the only excited fascial/epimysium tissue, it is reasonable to suggest that the spread of the saline pool must have predominantly affected this tissue given the proximity of the injection to the fascia.

It is possible that hypertonic saline through the mechanical bolus effect may have caused deformation of the fascial tissue thus causing greater pain from the mechanical deformation as this is the site of maximally sensitised tissue. Such a mechanism does, however, remain consistent with the proposed preferential increase in mechanosensitivity of fascial tissue. Under the present protocol there is no means of excluding this possibility and future studies should include isotonic saline injection as a control for this.

During DOMS there are acknowledged facilitated central pain mechanisms involved (Barlas et al. 2000; Bajaj et al. 2000; Weerakkody et al. 2003) in the perception of soreness. Ongoing nociceptive activity is associated with central facilitatory effects on pain perception (Woolf and Salter 2000). It could be proposed that if the DOMS event preferentially involves fascial/epimysium tissue and afferent fibre activity in this tissue contributes more to the persistent soreness, then the central synaptic processes associated with these specific afferent fibres will, over time be facilitated. Thus, noxious stimulation in the form of hypertonic saline injection to these tissues would result in greater pain perception, due to the central sensitisation, regardless of whether hypertonic saline response is or is not (as previously discussed) reflective of peripheral sensitisation. There is, therefore, the possibility that involvement of central mechanisms may provide some explanation for the observed differences in response to hypertonic saline between fascial-directed and deep muscle injections.

Referred pain

Referred pain is a centrally mediated mechanism (Hoheisel et al. 1993; Graven-Nielsen et al. 2000; Laursen et al. 1999). Interestingly, higher referred pain frequency was found following hypertonic saline injection in the deep muscle belly of the DOMS affected muscle compared to the control muscle. This occurred despite no significant difference in pain intensity induced by saline injection in the DOMS and control deep muscle belly. Referred pain occurrence is usually correlated with higher pain intensities (Graven-Nielsen 2006), and it is established that central sensitisation can overlap from somatotopically closely related regions; expansion of receptive fields and novel input to neurones becoming apparent (Hoheisel et al. 1993; Hoheisel et al. 1994). The neuroplastic changes induced by ongoing DOMS related nociceptive activity may be responsible for facilitation of prior ineffective dorsal horn neurone connections (Hoheisel et al. 1993) thus facilitating referred pain in deep muscle tissue in the absence of increased hypertonic saline pain intensity. Similar findings have previously been reported in a DOMS/hypertonic saline model (Gibson et al. 2006). Despite fascial injection of the DOMS affected muscle resulting in significantly higher pain intensity, referred pain was not seen as frequently following hypertonic saline injection to this site. This may simply be reflective of fascial tissue characteristics. Kellgren (1938) described fascial tissue experimental pain as primarily local in nature.

Conclusion

This study demonstrates increased pain in response to hypertonic saline injection directed to fascial/epimysium tissue as compared with deep muscle injections following eccentric exercise. The lack of increased pain response following deep muscle injection (at the site of maximal perceived sensitivity) implies that tissue specificity is important in the pain perception associated with DOMS. This argument is supported by our current animal study which demonstrated no increased afferent response following hypertonic sodium application to mechanically sensitised muscle receptive fields post-eccentric exercise. As such, this study suggests an important role of fascial tissue in delayed onset muscle soreness perception.

Acknowledgments The Danish Research Council supported this work.

References

Andersen H, Arendt-Nielsen L, Danneskiold-Samsoe B, Graven-Nielsen T (2005) Muscle hardness and spatial pressure sensitivity in delayed onset muscle soreness. Program No. 454-P60. In: Abstracts 11th world congress on pain. IASP Press, Seattle, pp 174

Bajaj P, Graven-Nielsen T, Wright A, Davies II, Arendt-Nielsen L (2000) Muscle hyperalgesia in postexercise muscle soreness assessed by single and repetitive ultrasound stimuli. J Pain 1:111–121

Barlas P, Walsh DM, Baxter GD, Allen JM (2000) Delayed onset muscle soreness: effect of an ischaemic block upon mechanical allodynia in humans. Pain 87:221–225

Bennell K, Hodges P, Mellor R, Bexander C, Souvlis T (2004) The nature of anterior knee pain following injection of hypertonic saline into the infrapatellar fat pad. J Orthop Res 22:116–121

Birtles DB, Rayson MP, Jones DA, Padhiar N, Casey A, Newham DJ (2003) Effect of eccentric exercise on patients with chronic exertional compartment syndrome. Eur J Appl Physiol 88:565–571

Butterfield TA, Herzog W (2006) The magnitude of muscle strain does not influence serial sarcomere number adaptations following eccentric exercise. Pflugers Arch 451:688–700

Friden J, Lieber RL (1998) Segmental muscle fiber lesions after repetitive eccentric contractions. Cell Tissue Res 293:165–171

Friden J, Lieber RL (2001) Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components. Acta Physiol Scand 171:321–326

Gibson W, Arendt-Nielsen L, Graven-Nielsen T (2006) Delayed onset muscle soreness at tendon-bone junction and muscle tissue is associated with facilitated referred pain. Exp Brain Res 174(2):351–360

Graven-Nielsen T (2006) Fundamentals of muscle pain, referred pain, and deep tissue hyperalgesia. Scand J Rheumatol 35(122):1–43

Graven-Nielsen T, McCardle A, Phoenix J, Arendt-Nielsen A, Jensen TS, Jackson A, Edwards RHT (1997) In vivo model of muscle pain: quantification of intramuscular, electrical and pressure changes associated with saline muscle pain in humans. Pain 69:137–143

Graven-Nielsen T, Babenko V, Svensson P, Arendt-Nielsen L (1998) Experimentally induced muscle pain induces hypoalgesia in heterotopic deep tissues, but not in homotopic deep tissues. Brain Res 787:203–210

Graven-Nielsen T, Aspegren KS, Henriksson KG, Bengtsson M, Sorensen J, Johnson A, Gerdle B, Arendt-Nielsen L (2000) Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients. Pain 85:483–491

Hiyama TY, Watanabe E, Ono K, Inenaga K Tamkun MM, Yoshida S (2002) Na(x) channel involved in CNS sodium-level sensing. Nat Neurosci 5:511–512

Hodges PW, Moseley GL, Gabrielsson A, Gandevia SC (2003) Experimental muscle pain changes feedforward postural responses of the trunk muscles. Exp Brain Res 151:262–271

Hoheisel U, Mense S, Simons DG, Yu XM (1993) Appearance of new receptive fields in rat dorsal horn neurons following noxious stimulation of skeletal muscle: a model for referral of muscle pain? Neurosci Lett 153:9–12

Hoheisel U, Koch K, Mense S (1994) Functional reorganization in the rat dorsal horn during an experimental myositis. Pain 59:111–118

Hoheisel U, Unger T, Mense S (2005) Excitatory and modulatory effects of inflammatory cytokines and neurotrophins on mechanosensitive group IV muscle afferents in the rat. Pain 114:168–176

Itoh K, Kawakita K (2002) Effect of indomethacin on the development of eccentric exercise-induced localized sensitive region in the fascia of the rabbit. Jpn J Physiol 52:173–180

Itoh K, Okada K, Kawakita K (2004) A proposed experimental model of myofascial trigger points in human muscle after slow eccentric exercise. Acupunct Med 22:2–12

Kawakita K, Miura T, Iwase Y (1991) Deep pain measurement at tender points by pulse algometry with insulated needle electrodes. Pain 44:235–239

Kellgren JH (1938) Observations on referred pain arising from muscle. Clin Sci 3:175–190

Laursen RJ, Graven-Nielsen T, Jensen TS, Arendt-Nielsen L (1999) The effect of compression and regional anaesthetic block on referred pain intensity in humans. Pain 80:257–263

Lieber RL, Shah S, Friden J (2002) Cytoskeletal disruption after eccentric contraction-induced muscle injury. Clin Orthop Relat Res (403):S90–S99

Malm C, Sjodin B, Sjoberg B, Lenkei R, Renstrom P, Lundberg IE, Ekblom B (2004) Leukocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. J Physiol-Lond 556:983–1000

Morgan DL, Proske U (2004) Popping sarcomere hypothesis explains stretch-induced muscle damage. Clin Exp Pharmacol Physiol 31:541–545

Newham DJ (1988) The consequences of eccentric contractions and their relationship to delayed onset muscle pain. Eur J Appl Physiol Occup Physiol 57:353–359

Nie H, Kawczynski A, Madeleine P, Arendt-Nielsen L (2005) Delayed onset muscle soreness in neck/shoulder muscles. Eur J Pain 9:653–660

Prasartwuth O, Taylor JL, Gandevia SC (2005) Maximal force, voluntary activation and muscle soreness after eccentric damage to human elbow flexor muscles. J Physiol 567:337–348

Reeh PW (1986) Sensory receptors in mammalian skin in an in vitro preperation. Neurosci Lett 66:141–186

Saxton JM, Donnelly AE (1996) Length-specific impairment of skeletal muscle contractile function after eccentric muscle actions in man. Clin Sci 90:119–125

Schumacher MA, Jong BE, Frey SL, Sudanagunta SP, Capra NF, Levine JD (2000) The stretch-inactivated channel, a vanilloid receptor variant, is expressed in small-diameter sensory neurons in the rat. Neurosci Lett 287:215–218

Slater H, Arendt-Nielsen L, Wright A, Graven-Nielsen T (2003) Experimental deep tissue pain in wrist extensors: a model of lateral epicondylalgia. Eur J Pain 7:277–288

Slater H, Arendt-Nielsen L, Wright A, Graven-Nielsen T (2005) Sensory and motor effects of experimental muscle pain in patients with lateral epicondylalgia and controls with delayed onset muscle soreness. Pain 114:118–130

Taguchi T, Matsuda T, Tamura R, Sato J, Mizumura K (2005a) Muscular mechanical hyperalgesia revealed by behavioural pain test and c-Fos expression in the spinal dorsal horn after eccentric contraction in rats. J Physiol-Lond 564:259–268

Taguchi T, Sato J, Mizumura K (2005b) Augmented mechanical response of muscle thin-fiber sensory receptors recorded from rat muscle-nerve preparations in vitro after eccentric contraction. J Neurophysiol 94:2822–2831

Watanabe E, Fujikawa A, Matsunaga H, Yasoshima Y, Sako N, Yamomoto T (2000) Nav2/NaG channel is involved in control of salt-intake behavior in the CNS. J Neurosci 20:7743–7751

Weerakkody NS, Whitehead NR, Canny BJ, Gregory JE, Proske U (2001) Large-fiber mechanoreceptors contribute to muscle soreness after eccentric exercise. J Pain 2:209–219

Weerakkody NS, Percival P, Hickey MW, Morgan DL, Gregory JE, Canny BJ, Proske U (2003) Effects of local pressure and vibration on muscle pain from eccentric exercise and hypertonic saline. Pain 105:425–435

Whitehead NP, Weerakkody NS, Gregory JE, Morgan DL, Proske U (2001) Changes in passive tension of muscle in humans and animals after eccentric exercise. J Physiol-Lond 533:593–604

Woolf CJ, Salter MW (2000) Neuronal plasticity: increasing the gain in pain. Science 288:1765–1769

Yu JG, Thornell LE (2002) Desmin and actin alterations in human muscles affected by delayed onset muscle soreness: a high resolution immunocytochemical study. Histochem Cell Biol 118:171–179

Yu JG, Malm C, Thornell LE (2002) Eccentric contractions leading to DOMS do not cause loss of desmin nor fibre necrosis in human muscle. Histochem Cell Biol 118:29–34