European Journal of Applied Physiology (2019) 119:1183–1194
https://doi.org/10.1007/s00421-019-04108-7

ORIGINAL ARTICLE

The relationship between stiffness and pain following unaccustomed eccentric exercise: the effects of gentle stretch and repeated bout

Pornpimol Muanjai[1,2] · **Mantas Mickevicius**[1] · **Sigitas Kamandulis**[1] · **Audrius Snieckus**[1] · **David A. Jones**[3]

Received: 6 December 2018 / Accepted: 20 February 2019 / Published online: 25 February 2019

Abstract

Purpose To determine how muscle stiffness and pain which develop after eccentric exercise are affected by gentle stretching and repeated exercise.

Methods Twenty-one healthy female participants undertook eccentric exercise of the elbow flexors and changes in resting elbow flexion angle (REFA; a measure of muscle stiffness), pain on stretch scale, pain elicited by pressure (PPT pain, a measure of mechanoreceptor hypersensitivity), and upper arm girth were followed for 7 days after exercise. The effects of gentle passive stretching on pain and muscle stiffness were investigated 2 and 4 days after exercise. Eleven participants also repeated the exercise with the same arm 6 weeks after the first bout.

Results There was a significant relationship between the pain on stretch scale and increased REFA (day 4; $R^2 = 0.65$, $p < 0.001$), whereas there was no relationship between REFA and PPT pain. REFA was reduced by passive stretching and pain on stretch scale was also reduced from 3.0 (1.4, 5.1) to 0.75 (0.0, 2.0) [median (IQR), $p = 0.01$]. PPT pain was unaffected by the passive stretching, as was muscle swelling. Following the repeated bout, increases in REFA were much reduced, as was pain on stretch scale ($p = 0.02$). However, PPT pain was not significantly different between the two bouts of exercise.

Conclusions The results indicate that reductions in pain on stretch scale, either by gentle passive stretching or as the result of repeated exercise, are primarily due to reductions in muscle stiffness which develops after eccentric exercise, whereas mechanoreceptor hypersensitivity is relatively unaffected.

Keywords Eccentric exercise · Stretching exercise · Damage response · Elbow flexor muscle

Abbreviations

CK	Creatine kinase
DOMS	Delayed onset of muscle soreness
ECC	Eccentric exercise
MVC	Maximum voluntary isometric contraction
PPT	Pressure pain threshold
REFA	Resting elbow flexion angle
VAS	Visual analog scale

Communicated by Olivier Seynnes.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00421-019-04108-7) contains supplementary material, which is available to authorized users.

✉ Pornpimol Muanjai
pornpimolm@buu.ac.th

[1] Institute of Sport Science and Innovations, Lithuanian Sports University, 44221 Kaunas, Lithuania

[2] Department of Physical Therapy, Allied Health Sciences Faculty, Burapha University, Saensuk, Chonburi 20131, Thailand

[3] School of Healthcare Science, Manchester Metropolitan University, Manchester M15 6BH, UK

Introduction

Unaccustomed eccentric exercise has a wide range of consequences including a serious loss of strength that recovers slowly over 2–3 weeks and the release into the circulation of soluble proteins from the damaged muscle. These aspects of eccentric exercise have received considerable attention in recent years (Carmona et al. 2018; Souron et al. 2018; Yamada et al. 2018), but the features which are most apparent to the unfortunate participant are a characteristic stiffness and pain (delayed onset muscle soreness; DOMS), felt when the muscle is stretched or palpated and which develops in the days shortly after the exercise.

The stiffness is evident as an electrically silent contracture of the muscle which, following damage to the elbow flexors,

can be quantified as degrees of flexion at the elbow (Jones et al. 1987; Reisman et al. 2005) or as the force required to extend the elbow (Howell et al. 1993). There was speculation that it is the swelling, possibly within a tight facial sheath around the muscle, that accounts for the stiffness (Howell et al. 1985; Jones et al. 1987), but in a later study, Howell et al. (1993) point out that the development of swelling and stiffness have different time courses with a peak in stiffness at 1–2 days, while swelling is maximal 3–4 days after elbow flexor eccentric exercise (Chleboun et al. 1998; Whitehead et al. 2001), suggesting that the stiffness is a property of the muscle fibres themselves, probably as a result of calcium entry into the fibres (Allen et al. 2005). Howell et al. (1993) also discuss the possibility that the swelling and pressure might stimulate mechanoreceptors within the damaged muscle and be the cause of the pain, but concluded that the pain is more likely to be a consequence of sensitisation of mechanoreceptors by locally released chemicals, such as the products of mast cell degranulation (Stauber et al. 1990).

One interesting feature of the pain and stiffness that has received very little attention is that both can be almost completely abolished by gentle passive stretching of the affected limb, although both return within about 30 min once the limb is rested (Jones et al. 1987; Reisman et al. 2005). In many ways, this is consistent with the view that the pain is due to locally released substances that are washed away in the blood, or lymphatic drainage, during the passive stretching (Kruse et al. 2016), but build up again when the muscle is rested. However, evidence from animal muscle suggests that the mechanical hypersensitivity is due to activation of ASIC, TRPV1, and TRPV4 ion channels rather than the continued presence of algesic substances in the muscle (Fujii et al. 2008; Ota et al. 2013; Mizumura and Taguchi 2016). It seems a little unlikely, therefore, that a few passive stretches of the affected muscle will reverse the activation of these ion channels. The fact that the reduction in DOMS by passive stretching is associated with reduction in muscle stiffness suggests an alternative mechanism that the hypersensitivity may remain, but the mechanoreceptors are only stimulated by stretching when the muscle fibres are stiff.

There are many studies of the "repeated bout" effect showing that not only does the muscle damage recover more rapidly and plasma creatine kinase response is much reduced, but the DOMS pain is also reduced following a repeated bout of exercise as is the contracture, or stiffness of the muscle (Newham et al. 1987; Miyama and Nosaka 2007; Chen et al. 2012; Deyhle et al. 2015; Lau et al. 2015; Margaritelis et al. 2015; Goodall et al. 2017; Souron et al. 2018; Pincheira et al. 2018). Despite this interest, the reason why the pain is diminished following a repeated bout of exercise, remain largely unknown. However, the coincidence of the reduction in pain and stiffness following a repeated bout suggests a similar mechanism to the reduction in pain following passive stretching, namely, that the major change might be in the stiffness of the muscle.

There are several ways to assess DOMS pain (Lau et al. 2015), probably the most commonly used method is to stretch the affected muscle, in the case of elbow flexors by extending the elbow or, with quadriceps damage, having the participant perform a squat, in both cases assessing the pain with a 10 point scale (Skurvydas et al. 2011). Less commonly used is measurement of the pressure pain threshold (PPT), where the muscle is probed and the minimum pressure required to elicit pain recorded (Newham et al. 1983; Rocha et al. 2012). Pain on stretch may reflect both the sensitivity of the mechanoreceptors and stiffness of the muscle, while PPT may be a more direct measure of mechanoreceptor sensitivity. It was, therefore, hypothesised that while passive stretching and a repeated bout of eccentric exercise would both result in a reduction in muscle stiffness and pain scale when stretched, mechanical hypersensitivity, assessed as pain in response to pressure, would not be affected to the same extent.

Methods

Participants

Twenty-one healthy and recreationally active women were recruited (22.5 ± 3.4 years; 61.2 ± 11.9 kg; 170.3 ± 8.2 cm). Exclusion criteria were an involvement in resistance exercise during the last 6 months, neuromuscular or skeletal problems associated with upper extremities or regular use of analgesic or anti-inflammatory drugs. Participants were asked about the phase of their menstrual cycle; none were using oral contraception. After the procedures had been carefully explained the participants provided written informed consent to the study which had been approved by the Kaunas Regional Biomedical Research Ethics Committee.

Study design

Sample size was selected based on published human studies of eccentric exercise concerned with changes in muscle pain as a result of a repeated bout effect or some intervention, where the numbers of participants ranged from 7 to 18 (Jones et al. 1987; Clarkson et al. 1987; Newham et al. 1987; Howell et al. 1993; Hilbert et al. 2003; Pearcey et al. 2015). Participants in study 1 exercised first one arm and then 2–3 weeks later the second. Participants in Study 2 exercised first arm then went on to exercise the first arm again in a repeated bout study 4 weeks later with second arm exercise after a further 2 weeks (Fig. 1). Combined data for from first and second arms were used to assess possible influence of speed of arm extension, arm dominance, and phase of the

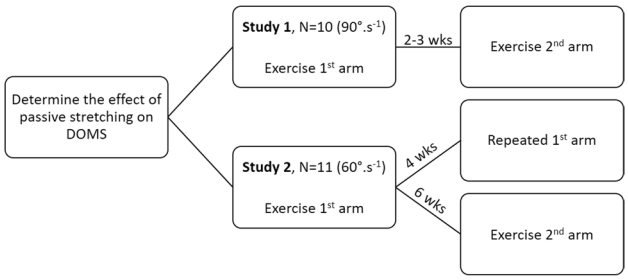

Fig. 1 Study design. Study 1: both arms exercised at 90 s^{-1} 2–3 weeks apart ($N = 10$). Study 2: exercise of first arm (60 s^{-1}) repeated 4 weeks later with second arm exercise after a further 2 weeks. Data from the first and second arms combined in supplementary data

menstrual cycle; these data are presented in Table 1, Supplementary Data. Combined data from studies 1 and 2 for the first bout of exercise were used to examine the effect of passive stretching on DOMS, while the data for the first arms in Study 2 examined the repeated bout effect.

Participants first attended a laboratory session in which they were familiarized with the apparatus and measurements to be made and then baseline data were collected. Subsequently, measurements were made of maximal voluntary contraction (MVC), plasma creatine kinase (CK), resting elbow flexion angle (REFA), girth of the upper arm, pain scale on passive extension of the elbow (pain on stretch) and mechanical hypersensitivity by the pressure pain threshold method (PPT), before, immediately after eccentric exercise, and then at 2, 4, and 7 days. All measurements were made by the same investigators.

Eccentric exercise of the elbow flexors and maximal isometric strength testing

The participants were seated on a Biodex System 3 isokinetic dynamometer (Biodex Medical Systems, Inc., Shipley, New York, USA) with the back rest fixed at 90° and with the trunk, pelvis and upper arm stabilized with Velcro straps. The axis of rotation was fixed at the elbow joint and the lever arm pad attached at the wrist. Participants were instructed to perform 6 sets of ten maximal voluntary eccentric contractions of the elbow flexors starting from 60° elbow flexion until full extension (180°) with 1-min rest between sets. In the first study, an angular velocity of 90 s^{-1} was used, while 60 s^{-1} was used for the second, repeated bout, study. Verbal encouragement to maintain a maximal effort was given throughout each stretch. Peak torque and work done during each set were recorded. Measurements of maximal isometric muscle strength were made with the subjects stabilized in the Biodex chair, as for the exercise, and with the elbow at 90°. Subjects were encouraged to make maximum voluntary contractions (MVC) for 2–3 s with 60 s rest between contractions. Provided the two values differed by no more that 5%, the highest torque was recorded; otherwise, the test was repeated and the highest value accepted for further analysis.

Measurements

Resting elbow flexion angle (an index of muscle contracture and stiffness) was recorded as the angle between the reference points of the acromion process, lateral epicondyle of the humerus, and the mid-point of the styloid process of the ulna and radius with the projection of the lateral epicondyle of the humerus as the axis by goniometer, with the participant standing with the arm hanging freely beside the trunk. The reference points were marked with a permanent marker for repeated testing. The average of two measures was used for analysis.

The circumference, or girth, of the upper arm (60% the distance between the acromion process and the lateral epicondyle of the humerus) was measured with an inextensible tape measure with the participant standing with the arm hanging freely (Kulig et al. 2001). The measurement point was marked with a permanent marker for repeated testing. The average of two measures was used for analysis.

Pain elicited by taking the elbow to full extension (pain on stretch scale) was evaluated using a 10 point scale, where 0 represented "no pain" and 10 "intolerably intense pain" (Skurvydas et al. 2011). The pressure pain threshold (PPT) of the mid-belly of the elbow flexors muscle group was assessed with an algometer (1 cm diameter probe, Wagner Instruments, Greenwich, USA) at the position, where arm circumference was measured, with the elbow flexed to 90° and the lower arm supported on a table. During the measurement, the investigator applied the tip of the device, slowly increasing pressure perpendicular to the skin up to a maximum force of 30 N. The participants reported the end point when the pressure first turned into a painful sensation (Newham et al. 1983; Rocha et al. 2012). The applied force at the end point of the two trials was averaged and subtracted from 30 (Newham et al. 1987) and is referred to as "PPT pain".

Approximately 0.25 mL of capillary blood was drawn from the finger. Samples were immediately centrifuged and plasma analysed for creatine kinase activity using the biochemical analyser SpotchemTM EZ SP-4430 (Menarini Diagnostics, UK) with soft reagent strips (ARKRAY Factory, Inc., Shiga, Japan) and plasma enzyme activity reported as micro-Katal per Litre (μkat L^{-1}). One μkat L^{-1}, the SI unit of CK activity, is equivalent to 60 of the more commonly used International Units.

Gentle passive stretching

On days 2 and 4 after the eccentric exercise, participants sat in the Biodex chair as for the eccentric exercise as described above, and the dynamometer passively and slowly (5 s^{-1}) extended the relaxed elbow from 60° flexion to full extension (180°). The procedure was repeated 6 times with 20 s hold at full extension, but otherwise with no rest. If, on standing, the resting elbow flexion angle had not returned to the baseline value, the passive stretching was repeated. Pain on stretch scale, muscle girth, and PPT pain was measured before, and immediately after the passive stretching procedure and, in some cases, following 30 min recovery.

Data and statistical analysis

Data were tested for normality by a Shapiro–Wilk test, where normally distributed descriptive data are presented as mean ± SD; SEM is given when comparing means. Where not normally distributed, data are reported as median and interquartile range (IQR). Data for MVC torque were examined with repeated measures ANOVA to assess differences over time, other data, which were not normally distributed, were investigated with a non-parametric Kruskal–Wallis test. Relationships between the two measures of pain scale and changes in CK, muscle stiffness, and girth were examined by linear regression. For data that were not normally distributed, the effect size (ES) for the passive stretching procedure was calculated from log-transformed data and are presented ± 95% confidence intervals. Statistical significance was assumed at $p \leq 0.05$. Statistical analysis was performed with SPSS for Window (version 24.0, Chicago, IL, USA).

Results

After the first stage of the study, it was thought that participants might be having problems maintaining a maximal voluntary effort with arm extension at 90 s^{-1}, and therefore, for the second, repeated bout, stage, the speed was reduced to 60 s^{-1}. There are a number of factors which might affect the response to eccentric exercise and the perception of pain, these include arm dominance, the speed of the eccentric action, and the phases of the menstrual cycle in the case of the female participants in this study. Data for both arms in the two studies were pooled (Fig. 1) and analysed for possible differences between eccentric exercise at 60 and 90 s^{-1}, for dominant and non-dominant arms as well as the possible effects of phase of the menstrual cycle, but none were found to affect the extent or time course of the changes in torque and CK responses, REFA or perceived pain; these data are given in Supplementary material. Consequently, data for the first bout of exercise of participants in Studies 1 and 2 were combined and the results in Figs. 2, 3, 4, 5 and Table 1 are from 14 dominant and 7 non-dominant arms, 10 for whom the eccentric exercise was at 90 s^{-1}, and 11 participants at 60 s^{-1}.

Time course of change

Figure 2a shows data for the time course of torque loss and partial recovery following the first bout of eccentric exercise, while data for plasma creatine kinase are given in Fig. 2b. Very soon, after the end of the eccentric exercise, a flexion contracture of the elbow developed (Fig. 3a). The contracture remained roughly constant or increased slightly over the course of the next 2–4 days before largely resolving by 7 days ($p < 0.05$). There was a small increase in girth of the upper arm (Fig. 3b) which increased steadily to reach

Table 1 Correlations between outcome measures

	Peak CK		PPT D2		PPT D4		REFA D2		REFA D4		Girth D2		Girth D4	
	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p
Pain on stretch D2	0.448	0.001	0.003	0.800			0.386	0.003			0.131	0.107		
Pain on stretch D4	0.425	0.001			0.002	0.854			0.652	< 0.001			0.322	0.007
PPT D2	0.028	0.464					0.002	0.840			0.003	0.804		
PPT D4	0.056	0.300							0.002	0.840			0.100	0.157
REFA D2	0.437	0.001												
REFA D4	0.391	0.002												
Girth D2	0.404	0.002					0.138	0.097						
Girth D4	0.700	< 0.0001							0.317	0.008				

Pain on stretch scale on days 2 (D2) and 4 (D4) after eccentric exercise

PPT pressure pain threshold, *PPT* pain, *REFA* change in resting elbow flexion angle, *Girth* change in girth of upper arm, *Peak creatine kinase (CK)* creatine kinase measured at day 4

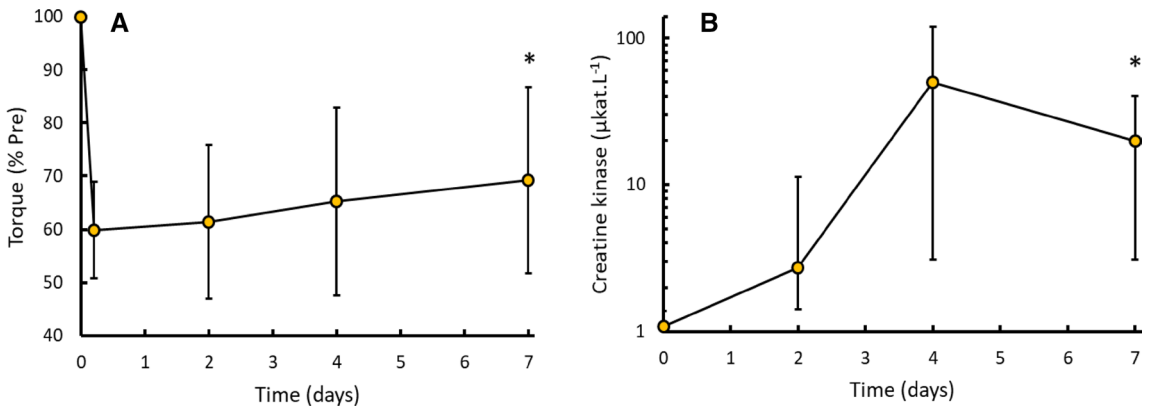

Fig. 2 Changes in torque and creatine kinase following eccentric exercise. Combined data for all subjects following the first bout of exercise ($N=21$). **a** Torque expressed as percentage of pre-exercise value; data are mean (SD). **b** Plasma creatine kinase as µkat L^{-1}, data are median and IQR; note the log scale. *Significant difference from pre-exercise values at all timepoints ($p<0.05$)

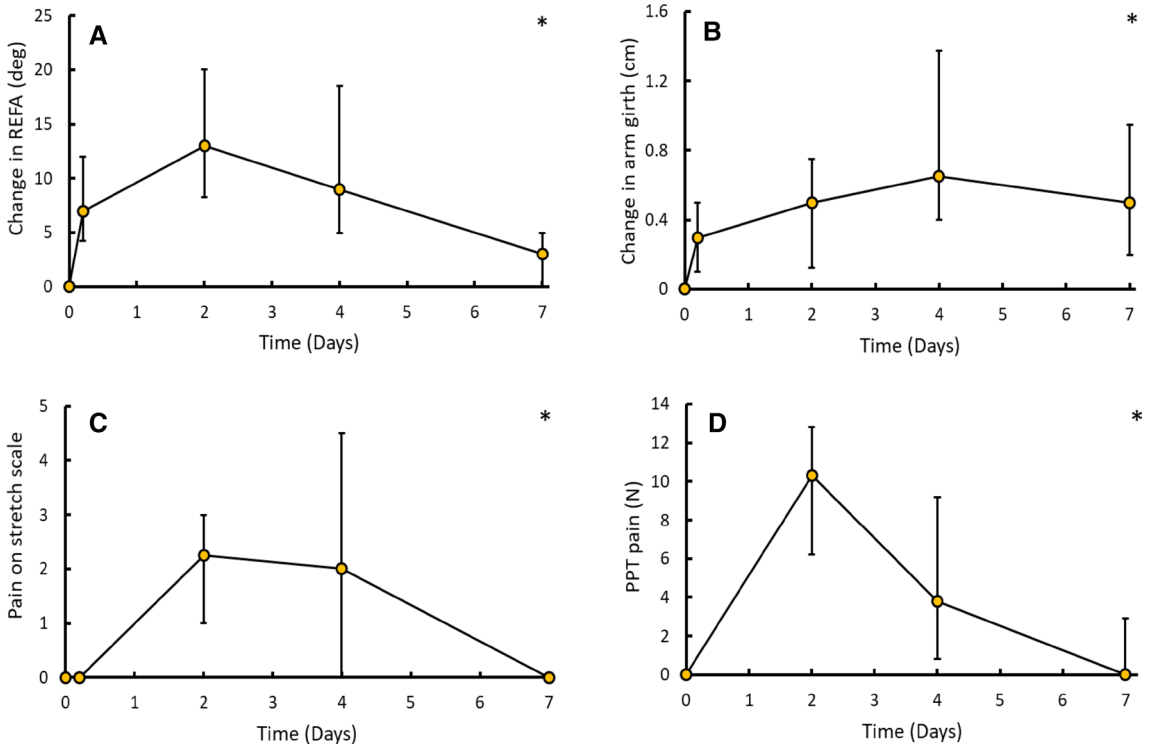

Fig. 3 **a** Change in resting elbow flexion angle (REFA). **b** Change in upper arm girth. **c** Pain on stretch scale. **d** PPT (pressure pain threshold) pain following eccentric exercise. Data are given as median and IQR ($N=21$). *Significant difference from pre-exercise values at all timepoints ($p<0.05$)

a peak at day 4, but was still elevated 7 days after the exercise ($p<0.05$). Pain scale, experienced, while the elbow was manually extended (pain on stretch) (Fig. 3c) increased, most commonly reaching a peak at day 2, although with a great deal of variation, some reaching a peak at 4 days, while others reported no pain at that time. Pain assessed by mechanical pressures (Fig. 3d; PPT pain) was maximal at day 2 after the exercise and decreased steadily over the next 5 days.

There were significant, but modest correlations between peak CK and pain on stretch scale, increased elbow flexion angle and change in arm girth at 2 and 4 days following the exercise (Table 1; Fig. 4a). PPT pain was not related to creatine kinase activity (Table 1; Fig. 4b). There were

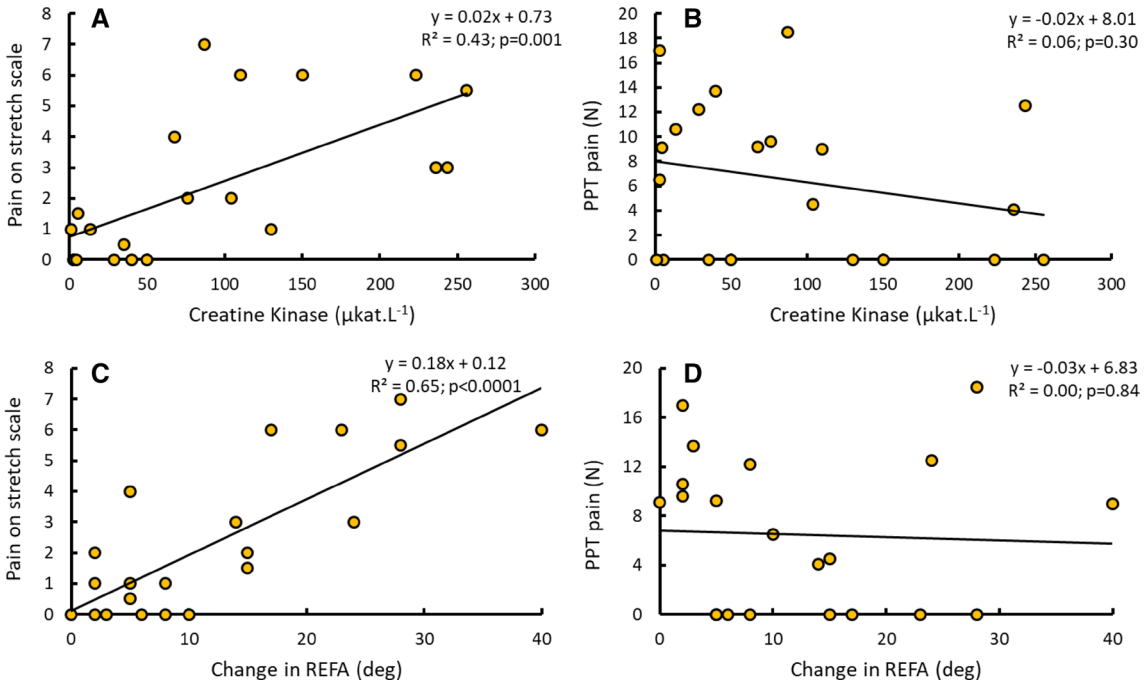

Fig. 4 Selected correlations of outcome measures. **a** Pain on stretch scale as a function of peak creatine kinase (µkat L^{-1}); **b** PPT (pressure pain threshold) pain as a function of peak creatine kinase; **c** pain on stretch scale as a function of change in resting elbow flexion angle (REFA); **d** PPT pain as a function of change in REFA. Data are from 21 arms with measurements made at day 4; for measurements at day 2 and other outcome measures, see Table 1

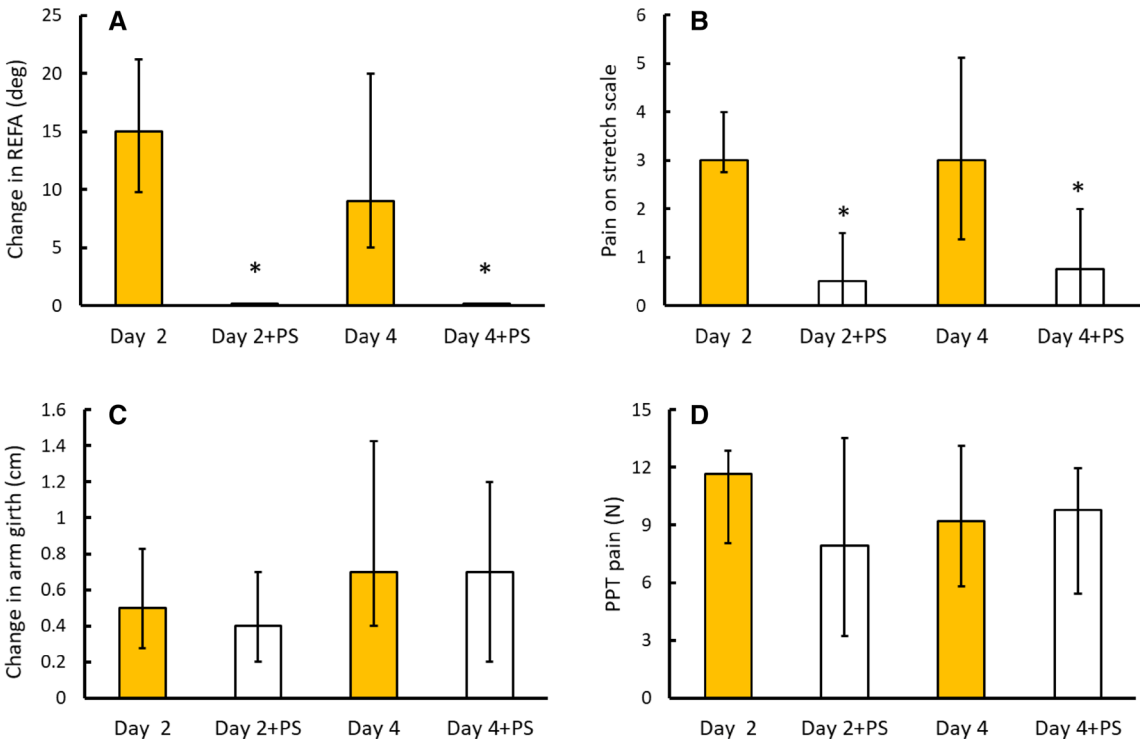

Fig. 5 Effects of passive stretching on damaged muscle. **a** Change in resting elbow flexion angle (REFA); **b** pain on stretch scale; **c** change in upper arm girth; **d** PPT (pressure pain threshold) pain. Measurements were made at days 2 and 4 after eccentric exercise and after passive stretching (+PS). Data are given as median and IQR ($N=21$). *Significant difference after passive stretching ($p<0.05$)

significant correlations between pain on stretch and the extent of elbow flexion which was particularly evident at day 4 (Table 1; Fig. 4c). In contrast, PPT pain was not related to change in elbow angle on either day (Table 1; Fig. 4d). There was no correlation between the two methods of assessing pain, by stretching or PPT either on day 2 or 4 (Table 1).

The effect of gentle passive stretching on stiffness, swelling, and pain

The pain experienced was very variable between participants. All but one reported some pain on day 2 but by day 4, four participants reported no pain on stretch scale, while 5 had no PPT pain. The data in Fig. 5b, d refer only to those participants who had pain before the passive stretching. The effect of gentle passive stretching was to abolished the flexion contracture (Fig. 4a; day 2 + PS ES 2.91 ± 0.49; and day 4 + PS, ES 2.26 ± 0.42). If the arm was then rested for 30 min, the resting elbow flexion angle returned to the same value as before the passive stretching [13° (2, 2°)]. Swelling of the upper arm was not affected by this procedure (Fig. 5c; day 2 + PS, ES 0.031 ± 0.025; and day 4 + PS, ES 0.028 ± 0.024). Pain on stretch scale was very much reduced by the passive stretching on day 2 [from 3 (1, 0.25) to 0.5 (1, 0.5) ES 1.31 ± 0.40] and likewise on day 4 [from 3 (2.1, 1.6) to 0.75 (1.25, 0.75) ES 0.65 ± 0.30] (Fig. 4b; day 2 + PS, ES 0.17 ± 0.22; and day 4 + PS, ES 0.12 ± 0.23) ($p < 0.001$). Although no formal measurements were made, the impression was that when the arm was rested, pain on stretch returned with a similar time course to resting elbow flexion angle. In marked contrast, PPT pain was largely unaffected by the passive stretching procedure (Fig. 5d), although there was a small reduction on day 2 ($p = 0.072$). The standard deviations of the log-transformed data indicate that the sample size of 22 was sufficient to detect changes of 14% for pain on stretch, 11% for PPT pain, and 9% resting elbow flexion angle, with 95% confidence.

Repeated bout effect

The responses to the first bout of exercise, both in terms of torque and CK, of the 11 participants who participated in the repeated bout experiment were very similar to those of the larger group (compare Figs. 2 and 6). Following the repeated bout of exercise 21 days after the first, the recovery of torque was much faster than after the first bout (Fig. 6a), while the large delayed CK response following the first bout was virtually abolished (Fig. 6b). Following the repeated bout of eccentric exercise, there was an increase in resting elbow flexion angle shortly after the exercise which was not significantly different to that after the first bout, but in subsequent days, the resting elbow flexion angle was much reduced (Fig. 7a). Quantifying the change, the sum of the angles,

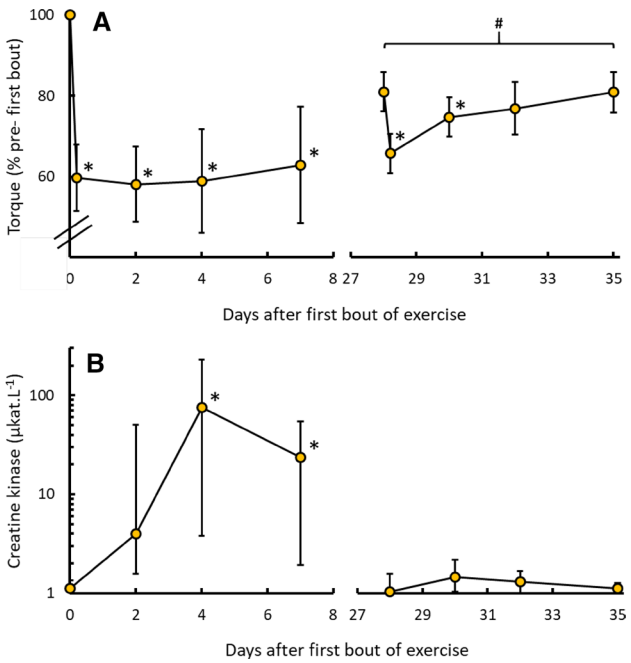

Fig. 6 Changes in torque and creatine kinase in the repeated bout experiment. Data for the subjects in Study 2 who repeated the eccentric exercise with the same arm 28 days after the first bout of exercise. **a** Torque expressed as percentage of torque measured before the first bout. Data are mean and SD ($N = 11$). **b** Plasma creatine kinase. Data are median and IQR; note the log scale for CK. *Significant difference from baseline ($p < 0.05$). #Significant difference from the first bout ($p < 0.05$)

essentially area under the curve in Fig. 7a was 35.5° (16.0, 66.8°) after the first bout and 12.5° (7.3, 16.8°) after the repeated bout (median and IQR, $p = 0.02$). Pain on stretch scale was also considerably reduced following the repeated bout (Fig. 7c); the sum of the pain scores being 7 (2.5, 10.5) after the first bout and 2 (1, 3) after the repeated bout of exercise (median and IQR, $p = 0.02$). In contrast, the PPT did not differ significantly between the first and repeated bouts of exercise (Fig. 7d). The increase in upper arm girth seen after the first bout of exercise was almost completely absent following the repeated bout (Fig. 7b).

Discussion

The starting point for this study was the observations familiar to anyone involved in sport and formally documented by Jones et al. (1987) and Reisman et al. (2005) that the pain and stiffness experienced when stretching a muscle that has been subjected to eccentric exercise is much reduced if the muscle is gently stretched a number of times. Despite the familiarity of this observation, two basic questions remain unanswered; why the stiffness should be reduced in this way and, likewise, why the pain should be reduced. The results

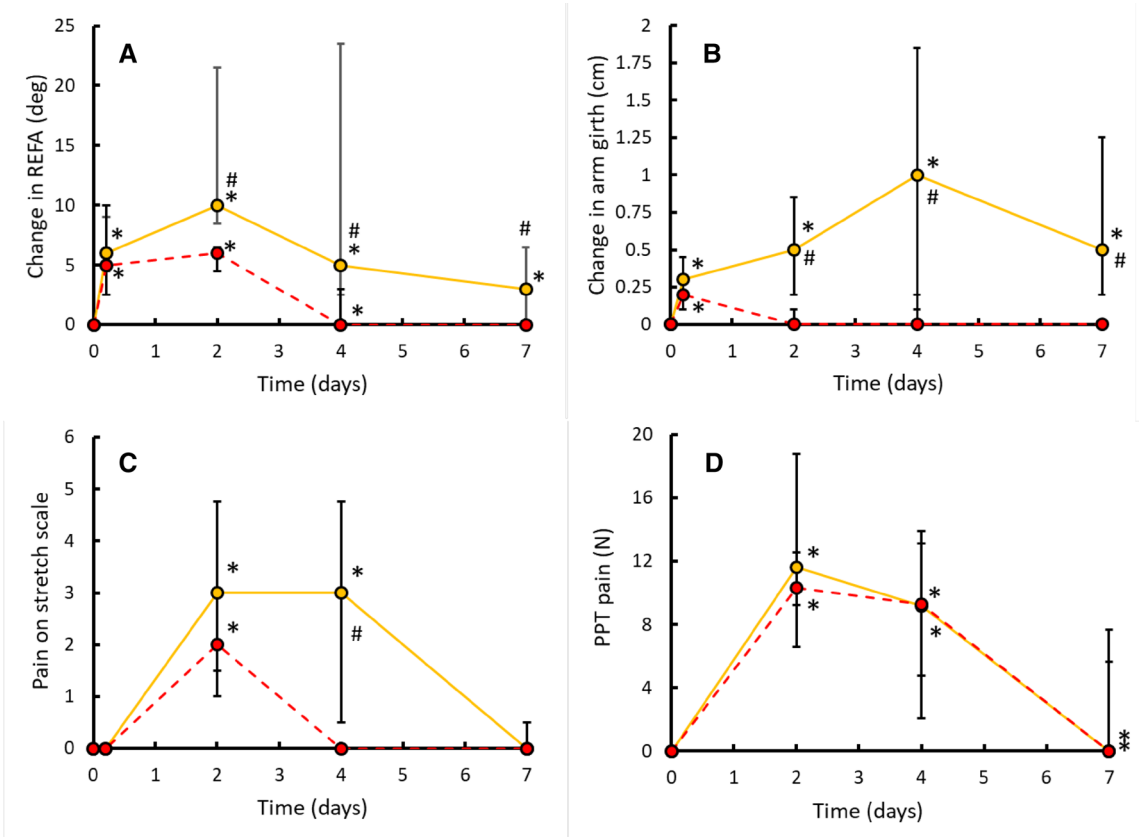

Fig. 7 Consequences of a repeated bout of eccentric exercise for elbow flexion, muscle swelling and pain. **a** Change in resting elbow flexion angle (REFA); **b** change in upper arm girth; **c** pain on stretch scale; **d** PPT (pressure pain threshold) pain. All data are given as median and IQR ($N=11$). *Significant difference from baseline ($p<0.05$). #Significant difference from the first bout ($p<0.05$)

presented here confirm what was already suspected that oedema and swelling of the muscle are unlikely to be the cause of the muscle stiffness nor is it the cause of the pain but, otherwise, throw no new light on the cause of stiffness. In respect of the pain, however, the results provide a new insight, suggesting that the pain on stretch is a combination of the stiffness of the muscle and mechanical hypersensitivity and that the reduction of the pain on stretch, either with gentle passive stretching, or following a repeated bout of exercise, is primarily due to a reduction in muscle stiffness, while the mechanical hypersensitivity assessed as PPT pain remains largely unaffected.

It is a common observation that the responses to eccentric exercise vary considerably from person to person and this was the case in the present study; hence, most of the data were not normally distributed and are presented as median and IQR, although it interesting that in most other studies, the data appear to be normally distributed. A wide range of factors might affect the response to eccentric exercise including the protective effect that exercising one arm might have for the contralateral arm (Chen et al. 2018). The data reported here in Figs. 2, 3, 4, and 5 are all for arms that were exercised the first time. Other factors which might influence the muscle damage include arm dominance, phase of the menstrual cycle, and the speed of the damaging eccentric movement, but no evidence was seen of any such influence (Supplementary Data, Table 1). Although the recovery of muscle strength following eccentric exercise damage of the elbow flexors is known to be slow (Newham et al. 1987), it was surprising that participants in the repeated bout study only recovered to 80% of their initial MVC torque (Fig. 6a). It is possible that the lower torque generated during the repeated bout of exercise might have resulted in the lower pain on stretch scale but, on the other hand, PPT pain was unaffected (Fig. 7d). In other studies, such as of drop jumps (Venckunas et al. 2012), torque did fully recover before the second bout, but the subsequent pain was reduced, suggesting that the extent of MVC torque recovery was probably not an important factor accounting for the reduced pain on stretch scale. Although there has been speculation that muscle oedema might be the cause of the increased stiffness and elbow flexion (Howell et al. 1985; Jones et al. 1987) the fact that swelling developed more slowly and continued for longer than the flexion (compare Fig. 3a and 3b) makes it

unlikely that it is the cause. In addition, the data in Fig. 5a, c show quite clearly that while resting elbow flexion angle, and by implication muscle stiffness, was reduced by passive stretching, the swelling was unaffected. Similar evidence comes from the work of Lau et al. (2015a, b, c) who found that while arm flexion and swelling were both present after a first bout of exercise, following a repeated bout there was no swelling but there was arm flexion, although this resolved more rapidly than after the first bout (see also Fig. 7a, b). The cause of the increased stiffness is not known, although there is interest in the idea of Ca^{2+} entering via stretch activated channels (Allen et al. 2005) which would increase cross bridge interaction and may also lead to conformational changes in titin (Labeit et al. 2003), but the present results do not throw any light on this aspect of muscle damage. The reason why gentle stretching can reverse the flexion and, further, why the flexion returns on resting for around 30 min, is equally obscure, although Riesman et al. (2005) have discussed a number of possible mechanisms. The pain generally noticed by participants following eccentric exercise is that which occurs when the muscle is stretched. The data presented here show a clear relationship between the pain on stretch scale and the extent of the resting elbow flexion angle (Fig. 4c). The relationship is further emphasised by the decrease in pain on stretch scale when the flexion contracture is reduced, either by the gentle passive stretching procedure (Fig. 5b) or after the repeated bout of exercise, where both pain on stretch scale and resting elbow flexion angle were much reduced (Fig. 7a, c). However, this is quite different to the PPT pain that was unaffected either by the passive stretching (Fig. 5d) or the repeated bout effect (Fig. 7d). The pain associated with PPT following the first bout of exercise was unrelated to plasma creatine kinase (Fig. 4b), indicting that it is not a consequence of inflammation, unlike swelling of the upper arm which was highly correlated with CK ($R^2 = 0.7$ at day 4, Table 1). In addition, unlike pain on stretch, PPT pain was not correlated with increased resting elbow flexion angle (Fig. 4d), was not influenced by gentle passive stretching of the damaged muscle (Fig. 4d) and was not significantly reduced following the repeated bout of exercise (Fig. 7d). Unlike other aspects of muscle damage, the resolution of the flexion contracture by gentle passive stretching and the relationship to muscle pain has received little attention. Previously, Jones et al. (1987) assessed the development of contracture and pain, measured both pain on stretch and PPT pain following eccentric exercise, but did not document the change in pain following the gentle stretching. Reisman et al. (2005) also assessed pain in the same two ways, together with the response to vibration, and showed that following five stretches which reduced the flexion there was a significant reduction in pain on stretch scale and the pain in response to vibration but possible changes in PPT were not reported. The present study is, therefore, the first to report the discrepancy between the two methods, stretching and pressure, for assessing pain following eccentric exercise. One interpretation of the results is that the two methods of assessing pain are measuring two different types of pain and it is notable that while the two measures have similar time courses (Fig. 3c, d), there was, in fact, no correlation between the two measures, an observation also made by Lau et al. (2015). There are, however, other explanations. The observations that when the flexion contracture, or muscle stiffness, is reduced by gentle passive stretching, the pain on stretch is also reduced has two possible explanations. It is generally agreed that the DOMS pain represents hypersensitivity of mechanoreceptors so the stretching process must either have reduced the hypersensitivity or, stretching no longer activates those mechanoreceptors. Animal work suggest hypersensitivity of mechanoreceptors is induced by a variety of factors including bradykinin and NGF, although these are not necessarily present in the tissue at the time when the pain is measured (Murase et al. 2010, 2013; Mizumura and Taguchi 2016), but lead to activation of ASIC and TRPV1 and TRPV4 ion channels by phosphorylation of various serine and tyrosine sites (Fujii et al. 2008; Ota et al. 2013). It seems unlikely that 5 min of gentle passive stretching will dephosphorylate critical sites and down regulate ion channels. Thus the most likely explanation for the reduction in pain is that stretching the muscle no longer activates the mechanoreceptors. When a muscle tendon unit is stretched the extent to which the length change is taken up by the muscle fibres and connective tissue and tendon will depend on their relative stiffness. In a relaxed muscle the tendon and connective tissue is much stiffer than the muscle fibres and the length change is taken up by the muscle fibres. However, when the muscle fibres are stiff, as during a contraction or contracture, the connective tissue and tendon will be stretched. Consequently, if the hypersensitive mechanoreceptors are located on connective tissue in series with the muscle fibres, they will only be stimulated when the muscle fibres are stiff as when a contracture develop after damaging exercise. Reducing the muscle contracture will thereby reduce the strain on the hypersensitive mechanoreceptors and the pain experienced when the muscle is stretched. This is consistent with the results shown in Fig. 5, where the pain on stretch was reduced by gentle stretching, which reduced the stiffness of the muscle, while PPT pain was unaffected. Andersen et al. (2013) have shown massage to be as equally effects as exercise in temporarily relieving DOMS and one form of self-massage, foam rolling, has also been shown to be effective (Pearcey et al. 2015). Although the benefits of exercise and massage probably involve peripheral mechanisms, a study of foam rolling for tender spots in the calf showed that contralateral PTT can be affected (Aboodarda et al. 2015) raising the possibility of central adaptations.

The question then arises as to whether the same explanation applies to the reduction in pain on stretch scale that is a well-known feature of the repeated bout effect. The observations reported here for the repeated bout of exercise are typical of many similar studies, where it has been shown that following the repeated bout there is faster recovery of torque, reduced pain scale and CK responses and, where measured, a reduction in the flexion contracture (Newham et al. 1987; Chen et al. 2012; Janecki et al. 2014; Lau et al. 2015a, b, c). The most direct comparison with the present study is with Newham et al. (1987), where muscle tenderness was assessed as a pain threshold. In that study the peak PPT pain was reduced by around 20% following the repeated bout of exercise. The present study was powered to detect an 11% change in PPT pain but given the wide variation in responses and the relatively small number of participants in both studies it is not possible to decide whether there is any significant difference between the previous and present studies. However, it is clear that both studies show there to be substantial PPT pain after the repeated bout and this contrasts with the major reductions in pain on stretch scale and muscle stiffness. In contrast, a study of eccentric exercise damage of the human tibialis anterior reports a significant reduction in PPT pain following a repeated bout when measured 1 day after exercise (Hosseinzadeh et al. 2013) and a study by Lau et al. (2015) reporting a substantial decrease in pain assessed by PPT in a repeated bout of exercise of the forearm flexors. There are also several studies, where a reduction in pain has been reported following a repeated bout of eccentric exercise using a variety of palpation techniques, where it is not clear how this relates to a pain threshold measurement (Lau et al. 2015a, b, c; Souron et al. 2018) and, likewise, a study, where participants rated their responses to a brief standard pressure stimulus (Janecki et al. 2014). While in the present study it is quite clear that the PPT pain was the same, or very similar, following the first and repeated bouts of exercise, there is an obvious discrepancy with some other studies, the reasons for which are not clear. Nevertheless, the conclusion of the present study is that the marked reduction in pain on stretch scale in response to the repeated bout of eccentric exercise was a consequence of the reduction in muscle fibre stiffness, while mechanical hypersensitivity assessed by PPT is largely unaffected. The main limitation, or at least reservation, concerning this study concerns the repeated bout study and the fact that the observation that PPT is unaffected and runs counter to several other studies. The reasons for this discrepancy may need to be explored but the central argument that the pain on stretch scale is a function of both stiffness and mechanical hypersensitivity does not depend on the repeated bout experiment; these results are simply consistent with the observations of the effects of passive stretching on pain and stiffness.

In conclusion, the present results have confirmed previous observations, which have largely been overlooked, that passive stretching reduces the stiffness that develops soon after eccentric exercise and demonstrates this is most unlikely to be due to tissue swelling. Furthermore, the pain on stretch scale is also most unlikely to be due to swelling but is reduced in proportion to the reduction in stiffness when the muscle is subjected to gentle passive stretching. The fact that PPT pain is not affected by this procedure indicates that mechanical hypersensitivity remains and suggests that the decrease in the pain on stretch scale is due to more compliant muscle fibres placing less strain on mechanoreceptors situated on connective tissue elements in the muscle. We have shown that much of the reduction in the pain on stretch scale associated with the repeated bout of eccentric exercise can also be ascribed to reductions in the stiffness of the muscle rather than changes in hypersensitivity of the mechanoreceptors. These observations focus attention on the nature of the increased stiffness of damaged muscle, its cause and how it can be modified by gentle stretching and repeated exercise.

Acknowledgements The present study does not constitute any financial funding. We would like to thank all of participants.

Author contributions PM was responsible the experimental design, data collection, and analysis drafting the manuscript. DAJ and SK were concerned with the design of the study, data analysis and drafting the manuscript. MM and AS assisted with data collection and drafting the manuscript and blood examination. All authors approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work.

Compliance with ethical standards

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

Ethical approval All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Aboodarda SJ, Spence AJ, Button DC (2015) Pain pressure threshold of a muscle tender spot increases following local and non-local rolling massage. BMC Musculoskelet Disord 16:265. https://doi.org/10.1186/s12891-015-0729-5

Allen DG, Whitehead NP, Yeung EW (2005) Mechanisms of stretch-induced muscle damage in normal and dystrophic muscle: role of ionic changes. J Physiol 567:723–735. https://doi.org/10.1113/jphysiol.2005.091694

Andersen LL, Jay K, Andersen CH et al (2013) Acute effects of massage or active exercise in relieving muscle soreness: randomized controlled trial. J Strength Cond Res 27:3352–3359. https://doi.org/10.1519/JSC.0b013e3182908610

Carmona G, Mendiguchía J, Alomar X et al (2018) Time course and association of functional and biochemical markers in severe semitendinosus damage following intensive eccentric leg curls: differences between and within subjects. Front Physiol 9:54. https://doi.org/10.3389/fphys.2018.00054

Chen TC-C, Chen H-L, Pearce AJ, Nosaka K (2012) Attenuation of eccentric exercise-induced muscle damage by preconditioning exercises. Med Sci Sports Exerc 44:2090–2098. https://doi.org/10.1249/MSS.0b013e31825f69f3

Chen TC, Lin MJ, Chen HL et al (2018) Muscle damage protective effect by two maximal isometric contractions on maximal eccentric exercise of the elbow flexors of the contralateral arm. Scand J Med Sci Sports 28:1354–1360. https://doi.org/10.1111/sms.13042

Chleboun GS, Howell JN, Conatser RR, Giesey JJ (1998) Relationship between muscle swelling and stiffness after eccentric exercise. Med Sci Sports Exerc 30:529–535

Clarkson PM, Byrnes WC, Gillisson E, Harper E (1987) Adaptation to exercise-induced muscle damage. Clin Sci Lond Engl 1979 73:383–386

Deyhle MR, Gier AM, Evans KC et al (2015) Skeletal muscle inflammation following repeated bouts of lengthening contractions in humans. Front Physiol 6:424. https://doi.org/10.3389/fphys.2015.00424

Fujii Y, Ozaki N, Taguchi T et al (2008) TRP channels and ASICs mediate mechanical hyperalgesia in models of inflammatory muscle pain and delayed onset muscle soreness. Pain 140:292–304. https://doi.org/10.1016/j.pain.2008.08.013

Goodall S, Thomas K, Barwood M et al (2017) Neuromuscular changes and the rapid adaptation following a bout of damaging eccentric exercise. Acta Physiol Oxf Engl 220:486–500. https://doi.org/10.1111/apha.12844

Hilbert J, Sforzo G, Swensen T (2003) The effects of massage on delayed onset muscle soreness. Br J Sports Med 37:72–75. https://doi.org/10.1136/bjsm.37.1.72

Hosseinzadeh M, Andersen OK, Arendt-Nielsen L, Madeleine P (2013) Pain sensitivity is normalized after a repeated bout of eccentric exercise. Eur J Appl Physiol 113:2595–2602. https://doi.org/10.1007/s00421-013-2701-0

Howell JN, Chila AG, Ford G et al (1985) An electromyographic study of elbow motion during postexercise muscle soreness. J Appl Physiol Bethesda Md 1985 58:1713–1718

Howell JN, Chleboun G, Conatser R (1993) Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. J Physiol 464:183–196

Janecki D, Jaskólska A, Marusiak J et al (2014) Twitch mechanical properties after repeated eccentric exercise of the elbow flexors. Appl Physiol Nutr Metab Physiol Appl Nutr Metab 39:74–81. https://doi.org/10.1139/apnm-2013-0097

Jones DA, Newham DJ, Clarkson PM (1987) Skeletal muscle stiffness and pain following eccentric exercise of the elbow flexors. Pain 30:233–242

Kruse NT, Silette CR, Scheuermann BW (2016) Influence of passive stretch on muscle blood flow, oxygenation and central cardiovascular responses in healthy young males. Am J Physiol Heart Circ Physiol 310:H1210–H1221. https://doi.org/10.1152/ajpheart.00732.2015

Kulig K, Powers CM, Shellock FG, Terk M (2001) The effects of eccentric velocity on activation of elbow flexors: evaluation by magnetic resonance imaging. Med Sci Sports Exerc 33:196–200

Labeit D, Watanabe K, Witt C et al (2003) Calcium-dependent molecular spring elements in the giant protein titin. Proc Natl Acad Sci USA 100:13716–13721. https://doi.org/10.1073/pnas.2235652100

Lau WY, Blazevich AJ, Newton MJ et al (2015a) Reduced muscle lengthening during eccentric contractions as a mechanism underpinning the repeated-bout effect. Am J Physiol Regul Integr Comp Physiol 308:R879–R886. https://doi.org/10.1152/ajpregu.00338.2014

Lau WY, Blazevich AJ, Newton MJ et al (2015b) Changes in electrical pain threshold of fascia and muscle after initial and secondary bouts of elbow flexor eccentric exercise. Eur J Appl Physiol 115:959–968. https://doi.org/10.1007/s00421-014-3077-5

Lau WY, Blazevich AJ, Newton MJ et al (2015c) Assessment of muscle pain induced by elbow-flexor eccentric exercise. J Athl Train 50:1140–1148. https://doi.org/10.4085/1062-6050-50.11.05

Margaritelis NV, Theodorou AA, Baltzopoulos V et al (2015) Muscle damage and inflammation after eccentric exercise: can the repeated bout effect be removed? Physiol Rep. https://doi.org/10.14814/phy2.12648

Miyama M, Nosaka K (2007) Protection against muscle damage following fifty drop jumps conferred by ten drop jumps. J Strength Cond Res 21:1087–1092. https://doi.org/10.1519/R-21056.1

Mizumura K, Taguchi T (2016) Delayed onset muscle soreness: involvement of neurotrophic factors. J Physiol Sci 66:43–52. https://doi.org/10.1007/s12576-015-0397-0

Murase S, Terazawa E, Queme F et al (2010) Bradykinin and nerve growth factor play pivotal roles in muscular mechanical hyperalgesia after exercise (delayed-onset muscle soreness). J Neurosci 30:3752–3761. https://doi.org/10.1523/JNEUROSCI.3803-09.2010

Murase S, Terazawa E, Hirate K et al (2013) Upregulated glial cell line-derived neurotrophic factor through cyclooxygenase-2 activation in the muscle is required for mechanical hyperalgesia after exercise in rats. J Physiol 591:3035–3048. https://doi.org/10.1113/jphysiol.2012.249235

Newham DJ, Mills KR, Quigley BM, Edwards RH (1983) Pain and fatigue after concentric and eccentric muscle contractions. Clin Sci Lond Engl 1979 64:55–62

Newham DJ, Jones DA, Clarkson PM (1987) Repeated high-force eccentric exercise: effects on muscle pain and damage. J Appl Physiol Bethesda Md 1985 63:1381–1386

Ota H, Katanosaka K, Murase S et al (2013) TRPV1 and TRPV4 play pivotal roles in delayed onset muscle soreness. PLoS One 8:e65751. https://doi.org/10.1371/journal.pone.0065751

Pearcey GEP, Bradbury-Squires DJ, Kawamoto J-E et al (2015) Foam rolling for delayed-onset muscle soreness and recovery of dynamic performance measures. J Athl Train 50:5–13. https://doi.org/10.4085/1062-6050-50.1.01

Pincheira PA, Hoffman BW, Cresswell AG et al (2018) The repeated bout effect can occur without mechanical and neuromuscular changes after a bout of eccentric exercise. Scand J Med Sci Sports. https://doi.org/10.1111/sms.13222

Reisman S, Walsh LD, Proske U (2005) Warm-up stretches reduce sensations of stiffness and soreness after eccentric exercise. Med Sci Sports Exerc 37:929–936

Rocha CS, Lanferdini FJ, Kolberg C et al (2012) Interferential therapy effect on mechanical pain threshold and isometric torque after delayed onset muscle soreness induction in human hamstrings. J Sports Sci 30:733–742. https://doi.org/10.1080/02640414.2012.672025

Skurvydas A, Brazaitis M, Venckūnas T, Kamandulis S (2011) Predictive value of strength loss as an indicator of muscle damage across multiple drop jumps. Appl Physiol Nutr Metab Physiol Appl Nutr Metab 36:353–360. https://doi.org/10.1139/h11-023

Souron R, Nosaka K, Jubeau M (2018) Changes in central and peripheral neuromuscular fatigue indices after concentric versus eccentric contractions of the knee extensors. Eur J Appl Physiol. https://doi.org/10.1007/s00421-018-3816-0

Stauber WT, Clarkson PM, Fritz VK, Evans WJ (1990) Extracellular matrix disruption and pain after eccentric muscle action. J Appl Physiol Bethesda Md 1985 69:868–874. https://doi.org/10.1152/jappl.1990.69.3.868

Venckunas T, Skurvydas A, Brazaitis M et al (2012) Human alpha-actinin-3 genotype association with exercise-induced muscle damage and the repeated-bout effect. Appl Physiol Nutr Metab Physiol Appl Nutr Metab 37:1038–1046. https://doi.org/10.1139/h2012-087

Whitehead NP, Weerakkody NS, Gregory JE et al (2001) Changes in passive tension of muscle in humans and animals after eccentric exercise. J Physiol 533:593–604

Yamada R, Himori K, Tatebayashi D et al (2018) Preconditioning contractions prevent the delayed onset of myofibrillar dysfunction after damaging eccentric contractions. J Physiol 596:4427–4442. https://doi.org/10.1113/JP276026