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Wing Yin Lau
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Doctor of Philosophy Thesis

Pain Assessments and Possible Mechanisms of Delayed Onset Muscle Soreness

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ABSTRACT

Muscle pain is felt during exercise or daily activities for several days after performing unaccustomed exercise, which is referred to as delayed onset muscle soreness (DOMS). Many people experience DOMS, but its underlying mechanisms are not fully understood. One of the challenges in the investigation of DOMS is its subjective nature, which makes the assessment ambiguous, thus establishing a standardised protocol is necessary. The present thesis scrutinised muscle pain assessments (Study 1, Study 2), developed a new assessment of muscle pain focusing on muscle fascia (Study 3), and investigated why DOMS is reduced after the second than the first bout of eccentric exercise (Study 4). From these studies, DOMS was thought to be more associated with connective tissue than muscle fibre damage and inflammation.

In **Study 1**, the relationship between pain level assessed by a visual analogue scale (VAS) and pain sensitivity assessed by pressure pain threshold (PPT) was examined. Thirty-one healthy young men performed 10 sets of 6 maximal isokinetic eccentric contractions with their non-dominant arm. Before and 1 - 4 days after the exercise, muscle pain perceived upon palpation of the biceps brachii at three sites (5, 9, and 13 cm above the elbow crease) was assessed by VAS with a 100 mm line (0 = no pain, 100 = extremely painful), and PPT of the same sites was determined by an algometer. The VAS increased after exercise and peaked two days post-exercise, while the PPT decreased most at 1 day post-exercise and did not return to baseline for 4 days following exercise ($P<0.05$). No significant difference among the three sites was found for VAS ($P=0.62$) or PPT ($P=0.45$). The magnitude of change in VAS did not significantly correlate with that of PPT ($r=-0.20$, $P=0.28$). These results suggest that the

level of muscle pain is not region specific, at least among the three sites investigated in the study, and VAS and PPT provide different information about DOMS, indicating that VAS and PPT represent different aspects of pain.

Muscle pain induced by elbow flexor eccentric exercise was investigated using different assessments in **Study 2**. Ten untrained men performed 10 sets of 6 maximal isokinetic eccentric contractions of the elbow flexors with one arm. Maximal voluntary isometric contraction torque (MVC), range of motion (ROM) and serum creatine kinase (CK) activity were measured before, immediately after, and 1 to 5 days after exercise as indirect markers of muscle damage. PPT of 50 sites over an exercised upper arm, VAS with a 100-mm line for pain level upon static pressure by a cuff and fingers, and palpation of the biceps brachii at three sites (3, 9, and 15 cm above the elbow crease) and different palpation methods (longitudinal, transverse and circular movements) on the mid-belly of biceps were assessed. Large decreases in MVC and ROM, and significant increases in serum CK activity indicated muscle damage. A significant difference ($P<0.05$) was found among 50 sites before exercise such that the distal and medial regions showed lower thresholds than the other regions. However, after eccentric exercise, the pain sensitive regions shifted ($P<0.05$) to the central regions of the mid-belly at 1 day post-exercise, plus the distal regions at 2 days post-exercise. Compared with static pressure, palpation induced greater pain; longitudinal and transverse movements induced greater pain than circular movements. The magnitude of change in VAS did not significantly correlate with that of PPT ($r=-0.08$ to -0.34, $P=0.45$ to 0.81) for three sites at 1-3 days after exercise. These results suggest that how to palpate muscle affects the pain level, and central and distal regions should be included for the DOMS assessment after elbow flexor eccentric exercise.

In **Study 3,** changes in the electrical pain threshold (EPT) of the biceps brachii fascia, biceps brachii muscle and brachialis fascia following eccentric elbow flexor contractions, and the relationship between EPT and VAS or PPT were investigated. Ten healthy untrained men performed two eccentric exercise bouts (ECC1, ECC2) consisting of 10 sets of 6 maximal isokinetic eccentric contractions of the elbow flexors with the same arm separated by 4 weeks. Changes in MVC, ROM, VAS and PPT were smaller ($P<0.05$) following ECC2 than ECC1, showing the repeated bout effect. EPT decreased ($P<0.05$) immediately after exercise in both bouts; however, the magnitude of the decrease in EPT was significantly greater ($P<0.05$) in ECC1 than ECC2. Comparing the biceps brachii fascia, biceps brachii muscle and brachialis fascia, EPT showed significantly ($P<0.05$) decrease sensitivity for biceps brachii fascia (from 0.13 ± 0.11 mA to 0.67 ± 0.28 mA) and brachialis fascia (from 0.28 ± 0.19 mA to 0.86 ± 0.49 mA) than biceps brachii muscle (from 0.69 ± 0.32 mA to 1.32 ± 0.37 mA) at 1, 2 and 4 days post-ECC1. However, no significant difference was found between the biceps brachii and brachialis fascia after both bouts. The magnitude of change in EPT and PPT was correlated at 1 day post-exercise ($r=0.77$, $P<0.05$), but no significant correlation was found between EPT and VAS. These results suggest that fascia became more sensitive than muscle to electrical stimulation after eccentric exercise.

The purpose of **Study 4** was to investigate the magnitude of muscle lengthening during the first and second bout of eccentric exercise bouts and whether the muscle length changes are associated with the magnitude of DOMS and changes in other indirect markers of muscle damages between bouts. Ten healthy untrained men performed two eccentric exercise bouts (ECC1, ECC2) consisting of 10 sets of 6 maximal isokinetic eccentric contractions of the elbow flexors using the same arm separated by 4 weeks. Changes in MVC, ROM, muscle thickness, ultrasound echo

intensity, serum CK activity and muscle soreness (VAS) were smaller ($P<0.05$) following ECC2 than ECC1, showing less muscle damage after ECC2 than ECC1. The magnitude of myotendinous junction (MTJ) displacement (average of 6 contractions) increased from 1st (8.2 ± 4.7 mm) to 10th set (16.4 ± 4.7 mm) during ECC1 ($P<0.05$), but no significant changes over sets were evident during ECC2 (1st set: 8.5 ± 4.0 mm; 10th set: 9.3 ± 3.1 mm). These results suggest that a lack of change in muscle lengthening as exercise progresses in a repeated bout of eccentric contractions may be an important factor in the attenuation of DOMS and muscle damage.

DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

1. incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education;

2. contain any material previously published or written by another person except where due reference is made in the text; or

3. contain any defamatory material.

Signed: **Wing Yin LAU**

Date: 28 June 2014

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PUBLICATIONS

1. **Lau WY**, Muthalib M, Nosaka K. Visual analogue scale and pressure pain threshold for delayed onset muscle soreness assessment. *J Musculoskelet Pain*. 21:320-326, 2013. (Appendix VII)
2. **Lau WY,** Blazevich A, Newton M, Wu SSX, Nosaka K. Assessments of muscle pain induced after elbow flexor eccentric exercise. (Submitted to a journal)
3. **Lau WY,** Blazevich A, Newton M, Wu SSX, Nosaka K. Changes in electrical pain threshold of biceps brachii and brachialis fascia and muscle after eccentric elbow flexor exercise. (To be submitted to a journal)
4. **Lau WY,** Blazevich A, Newton M, Wu SSX, Nosaka K. Reduced muscle lengthening during a second bout of eccentric contractions as a mechanism underpinning the repeated bout effect. (Submitted to a journal and under review)

LIST OF CONFERENCE PRESENTATIONS

1. 6th Exercise and Sports Science Australia Conference and Sports Dieticians Australia Update: Research to Practice, 10-12 April 2014, Adelaide – Australia. Oral presentation – (1st Price Winner: Young Investigator Award: Sports Science): **Lau WY,** Blazevich A, Newton M, Wu SSX, Nosaka K. Changes in fascia and muscle pain threhold after eccentric contractions (Appendix VIII).

2. 16th annual Congress of the European College of Sport Science, 6-9 July 2011, Liverpool – UK. Oral presentation: **Lau WY**, Blazevich A, Newton M, Nosaka K. Difference in aponeurosis elongation during eccentric contractions between the first and second exercise bouts of the elbow flexors (Appendix IX).

CHAPTER ONE

1. INTRODUCTION

1.1. Background and Literature Review

This section provides the background of the study based on reviewing articles that are related to the present research project.

1.1.1 Delayed Onset Muscle Soreness

Humans often experience muscle pain for several days after performing exercise, especially "unaccustomed" exercise. This type of muscle pain is referred to as delayed onset muscle soreness (DOMS), which generally develops several hours after exercise, peaks at 1-3 days, and disappears by 7 days after exercise (6, 26). DOMS is characterised by a sensation of dull, aching pain, usually felt during movement or palpation of the affected muscle, and is combined with tenderness and stiffness (6, 31). DOMS is regarded as mechanical hyperalgesia (92), since stimuli (e.g. muscle contraction, stretching, palpation) that do not typically induce pain in normal condition evokes pain (16). In particular, DOMS is considered as one of the symptoms of muscle damage induced by exercise consisting of eccentric contractions (31, 117).

1.1.2 Eccentric Exercise-Induced Muscle Damage and Muscle Soreness

Skeletal muscles are damaged by physical, chemical and mechanical stimuli, but have remarkable regenerative ability (32, 66). According to Safran et al. (122) who classified muscle injury in sports based on clinical presentation (pain), DOMS is placed as a type 1 injury, whereas a type II injury is an acute disabling pain from a muscle tear,

ranging from a tear of a few fibres with fascia remaining intact to a complete tear of the muscle and fascia, and a type III injury is related to the muscle soreness or cramping that occurs during or immediately after exercise. DOMS is induced after unaccustomed and/or strenuous exercise consisting of eccentric contractions, where muscles are lengthened during force generation (6, 30). Clarkson and Hubal (29) documented that eccentric contractions induce microtrauma to muscle fibres and/or extracellular matrix leading to DOMS following the upregulation of inflammation responses. Proske and Morgan (117) have proposed that the primary damage originates from disrupted sarcomeres (popping sarcomeres hypothesis) that shift the optimum muscle fibre length to longer lengths, which further increases muscle fibre damage that is followed by inflammatory responses, in which nociceptors are sensitised, causing DOMS. However, Allen et al. (2) described that one of the key events in the muscle damage process was an increased intracellular Ca^{2+} concentration, mediated through stretch-activated channels stimulated by lengthening (eccentric) contractions, followed by increased membrane permeability to release muscle proteins such as creatine kinase (CK) and reduce the force production due to a decreased tetanic Ca^{2+} (Figure 1). Damage to muscle and connective tissue is followed by an inflammatory response that is necessary for regeneration (73). During this process, neutrophils and macrophages infiltrate damaged muscle fibres and degrade damaged proteins (134).

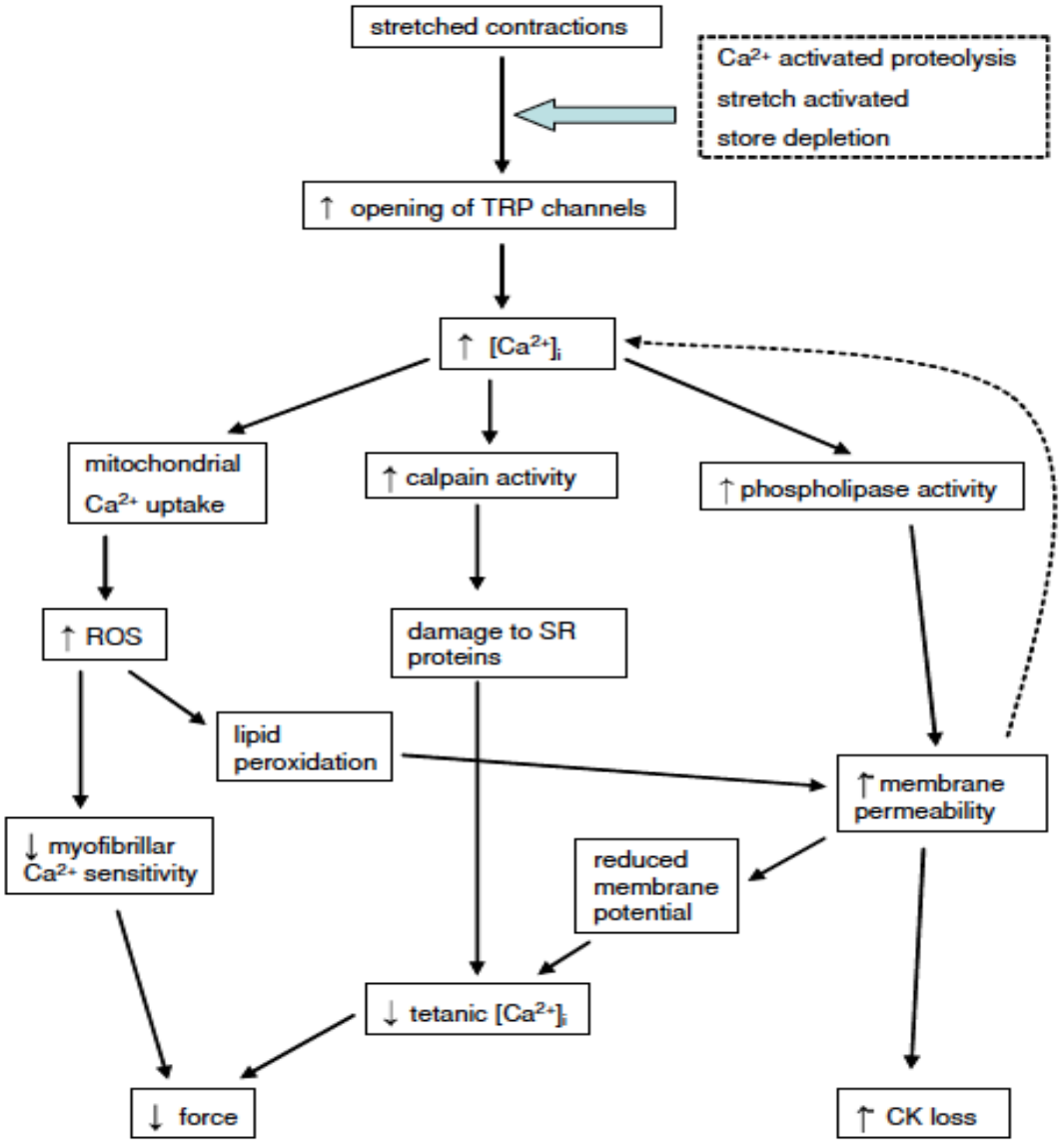

Figure 1. Pathways involved in eccentric exercise-induced muscle damage. Dashed box indicates hypothetical mechanisms that may be involved in activating channels for Ca^{2+} entry. Dashed arrow indicates positive feedback pathway that would occur when increased membrane permeability causes elevated (Ca^{2+}). Adapted from Allen et al. (2).

Armstrong et al. (6) documented four possible sequences for DOMS: 1) the high mechanical force produced during eccentric exercise causes disruption of structural proteins in muscle fibres and connective tissue, 2) structural damage to sarcolemma or alterations in permeability of the cell membrane increase the influx of Ca^{2+}, by which

mitochondria accumulate Ca^{2+}, inhibiting cellular respiration, and Ca^{2+} calcium-dependent proteolytic enzymes are activated, 3) the progressive degeneration of muscle fibres and collagens attract monocytes that convert to macrophages to activate mast cells and histocytes in the injury area, and 4) the accumulation of histamine, kinins and potassium in the interstitium trigger nociceptor activation, inducing DOMS.

1.1.2.1 Direct Markers of Muscle Damage

As mentioned above, eccentric contractions could result in muscle damage that is directly presented by histological changes in myofilaments and/or intermediate filaments observed under electron microscope, and/or muscle fibres and their surrounding connective tissue observed under light microscope (46, 131). Lauritzen et al. (77) showed that ultrastructural changes such as myofibrillar disruptions, Z-disc disruption, autophagic vacuoles and necrotic segments were observed in biceps brachii muscle samples taken after 70 maximal eccentric elbow flexor contractions. Paulsen et al. (112) reported myofilament disorganisation such as loss of Z-disk integrity and muscle fibre inflammation after 300 eccentric quadriceps femoris contractions. Crameri et al. (32) compared vastus lateralis muscle damage between 210 maximal eccentric contractions with electrical muscle stimulation (EMS) and 210 voluntary maximal eccentric contractions (VOL) in the knee extensors, and showed that larger Z-lines disruption was found in EMS (40%) compared with VOL (10%) in the biopsy samples from the vastus lateralis muscle. However, at the muscle fibre level, only 1% of the fibres observed under light microscope showed degeneration (112). Therefore, it appears that muscle fibres are damaged during and/or after eccentric exercise, but the extent of muscle fibre damage is not large, and the damage is more limited to the myofilament level than muscle fibre level.

1.1.2.2 Indirect Markers of Muscle Damage

Muscle damage is more often indirectly assessed by quantifying changes in maximal voluntary contraction (MVC) torque and range of motion (ROM), swelling of muscle represented by increases in muscle thickness or limb circumference measured using magnetic resonance or B-mode ultrasound imaging technique, and muscle proteins in the blood such as serum creatine kinase (CK). Figure 2 shows four main symptoms of muscle damage: muscle weakness, muscle pain, muscle stiffness and swelling, and how these symptoms are assessed (99).

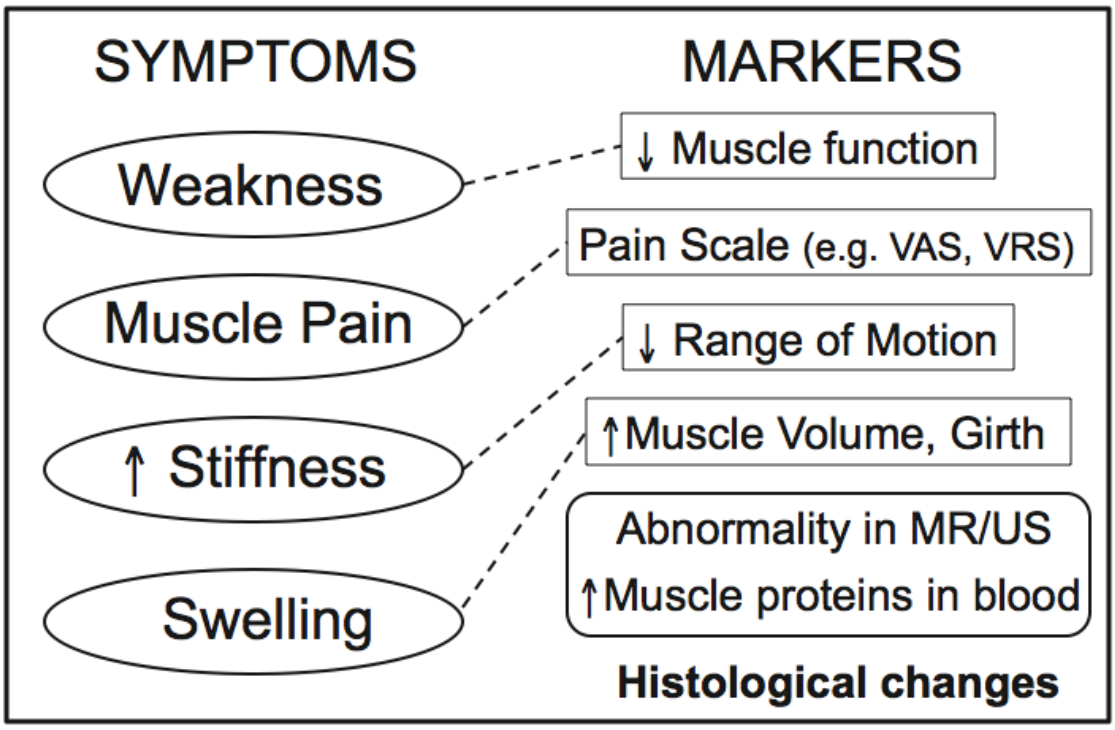

Figure 2. Four main symptoms and several commonly used markers of muscle damage (some measures are used to quantify the symptoms as shown by dotted lines). Histological changes are direct markers of muscle damage, and magnetic resonance/ultrasound images (MR/US) and increases in muscle proteins in the blood (e.g creatine kinase activity, myoglobin concentration) are used as other indirect markers of muscle damage. VAS: visual analogue scale, VRS: visual rating scale. Adapted from Nosaka et al. (99).

1.1.2.3 Maximal Voluntary Contraction (MVC) Torque/Force

It is well documented that MVC torque or force decreases immediately after unaccustomed eccentric exercise, and does not fully recover for several days, or weeks in some cases (30). The magnitude of MVC force loss immediately after high force eccentric exercise of the elbow flexors has been shown to be 40 to 60% (29, 104) and this impairment could remain for two weeks or longer after eccentric exercise (29, 59). The underlying mechanisms of the force loss are not fully understood; however, it is thought to be associated with the excitation-contraction (E–C) coupling failure and structural damage. Warren et al. (137) documented that MVC decreased in the first 3 days following eccentric exercise was largely associated with E-C coupling failure located at somewhere between the T-tubule voltage sensor (dihydropyridine or L-type Ca^{2+} channel) and SR Ca^{2+} release channel (ryanodine) receptors. After that, MVC loss is more ascribed to the decrease in contractile protein content resulting from the structural damage of contractile proteins.

1.1.2.4 Range of Motion (ROM)

Elbow joint ROM is determined by the difference between the flexed (FANG) and relaxed (RANG) or stretched (SANG) elbow joint angle. It has been shown to decrease immediately following novel eccentric exercise of the elbow flexor muscles, reaching the smallest angle around three days after exercise, and slowly recovering to the baseline (normal condition) over the next several days (30, 101). Relaxed elbow joint angle (RANG) is determined by the angle at the elbow while the arm is hanging freely by the side of the body, and it found to be at its most acute three days after exercise and slowly recovering to the baseline level by approximately 10 days following exercise (30). Reduced ROM following eccentric contractions remains to be fully

elucidated; however, a previous research suggested that it might be due to the accumulation of fluid in the muscle (59) or attributed to connective tissue shortening and/or muscle contractures due to changes in calcium homeostasis (7).

1.1.2.5 Limb Circumference (CIR) and Muscle Thickness

Following unaccustomed eccentric elbow flexor contractions, upper arm circumference increases, and peaking three to five days after exercise (30, 59). The underlying mechanism explaining the increased circumference is not fully known, but it has been suggested that accumulation of water at connective tissue (30) and/or between muscle fibres (33) may be related to the swelling, or the increased synthesis of connective tissue (128) is associated with the increased limb circumference. Muscle thickness assessed by ultrasound B-mode images is also used as an indicator of muscle swelling (59, 107, 103).

1.1.2.6 B-mode Ultrasound and Magnetic Resonance Imaging Technique

B-mode ultrasound and magnetic resonance (MR) imaging techniques have been used to visualise muscle damage. In ultrasound images, echo intensity increases when muscle damage is induced (23, 107). For MR images, it has been reported that T_2 relaxation time increases following eccentric exercise when muscle damage is induced and probably reflects the level of muscle oedema (63, 115). The increases in the echo intensity or T_2 relaxation time appear to be associated with oedema or destruction of proteins in the exercised muscle (27, 30). For elbow flexor eccentric exercise, it has been reported that echo intensity and MRI T_2 relaxation time increase and peak 3-7 days after exercise (63, 115).

1.1.2.7 Intracellular Protein Release

It is well known that when skeletal muscle is damaged, intracellular muscle proteins such as creatine kinase (CK), aspartate aminotransferase, lactate dehydrogenase, alanine aminotransferase, and myoglobin increase in the blood (100). Among them, CK is most commonly used. Since the CK molecules are relatively large (80 kD), it is assumed that they cannot escape from muscle fibres unless the cell membrane is damaged; thereby, increases in CK in the blood are thought to be due to plasma membrane damage (63, 123). Serum or plasma CK activity peaks between four to seven days after eccentric elbow flexor exercise (76, 107) and slowly returns to the baseline level thereafter. It should be noted that the magnitude of CK released in the blood is affected by the type and intensity of the eccentric exercise (63), and the magnitude of CK released is variable among individuals (28).

1.1.2.8 Relationship between Direct and Indirect Markers of Muscle Damage

The relationship between the direct and indirect muscle damage markers does not appear to be strong. As shown in Figure 3 (112), the number of damaged muscle fibres that are filtrated by CD16- and CD68-positive cells is small (0.20–1.35%); however, the inflammatory cells are located more at the endomysium. Paulsen et al. (112) have reported no association between DOMS and inflammation of muscle fibres after 300 eccentric contractions of the quadriceps femoris.

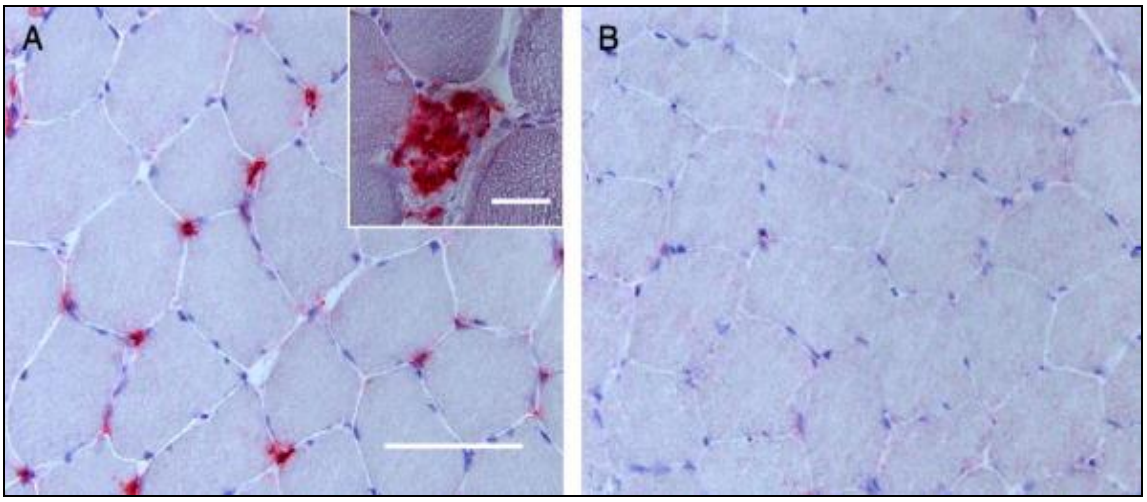

Figure 3. *A*:CD16+ cells (red stain) were observed in the interstitial spaces of the exercising leg (musculus vastus lateralis) shown here at 96 h (4 d) after exercise. The inserted picture shows CD68+ cells (red) inside a muscle cell (scale bar = 50 μm). *B*:A small number of CD16+ cells were noted in the control leg. The blue stain (hematoxylin) shows nuclei. Scale bar = 100 μm. Adapted from Paulsen et al. (112).

Raastad et al. (118) showed that the myofibrillar (Z-line structure) disruptions are related to the magnitude of force loss after eccentric contractions. However, it is not clear whether the extent of muscle fibre degeneration is associated with the magnitude of force loss after eccentric exercise. Crameri et al. (32) found greater force loss after voluntary maximal eccentric contractions (VOL) than eccentric contractions with

electrical muscle stimulation (EMS) of the knee extensors, although damaged muscle fibres were less after VOL than EMS (Figure 4). They also found that the magnitude of DOMS developed after exercise and increased staining of the intramuscular connective tissue (tenascin C) were similar between EMS and VOL.

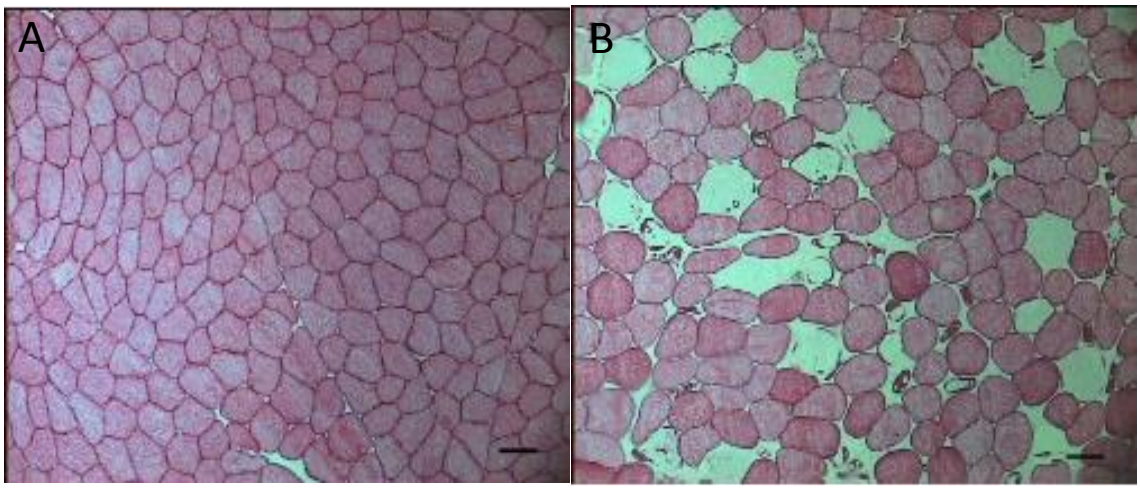

Figure 4. Detection of gross disturbance to the myofibre. No desmin-negative muscle fibres are noted after voluntary eccentric exercise (A) at any time point measured. In contrast, there was a significant increase in the number of myofibres that were not immunoreactive to desmin after voluntary eccentric exercise plus electrical stimulation, showing classic signs of myofibre necrosis (B). Scale bars represent 50 μm. Adapted from Crameri et al. (32).

The relationship between histological changes and other symptoms of muscle damage (i.e. increased stiffness, swelling) is less clear, and to the best of my knowledge, this has not been investigated. Thus, it is important to note that histological changes observed in muscle biopsy samples do not necessarily represent whole picture of muscle damage. Warren et al. (138) stated that the needle biopsy only represents a small fraction of the involved muscle and questioned whether this small sample biopsy actually presents the changes in the exercise muscle; and it also suggested that the

measurement of voluntary contraction torque and range of motion are the best methods for quantifying muscle injury.

1.1.2.9 Relationship between DOMS and Other Indirect Markers of Muscle Damage

Some studies have investigated the relationship between DOMS and other indirect markers of muscle damage such as MVC, ROM and CK activity in the blood following eccentric contractions. Rodenburg et al. (120) found that there was a low correlation (r=-0.38) between DOMS assessed by a scale ranging from 0 to 6 (0: no soreness, 6: intolerable soreness) and MVC, and DOMS and CK activity at day 2 post-exercise (r=0.58) following 120 maximal eccentric contractions of the forearm flexors. Smith et al. (129) investigated the impact of a repeated bout of eccentric chest press exercises on DOMS and serum CK activity and reported that DOMS and serum CK activity were not associated, such that DOMS was the same between bouts but the increases in serum CK activity were significantly less in the repeated bout. Nosaka et al. (115) showed the dissociation between the time course and the magnitude of DOMS and other indirect markers of muscle damage (Figure 5).

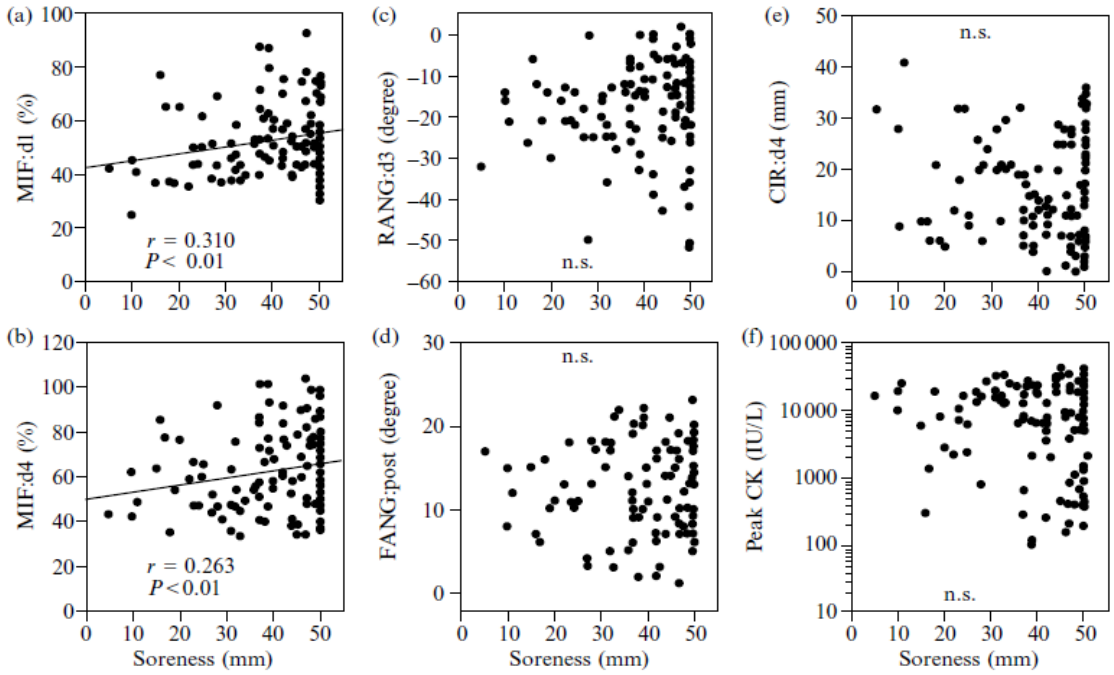

Figure 5. Correlations between peak muscle soreness when extending the elbow joint and other indicators of muscle damage (a, b: maximal isometric force, c: relaxed elbow joint angle, d: flexed elbow joint angle, e: upper arm circumference, f: peak plasma CK activity). Post: immediately post-exercise, d1: 1 day post-exercise, d3: 3 days post-exercise, d4: 4 days post-exercise, ns: not significant, *n*=110. Adapted from Nosaka et al. (115).

1.1.3 Repeated Bout Effect

It is well documented that a repeated bout of the same eccentric exercise performed within several weeks to months results in less muscle damage than the first bout, and this protective adaptation is referred to as the repeated bout effect (57, 83). The repeated bout effect has been investigated using eccentric exercise of the knee extensors (69, 88) and the elbow flexors (56, 93). The repeated bout effect is characterised by a faster recovery of muscle function such as MVC and ROM, and smaller increases in DOMS and CK activity in the blood, less swelling and abnormality shown by ultrasound and/or magnetic resonance images (83, 98). Figure 6 shows a typical repeated bout effect for changes in indirect markers of muscle damage following two bouts of eccentric contractions of the elbow flexors separated by 4 weeks (93).

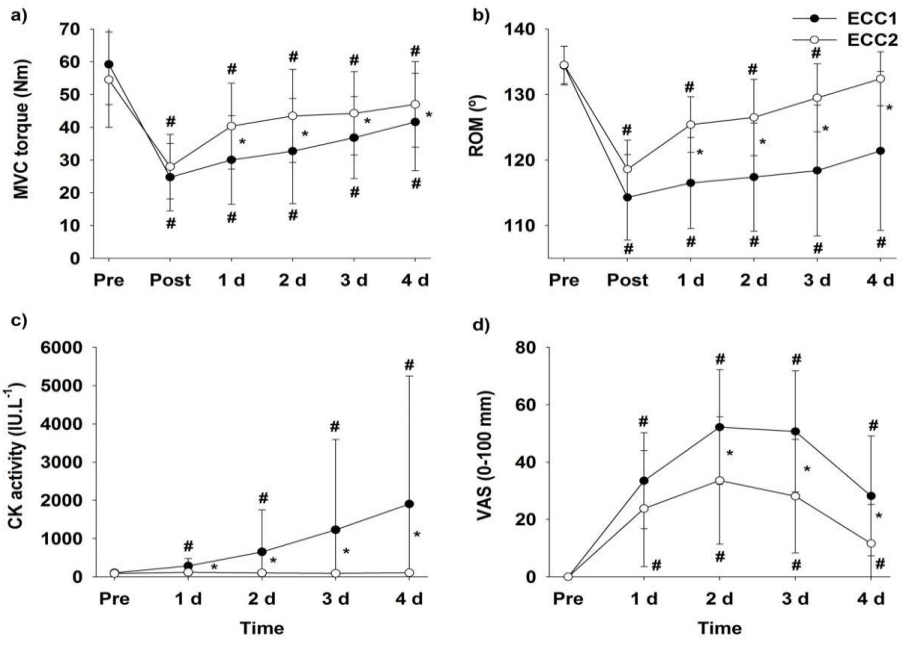

Figure 6. Time course of changes (mean values ± SD; N = 10) in (a) maximal voluntary isometric contraction (MVC) torque, (b) range of motion (ROM), (c) plasma CK activity, and (d) muscle soreness by VAS measured at pre-exercise (Pre), immediately post-exercise (Post), and 1 – 4 days after the first (ECC1) and second (ECC2) eccentric exercise bouts. *: significantly ($P<0.05$) different between ECC1 and ECC2. #: significantly ($P<0.05$) different from Pre. Adapted from Muthalib et al. (93).

The repeated bout adaptation has been shown to occur after an initial eccentric exercise bout, after as little as two maximal eccentric contractions (106) or even after low intensity eccentric contractions (18) or maximal isometric contractions at a long muscle length (22). For example, Nosaka et al. (106) demonstrated that two maximal eccentric contractions can confer a protective effect against the subsequent bout of 24 maximal eccentric contractions performed two weeks later. Chen et al. (18) reported that low-intensity eccentric contractions (10% of MVC) conferred protective effect against muscle damage induced by maximal eccentric contractions performed either 2, 7 or 14 days later. Recently, Chen et al. (19) reported that two maximal isometric contractions performed 2 and 4 days before 30 maximal eccentric contractions significantly attenuated the magnitude of muscle damage. Previous studies (26, 105) showed that the protective effect starts as early as 1-2 days and lasts for at least 6 months for most damage markers, but it disappears between 9 and 12 months after an initial eccentric exercise bout.

1.1.3.1 Possible Mechanisms of Repeated Bout Effect

The exact mechanisms underpinning the repeated bout effect are not fully elucidated; however, it has been speculated to be associated with a combination of neural, mechanical and cellular adaptations (83).

1.1.3.1.1 Neural Adaptation

It has been proposed in previous studies (26, 82, 83) that neural adaptations include more efficient recruitment of motor units, increased synchrony of motor unit firing, better distribution of the workload among muscle fibres, improved usage of the workload among muscle fibres, improved usage of synergist muscles, and increased

slow-twitch fibre recruitment. For instance, Dartnall et al. (35) showed that the motor unit synchronisation increased by 34% at 24 h after a single bout of eccentric contractions. Dartnall et al. (36) also found that the motor unit synchronisation was elevated immediately after and remained elevated by 57% at 7 days after the first bout of eccentric exercise, and the motor unit synchronisation still remained higher than the baseline (before the first eccentric bout) when the same bout of eccentric exercise was repeated 7 days after the initial bout. Therefore, changes in the motor unit recruitment could limit the extent of damage in the second bout.

1.1.3.1.2 Mechanical Adaptation

McHugh et al. (83) speculated that increases in the extensibility of relaxed muscle (passive stiffness) and active muscle (dynamic stiffness), remodelling of the intermediate filament system, and increased intramuscular connective tissue following eccentric training are mechanical adaptations that could protect against damage from the repeated bout. For example, Lapier et al. (74) examined the intramuscular connective tissue of rat extensor digitorum longus muscles after immobilising them for 3 weeks at either a shortened or lengthened position, and found that the intramuscular connective tissue concentration was increased for both conditions, and that muscle damage was attenuated in these muscles after electrically stimulated eccentric contractions of the plantar flexors.

1.1.3.1.3 Cellular Adaptation

Cellular adaptation theory includes addition of sarcomeres, excitation-contraction coupling changes and adaptations in the inflammatory response to eccentric contractions following the initial bout. Then, the repeated bout adaptation is the result of reduced

sarcomere strain and/or adaptation to structures involved in E-C coupling in the subsequent bout. For example, Hubal et al. (60) examined the changes in mRNA levels and protein localisation of inflammatory genes following two bouts of eccentric exercise separated by 4 weeks. They found that several inflammatory genes were transcriptionally unregulated (rather than attenuated) after the subsequent eccentric bout, potentially indicating a role for these genes in the adaptation process.

1.1.3.1.4 Other Adaptations

Other possible adaptations include increases in heat shock protein activities (111), and remodelling of sarcomeres (140) or ECM (79) following the initial bout of eccentric exercise. Paulsen et al. (110) investigated the expression of heat shock protein (HSP27), αB-crystallin and HSP70 after two bouts of 70 eccentric elbow flexor contractions in humans, and found that a large amount of the HSP27, αB-crystallin and HSP70 in the cytoskeletal myofibrillar faction after a repeated bout of exercise, and indicated an increase in these proteins is a protective role as part of the repeated bout effect. Furthermore, Mackey et al. (79) investigated the ECM in the gastrocnemius muscles following a single bout or repeated bout of electrical stimulation, and found that ECM laminin-β1 and collagen types I and III were elevated after the first bout of stimulation, and concluded that the strengthening of ECM plays a role in protecting against muscle damage.

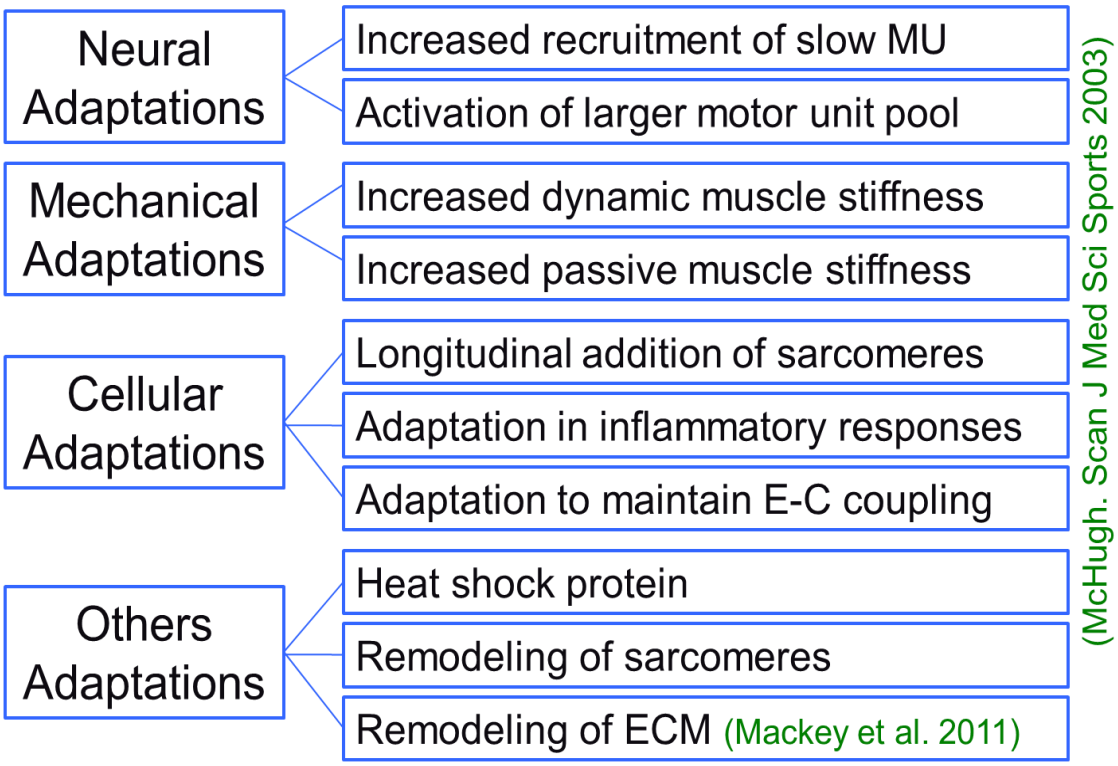

Figure 7. Summary of the possible mechanisms of repeated bout effect. Based on the review of previous studies (79, 83).

1.1.4 Pain and Pain Receptors (Nociceptors)

Pain is an unpleasant sensory and emotional experience (86). It is generally considered a warning signal of actual or perceived tissue damage (45, 136). However, pain often develops without clear evidence of tissue damage, and its onset, magnitude and duration do not necessarily correspond to tissue damage (84).

It is known that skeletal muscles contain four types of afferent fibres: group I (Aα), II (Aβ), III (Aδ), and IV (C), and the free nerve endings of the latter two respond to noxious stimuli such as mechanical pressure, heat, cold, and algesic substances such as bradykinin, potassium, serotonin and histamine (45). Group III (Aδ) afferent fibres are wide in diameter with thin myelinated fibres with a relatively fast conducting

velocity (5-30 $m \cdot s^{-1}$), and these fibres respond to muscle stretch, contractions, and noxious pressure and are sensitised by thermal and chemical stimuli (45). Group IV (C) fibres are thin and unmyelinated, and transmit signals more slowly (0.5-2 $m \cdot s^{-1}$) than group III fibres (45). Similarly, Group IV (C) fibres respond to thermal stimuli and ischemia, and are sensitised by chemical stimuli (45). Stimulation of Aδ fibres in the skin results in a sharp, pricking and stabbing pain (110) However, stimulation of Aδ and C fibres in muscle elicits a dull, aching and cramping pain (45). It is important to note that pain sensation from muscle is thought to be mainly mediated by group IV fibres, and group III fibres are secondary (75).

It has been documented that free nerve endings (nociceptors) are located along the walls of arteries and mostly in the surrounding connective tissue (42, 53). Figure 8 shows the illustration of afferent fibres in cat skeletal muscle.

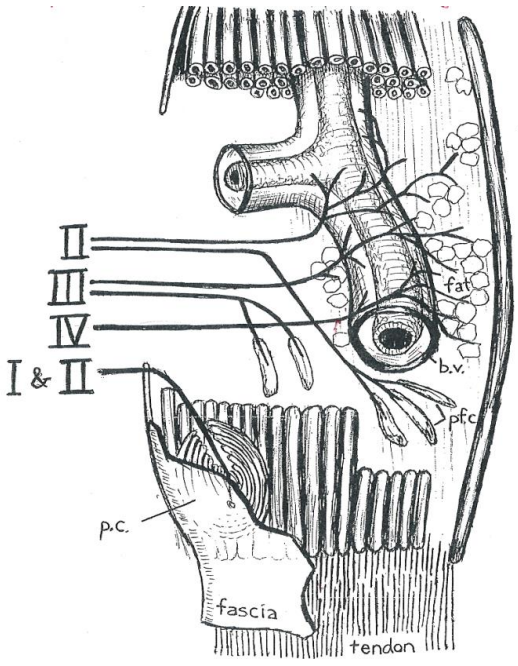

Figure 8. An illustration of afferent fibres in cat skeletal muscle. Type I and II afferents do not respond to noxious stimuli, while types III and IV are nociresponsive. Abbreviations are as follows: b.v. = blood vessel, p.c. = pacinian corpuscle, pf.c = paciniform corpuscle. Adapted from O'Connor and Cook (110).

The difference in the density of nociceptors between connective tissue and muscle belly has been reported in previous studies (85, 130). Mense and Simons (85) reported that the innervation density of nociceptors in the connective tissue surrounding the calcaneal tendon of a cat was approximately five times higher than in the gastrocnemius-soleus muscle but no difference was found in the innervation density throughout normal muscle tissue. Tesarz et al. (40) investigated the density and distribution of nerve fibres in rats as well as human thoracolumbar fascia by immunohistological technique, and documented that muscle fascia had a dense neuronal (PGP9.5-positive) innervation with nonpeptidergic nerve fibre endings and encapsulated mechanoreceptors. It appears that connective tissue such as fascia, which contains high density of nociceptors, is responsible for muscle pain.

1.1.5. Assessments of Muscle Pain

There is no generally accepted single best measure of pain (110). To quantify the level of muscle soreness is a challenge due to the subjective nature of pain (110). Therefore, it is necessary to integrate information from different pain measures to understand pain (132). There are two different pain assessment methods for evaluating pain, pain threshold assessment and the suprathreshold pain rating method. Pain threshold assessment is based on the onset of pain sensation evoked by pressure, heat, cold or electrical stimulus (49). The suprathreshold pain rating method detects the magnitude of pain in response to the stimulus that is generally above the pain threshold using a scale, including intensity visual analogue scale (VAS), verbal rating scale, numerical rating scale, and descriptor differential scale (49, 132) or questionnaire like Mc Gill questionnaire and quantity by assessing pain locations.

1.1.5.1. Pressure Pain Threshold (PPT)

Pressure pain threshold (PPT) is a single point method that detects the pain threshold by using a pressure algometer applying a minimum stimulus intensity to perceive a painful sensation (49, 110). The pain sensation of PPT depends on the degree of stimulus intensity or the duration of time corresponding to a fixed response to pain threshold. Previous studies (4, 43, 72) documented that the stimulating area (size of the probe), the skin sensitivity, muscle and subcutaneous tissue thickness also influence the PPT assessments. Andersen et al. (4) suggested that using a larger stimulated area (probe) to detect muscle pain threshold could reduce the cutaneous sensitisation during measurement because the pressure is spread over a larger area of the tissue. Kosek et al. (72) reported that skin pressure pain sensitivity influenced PPT values. Fischer (43) also reported that PPT values were influenced by a variation in muscle thickness and subcutaneous tissues among subjects or by the inherent pain sensitivity difference between subjects. PPT has been demonstrated to be reliable for measuring pain threshold (25, 109) and used to assess DOMS following eccentric contractions (76, 95, 127).

Rice et al. (119) reported that PPT significantly decreased at one day post-exercise and no further change was seen at two days following four sets of 15 eccentric and concentric contractions of the knee extensors. Peake et al. (113) showed that PPT decreased at one day post-exercise, and no further decrease was evident at two days post-exercise following 10 sets of three eccentric contractions of the elbow flexors. Some studies (39, 46) investigated the distribution of PPT in response to DOMS on the lower limb muscles, forearm (41, 126) or shoulder muscle (70); and found that the pain sensation is unevenly distributed. For example, Hedayatpour et al. (71) have recently investigated DOMS by using a PPT mapping method on 15 sites on the knee extensor

muscle following 4 bouts of 25 sets eccentric knee extension contractions, and found that a greater reduction in PPT is located at the distal region than the proximal region of quadriceps muscle following exercise.

1.1.5.2. Ratings of Pain Intensity

The rating of pain intensity using different scales is used to quantify muscle pain by applying stimuli that could evoke pain such as muscle contraction, stretching, palpation, or hypertonic saline injection (15, 47). There are different pain scales such as a visual analogue scale (VAS) (141), verbal rating scale (1), numerical rating scale (65), and descriptor differential scale (54) which have been used in previous studies to assess DOMS. Among them, VAS is most often used for DOMS assessment (9) with a certain length of line (e.g., 100 mm) in which one end of the line indicates no pain and the other end indicates extreme pain. A previous study (38) documented that VAS is a sensitive, simple, reproducible and universal self-rating pain scale. Since this method includes sensations over the whole perceptual range and does not detect only a single point of the threshold level, subjects can quantify the evoked pain sensation on a scale, and this rating method is classified as a response-dependent method (49). The use of VAS to assess musculoskeletal pain has been reported to be reliable (14, 116); however, the assessment of palpation soreness by VAS is often criticised because the pain sensation can vary among subjects and the ambiguity in the palpation procedure such as the pressure applied to the muscle (11).

1.1.5.3. Electrical Pain Threshold (EPT)

EPT is an invasive intramuscular electrical stimulation technique, which is assessed by inserting a needle electrode into the muscle with the electrical current intensity being increased gradually to quantify the pain threshold. The electronic current excites afferent pathways in an unnatural synchronised fashion, bypasses the afferent receptors, and activates and excites all nociceptive afferent fibres inside the tissue (49). Itoh et al. (70) measured EPT of the skin, fascia and muscle separately, while the intensity of the current stimulus increases at a constant rate until pain is felt, and reported that EPT was significantly lower in the fascia compared with the muscle and skin of the forearm at 2 days after eccentric exercise of the middle finger (Figure 9).

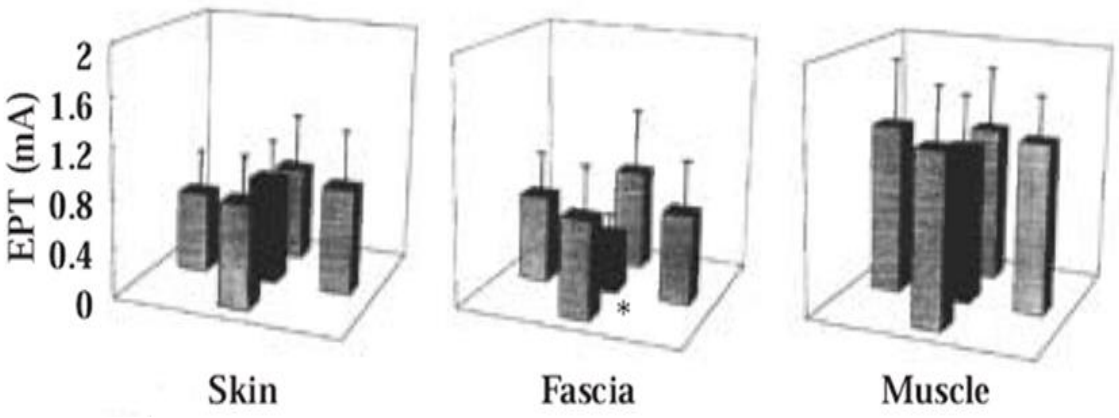

Figure 9. The distribution of electrical pain thresholds (EPTs) of the different tissues readings taken on the second day after eccentric exercise of the middle finger. Adapted from Itoh et al. (70).

1.1.6. Mechanisms of DOMS

As described above, the damage to contractile proteins, intermediate filaments, and/or connective tissue surrounding muscle fibres, and the subsequent inflammatory responses are thought to be responsible for DOMS (26, 30, 57). However, the exact underlying mechanisms of DOMS are still not fully understood.

Historically, a lactic acid theory was proposed, but this theory was rejected as no correlation was found between lactic acid levels and DOMS following exercise (124). A muscle spasm theory was also proposed, but no correlation was found between an increase in EMG and a perception of soreness (1, 96). A muscle damage theory was also introduced, which focuses on the disruption of the contractile component of the muscle tissue, particularly at the level of the Z-line (6, 66). The disruption of myofilaments (e.g. Z-line) and sarcolemma were thought to result in muscle fibre damage and inflammation, inducing DOMS. However, previous studies (115, 129) found no correlation between DOMS and the amount of increase in CK activity in the blood following eccentric exercise. As described above, several studies did not find extensive muscle fibre damage after eccentric exercise, and failed to find an association between muscle fibre damage and DOMS (32, 118).

A connective tissue damage-inflammation theory has been proposed relatively recently. Paulsen et al. (112) found no correlation between DOMS and leukocyte accumulation in the muscle fibres, and stated that damage and remodelling of the extracellular matrix (ECM) were related to DOMS. Other studies also found the evidence to support that ECM or endomysium inflammation would be more closely associated with DOMS (32, 118) Gibson et al. (15) showed that fascia rather than muscle tissue in the tibialis anterior muscle became more sensitive to hypertonic saline injection when DOMS existed (Figure 10).

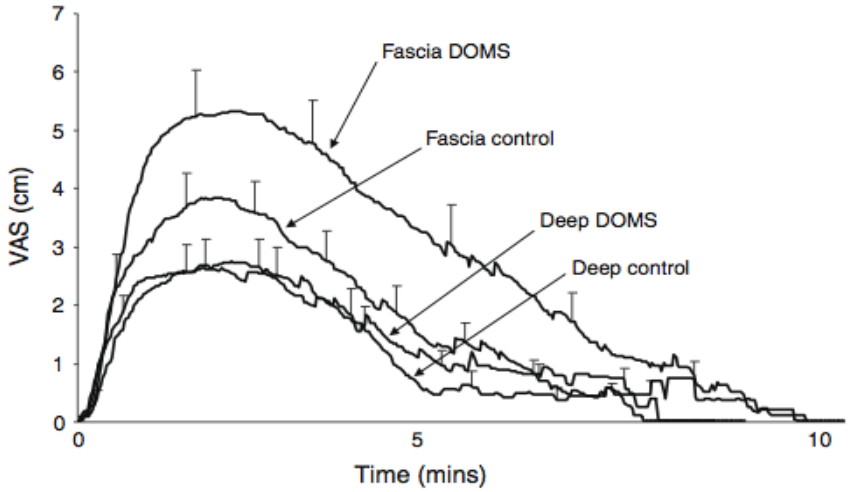

Figure 10. Average (±SE) visual analogue scale (VAS) profiles following hypertonic saline injection (0.5 ml, 5.8%) to the fascia and deep structures in the muscles with DOMS and without DOMS (control). Adapted from Gibson et al. (15).

The understanding of molecular mechanisms of DOMS has been expanded in the last several years based on animal studies. As summarised in Figure 11, it has been found that the release of bradykinin from the damaged tissues not only sensitise nociceptors, but also changes the expression of neuropeptides and channels in several types of cell (10, 62). Recently, nerve growth factor (NGF) has been given some attention to mechanical hyperalgesia. NGF has been shown to be produced by either degenerated tissues or skeletal muscle after ischemia (135) and nerve injury (3). Some studies reported that NGF could excite and sensitise the cutaneous (12, 125) and muscular nociceptors (81, 92). Furthermore, muscular mechanical hyperalgesia has also been shown to be induced after intramuscular NGF injection (92, 133). Murase et al. (92) have recently investigated the bradykinin and nerve growth factor in mechanical hyperalgesia after eccentric exercise of rats' extensor digitorum longus (EDL) muscles and found that bradykinin was released during exercise from vascular endothelial cells,

and triggered upregulation of NGF through B_2 receptors, and then the NGF increased nociceptors sensitisation and continuously changed the expression of neurotransmitter and ion channels in dorsal root ganglion (DRGs) neurons (114, 142). Another neurotrophic factor glial cell line-derived (GDNF) has been recently reported (90, 91) to increase the response of muscular Aδ-fibre afferents to mechanical stimuli, resulting in muscular mechanical hyperalgesia. Murase et al. (91) found that cyclooxygenase (COX)-2 was upregulated shortly after exercise, thus increasing the prostaglandins and was followed by triggered upregulation of GDNF in the muscle which increased nociceptor sensitisation associated with DOMS.

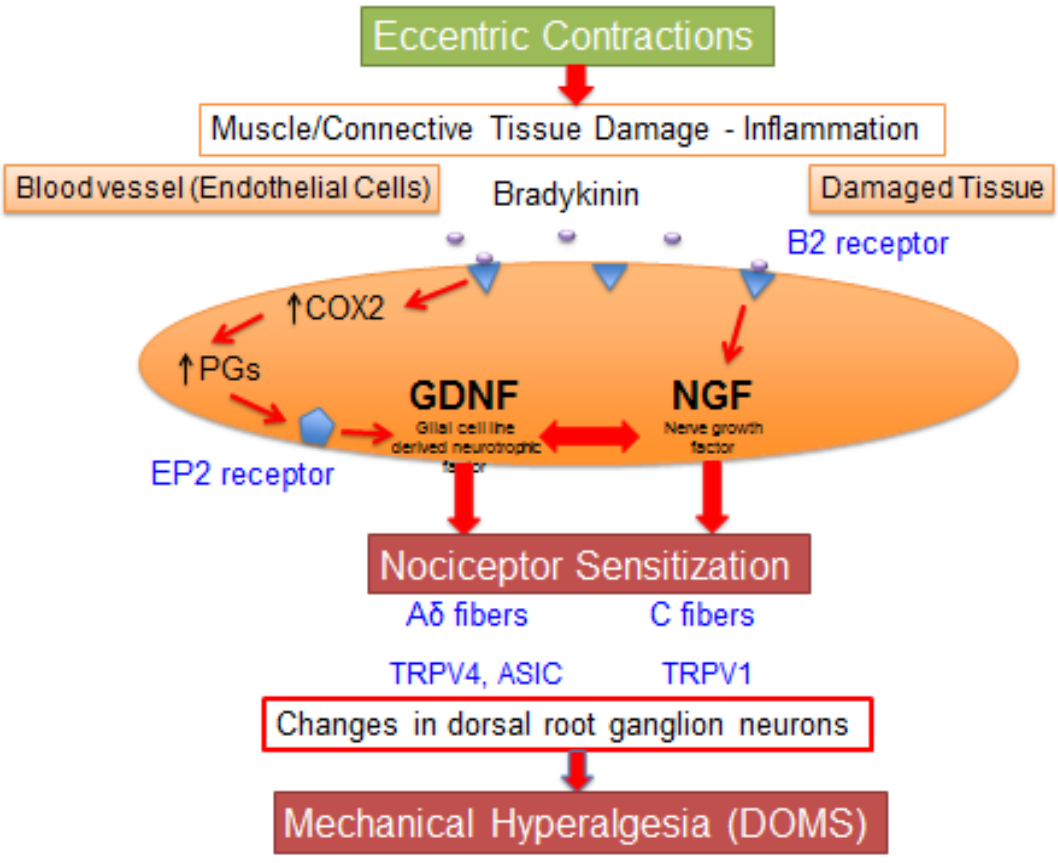

Figure 11. The possible mechanisms of mechanical hyperalgesia (DOMS) following eccentric exercise induced muscle/connective tissue damage. Based on the findings from two recent animal studies (91, 92).

1.1.7. Focus of This Research Project

From the literature review described above, the following areas of research were identified to be necessary. Previous studies applied both VAS and PPT to evaluate DOMS (9, 113, 119), but no previous study has investigated whether DOMS assessed by a VAS upon palpation and PPT assessments is related, although both measures assess the pain induced by pressure. Uneven pain sensation distribution on the lower limb muscles assessed by PPT mapping method has been reported in previous studies (39, 46, 71); however, no previous studies have examined the pain distribution of the elbow flexor muscles after elbow flexor eccentric exercise that is often used as a model to investigate DOMS (20, 76, 93). Since previous studies showed that damage to connective tissue is associated with DOMS, it seems that the changes in the pain sensation in the connective tissue such as fascia would be different than muscle. Itoh et al. (70) measured the electrical pain threshold (EPT) as a way to assess the pain threshold of the fascia and muscle of extensor digital muscle. However, no previous studies have investigated the pain sensation of biceps brachii fascia, biceps brachii and brachialis fascia by EPT, and whether the changes in EPT are associated with the magnitude of muscle soreness assessed by other methods (i.e. VAS, PPT) following a repeated bout of eccentric elbow flexor contractions. Previous animal (61, 80) and human studies (67, 108) have shown that muscle length change is a key factor influencing the magnitude of muscle damage and muscle soreness induced by eccentric exercise. If these mechanisms are indeed pivotal, then a greater muscle lengthening during eccentric contractions should result in greater muscle damage and soreness. However, no previous study has investigated whether the magnitude of muscle lengthening during eccentric contractions is associated with the magnitude of muscle

damage and DOMS after eccentric exercise. From these studies, the possible connective tissue damage-inflammation theory to explain the cause of DOMS will be discussed.

1.2. Purpose of Research

The scope of the thesis was to investigate delayed onset muscle soreness (DOMS) after elbow flexor eccentric exercise using several different pain assessments and to test the hypothesis that DOMS would be more associated with connective tissue than muscle fibre damage-inflammation. The thesis comprises four separate studies, which have their own purpose as shown below.

Study 1 investigated the relationship between two commonly used pain assessments to quantify delayed onset muscle soreness by visual analogue scale (VAS) and pressure pain threshold (PPT) after a single bout of maximal eccentric exercise. The subsequent study (Study 2) investigated the distribution of PPT over upper arm (biceps brachii/brachialis muscles) using a grid method to clarify which region of the muscle became more sensitive after elbow flexor eccentric exercise. Furthermore, it also investigated whether different stimuli: static pressure and palpation (circular, longitudinal or transverse movements) would induce different levels of pain assessed by VAS. The third study (Study 3) investigated the changes in electrical pain threshold (EPT) on biceps brachii fascia, biceps brachii and brachialis fascia after eccentric exercise to test the hypothesis that fascia would become more sensitive than muscle. The last study of this thesis (Study 4) investigated the magnitude of muscle lengthening during the first and second bout of eccentric exercise bouts by using real-time ultrasound, and whether the magnitude of muscle length changes would be associated

with the magnitude of DOMS and changes in other indirect markers of muscle damage between bouts.

1.3. Research Questions

The present thesis will provide the answers to the following specific questions.

1. Do VAS and PPT pain assessments represent different aspects of DOMS?
2. Does palpation induce greater pain than static pressure assessment?
3. Does DOMS develop at specific regions of the biceps brachii after eccentric exercise of the elbow flexors?
4. Do different palpation movements induce different pain sensation?
5. Does EPT decrease greater at the fascia than the muscle?
6. Is the magnitude of the biceps brachii myotendinous junction movement less during the second bout when compared with the first eccentric exercise bout of the elbow flexors?
7. Is the change in the myotendinous junction displacement during the course of eccentric exercise associated with DOMS?

CHAPTER TWO

STUDY 1

2.1 INTRODUCTION

People often experience muscle pain in the days following exercise or daily activities, and this type of pain is referred to as delayed onset muscle soreness (DOMS) (26). DOMS is characterised by the sensation of a dull, aching pain, usually felt during movement or palpation of the affected muscles, develops within 24 hours after performing exercise, and peaks 1-3 days post-exercise (26, 92). The underlying mechanisms of DOMS have not been fully understood, but it has been documented that damage to contractile proteins, intermediate filaments and/or connective tissue surrounding muscle fibres, and subsequent inflammatory processes are associated with it (26, 58). DOMS is considered a mechanical hyperalgesia, which is characterised by an increased sensitivity of nociceptors (type III and IV afferents) to a stimulus (92) and/or allodynia in which pain is induced by a stimulus that does not normally provoke pain (16, 34).

To quantify the level of muscle soreness is a challenge due to the subjective nature of pain (110). Different pain scales such as a visual analogue scale (VAS) (141), verbal rating scale (1), numerical rating scale (65), and descriptor differential scale (54) have been used in previous studies to assess DOMS. Among them the VAS is most often used for DOMS assessment (9, 141) consisting of a certain length of line (e.g., 100 mm) in which one end of the line indicates no pain and the other end indicates the worst pain. Since DOMS is not felt when the affected muscle is still, it is necessary to

provide a mechanical stimulus to induce the pain such as palpation, contraction, or stretching of the muscle (110, 127). The use of VAS to assess musculoskeletal pain has been reported to be reliable (14, 116); however, the assessment of palpation soreness by VAS is often criticised because of the ambiguity in the palpation procedure (11).

An alternative way to quantify muscle pain is the use of a pressure algometer that assesses the point where a sensation of pressure changes into a sensation of pain in the muscle, which is referred to as the pressure pain threshold (PPT) (52, 68). PPT has been demonstrated to be reliable for measuring pain threshold (25, 109). Previous studies used PPT to assess DOMS (13, 41) and some of the studies applied both VAS and PPT to evaluate DOMS (9, 113, 119). Previous studies showed that muscle soreness assessed by VAS peaked at two days, and PPT decreased the most at one day post-exercise and no further decrease was seen at two days following eccentric exercise of the elbow flexors (76, 113).

It appears that DOMS assessed by VAS upon palpation and that by PPT are related, since both measures assess the pain induced by pressure. However, no correlation analysis between VAS and PPT has been performed in previous studies. It is necessary to clarify how the VAS and PPT measures are associated with each other and whether they provide different information about DOMS. Therefore, the purpose of this study was to examine the relationship between VAS upon palpation and PPT of the elbow flexors following eccentric exercise of the elbow flexors.

2.2 METHODS

2.2.1 Subjects

This study was approved by the Institutional Human Research Ethics Committee and complied with the Declaration of Helsinki. Thirty-one healthy men with no current or previous upper arm injuries and who had not performed resistance training of the upper limbs for at least six months prior to the present study were recruited. Their mean ± standard deviation (SD) age, body weight, and height were 25.8 ± 5.5 y, 70.2 ± 9.5 kg, and 173.4 ± 7.2 cm respectively. All subjects completed an informed written consent form and a medical questionnaire before participating in the study. Subjects were requested not to change their lifestyle and diet, not take any anti-inflammatory drugs or nutritional supplements and not perform unaccustomed exercise during the experimental period.

2.2.2 Eccentric Exercise

The exercise consisted of 10 sets of six maximal voluntary eccentric contractions of the elbow flexors on an isokinetic dynamometer (Cybex 6000, Ronkonkoma, NY. USA). For each eccentric contraction, the elbow joint was forcibly extended from a flexed (90°) to a fully extended position (~0°) in 1s at an angular velocity of $90°\cdot s^{-1}$ in a supinated wrist position. The subjects were verbally encouraged to generate maximal force at the flexed position and to maximally resist against the elbow extending action throughout the range of motion. After each eccentric contraction, the isokinetic dynamometer returned the arm to the flexed position at a velocity of $9°\cdot s^{-1}$, which provided a 10-s rest between contractions. The rest period between sets was three minutes. Torque and displacement signals were obtained directly

from the dynamometer output and captured using a data acquisition system (PowerLab with a Chart 7 software, ADinstruments, Bella Vista, Australia).

2.2.3 Muscle Damage Markers

2.2.3.1 Maximal Voluntary Isometric Contraction (MVC) Torque

As a marker of muscle damage, maximal voluntary isometric contraction (MVC) torque of the elbow flexors was measured before, immediately after, and 1 to 4 days following exercise. Using the same isokinetic dynamometer (Cybex 6000) and the same positioning of the subjects as described for the eccentric exercise, subjects performed two 3-s maximal voluntary isometric contractions at an elbow joint angle of 90° with a 60-s rest between contractions. The higher torque of the two measures was used for further analysis.

2.2.3.2 Visual Analogue Scale (VAS)

The level of muscle soreness was quantified using a 100 mm VAS in which 0 indicated “no pain” and 100 represented “extreme pain”. The subjects were asked to mark the level of perceived soreness on the VAS, when the elbow flexors were palpated in a circular motion by the investigator before and one, two, three and four days after exercise (76). During the palpation, the investigator placed his index and middle fingers over the mid-belly of the biceps brachii at 5, 9 and 13 cm above the elbow crease while the subject placed his forearm on an armrest that supported the elbow joint angle at approximately 90°. The investigator applied pressure (approximately 40 kPa) and palpated in a clockwise direction with the tips of the two fingers toward the deeper tissues at each site for approximately 3 s. The pressure (40 kPa) was measured by a handheld dynamometer in the pilot testing, and showing that this pressure induced pain

when DOMS existed but not when DOMS was absent for most subjects, and it was close to the PPT for biceps brachii muscles for most subjects before exercise. The investigator practised more than 100 times to reproduce the pressure, and it was confirmed that the investigator could apply this pressure constantly. The palpation pressure given to the sites was kept as constant as possible between days and among subjects, and all measurements were taken by the same investigator throughout the experiment. The measurement at the 5 cm site was performed first followed by the measurements at the 9 and 13 cm sites in this order. One measurement was taken from each site with a 10-s interval between measurements. It should be noted that the arm length was not considered for the measurement sites, thus the relative distribution of the measurement sites was different among the subjects depending on the arm length in the present study.

2.2.3.3 Pressure Pain Threshold (PPT)

After the VAS evaluation, pressure pain threshold was measured using an electronic algometer (Somedic AB, Sweden) before, and 1 to 4 days after exercise. The probe head of the algometer (area of 1.0 cm^2) was placed perpendicular to the mid-belly of the biceps brachii at 5, 9, and 13 cm above the elbow crease (the same sites as the palpation muscle soreness measures by VAS) and force was gradually applied at a rate of 50 $kPa \cdot s^{-1}$ until the subject reported the first feeling of noticeable pain of the muscle. The value (in kPa) corresponding to the force applied to elicit pain was recorded. In the same way to that of the VAS assessment, the 5 cm site was measured first followed by the 9 and 13 cm sites with a 30 s interval between measurements. Two minutes after completing the first round of the PPT assessment, the second round of the PPT

assessment was performed in the same order and interval between sites. The average of the two measures for each site was used for further analysis.

2.2.4 Statistical Analysis

Changes in MVC torque over time were analysed by a one-way repeated measures analysis of variance (ANOVA). When the ANOVA showed a significant time effect, a Tukey's post-hoc test was followed for multiple comparisons. Changes in VAS and PPT over time were compared amongst the three measurement sites (5, 9, 13 cm) by a two-way repeated measures ANOVA. Pearson's product moment correlation coefficient was used to analyse the relationship between the changes in VAS and PPT measures following eccentric contractions. A statistical significance was set at $P<0.05$, and all data were presented as mean ± standard error of mean (SEM), unless otherwise stated.

2.3 RESULTS

2.3.1 Reliability of the Measurements

Intra-class correlation (r) and coefficient variation (CV) were used to analyse the reliability of the VAS and PPT measurements using the data obtained from 10 subjects used in the study who had two pre-exercise measurements taken at one day prior to and immediately before exercise. The r of the intra-class correlation for 5, 9, and 13 cm sites ranged from 0.98-0.99 for VAS and from 0.92-0.98 for PPT, and the CV for 5, 9, and 13 cm sites ranged from 2.2-4.5% for VAS and from 5.6-8.9% for PPT.

2.3.2 MVC Torque

The baseline MVC torque was 55.5 ± 2.0 Nm. MVC torque decreased significantly ($P<0.05$) at 1 day post-exercise by approximately 40% to 32.9 ± 1.9 Nm, recovered to 71% of the pre-exercise level at 3 days (39.6 ± 1.9 Nm), and remained significantly ($P<0.05$) below the baseline by 23% at 4 days post-exercise (42.8 ± 2.0 Nm).

2.3.3 VAS

Figure 12 shows changes in VAS upon palpation of the biceps brachii muscle at the 5, 9, and 13 cm sites following eccentric exercise. The VAS significantly ($P<0.05$) increased after exercise and peaked at two days post-exercise. No significant ($P=0.62$) difference in the changes in VAS was evident among the three sites.

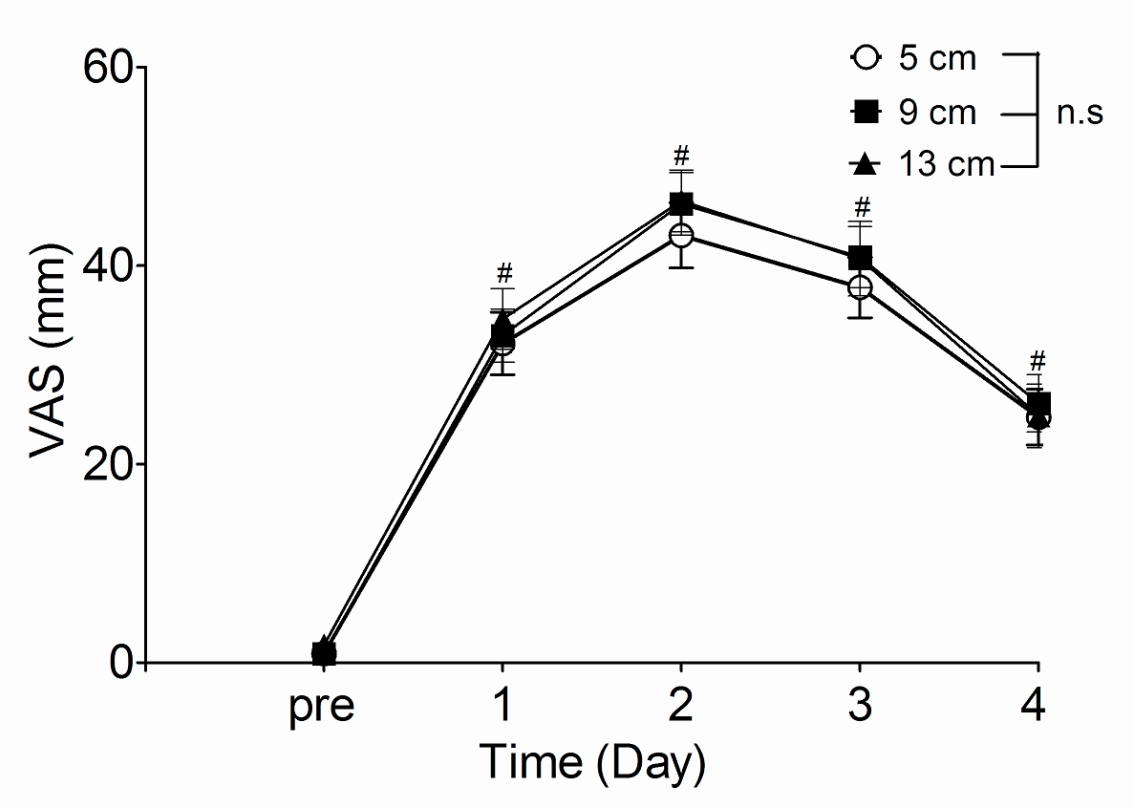

Figure 12. Changes (mean ± SEM) in visual analogue scale (VAS) upon palpation at three sites (5, 9, and 13 cm) before (pre) and 1 to 4 days following eccentric exercise. # = significant ($P<0.05$) difference from the pre-exercise value, n.s. = not significantly different among the groups.

2.3.4 PPT

Changes in PPT at the 5, 9, and 13 cm sites are shown in Figure 13. No significant (P=0.87) difference in the pre-exercise PPT was found among the sites. The pressure to elicit pain decreased significantly (P<0.05) from the baseline (368.4 ± 23.7 kPa) to one day after eccentric exercise (262.7 ± 21.3 kPa), and remained significantly (P<0.05) below the baseline (328 ± 26.7 kPa) by 11% at four days post-exercise. No significant (P=0.45) difference in the changes in the PPT was evident amongst the three sites.

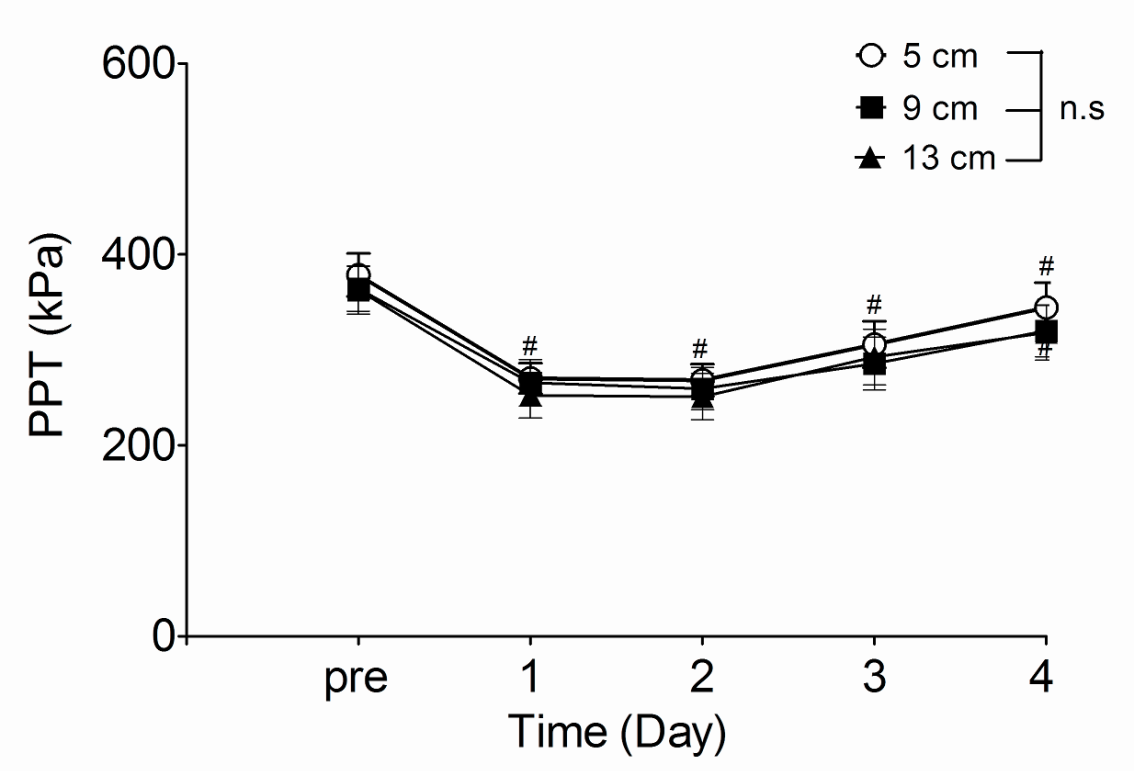

Figure 13. Changes (mean ± SEM) in pressure pain threshold (PPT) of biceps brachii muscle at three sites (5, 9, and 13 cm) before (pre) and 1 to 4 days following eccentric exercise. # = significant (P<0.05) difference from the pre-exercise value, n.s. = not significantly different among the groups.

2.3.5 Correlation between VAS and PPT

Figure 14 shows correlation between the amount of changes in VAS and PPT at the 9 cm site at two days post-exercise from the baseline values. No significant (r=-0.20, P=0.28) correlations were found between VAS and PPT. No significant correlations were evident between the changes in VAS and PPT for other days (days 1, 3, and 4) and other sites (5 and 13 cm).

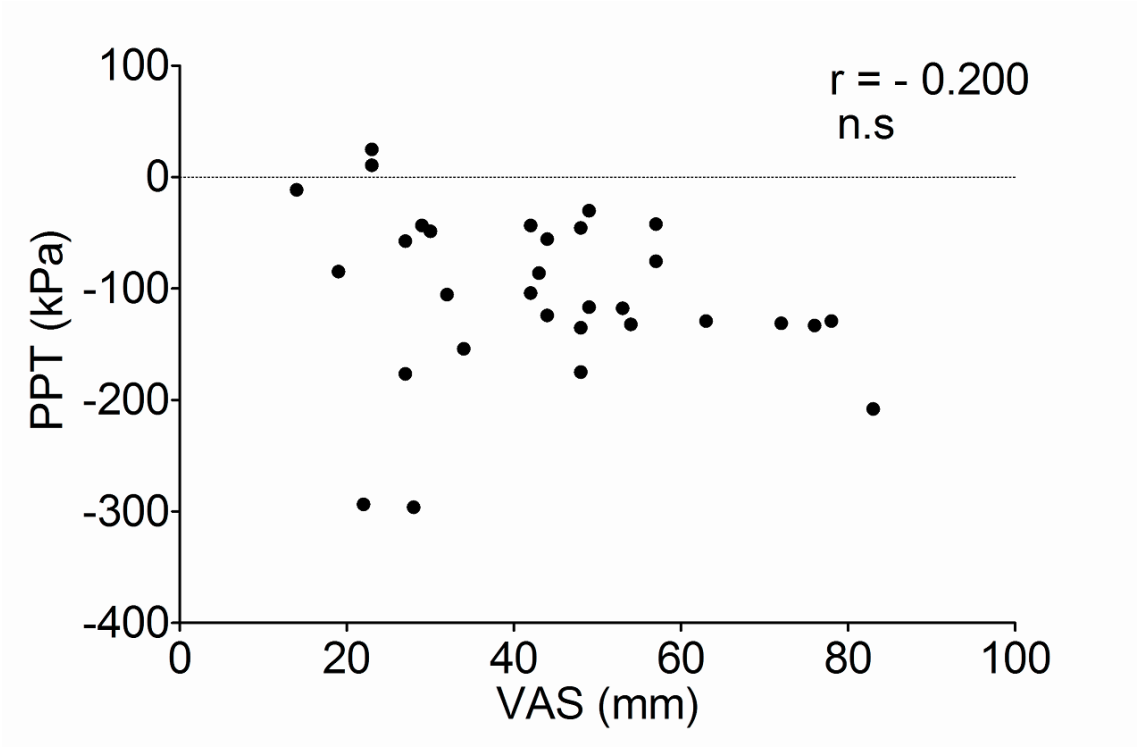

Figure 14. Correlation between the changes in visual analogue scale (VAS) and pressure pain threshold (PPT) at 9 cm site measured at two days post-exercise, n.s. = no significant correlation

2.4 DISCUSSION

To the best of our knowledge, this is the first study to investigate the correlation between VAS and PPT for DOMS assessment of the elbow flexors after eccentric exercise. The results showed 1) no significant difference between the three assessment sites on the biceps brachii muscle (5, 9, and 13 cm above the elbow crease) for the changes in VAS and PPT following eccentric exercise, and 2) no significant correlation between VAS and PPT. Although some similarities exist for VAS upon palpation and PPT measurements, the time course of changes in VAS and PPT was different, and the changes were not correlated, thus the two forms of measurements appear to present different aspects of DOMS.

Both VAS and PPT have been widely used in previous studies (9, 54, 119) to quantify DOMS after eccentric exercise. The changes in VAS, PPT and muscle strength after eccentric exercise in the present study were similar to those reported in previous studies (76, 113) in which the elbow flexor eccentric exercise was performed in a similar way to that of the present study. Thus, the changes reported in this study are considered "typical" examples that are seen after eccentric exercise of the elbow flexors.

In the present study, DOMS assessments were taken from three sites on the biceps brachii muscle, which were assumed to represent the distal myotendinous junction (5 cm), mid-belly (9 cm), and proximal myotendinous junction (13 cm). However, the chosen sites did not appear to be matched with the assumed region. It is important to note that the sites relative to the arm length were not the same amongst the subjects, and it was a limitation that the relative location of the sites was not considered in the present study. It should be noted that where the sites on the biceps brachii muscle, especially the 9 cm and 13 cm sites, were located was dependent on the arm length of

the subject. However, this does not appear to affect the analysis to compare VAS and PPT, and that the results demonstrate no significant differences amongst the sites for VAS (Figure 12) and PPT (Figure 13).

Our recent study (unpublished data: Study 2) showed that the most painful region of the biceps brachii muscle was located at the distal myotendinous junction following a similar eccentric exercise of the elbow flexors to that used in this present study. In the study, the whole surface covering the biceps brachii was divided into fifty regions by a grid method (5 x 10 matrix), and the PPT of the 50 sites were assessed and compared. The difference in PPT between the regions that showed the highest sensitivity was located at the distal myotendinous junction and other regions was 27–171 kPa (73.7 ± 5.3 kPa) at 1 day post-exercise and 9–162 kPa (52 ± 6.1 kPa) at 2 days post-exercise. However, in the present study, there was no difference between the estimated distal myotendinous junction region (5 cm site) and other sites (9 and 13 cm sites). It appears that the 5 cm region was not exactly the distal myotendinous junction site. In fact, more than 40 kPa difference existed between the most sensitive region (197.3 ± 20.2 kPa) and the regions surrounding the distal myotendinous junction in the 50 grid method with a range of 214–257 kPa (237.1 ± 5.7 kPa) in our recent study (unpublished data: Study 2). Thus, the 5 cm site did not appear to exactly match with the distal myotendinous junction. It seems likely that pain sensation of the biceps brachii is similar across the regions except for the distal myotendinous junction. It is necessary to identify the exact region corresponding to the distal myotendinous junction and include it in the pain assessment following eccentric exercise of the elbow flexors in future studies.

It should be noted that the time course of changes in the VAS and PPT was different following eccentric exercise, such that muscle soreness assessed by VAS

peaked two days post exercise (Figure 12), but the reduction of PPT was greatest at one day post-exercise (Figure 13). This was also reported in previous studies from other laboratories (9, 119) and in our previous studies (76, 113). For example, Rice et al. (119) reported that muscle soreness assessed by VAS significantly increased at 1 day and peaked at 2 days after exercise, but PPT significantly decreased at one day post-exercise and no further change was seen at two days following four sets of 15 eccentric and concentric contractions of the knee extensors. Peake et al. (113) showed that muscle soreness assessed by VAS peaked at two days post-exercise, but PPT decreased at one day post-exercise, and no further decrease was evident at two days post-exercise following 10 sets of three eccentric contractions of the elbow flexors. However, there was no discussion in these studies as to why the time course of the changes was different between VAS and PPT.

It is speculated that the different time course between VAS and PPT is associated with the different ways to quantify pain sensation. It is important that the minimum pressure to induce pain is assessed in PPT measurements, whereas the magnitude of pain felt with a standardised pressure is assessed in VAS measurements (approximately 40 kPa in the present study). It is assumed that PPT decreases with the development of DOMS; however, it is possible that the threshold to feel the "first discernible sensation of pain" in the muscle does not decrease further, even if the magnitude of the pain to a standardised pressure increases. It should also be noted that the subjects rated the magnitude of pain using VAS after the muscles were palpated by the investigator who placed his index and middle fingers over the biceps brachii muscle and moved the muscles in a circular motion for 3 s. It has been found (unpublished data: Study 2) that palpating the muscle during DOMS induces greater pain than only

applying a static pressure with the tips of the fingers toward the deeper tissues. Thus, it may be that the time course of changes in DOMS is better represented in VAS than PPT.

The present study showed no significant correlation between VAS and PPT (Figure 14). Although both measurements used “pressure” to induce pain, there are some differences between the measurements. As discussed above, PPT assessment is a single point method that detects the pain threshold by applying a minimum stimulus intensity to perceive a painful sensation (110). The pain sensation of PPT depends on the stimulus intensity or the duration of time corresponding to a fixed response to pain threshold; therefore, this method is considered to be a stimulus-dependent method (49). However, VAS is a suprathreshold pain intensity rating method to detect the pain intensities by a standardised stimulus (110). Since this method includes sensations over the whole perceptual range and does not detect only a single point of the threshold level, subjects can quantify the evoked pain sensation on the scale, and this rating method is classified as a response-dependent method (49). It can be said that PPT detects a pain threshold for “minimum stimulus intensity”, but VAS represents pain intensity through “subject responses to a whole perceptual range of pain intensity” (49).

It is also important to point out that the interval between assessments was different between VAS and PPT in the present study. The interval for the VAS assessment between sites was 10 s, but the interval between sites in the PPT assessment was 30 s. Ruscheweyh et al. (121) reported that pain perception was reduced by three different distraction strategies (i.e. two minutes of mental imagery, music and brush tasks), and pain reduction was due to descending pain inhibition. It is possible that the longer interval (30 s) between measures in the PPT assessment resulted in different pain perception than that in the VAS assessment that used a shorter interval (10 s) between measures. It would have been better to match the interval time between the VAS and

PPT measures. However, Nie et al. (97) investigated the temporal summation of pressure pain during four (1, 5, 10 and 30 s) different inter-stimulus intervals (ISI) over ten sequential pressure stimulations after the induction of DOMS of the trapezius muscle, and found that a 1 s stimulus duration showed significantly higher VAS scores than 5, 10, and 30 s ISI, but no significant difference among 5, 10 and 30 s. Therefore, it seems unlikely that the different measurement intervals between the VAS (10 s) and PPT (30 s) assessments can fully explain no significant relationship between the two measures shown in Figure 14.

In the present study, the stimulated area for VAS assessment (index and middle fingers) was approximately 3 to 4 cm^2, whereas the head of the probe for PPT assessment was 1 cm^2. Andersen et al. (4) suggested that using a larger stimulated area (probe) to detect muscle pain threshold could reduce the cutaneous sensitisation during measurement because the pressure is spread over a larger area of the tissue. Previous studies (52, 51) found that increasing the size of the stimulated area could increase the pain thresholds detected from the skin or from the deep tissue such as muscle and fascia. Thus, it seems that a larger stimulated area in VAS affected more nociceptors than PPT. It is possible that the movement in the VAS assessment not only stimulates a larger area of the muscle at the specific measurement site, but also stimulates the surrounding tissue including skin, connective tissues, and muscles, stimulating more nociceptors and changing the sensitisation of the dorsal horn neurons of the spinal cord. Nie et al. (97) reported that 1 s of sequential suprathreshold stimuli facilitated temporal summation of pressure pain on sore muscle. It is possible that the suprathreshold stimuli during VAS assessment enhanced dorsal horn temporal summation, whereas the stimuli applied during PPT assessment did not have such an effect. Further studies are necessary to

understand the underpinning mechanisms of DOMS, and how the mechanisms are associated with the difference in VAS and PPT.

In conclusion, the present study has shown that muscle pain assessed by VAS upon palpation and PPT is different. This indicates that VAS and PPT assessments represent different aspects of pain. Therefore, it is better to include both VAS and PPT to assess DOMS; however, if it is necessary to choose one method of assessment, once the protocol of the VAS measure is carefully standardised, VAS would indicate the time course of changes in DOMS more accurately than PPT.

This chapter showed that VAS and PPT represent different aspects of DOMS, and the next chapter was focused on established standardised pain assessment protocol.

CHAPTER THREE

STUDY 2

3.1 INTRODUCTION

Delayed onset muscle soreness (DOMS) is a common form of musculoskeletal pain that occurs from several hours to several days or a week after performing unaccustomed exercise, especially when eccentric (lengthening) contractions are involved (6, 26). DOMS is characterised by a dull, aching pain, usually felt during movement or palpation of the affected muscle, and when combined with tenderness and stiffness (6, 31) is regarded as mechanical hyperalgesia (41, 92). It has been documented that damage to contractile proteins, intermediate filaments, and/or connective tissue surrounding muscle fibres, and subsequent inflammatory processes, are associated with DOMS (26, 58). However, the mechanisms underpinning DOMS have not been fully elucidated.

One factor influencing our understanding is the difficulty in assessing pain, which is subjective by nature. Visual analogue scales (VAS) are widely used to quantify musculoskeletal pain (56, 127), and many studies have used VAS for DOMS assessment. Since DOMS is not felt without a mechanical stimulus such as palpation, stretching or contracting muscles, to quantify muscle pain requires a standardised stimulation. However, it is not clearly documented how stimuli should be imposed to quantify the pain level using VAS, and no standardised protocols are documented. Hence, DOMS assessment using palpation is often criticised because of the ambiguity associated with the process (11). Based on the observation from our pilot testing, it

appears that different palpation movement affects pain sensitivity when DOMS exists. In fact, in terms of pressure and movement, no standardised protocol for pain upon palpation has been established in previous studies, which raises the question of whether this assessment is reproducible. Moreover, the protocols for assessing muscle pain using VAS vary among studies making it difficult to compare results.

An alternative method of quantifying muscle pain is to assess pain threshold from pressure exerted using a pressure algometer. This objective quantification method is referred to as the pressure pain threshold (PPT) (52, 68). PPT has been demonstrated to be reliable for measuring the pain threshold (25, 109) and has often been used to assess DOMS (13, 75). Some studies investigating the muscular distribution of PPT in response to DOMS in the lower limb muscles have found that the pain sensation is unevenly distributed (39, 46). For example, Edwards et al. (39) reported that muscle pain in the quadriceps femoris after 15 min of eccentric stepping exercise was located close to the distal insertion of the myotendinous junction of the vastus medialis and lateralis. Hedayatpour et al. (71) recently reported a greater reduction in PPT in the distal quadriceps region than the proximal region after 100 eccentric knee extensions. In contrast, Andersen et al. (4) found that tibialis anterior muscle belly sites became more sensitive to pressure stimulation than muscle-tendon junction sites following eccentric exercise. These studies suggest that the choice of PPT assessment sites may influence the results obtained, and thus the conclusions drawn. However, no previous studies have examined the PPT distribution in the elbow flexor muscles after elbow flexor eccentric exercise, which is one of the most frequently used models to investigate DOMS (20, 76, 93).

Therefore, the present study was designed to examine the distribution of PPT in the biceps brachii and brachialis using a grid method to clarify region-specific changes in sensitivity after eccentric elbow flexor exercise, and compared the changes in pain levels using VAS with static pressure and palpation (circular, longitudinal or transverse movements) after the eccentric exercise. The relationship between the pain levels assessed by VAS, category-ratio 10 (CR-10) scales, and pain sensitivity (PPT) methods was also examined. From these approaches, an attempt was made to establish a standardised pain assessment protocol for DOMS induced by elbow flexor eccentric exercise.

3.2 METHODS

3.2.1 Subjects

This study was approved by the Institutional Human Research Ethics Committee and complied with the Declaration of Helsinki. Ten healthy young men with no current or previous upper arm injuries, who were not suffering from any present or ongoing upper arm pain, and had not performed resistance training of the upper limbs for the previous six month, were recruited for this study. Their mean (±SD) age, body mass, height and maximal voluntary isometric elbow flexor contraction (MVC) torque were 24.9 ± 5.4 y, 69.2 ± 8.3 kg, 169.8 ± 6.2 cm, and 60.0 ± 12.0 Nm respectively. All subjects provided informed written consent, and a medical questionnaire was completed before participating in the study. They were requested not to change their lifestyle and dietary habits, not to take any anti-inflammatory drugs or nutritional supplements, and not to perform unaccustomed exercise during the experimental period.

3.2.2 Eccentric Exercise

All subjects performed 10 sets of 6 maximal isokinetic eccentric contractions of the elbow flexors with a randomly chosen arm (dominant arm: n=6, non-dominant arm: n=4) on an isokinetic dynamometer (Cybex 6000, Ronkonkoma, NY, USA). They were individually positioned on a seated preacher arm curl bench that secured the shoulder joint at 45° flexion in front of the body, with the elbow joint being aligned with the axis of rotation of the dynamometer and the lever arm of the dynamometer attached to the wrist in a supinated position. For each eccentric contraction, the elbow joint was forcibly extended from a flexed (60°) to a fully extended position (0°) in 1 s at an angular velocity of $60°\cdot s^{-1}$, while the subjects were verbally encouraged to generate maximal force in the flexed position and to maximally resist against the elbow-extending action throughout the full range of motion. After each eccentric contraction the isokinetic dynamometer was programmed to return the arm to the flexed position at a velocity of $6°\cdot s^{-1}$, which provided a 10-s rest between contractions. The rest period between sets was set at 3 min. Torque signals were recorded via a data acquisition system (Powerlab with a Chart 7 software, ADinstrument, Bella Vista, Australia) at a sampling rate of 200 Hz, and real-time visual feedback of torque was displayed on a computer monitor.

3.2.3 Muscle Damage Markers

Indirect markers of muscle damage included maximal voluntary isometric contraction (MVC) torque and range of motion (ROM), and they were measured before, immediately after, and 1 – 5 days following exercise. Serum creatine kinase (CK) activity was measured before, and 4 and 5 days after exercise, since it has been reported

that CK activity in the blood peaks 4-5 days after eccentric elbow flexor exercise (76, 107).

3.2.3.1 Maximal Voluntary Isometric Contraction (MVC) Torque

MVC torque of the elbow flexors was measured using the isokinetic dynamometer with the same subject positioning described above for the eccentric exercise. Each subject performed two 3-s maximal voluntary isometric contractions at an elbow joint angle of 90° with a 30-s rest between contractions. Measurements were taken twice and the peak torque of the two contractions was used as the MVC torque (76, 95).

3.2.3.2 Range of Motion (ROM)

A plastic goniometer was used to measure extended (EANG) and flexed elbow joint angles (FANG). The EANG was determined when subjects attempted to fully extend the elbow joint while standing and hanging the arm by their side, and the FANG was determined when subjects attempted to fully flex the elbow joint to touch the shoulder of the same side with the palm (76, 93). A semi-permanent ink pen was used to mark the lateral epicondyle of the humerus, the acromion process and the mid-point of the styloid process of the ulna and radius. Measurements were taken twice for each joint angle and the mean value of the two measurements was used to calculate the ROM by subtracting FANG from EANG (76, 93).

3.2.3.3 Serum CK Activity

Approximately 8 ml of blood was taken from the antecubital vein by a standard venipuncture technique. The samples were allowed to clot at room temperature then centrifuged for 10 min at 4°C to obtain serum. Serum CK activity was determined by a

Hitachi Modular PT automated clinical chemistry analyser (Hitachi, Japan) with a commercially available Roche Diagnostics Reagent (Mannheim, Germany). The normal resting reference value using this method is < 200 $IU \cdot L^{-1}$ (76).

3.3 Muscle Pain Assessments

Pain in the exercised arm was assessed in several ways, as described below. The level of pain was assessed using VAS and Borg's category-ratio 10 (CR-10) scales when the exercised upper arm received pressure and palpation by fingers followed by application of a cuff, and PPT was measured from 50 sites as described below, before, immediately after and 1-5 days after exercise.

3.3.1 Visual Analogue Scale (VAS) and Borg's Category-Ratio 10 (CR-10) Scale

The level of muscle pain evoked by a standardised stimulus was assessed by a 100-mm VAS in which 0 indicated “no pain” and 100 represented “extreme pain” (76), and a CR-10 scale in which 0 indicated “no pain”, 1: “very faint pain”, 2: “weak pain”, 3: “mild pain”, 4: “slightly pain”, 5 “moderate pain”, 6: “above moderate pain”, 7: “somewhat strong pain”, 8: “strong pain”, 9: “very strong pain” and 10 “maximal pain” (44). Each subject was asked to mark the level of perceived pain on the VAS followed by the CR-10 scale when the investigator applied pressure by palpating the biceps brachii at 3, 9 and 15 cm above the elbow crease. In the pressure assessment, the investigator placed his index and middle fingers over the site and applied pressure (approximately 250 mmHg) for 3 s with the tips of the fingers toward the deeper tissues. The investigator practised reproducing the same pressure and the protocol was kept as consistent as possible between days and among subjects, and all measurements were taken by the same investigator throughout the study. In the palpation assessment, the

investigator moved his index and middle fingers clockwise 3 times to palpate the site while keeping the pressure as consistent as possible. In addition to these assessments, in order to compare different palpation protocols, the investigator moved his fingers upward and downward longitudinally and then transversely (left and right) to palpate the site, and the subjects were asked to report the pain of each assessment using VAS only.

Furthermore, a cuff (5 cm width) with an inflator (TD 312; Hokanson, Bellevue, USA) was placed over the exercised arm at 3, 9 and 15 cm above the elbow crease, a solid wooden ball (3 cm in diameter) was placed between the cuff and the skin, and pressure (250 mmHg) was applied to assess the pain level. This pressure was determined during pilot testing to be similar to the pressure induced by the finger method detailed above. The investigator gradually increased the cuff pressure to 250 mmHg and the subjects were asked to report the pain using VAS and CR-10 scales separately. A cuff with an inflator measures method has been used in the previous study for DOMS assessment (17).

After this measure, the investigator reset the pressure to 0 mmHg then reinflated the cuff to 250 mmHg, and the muscle was palpated with the ball under the cuff in circular, transverse and longitudinal movements respectively, as detailed above for the finger palpation procedure. The investigator palpated the site by moving the ball without applying any extra pressure. The pain level was again assessed using VAS and CR-10 scales (pressure and circular palpation only).

All of the above measurements were collected while the subject was lying on a massage table with their relaxed arms by their side on the table in a supinated forearm position. One measurement was taken for each assessment for each time point.

However, to examine the test-retest reliability of the VAS measures, the same assessments were repeated 1 hour later on either 1, 2, 3 or 4 days post-exercise (depending on subjects) using several subjects.

3.3.2 Pressure Pain Threshold (PPT)

A polythene sheet marked with a grid consisting of 50 squares (2 cm × 2 cm) was placed over the upper arm to assess the localisation of pain (Figure 15) using an electronic algometer (Somedic AB, Hörby, Sweden). Among the 50 sites, the VAS and CR-10 (3, 9 and 15 cm above the elbow crease) were the same sites as those for PPT assessments. The probe head of the algometer (area of 1.0 cm^2) was placed perpendicular to each site and the investigator gradually applied force at an application rate of 50 $kPa \cdot s^{-1}$ until the subject reported the first feeling of noticeable pain in the muscle. The value (in kPa) corresponding to the force applied to elicit pain was recorded, and this is referred to as pressure pain threshold (PPT). All measurements were taken while the subject was lying on a massage table with their arms relaxed in a supinated forearm position. The order of measurements was standardised from 1 to 50 sites with a 10-s interval between measurements. After completing the first round of the PPT assessment, the subsequent round was performed in the same order with a 5-min interval between rounds. The total duration of the two rounds was approximately 20 min, and the average of the two measures for each site was used for subsequent analysis.

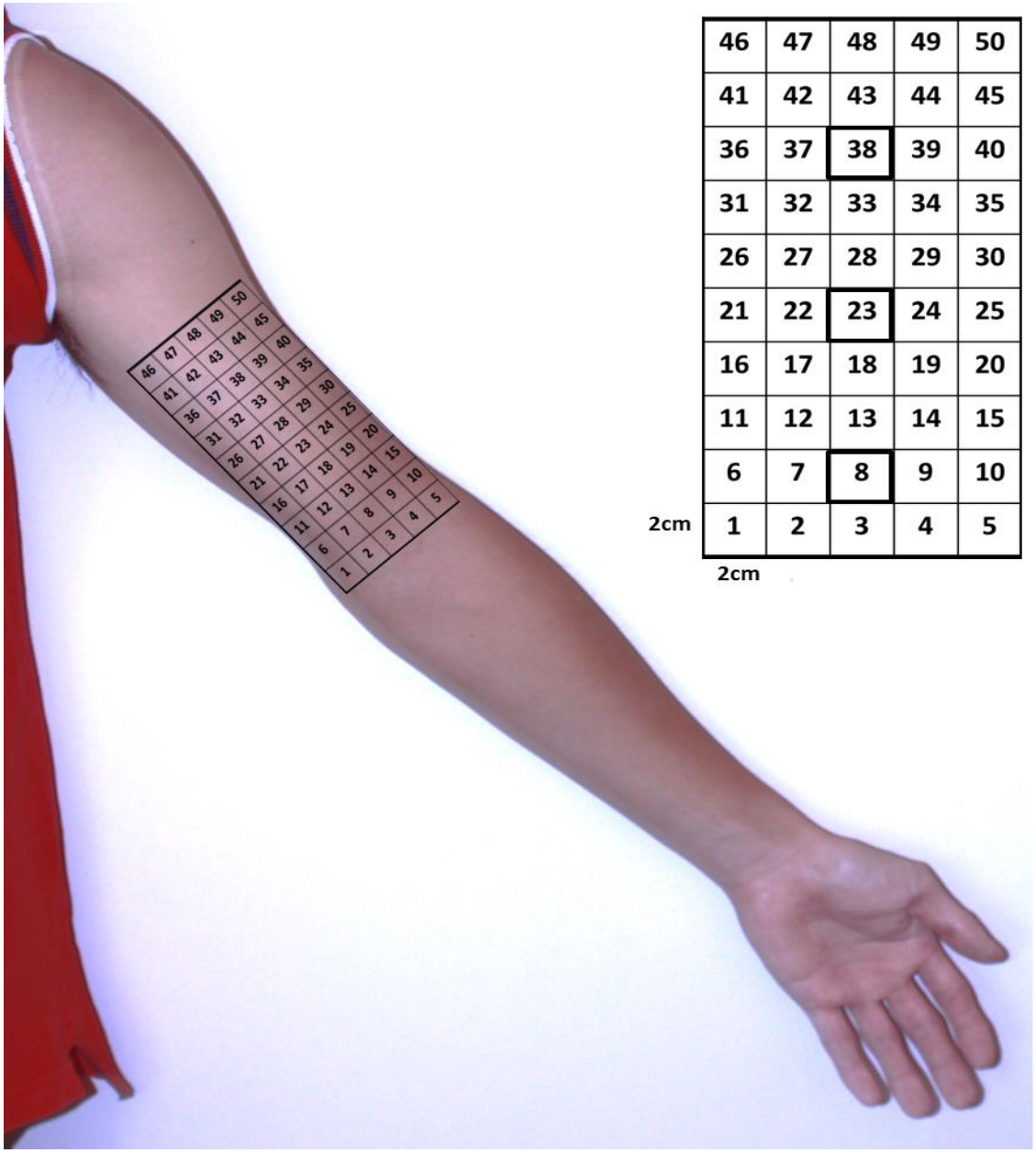

Figure 15. Pressure pain threshold (PPT) measured at 50 sites in the upper arm. A polythene sheet marked with a grid consisting of 5 × 10 (50) squares (each square is 2 × 2 cm = 4 cm^2) was placed on the upper arm. Sites 8, 23 and 38 represent the location at 3, 9 and 15 cm above the elbow crease respectively, used for the VAS assessments.

3.4 Statistical Analysis

Coefficient of variation (CV) and standard error of measurement (SEM) were used to determine the test-retest reliability of the VAS palpation measurements. CV and SEM were also used to determine the test-retest reliability of PPT measurements taken at 1 – 3 days after exercise using the first and second PPT measures (sites 8, 23 and 38 in Figure 15). CV and SEM were also used to determine the test-retest reliability of the MVC and ROM measurements.

One-way repeated measures ANOVA was used to analyse the changes in muscle damage markers (MVC, ROM, serum CK activity), VAS, CR-10 and PPT over time (pre, immediately post, 1 – 5 days after exercise). Changes in VAS and CR-10 over time were compared between the pressure and palpation, between finger and cuff protocols by two-way repeated measures ANOVA, and changes in VAS over time were also compared among three palpation protocols (circular, longitudinal and transverse movements) by two-way repeated measured ANOVA. PPT values for each day were compared among the 50 sites by one-way repeated measures ANOVA. When the ANOVA showed a significant main effect, a Tukey's post-hoc test was used for multiple comparisons. Pearson's product moment correlation coefficients were computed to determine the relationships between VAS and CR-10, and VAS and PPT measures. Statistical significance was set at $P<0.05$, and all data were presented as mean ± standard deviation (SD).

3.5 RESULTS

3.5.1 Reliability of the Measurements

The CV was 3.6% and the SEM was 2.6 mm for the two time points separated by one hour for VAS measurements. For PPT, the CV was 9.6% and the SEM was 23.3 kPa for the two assessments separated by 10 min. For MVC, CV was 6.4 % and the SEM was 3.3 % for the two assessments. For ROM, the CV was 1.2 % and the SEM was 0.3 degree.

3.5.2 MVC Torque, ROM and Serum CK Activity

MVC torque decreased from the baseline (60.2 ± 12.2 Nm) at 1 day post-exercise by approximately 50% (31.2 ± 11.2 Nm) and remained approximately 20% below the baseline (47.0 ± 10.7 Nm) at 5 days post-exercise ($P<0.05$). ROM decreased ($P<0.05$) immediately after exercise from the baseline (140 ± 6.7°) to 96.1 ± 16.4°, then slowly recovered to 134 ± 5.7° at 5 days following exercise. Serum CK activity increased significantly ($P<0.05$) from the baseline (181.0 ± 78.2 IU/L) to 5 days (926.1 ± 434.9 IU/L) after exercise.

3.5.3 VAS, CR-10 and PPT

Figure 16a shows changes in VAS upon biceps brachii palpation at the 3, 9 and 15 cm sites following eccentric exercise. VAS increased after exercise, peaked between 1 and 2 days, and slowly recovered to the baseline at 5 days following exercise ($P<0.05$). This was also the case for CR-10 (Figure 16b). No significant ($P=0.59$-0.84) difference in the changes was evident between the three sites for both VAS and CR-10. Changes in PPT at the same sites as those used for the VAS and CR-10 (i.e. 3, 9 and 15 cm sites) are shown in Figure 16c. The pressure to elicit pain decreased ($P<0.05$) from the baseline to 1 day after eccentric exercise and remained below the baseline at 3 days after exercise. No significant ($P=0.29$) difference in the change in PPT was evident between the three sites following exercise.

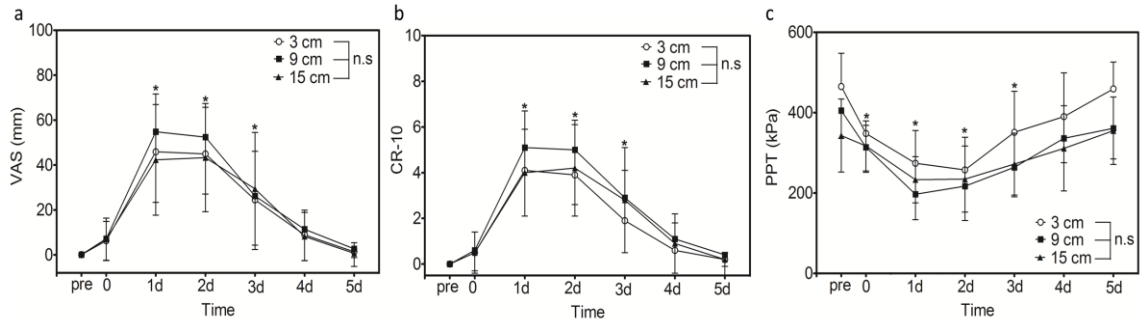

Figure 16. Changes in visual analogue scale (VAS) upon palpation (a), category-ratio (CR-10) scale upon palpation (b), and pressure pain threshold (PPT) of biceps brachii (c) at 3, 9 and 15 cm above the elbow crease before (pre), immediately after (0), and 1 to 5 days following eccentric elbow flexor exercise.

3.5.4 VAS and CR-10 – Pressure vs. Palpation and Finger vs. Cuff Measures

Figure 17a compares the VAS between pressure and palpation using fingers. VAS upon finger palpation was greater ($P<0.05$) than finger pressure on 1 day post-exercise; however, no significant difference ($P=0.11$-0.74) was evident 2 and 3 days after exercise. Figure 17b compares the VAS upon cuff pressure and cuff pressure plus palpation. VAS upon cuff palpation was greater ($P<0.05$) than pressure at 1-3 days after exercise. This was also the case for CR-10 (Figure 17c), with finger palpation inducing greater ($P<0.05$) pain than finger pressure, and cuff palpation being greater ($P<0.05$) than pressure (Figure 17d). No significant differences were evident, however, between the finger and cuff pressure measurements and the finger and cuff palpation measurements following exercise.

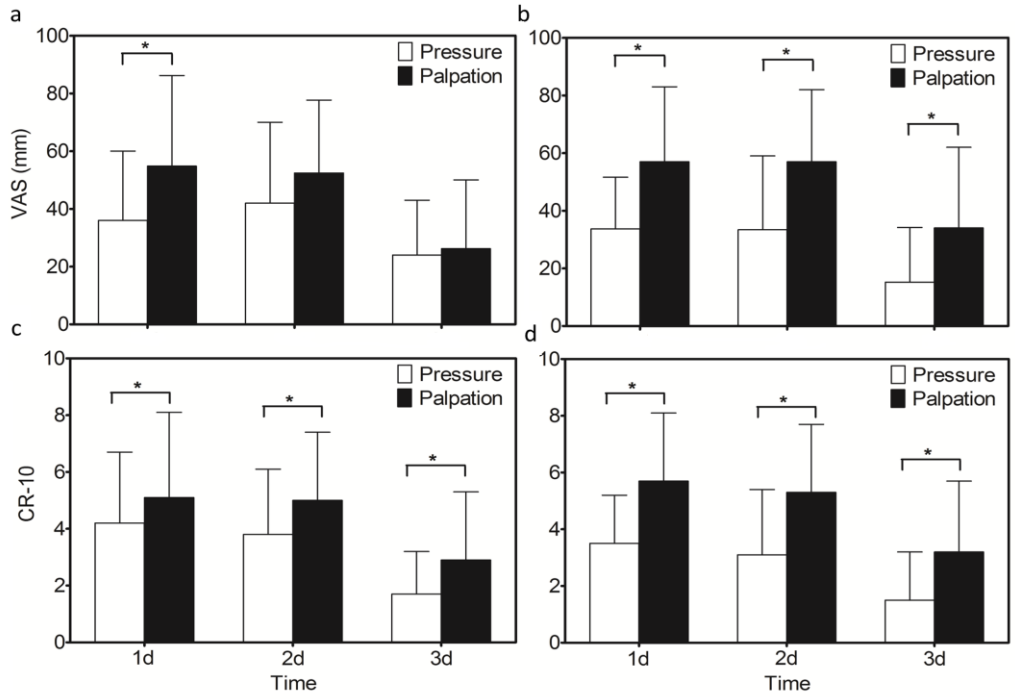

Figure 17. Comparison of the pain level using the visual analogue scale (VAS) and category-ratio 10 (CR-10) scale at 1 to 3 days after eccentric exercise between pressure and palpation using the fingers (a: VAS, c: CR-10) and a ball located between the pressure cuff and the skin (b: VAS, d: CR-10). * indicates significant ($P<0.05$) difference between pressure and palpation.

3.5.5 VAS – Palpation Methods (Circular, Longitudinal and Transverse)

VAS upon circular, longitudinal and transverse palpation measures at 1 to 3 days post-exercise are shown in Figure 18. VAS upon longitudinal (82.4 ± 22.3 mm) and transverse palpation (79.4 ± 22.6 mm) was greater ($P<0.05$) than circular palpation (54.8 ± 31.4 mm) at 1 day, but no significant difference was found between longitudinal and transverse palpations. This was also the case at 2 and 3 days post-exercise.

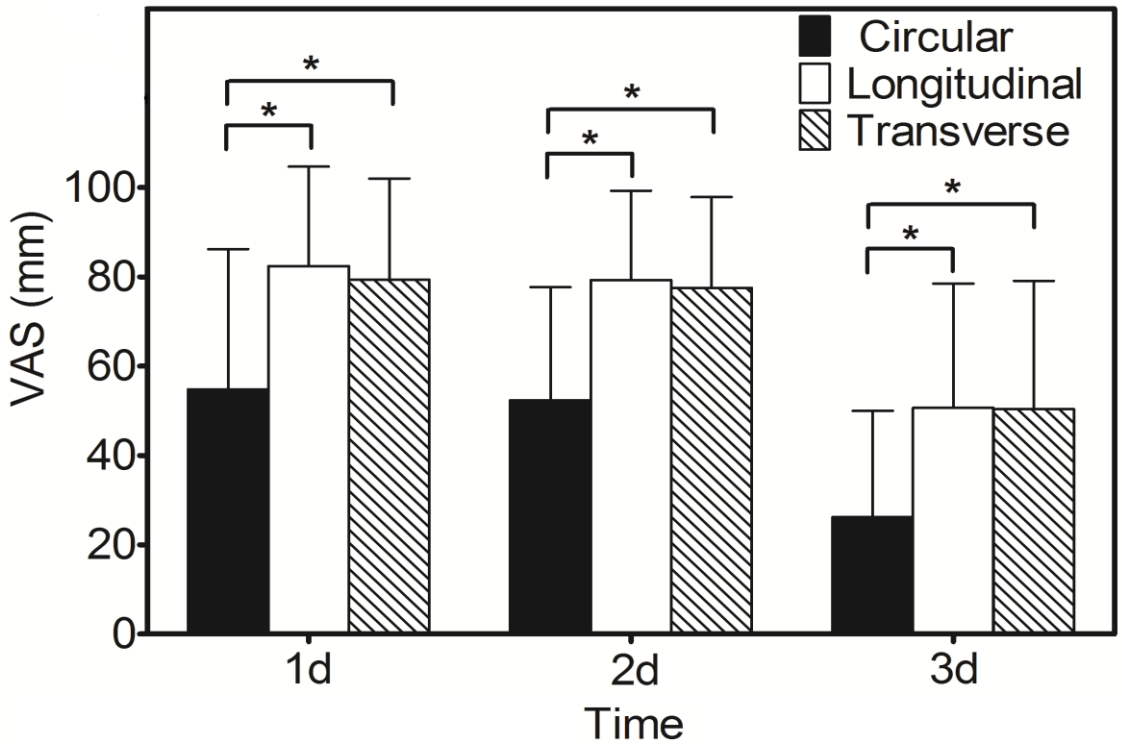

Figure 18. Comparison in the pain level recorded by a visual analogue scale (VAS) at 1 to 3 days after eccentric exercise between circular, longitudinal and transverse palpation assessments using the fingers. * indicates significant ($P<0.05$) difference between measures.

3.5.6 PPT at 50 Sites

A significant difference ($P<0.05$) was found between the 50 sites before exercise such that the sites located medially showed a lower threshold ($P<0.05$) than the other sites. After eccentric exercise, the pain sensitive sites were located centrally in the mid-belly ($P<0.05$) at 1 day post-exercise, and the distal sites became sensitive at 2 days post-exercise then returned to the baseline at 4 days after exercise. It is of interest that the sites used for palpation measures (3, 9 and 15 cm above the elbow crease) were among the sites showing lower PPT values than other sites at 1-3 days post-exercise when DOMS was evident.

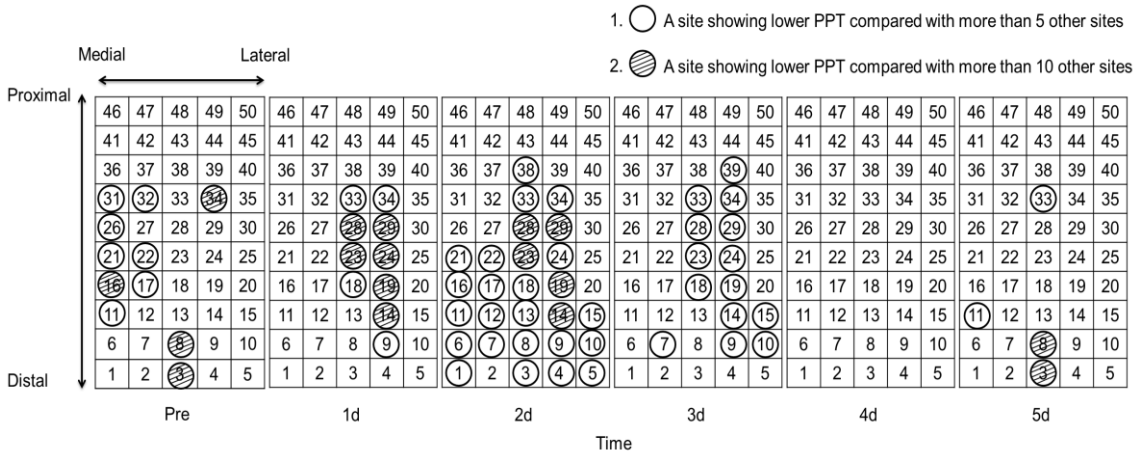

Figure 19. Absolute changes in pressure pain threshold (PPT) at 50 sites (average of 10 subjects) from the baseline values at 1 to 5 days after eccentric elbow flexor exercise. The sites that showed significant ($P<0.05$) difference from other 5-9 sites or from more than 10 other sites are shown in open circle and shaded circles respectively.

3.5.7 Correlation between VAS and CR-10, VAS and PPT

A significant (P<0.05) correlation was found between VAS and CR-10 (r=0.91) as shown in Figure 20a. Figure 20b illustrates that no significant (P=0.45) correlation was found between the changes in VAS and PPT from the baseline (r=-0.28).

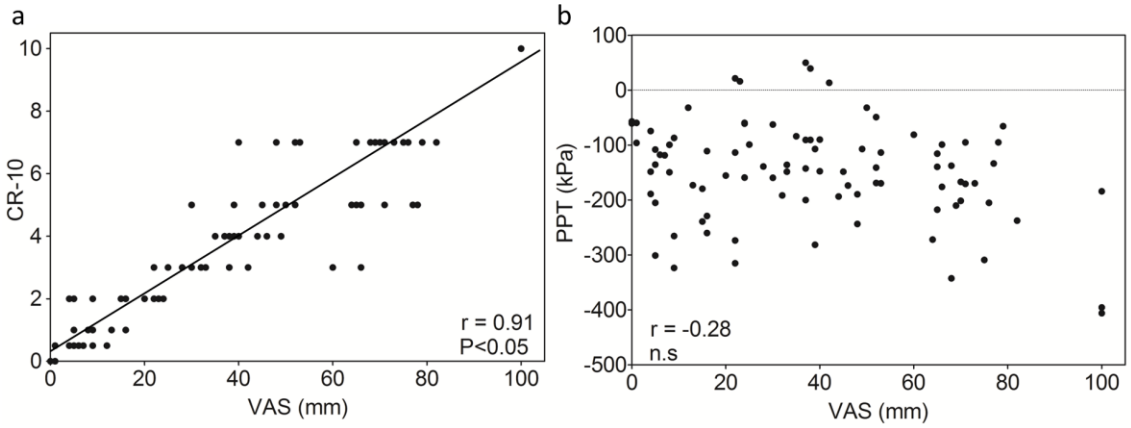

Figure 20. Correlations between the changes in visual analogue scale (VAS) and category-ratio 10 scale (CR-10) measurements of the pain level assessed using palpation at three sites (3, 9 and 15 cm above the elbow joint) between 1 and 3 days post-exercise (a), and correlations between VAS assessed during palpation and the pressure pain threshold assessed at three sites over 3 days (1-3 days post-exercise) for the absolute change from pre-exercise values (b).

3.6 DISCUSSION

The present study aimed to establish a standardised protocol to assess muscle pain (DOMS) induced by eccentric elbow flexor exercise. The main findings were that 1) the test-retest reliability of VAS and PPT assessments was high, 2) no significant difference was found at 3, 9, and 15 cm sites above the elbow crease for VAS and CR-10, 3) VAS and CR-10 values were greater upon muscle palpation than pressure, 4) no difference was evident between pressure and palpation by fingers and by a cuff when the pressure was standardised, 5) VAS values upon palpation were greater with longitudinal or transverse movements than circular movements, 6) distal and central sites showed increased PPT sensitivity during palpation compared with other sites at 1-3 days after exercise, and 7) the two pain rating scales (VAS and CR-10) were significantly correlated, but no significant correlation was found between VAS and PPT assessments. Based on these results, a standardised protocol will be described in the remainder of the discussion.

VAS and PPT methods have been widely used in previous studies (75, 113, 119) to quantify DOMS, which is a prominent symptom after exercise-induced muscle damage is induced, whilst the CR-10 scale is used to quantify pain subjectively during exercise exertion (44, 94). The changes in MVC, ROM and serum CK activity after eccentric exercise in the present study were similar to those reported in previous studies (21, 75, 93), and indicate that muscle damage was induced by the exercise. The changes in VAS and PPT were also similar to those reported after eccentric elbow flexor exercise in previous studies (76, 95). As shown in Figure 16, no significant differences between the three sites (3, 9 and 15 cm above the elbow crease) were evident for the changes in VAS and CR-10 upon palpation or the PPT. In a previous study (95) no

significant difference was observed between the three sites i.e. 5, 9 and 13 cm above the elbow crease. It should be noted that the sites were more dispersed in the present study than in the previous study; however, the results were the same and show that pain rated on the VAS upon palpation is similar along the central region of the biceps brachii, at least between 3 and 15 cm above the elbow crease. Thus, it is suggested that if VAS is used during palpation from any one of these three sites, it would provide the pain level of the biceps brachii. However, it should be noted that the relative location of the sites is affected by the length of the arm such that the 9 cm site could be close to the proximal tendon for some subjects but close to the mid-belly for others. It is possible to normalise the site placements to arm length; however, it may be that a non-significant difference would be observed for the "maximal" biceps brachii pain level along the arm if the measurements were taken close to the elbow joint (e.g. less than 10 cm), because distal regions become more sensitive to pressure after eccentric exercise, as discussed below. It is also important to note that the changes in VAS and CR-10 were very similar (Figure 16). Thus, either CR-10 or VAS can be used to assess pain level after eccentric elbow flexor exercise.

It may be of practical importance that the use of fingers and cuff for the VAS and CR-10 measures for pressure only and palpation obtained similar results (Figure 17), when the same pressure was applied. The finger palpation technique is often criticised for its potentially-poor reliability, because of possible differences in pressure application. The present study standardised the pressure and palpation measurements by using a cuff with an inflator which adjusted the pressure to approximately 250 mmHg during assessments. It is important to standardise the pressure for palpation assessment, ensuring the same pressure can be reproduced over measurements. The present results show that palpating the muscle in a circular motion induced greater pain than the

application of static pressure with the tips of the fingers. It seems possible that the movement activates more pain nociceptors in the skin, fascia and connective tissues surrounding muscle fibres. Furthermore, when comparing three different palpation movements (i.e. circular, longitudinal and transverse), it was found that longitudinal and transverse muscle palpation induced greater pain than circular palpation (Figure 18). It may be that longitudinal and transverse palpations impose greater mechanical pressure to a smaller area than circular palpation, where the application area can be larger. Therefore, this study suggests that a standard palpation method be used, where muscular pressure is applied using either a longitudinal or transverse palpation rather than a circular palpation, and the distance of the movement should be small (e.g. within 2 cm). It is also important that the stimulus (i.e. palpation) intensity is sufficiently large, which is close to a pressure that induces pain before exercise as this intensity will clearly induce pain after eccentric exercise.

Regarding PPT, measurements were taken at the 50 sites in the present study, which took approximately 20 min to complete when measuring each site twice. Thus, although this may not be time-efficient for some clinical or research uses, it appears to more precisely indicate pain sensitivity than other measures. As mentioned above, it is important to note that the location of the measurement sites (relative to the arm length) varied between subjects. However, this did not appear to substantially affect the PPT assessment, since the exercise typically affected PPT in distal regions (Figure 19). It may be of interest that the medial region was more sensitive to pressure before exercise than other regions, but central and distal regions were more sensitive after exercise. This may be related to the medial region being closer to the biceps brachii-brachialis muscle junction, the brachial artery and veins, and/or the medial antibrachial cutaneous nerve. Fischer (43) reported that PPT values were influenced by muscle and subcutaneous

tissues thickness, and also by the inherent pain sensitivity difference between individuals. The present study is the first study to report the pain distribution over the biceps brachii after eccentric elbow flexor exercise, although some studies (39, 46, 47) reported that pain sensitive regions were typically located in the distal regions of other muscles after eccentric exercise. For instance, Edwards et al. (39) found that quadriceps muscle pain after 15 min of eccentric stepping exercise was located close to the distal vastus medialis and lateralis myotendinous junction, and Hedayatpour et al. (71) reported a greater reduction in PPT in the distal quadriceps after 100 eccentric knee extensions. It is speculated that the distal region receives more mechanical stimulus during eccentric contractions, thus damage and inflammation would be more substantial than in other muscle regions. Mense and Simons (85) reported that the innervation density of nociceptors in the connective tissues surrounding the calcaneal tendon in cats was approximately five times higher than in the gastrocnemius-soleus muscle, but no difference was found in innervation density throughout the muscle tissue. Further studies are necessary to investigate whether any regional differences in histological changes within muscle fibres and surrounding connective tissues exist after eccentric exercise in the biceps brachii. It should be noted that the sites used for palpation (i.e. 3, 9, 15 cm above the elbow crease) became sensitive to pressure. Thus, it is necessary to include at least these sites for PPT assessment for the biceps brachii.

A strong and statistically significant correlation was observed between VAS and CR-10 measurements (Figure 20a). This is not surprising given that the two measurements were obtained using the same stimulus. As shown in Figure 20a, for the same CR-10 value, some spread of VAS values were seen, possibly due to the VAS being based on a continuous number scale. If only a single scale can be used, then the VAS may be a better option than the CR-10, because VAS could provide better

resolution of pain levels. In the present study, no significant correlation was observed between the changes in VAS and PPT assessments, which confirmed the results of our previous study (95) where no significant correlations were observed between VAS and PPT pain assessments made 5, 9 and 13 above the elbow crease at 1 to 4 days after 60 eccentric elbow flexor contractions. It should be noted that PPT is a pain threshold assessment used to quantify the minimum pressure intensity to evoke pain, whereas VAS uses a stimulus (either pressure or palpation in the present study) generally exceeding pain threshold. Thus, the two assessments are not the same because they represent different information regarding subjective pain. However, if a choice has to be made between VAS and PPT, then it may be a better option to obtain information regarding the level of pain rather than the threshold of pain, and thus the VAS can be recommended.

In conclusion, when DOMS in the biceps brachii is assessed after eccentric elbow flexor exercise, the following protocols should be considered: 1) VAS assessments should be included to rate pain level; however, it is also advisable to include PPT assessments in order to obtain information regarding pain thresholds, 2) CR-10 can be used instead of VAS to rate pain level; however, VAS is preferable, 3) it is better to include multiple sites (e.g. 3, 9, 15 cm above the elbow crease) covering the distal and central muscle regions for VAS and/or PPT assessments to account for region-specific differences in pain, and 4) the muscle should be palpated in either a longitudinal or transverse direction, rather than circular, and this should be standardised before the commencement of testing. The results of the present study suggested that the consistency of the stimulus for DOMS assessments is important and it also indicate that DOMS might be associated with damage and inflammation to connective tissues

surrounding the muscle fibres (i.e. the endomysium) and/or muscle bundles (i.e. the perimysium or fascia), especially close to the distal myotendinous junction.

This chapter established DOMS assessment protocols by using different pain assessment techniques and the next chapter focused on using electrical pain threshold (EPT) technique to detect the pain sensitivity on biceps brachii fascia, muscle and brachialis fascia.

CHAPTER FOUR

STUDY 3

4.1 INTRODUCTION

After performing unaccustomed exercise, people often experience muscle pain in the following days, which is referred to as delayed onset muscle soreness (DOMS). DOMS is characterised as mechanical hyperalgesia (92), and pain is felt when exercised muscles are moved or palpated. DOMS generally develops several hours after exercise, peaks at one to three days, and has disappeared by a week after exercise (6, 26). It has been documented that damage to contractile proteins, intermediate filaments, and/or connective tissue surrounding muscle fibre, and the subsequent inflammatory responses are responsible for DOMS (26, 58, 134); however, the mechanisms underpinning DOMS are still not fully understood.

Some studies have documented that connective tissue damage and inflammation are more responsible for DOMS than muscle fibre damage and inflammation (32, 112, 118). For example, Paulsen et al. (112) reported no association between DOMS and inflammation of muscle fibres after 300 eccentric contractions of the quadriceps femoris, and noted that damage and remodelling of the extracellular matrix (ECM) were related to DOMS. Crameri et al. (32) compared muscle damage between 210 maximal eccentric contractions with electrical muscle stimulation (EMS) and 210 voluntary maximal eccentric contractions (VOL) of the knee extensors, and found that the magnitude of DOMS and increased staining of tenascin C were similar between EMS and VOL, but muscle fibre damage was evident only after EMS. Malm et al. (89) also

reported that DOMS was related to a greater increase in the inflammatory markers such as T cells (CD3), granulocytes (CD11b) and leukaemia inhibitory factor (LIF) in the epimysium, but not in the skeletal muscle after 45 min of downhill running. These results indicate that the sensation of pain is not located within the muscle fibres but the connective tissue surrounding muscle fibres after eccentric exercise.

It is known that skeletal muscles contain four types of afferent fibres: group I (Aα), II (Aβ), III (Aδ) and IV (C), and the free nerve endings of the latter two fibres respond to noxious stimuli such as mechanical pressure, heat, cold and algesic substances (45). DOMS is thought to be mainly mediated by group IV fibres, whilst group III fibres play a secondary role (75). These free nerve ending (nociceptors) are located along the walls of arteries and mostly in the surrounding connective tissue (42, 53). It appears that connective tissue such as fascia, which contains a high density of nociceptors (85, 130), is responsible for muscle pain. In fact, Gibson et al. (15) showed that fascia, rather than muscle tissue, in the tibialis anterior muscle became more sensitive to hypertonic saline injection when DOMS was elicited.

Itoh et al. (70) introduced an intramuscular electrical pain threshold (EPT) technique to assess pain threshold of the skin, fascia and muscle separately. They reported that EPT was significantly lower in the fascia compared with the muscle and skin of the forearm 2 days after eccentric exercise of the middle finger, and suggested that the sensitised nociceptors at the fascia level were responsible for DOMS. However, no previous studies have applied this technique to the elbow flexors and then investigated changes in EPT in relation to the magnitude of muscle damage, which is largely different between the initial and secondary bouts of the same exercise.

Thus, the present study investigated changes in EPT at the biceps brachii fascia (BBF), muscle and brachialis fascia (BF) after the first and second bouts of maximal

eccentric elbow flexion exercise. It was hypothesised that EPT would decrease more at the fascia than the muscle after eccentric exercise, and that the magnitude of decrease would be greater after the first than the second exercise bout performed by the same arm 4 weeks later.

4.2 METHODS

4.2.1 Subjects

This study was approved by the Institutional Human Research Ethics Committee and complied with the Declaration of Helsinki. Ten young men with no current or previous upper arm injuries, who were not suffering from any upper arm pain and who had not performed resistance training of the upper limbs for at least six months prior to the present study, were recruited for this study. The number of subjects was determined by a sample size estimation using the data of a previous study (24) that reported the repeated bout effect of maximal eccentric exercise of the elbow flexors. Based on α-level of 0.05, a power (1-β) of 0.80, and an expected 20% difference in maximal voluntary contraction (MVC) torque recovery at 3 days after maximal eccentric elbow flexor exercise between the first and second bouts, at least 10 subjects were deemed necessary. Their mean (±SD) age, body mass, height and MVC torque were 24.0 ± 2.0 y, 69.7 ± 14.3 kg, 170.1 ± 8.6 cm, and 50.6 ± 8.1 Nm respectively. All subjects provided informed written consent, and a medical questionnaire was completed before participation in the study. Subjects were requested not to change their lifestyle and dietary habits, not to take any anti-inflammatory drugs or nutritional supplements, and not to perform unaccustomed exercise during the experimental period.

4.2.2 Eccentric Exercise

All subjects performed two exercise bouts separated by 4 weeks, consisting of 10 sets of 6 maximal isokinetic ($60°\cdot s^{-1}$) eccentric elbow flexor contractions on an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York, USA) using their non-dominant arm. Each subject was seated on a Biodex seat with the shoulder joint secured at 45° flexion, with the elbow being aligned with the axis of rotation of the lever arm of the dynamometer which was attached to the subject's wrist in a supinated position. For each eccentric contraction, the elbow joint was forcibly extended from a flexed (60°) to a fully extended position (0°) in 1 s at an angular velocity of $60°\cdot s^{-1}$, while the subjects were verbally encouraged to generate maximal force at the flexed position and to maximally resist against the elbow-extending action for the full range of motion. After each eccentric contraction, the isokinetic dynamometer was programmed to return the arm to the flexed position at a velocity of $6°\cdot s^{-1}$, which provided a 10-s rest between contractions. The rest period between sets was 3 min. Torque signals were recorded via a data acquisition system (Powerlab with a Chart 7 software, ADinstrument, Bella Vista, Australia) at a sampling rate of 200 Hz, and real-time visual feedback of torque was displayed on a computer monitor.

4.2.3 Muscle Damage Markers

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

4.2.3.1 Maximal Voluntary Isometric Contraction (MVC) Torque

Elbow flexion MVC torque was measured using the isokinetic dynamometer with the same positioning of the subject as that for the eccentric exercise described above. Each subject performed two 3-s maximal voluntary isometric contractions at an elbow joint angle of 90° with a 30-s rest between contractions. Measurements were taken twice and the peak torque of the two contractions was used as the MVC torque (76, 95).

4.2.3.2 Range of Motion (ROM)

A plastic goniometer was used to measure extended (EANG) and flexed elbow joint angles (FANG). The EANG was determined when subjects attempted to fully extend the elbow joint while standing and hanging the arm by their side, and the FANG was determined when subjects attempted to fully flex the elbow joint to touch the shoulder of the same side with the palm (76). A semi-permanent ink pen was used to mark the lateral epicondyle of the humerus, the acromion process and the mid-point of the styloid process of the ulna and radius. Measurements were taken twice for each joint angle and the mean value of the two measurements was used to calculate the ROM by subtracting FANG from EANG (76).

4.2.3.2 Visual Analogue Scale (VAS)

The level of muscle soreness was assessed using a 100-mm VAS in which 0 indicated “no pain” and 100 represented “extreme pain” (76, 95). The subjects were asked to mark the level of perceived soreness on the VAS when the elbow flexors were palpated by the investigator who placed his index and middle fingers over the mid-belly

of the biceps brachii at 9 cm above the elbow crease and applied pressure and palpated with the tips of the finger toward the deeper tissues for approximately 3 s, while the subject was lying on the massage table with his forearm in an armrest position. The pressure applied to the site was kept as constant as possible between days and among subjects, and the measurements were taken by the same investigator throughout the study.

4.2.3.3 Pressure Pain Threshold (PPT)

PPT was measured using an electronic algometer (Somedic AB, Hörby, Sweden). The probe head of the algometer (area of 1.0 cm^2) was placed perpendicular to the mid-belly of the biceps brachii at 9 cm above the elbow crease (the same site as the VAS measures) and force was gradually applied at a rate of 50 $kPa \cdot s^{-1}$ until the subject reported the first feeling of noticeable pain of the muscle. The value (kPa) corresponding to the force applied to elicit pain was recorded. A 10-s interval was provided between measurements. The average of the two measures was used for further analysis (95).

4.2.3.4 Electrical Pain Threshold (EPT)

EPT of biceps brachii fascia (BBF), muscle (biceps brachii in between the two fascias) and brachialis fascia (BF) that separated the biceps brachii and brachialis, were measured separately by a pulse algometer (UPA-301, Unique Medical Co Ltd, Tokyo, Japan) while the subject lied supine on a massage table and relaxed their arms in a supinated forearm position (Figure 21a and b). The frequency of the pulse algometer was adjusted to 40 Hz before each measurement. A stainless steel needle electrode insulated with acrylic resin (180 µm in diameter, Toyo Medical Institute, Osaka, Japan)

was inserted into the mid-belly of the biceps brachii muscle (approximately 9 cm above the elbow crease; Figure 21c); and the BBF, muscle and BF pain thresholds were assessed (Figure 21d). The location of the needle was confirmed using real-time B-mode ultrasonography (Aloka F75 with a 5 cm UST-567 transducer; Aloka Co., Japan) before each measurement (Figure 21d). The pain threshold was determined for BBF followed by muscle and BF. When the needle was inserted into BBF, the intensity of the current was increased from zero at a constant rate (0.05 $mA \cdot s^{-1}$) and the subject indicated the feeling of pain by pressing a button on a controller that records the stimulus current at that pain level. The pain threshold was automatically displayed on the digital display of the algometer in units of mA. The intensity of the current stimulus was reset (back to zero), and the second stimulation was given with a 30-s interval between measurements. Following this measurement, the needle was progressively inserted into the muscle with the needle location confirmed by monitoring needle depth, and EPT of muscle (in between biceps fascia and brachialis fascia), and subsequently BF was measured. A semi-permanent ink pen was used to mark the skin for the EPT insertion site to make sure the insertion site was consistent between days. The average of the two measures for each region was used for further analysis.

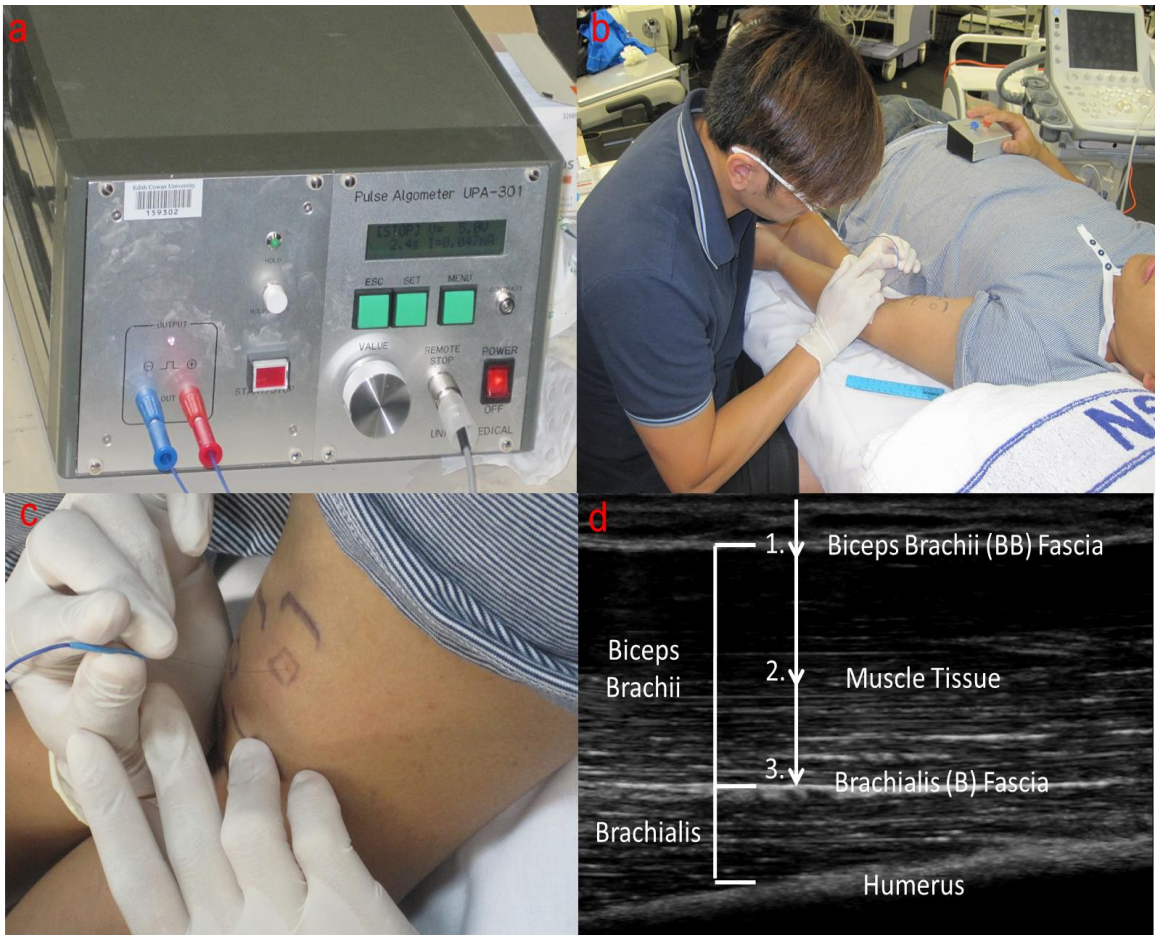

Figure 21. A pulse algometer used in the study (a) and the measurement protocol for electrical pain threshold (EPT) using the pulse algometer with a stainless steel needle electrode and terminate current controller (b). A needle electrode was inserted in the mid-belly of the biceps brachii (c), and EPT at biceps brachii fascia, muscle tissue (biceps brachii, between the two fascias) and brachialis fascia as shown in the B-mode ultrasound image (d) was assessed.

4.2.4 Statistical Analysis

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

4.3 RESULTS

4.3.1 Reliability for EPT Measurement

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

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

Time	Region	r	CV (%)	SEM (mA)
Baseline	BBF	0.96	4.3	0.05
Between - days	M	0.98	2.7	0.04
	BF	0.99	2.7	0.03
Baseline	BBF	0.94	5	0.06
Within - day (1h)	M	0.96	4.6	0.06
	BF	0.99	1.4	0.02
2 days post-Ex	BBF	0.97	3.2	0.03
Within - day (1h)	M	0.93	5.5	0.05
	BF	0.98	2.1	0.02

4.3.2 Eccentric Exercise

No significant differences in the changes in peak torque (P=0.38) and total work (P=0.92) over the 10 sets of 6 eccentric contractions were evident between the first and second bouts.

4.3.3 Muscle Damage Markers

There were no significant differences in the pre-exercise values between bouts; baseline MVC torque was 50.6 ± 8.1 Nm for ECC1 and 49.5 ± 7.9 Nm for ECC2, ROM was 140.5 ± 4.5° for ECC1 and 140.1 ± 4.7° for ECC 2, VAS was 0 cm (no pain) for both bouts, and PPT was 418.6 ± 45.6 kPa for ECC1 and 425.9 ± 45.6 kPa for ECC2. MVC torque decreased significantly immediately after exercise by approximately 58% for both bouts, but recovered significantly faster after ECC2 when compared with ECC1 (Figure 22a). ROM also decreased similarly between bouts immediately after exercise, with recovery being significantly faster after ECC2 than ECC1 (Figure 22b). VAS for muscle soreness increased significantly after both bouts; however, the magnitude of muscle soreness was significantly less after ECC2 than ECC1 (Figure 22c). PPT decreased significantly after both bouts, but the magnitude of the decrease was significantly smaller after ECC2 than ECC1 (Figure 22d).

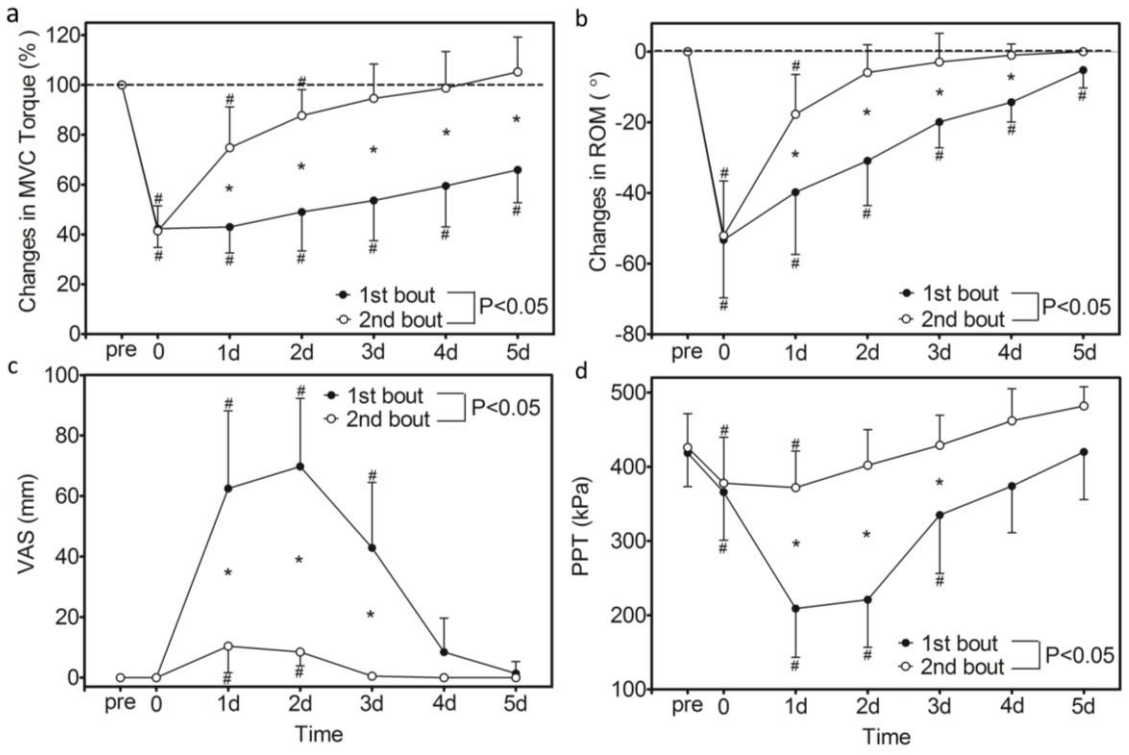

Figure 22. Changes in maximal voluntary isometric contraction torque (a), range of motion (b), muscle soreness using a visual analogue scale (c), and pressure pain threshold (d) before (pre), immediately after (0), and 1-5 days after the first and second eccentric exercise bouts. A significant ($P<0.05$) interaction effect is shown for all variables. * indicates a significant ($p<0.05$) difference between bouts. # indicates a significant different from pre-exercise value.

4.3.4 EPT

Figure 23 shows changes in EPT of BBF, muscle and BF after ECC1 and ECC2. EPT decreased significantly after both bouts; however, the changes were greater after ECC1 than ECC2 for the three regions. EPT decreased immediately after exercise and decreased further at 1-2 days after exercise for BBF (8-14%), muscle (43-55%) and BF (14-20%), and remained significantly below the baseline at 4 days post-ECC1. After ECC2, EPT decreased immediately after exercise, but did not show further large decreases and returned to the baseline at 4 days after exercise. When comparing the three regions, the magnitude of the decrease was significantly greater for both BBF and BF (54-92%) than muscle (16-57%) at 1, 2 and 4 days post-ECC1, without a significant difference between BBF and BF. After ECC2, the magnitude of the decrease was significantly greater for BBF than muscle, but no significant difference was found between BF and muscle.

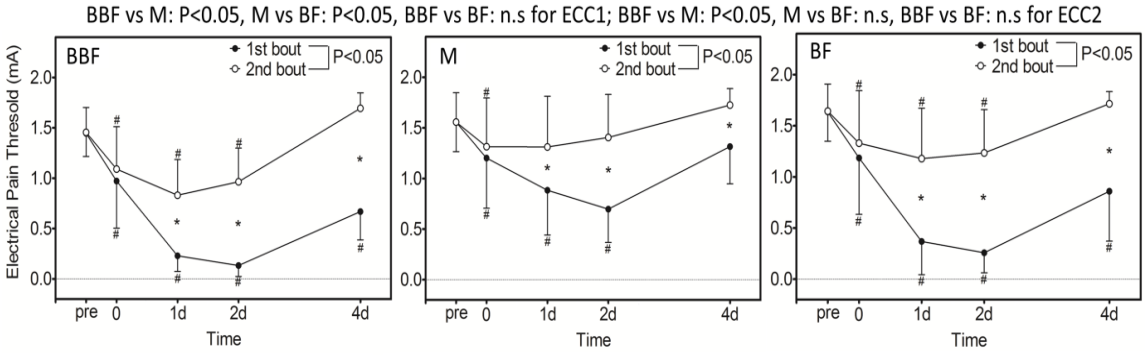

Figure 23. Changes in electrical pain threshold (EPT) at biceps brachii fascia (BBF), biceps brachii muscle (M) and brachialis fascia (BF) before (pre), immediately after (0), and 1, 2 and 4 days after the first and second eccentric exercise bouts. A significant ($P<0.05$) interaction effect is shown for all locations. * indicates a significant ($p<0.05$) difference between bouts. A significant ($P<0.05$) difference was found between biceps brachii fascia (BBF) and muscle (M); brachialis fascia (BF) and muscle (M); however,

no significant difference was found between BBF and BF. # indicates a significant ($P<0.05$) difference from pre-exercise value.

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

The correlations between EPT and VAS, EPT and PPT, and VAS and PPT measures are shown in Table 2. No significant correlation was found between the changes in EPT and VAS for all regions (r=-0.01-0.50) at 1, 2 and 4 day post-exercise. A significant correlation (0.63-0.87) was found between the changes in EPT for BBF and PPT, and for BF and PPT at 1 and 2 days post-exercise; however, no significant correlation was found at 4 days post-exercise. Furthermore, no significant correlation was found between the changes in VAS and PPT following eccentric contractions.

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

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

4.4 DISCUSSION

The present study was designed to test the hypothesis that fascia would become more sensitive to electrical stimulation (i.e. more painful) than muscle after eccentric exercise, and the change would be greater after the first compared with the second eccentric exercise bout. The indirect markers of muscle damage (MVC, ROM, VAS and PPT) indicated that the magnitude of muscle damage was less and the recovery was faster after the second eccentric exercise bout (Figure 22), indicating a typical repeated bout effect. The magnitude of decrease in EPT was greater for the biceps brachii fascia and brachialis fascia when compared with muscle after the first and second eccentric exercise bouts, and was smaller for the second bout than the first bout (Figure 23). These results, therefore, support the stated hypothesis. It should be noted that the changes in EPT at the fascia were significantly correlated with the changes in PPT at 1 and 2 days post-exercise, but a statistically significant correlation was not observed between the changes in EPT and VAS. This suggests that the pain threshold assessed by EPT is related to PPT, but different from the magnitude of pain expressed by VAS.

In the present study, EPT was not assessed at the same time points as those of VAS and PPT, because the invasiveness of the measurement could damage muscle fibres and connective tissue and thus affect pain sensation. The depth of the needle insertion was confirmed using B-mode ultrasonography before the measurements, thus the investigator was confident that the tip of the needle was located precisely for each measurement. The test-retest reliability of EPT measurements between days and within days was high. However, it should be noted that a large inter-individual variability in EPT was evident. To the best of our knowledge, this was the first study to investigate the changes in EPT at the fascia and muscle of the biceps brachii and brachialis. It is

interesting that there was no significant difference in EPT between fascia and muscle before exercise as it was assumed that fascia would be more sensitive than muscle at the baseline. The rationale for this hypothesis is that nociceptors are considered to be more numerous in fascia than muscle (42, 85). It is possible that the pain-responsive nociceptors are activated when damage and/or inflammation are induced, and thus the difference in EPT between fascia and muscle was only evident after exercise.

Itoh et al. (70) measured EPT at the skin, fascia and muscle at 2 and 7 days after eccentric exercise of the middle (3^{rd}) finger, and reported that EPT was 0.39-0.82 mA lower in the fascia compared with muscle and skin 2 days post-exercise, and suggested that the sensitised nociceptors at the fascia were responsible for DOMS symptoms. The results of the present study also showed that both the biceps brachii and brachialis fascia became more sensitive to electrical stimulation-induced pain than the biceps brachii muscle (Figure 23). This finding is consistent with previous studies showing that fascia (15) and other connective tissue such as tendon/tendon-bone junction (47) are more sensitive to hypertonic saline injection compared with muscle belly tissue following eccentric contractions. Gibson et al. (47) investigated the pain threshold sensitivity at the tendon, tendon-bone junction and muscle belly sites of the tibialis anterior muscle after 3 sets of 10 eccentric dorsiflexor contractions and reported that both the tibialis anterior tendon and tendon-bone junction became more sensitive to hypertonic saline injection compared with muscle tissue when assessed by visual analogue scale (VAS) and pressure pain threshold (PPT). In their subsequent study, Gibson et al. (15) also examined fascia and deep muscle sensitivities by hypertonic saline injection in the tibialis anterior following 3 sets of 10 eccentric contractions and found that fascia rather than muscle tissue was more sensitive to these saline injections at 2 days post-exercise when DOMS was prevalent. They suggested that the higher pain sensitivity found in the

fascia reflected fascial/epimysium receptor sensitisation and concluded that fascia rather than muscle tissue was most important in DOMS-associated sensitisation.

It has been documented that damage and inflammation to connective tissue surrounding muscle fibres are responsible for DOMS (26, 57, 134). Paulsen et al. (112) found a negative correlation between DOMS and leukocyte accumulation in inflamed muscle fibres after 300 eccentric quadriceps femoris contractions and concluded that damage and remodelling of the extracellular matrix (ECM) were associated with DOMS. Simultaneously, Raastad et al. (118) showed that tenascin-C and N-terminal propeptide of procollagen type III increased staining in the endomysium of the exercised muscle after performance of the same exercise and concluded that ECM was affected. Crameri et al. (32) compared muscle damage between 210 maximal eccentric contractions with electrical muscle stimulation (EMS) and 210 voluntary maximal eccentric contractions of the knee extensors, and found similar increases in the staining of tenascin C after EMS-induced and voluntary contractions, although muscle fibre damage was evident only after EMS. Thus, in the present study it seems likely that damage and inflammation occurred in the biceps brachii and brachialis fascia during and/or after the eccentric contractions. However, further studies are necessary to explicitly examine the histological changes in fascia after eccentric exercise.

It has been documented that the free nerve endings (nociceptors) are located along the walls of arteries and mainly in the surrounding connective tissue (42, 53), and the density of nociceptors is different between connective and muscle tissue (85, 130). Mense and Simons (85) reported that the innervation density of nociceptors in the connective tissue surrounding the calcaneal tendon of a cat was approximately five times higher than in the gastrocnemius-soleus muscle but no difference was found in innervation density throughout normal muscle tissue. Tesarz et al. (40) examined the

density and distribution of nerve fibres in rats as well as human thoracolumbar fascia using immunohistological techniques, and reported that muscle fascia was densely innervated with (PGP9.5-positive) non-peptidergic nerve fibre endings and encapsulated mechanoreceptors in the muscle fascia. Deising et al. (87) reported that the nociceptors in the fascia were sensitised and activated following nerve growth factor (NGF) injection to erector spinae at lumbar level (L4-L5), and suggested that the nociceptors in the fascia were particularly prone to sensitisation and this might contribute to acute or chronic muscle pain. Thus, it seems possible that damage to the connective tissues following eccentric contractions results in the activation of more nociceptors (increasing peripheral sensitisation) in the fascia, releasing sensitised noxious chemical substances through the axon reflex (neurogenic inflammation) in the damaged region and therefore enhancing temporal summation of nociceptive input (increasing central sensitisation) to the spinal cord at the dorsal horn, and in turn increasing the pain response to electrical stimuli at the fascia and inducing DOMS.

The time courses of changes in the VAS, PPT and EPT were different following eccentric exercise, such that muscle soreness assessed by VAS peaked 2 days post-exercise but the reduction in PPT was greatest at 1 day post-exercise, and both measures (VAS and PPT) returned to the baseline by 4 days post-exercise. However, the reduction in EPT was greatest at 2 days post-exercise in the fascia and remained below the baseline at 4 days after exercise. The present results showed a significant correlation between EPT and PPT at 1 and 2 days after exercise. It should be noted that PPT and EPT are based on pain thresholds despite the stimulation method being different (pressure vs. intramuscular electrical stimulation). This could explain the significant correlation between the two. In contrast, no significant correlation was found between EPT and VAS. It is important to note that VAS indicates the level of pain upon

mechanical stimulation (i.e. palpation, pressure) whereas PPT and EPT assess the minimum stimulus required to induce pain (i.e. pain threshold). It is possible that, even if the pain threshold is different, the level of pain induced by standardised stimuli (e.g. palpation) is the same. In fact, no correlation between VAS and PPT assessments was evident in the present study or in a previous study (95).

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

This chapter showed that Biceps brachii and brachialis fascia are more responsible for DOMS sensation than muscle following eccentric exercise. In the next chapter, it was focused on muscle lengthening during eccentric contraction and how the magnitude of lengthening affected the magnitude of DOMS and other markers of muscle damage.

CHAPTER FIVE

STUDY 4

5.1 INTRODUCTION

Muscle damage is often induced by the performance of unaccustomed eccentric exercise (58, 98). Typical symptoms of muscle damage include delayed onset muscle soreness (DOMS) and prolonged losses in muscle strength and range of motion (ROM), which are most prominent 1-3 days after exercise and can negatively impact daily activities and athletic performances (29, 98). In order to develop strategies to minimise this damage it is important to understand how it is induced, yet the factors influencing the magnitude of eccentric contraction-induced muscle damage have not been fully elucidated (58, 98). Previous evidence indicates that one of the key events in the muscle damage process is an increased intracellular Ca^{2+} concentration, mediated through stretch-activated channels stimulated by coincident muscle activation and lengthening (2). Another possibility is that damage results directly from the imposition of mechanical strain, triggering inflammation-dependent catabolic processes that weaken the muscle and trigger pain responses (2, 5, 48, 83). If these mechanisms are indeed pivotal, then a greater muscle lengthening during eccentric contractions should result in greater muscle damage and soreness. Indeed, previous animal (61, 80) and human studies (67, 108) have shown that muscle length change is a key factor influencing the magnitude of muscle damage induced by eccentric contractions.

Interestingly, a repeated bout of the same exercise performed within several weeks or months results in less muscle damage than the first bout, which is typically indicated by a faster recovery of muscle function and smaller increases in DOMS and creatine kinase (CK) activity in the blood (83, 98). This protective adaptation is referred to as the repeated bout effect, and has been investigated using models of eccentric exercise in the knee extensors (69, 88),elbow flexors (37, 56, 93) and shoulder muscle (71), with a clearer and stronger repeated bout effect being reported using elbow flexor exercise (57, 64). The repeated bout effect has been speculated to be associated with neural, mechanical and cellular adaptations, although its underpinning mechanisms have yet to be fully described (83). Based on the evidence presented above, however, a logical prediction is that muscle length change would be less during the second eccentric exercise bout than the initial bout, because the magnitude of muscle damage should be largely determined by the magnitude of muscle lengthening during the exercise bout. Nonetheless, this fundamental hypothesis has never been explicitly tested.

The present study used the B-mode ultrasound technique to assess biceps brachii muscle length changes during maximal eccentric elbow flexor contractions and compared these length changes between first and second bouts. It is assumed that the movement distance (displacement) of the distal biceps brachii myotendinous junction (MTJ) from the onset to the end of each eccentric contraction represented biceps brachii muscle, and fascicle length changes, thus a greater MTJ displacement indicated a greater muscle length change. A remarkable characteristic of the repeated bout effect is that its effects last for months after the first bout, so any potentially important mechanism has to be identifiable at least 1 month after the initial bout. Therefore, this

study imposed the second bout 4 weeks after the initial bout in order to specifically test the hypothesis that the magnitude of muscle length change would be less during the second bout when compared with the first eccentric exercise bout, and that this would be associated with a decrease in changes in indirect markers of muscle damage (i.e. the repeated bout effect). Changes in muscle length were examined between contractions performed within a set as well as across 10 complete sets of the exercise to determine whether muscle lengthening would vary as fatigue (damage) accumulates during the exercise.

5.2 METHODS

5.2.1 Experimental Design

Ten healthy men performed two exercise bouts consisting of 10 sets of 6 maximal isokinetic ($60°\cdot s^{-1}$) eccentric elbow flexor contractions using a randomly chosen arm separated by 4 weeks. Indirect markers of muscle damage, including maximal voluntary isometric contraction (MVC) torque, range of motion (ROM), muscle thickness, ultrasound echo intensity, serum CK activity and muscle soreness, were measured before, immediately after, and 1, 2, 3, 4, 5 and 7 days after exercise and were compared between bouts. Biceps brachii MTJ displacement during eccentric contractions (Figure 24) was recorded using B-mode ultrasonography, and the MTJ displacement from the beginning to the end of each eccentric contraction was assessed as explained below, and its changes within and over sets were compared between bouts.

5.2.2 Subjects

This study was approved by the Institutional Human Research Ethics Committee and complied with the Declaration of Helsinki. Ten young men with no current or previous upper arm injuries and who had not performed upper limb resistance training for at least six months prior to the present study were invited to participate. The number of subjects was determined by a sample size estimation using the data of a previous study (24), which reported on the repeated bout effect after maximal elbow flexors eccentric exercise. Based on an α-level of 0.05 and a power (1-β) of 0.80, and an expected 20% difference in MVC torque recovery at 3 days post-exercise between the first and second bouts, the analysis indicated that at least 10 subjects were required. Their mean (±SD) age, body mass, height and MVC torque were 24.9 ± 5.4 y, 69.2 ± 8.3 kg, 169.8 ± 6.2 cm, and 60.0 ± 12.0 Nm respectively. All subjects provided informed written consent, and a medical questionnaire was completed before participating in the study. Subjects were requested not to change their lifestyle and diet, not to take any anti-inflammatory drugs or nutritional supplements, and not to perform unaccustomed exercise during the experimental period.

5.2.3 Eccentric Exercise

The exercise consisted of 10 sets of 6 maximal voluntary eccentric elbow flexor contractions on an isokinetic dynamometer (Cybex 6000, Ronkonkoma, NY, USA) using one arm that was randomly chosen without considering arm dominance. Subjects were individually positioned on a seated preacher arm curl bench that secured the shoulder joint at 45° flexion in from of the body, with the elbow being aligned with the axis of rotation of the dynamometer and the lever arm of the dynamometer being attached to the subject's wrist in a supinated position. For each eccentric contraction,

the elbow joint was forcibly extended from a flexed (60°) to a fully extended position (0°) in 1 s at an angular velocity of $60°\cdot s^{-1}$, while the subjects were verbally encouraged to generate maximal force at the flexed position and to maximally resist against the elbow-extending action throughout the full range of motion. The smaller range of motion (60°) was set in the present study to obtain better ultrasound images during eccentric contractions (see below) and previous study (106) showed that greater damage was found when performed in longer muscle length. After each eccentric contraction, the isokinetic dynamometer was programmed to return the arm to the flexed position at a velocity of $6°\cdot s^{-1}$, giving 10 s of rest between contractions. The rest period between sets was 3 min. Torque signals were recorded via a data acquisition system (Powerlab with a Chart 7 software, ADinstrument, Bella Vista, Australia) at a sampling rate of 200 Hz, and real-time visual torque feedback was displayed on a computer monitor.

5.2.4 Muscle Damage Markers

5.2.4.1 Maximal Voluntary Isometric Contraction (MVC) Torque

MVC torque of the elbow flexors was measured using the isokinetic dynamometer with the same positioning of the subjects as that for the eccentric exercise described above. Each subject performed two 3-s maximal voluntary isometric contractions at an elbow joint angle of 90° with a 30-s rest between contractions. Measurements were taken twice and the peak torque of the two contractions was used as the MVC torque.

5.2.4.2 Range of motion (ROM)

A plastic goniometer was used to measure extended (EANG) and flexed elbow joint angles (FANG). The EANG was determined when subjects attempted to fully

extend the elbow joint while standing and hanging the arm by their side, and the FANG was determined when subjects attempted to fully flex the elbow joint to touch the shoulder of the same side with the palm. A semi-permanent ink pen was used to mark the lateral epicondyle of the humerus, the acromion process and the mid-point of the styloid process of the ulna and radius. Measurements were taken twice for each joint angle and the mean value of the two measurements was used to calculate the ROM by subtracting FANG from EANG (76, 93).

5.2.4.3 Muscle Thickness and Echo Intensity

B-mode ultrasound images were obtained using an Aloka SSD-α10 ultrasound system (Aloka Co, Ltd., Tokyo, Japan) using a frame rate of 47 Hz with a 10-MHz electronic flat T-head probe (6 cm, UST-5713) from the biceps brachii mid-belly at 9 cm above the elbow crease. The examiner placed the probe on this site to obtain longitudinal images. Images were recorded by the ultrasound system and transferred to a portable computer (Dell Laptop, MSK 1750, USA), and a software program (Image J, version 1.47, National Institute of Health, USA) was used to determine muscle thickness and echo intensity. Elbow flexor muscle thickness was measured as the distance between the subcutaneous fat layer and the edge of the humerus (63, 78). B-mode echo intensity of each image was determined by selecting a region of interest (1 cm × 1 cm) within the biceps brachii in each image. The echo intensity of a histogram of gray scale (0: black, 255: white) for the region was quantified using the software program (63).

5.2.4.4 Serum CK Activity

Approximately 8 ml of blood was drawn from the antecubital vein from the participants by a standard venipuncture technique. Since previous studies have shown that CK activity peaks 4 – 5 days after eccentric elbow flexor exercise (76, 107), blood samples were taken immediately before, and 4, 5 and 7 days after exercise. Blood samples were allowed to clot at room temperature, centrifuged for 10 min at 4°C to obtain serum and separated into four 1-ml aliquots. Serum CK activity was determined by a Hitachi Modular PT automated clinical chemistry analyser (Roche, Germany) with a commercially available Roche Diagnostics Reagent (Mannheim, Germany). The normal reference range using this method is < 200 $IU \cdot L^{-1}$.

5.2.4.5 Visual Analogue Scale (VAS)

The level of muscle soreness was assessed using a 100-mm VAS in which 0 indicated "no pain" and 100 represented "extreme pain". The subjects were asked to mark the level of perceived soreness on the VAS when the elbow flexors were palpated by the investigator before, immediately after and 1 – 5 and 7 days post-exercise. In the palpation, the investigator placed his index and middle fingers over the mid-belly of biceps brachii at 3, 9 and 15 cm above the elbow crease, and applied pressure and palpated with the tips of the finger toward the deeper tissues for approximately 3 s, while the subject was lying on the massage table with his forearm in an armrest position. The measurement at the 3 cm site was performed first followed by the measurements at the 9 and 15 cm sites. One measurement was taken from each site with a 10 s interval between measurements. The pressure given to the sites was kept as constant as possible between days and among subjects, and the measurements were taken by the same investigator throughout two bouts of testing. The mean of the three sites was used for further analysis (76).

5.2.4.6 Biceps Brachii Myotendinous Junction Displacement

The movements of the biceps brachii myotendinous junction (MTJ) were captured by a real time B-mode ultrasound apparatus with the specifications described above and recorded on a data acquisition system (Powerlab with a Chart 7 software, ADinstrument, Bella Vista, Australia). The ultrasound probe was firmly attached to the distal portion of the muscle over the MTJ above the elbow crease by tape and bandage. The investigator identified the probe position in a familiarisation session and a semi-permanent ink pen was used to mark this position on the biceps brachii to achieve

consistent measurements over two bouts of eccentric exercise. The video images were displayed in real time on the ultrasound and computer monitors during the exercise. The ultrasound images captured by a frame rate of 47 Hz and torque data on the LabChart 7.0 were synchronised and recorded by a computer (Dell Labtop, MSK 1750, USA) for further analysis. Changes in MTJ displacement from the beginning to the end of each contraction were analysed by a computer software (DartFish Prosuite 5.0, DartFish, Alpharetta, GA, USA), and corresponding elbow joint angles were checked using the LabChart 7.0 computer software program to ensure that a full ROM was achieved. During eccentric contractions, MTJ displacement (l) was determined by the following formula:

$$l = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$

where l is the MTJ displacement, x_1 and y_1 are the MTJ coordinates at the beginning of the contraction (60°), and x_2 and y_2 are the coordinates at the end of the contraction (0°) as depicted in Figure 24.

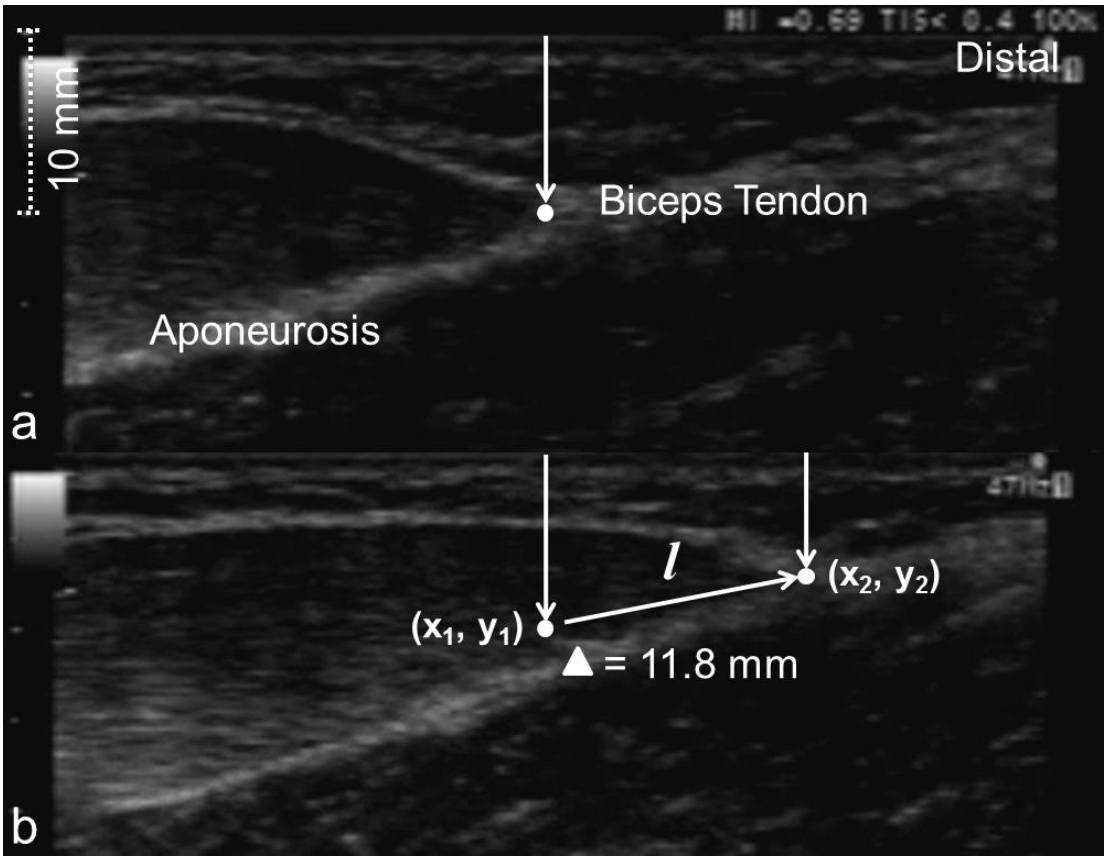

Figure 24. A typical B-mode ultrasound image of biceps brachii immediately before an eccentric contraction (maximal isometric contractions at 60˚ elbow flexion (a)), and the end of an eccentric contraction at 0˚ elbow flexion (b). The displacement of the myotendinous junction (shown in the white dot) was calculated from the two pictures based on the formula; $l = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$, showing the myotendinous junction (pointed by an arrow) moved. Displacement (l) is 11.8 mm in this example.

5.2.5 Statistical Analysis

A one-way repeated measures analysis of variance (ANOVA) was used to compare the changes in the biceps brachii MTJ displacement between contractions within each set. A two-way repeated measures ANOVA was then used to compare the changes in displacement over the 10 sets between the first (ECC1) and second (ECC2) bouts and for the changes in muscle damage markers (MVC, ROM, muscle thickness, echo intensity, serum CK activity and muscle soreness by VAS) over time (before, immediately after, 1 – 5, and 7 days post-exercise). When the ANOVA revealed significant time or interaction effects, a Tukey's post-hoc test was used for multiple comparisons. Linear relationships between the within-bout changes in MTJ displacement (i.e. between the 1^{st} and 10^{th} sets) and changes in muscle damage markers (MVC torque and ROM at 1 day post-exercise, muscle thickness and peak ultrasound echo intensity, peak serum CK activity, peak muscle soreness) were examined by computing Pearson's product moment correlation coefficients. For the relationship between the change in MTJ displacement and the peak echo intensity in ECC1, the strength of the curvilinear (logarithmic) relationship was calculated (see Figure 28b). The specific time points were chosen as they were considered to represent the magnitude of muscle damage most clearly. Statistical significance was set at $P<0.05$, and all data are presented as mean ± standard deviation (SD).

5.3 RESULTS

5.3.1 Peak Torque and Total Work

No significant between-bout differences in the changes in peak torque (P=0.12) or total work (P=0.35) were evident over the 10 sets of 6 eccentric contractions.

5.3.2 Muscle Damage Markers

There were no significant differences in pre-exercise values between bouts; baseline MVC torque was 60.2 ± 12.2 Nm for ECC1 and 56.3 ± 10.8 Nm for ECC2, ROM was 139.5 ± 6.6° for ECC1 and 139.3 ± 6.7° for ECC 2, and muscle thickness was 27.3 ± 5.6 mm for ECC1 and 27.8 ± 5.2 mm for ECC2. MVC torque decreased significantly immediately after exercise by approximately 50% in both bouts, but recovered significantly faster following ECC2 when compared with ECC1 (Figure 25a). ROM also decreased similarly between bouts immediately after exercise, but the recovery was significantly faster after ECC2 than ECC1 (Figure 25b). A significant increase in muscle thickness was observed after exercise; however, the magnitude of increase was significantly less in ECC2 than ECC1 at 2-7 days post-exercise (Figure 25c). Figure 2d shows the relative changes in ultrasound echo intensity from the baseline (100%). In the figure it is clear that echo intensity increased significantly from pre-exercise values after ECC1, but did not change after ECC2 (Figure 25d). There was a tendency (P=0.06) for the increases in serum CK activity to be smaller after ECC2 compared with ECC1 (Figure 25e). Muscle soreness increased significantly after both bouts, but the magnitude of muscle soreness was significantly less after ECC2 than ECC1 (Figure 25f).

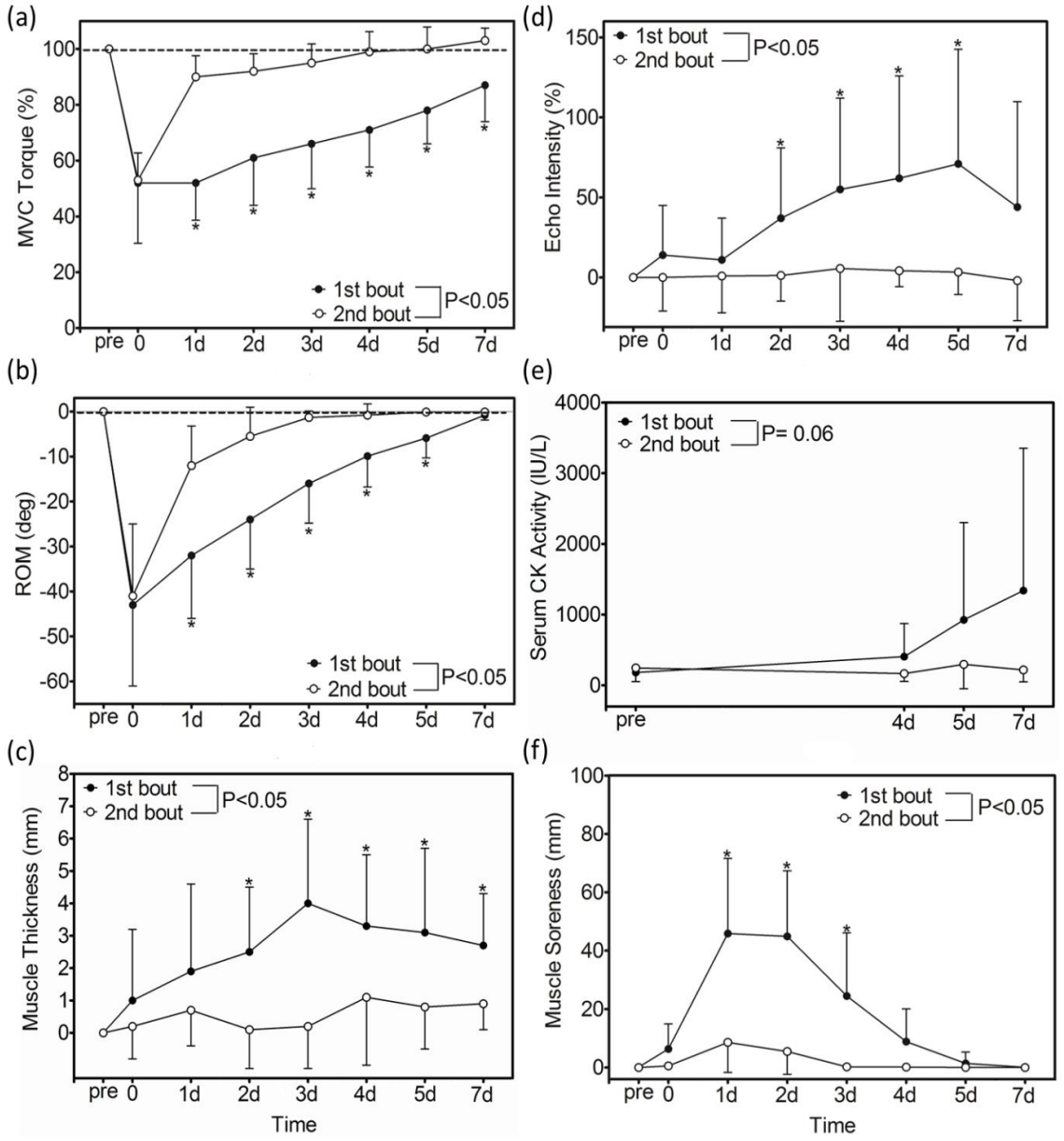

Figure 25. Changes (mean ± SD) in maximal voluntary isometric contraction torque (a), range of motion (b), elbow flexor muscle thickness (c), B-mode ultrasound echo intensity from baseline (d), serum CK activity (e) and muscle soreness by visual analogue scale (f) before (pre), immediately after (0), and 1-7 days following the first and second eccentric exercise bouts. A significant ($P<0.05$) interaction effect is shown for all variables. * indicates a significant ($p<0.05$) difference between bouts.

5.3.3 MTJ Displacement

Figure 26 compares the changes in the biceps brachii MTJ displacement from the beginning to the end of each contraction over 6 contractions in sets 1, 5 and 10 for ECC1 and ECC2. No significant changes over 6 contractions were evident within each set, and this was also the case for other sets that are not included in the figure (i.e. sets 2-4, 6-9). During ECC1, the displacement was significantly greater during sets 5 and 10 when compared with set 1, and for set 10 compared to set 5 (Figure 26a); however, no significant difference between the sets was evident during ECC2 (Figure 26b). No significant difference between ECC1 and ECC2 was evident in set 1, but there was a significant difference between bouts in sets 2-10.

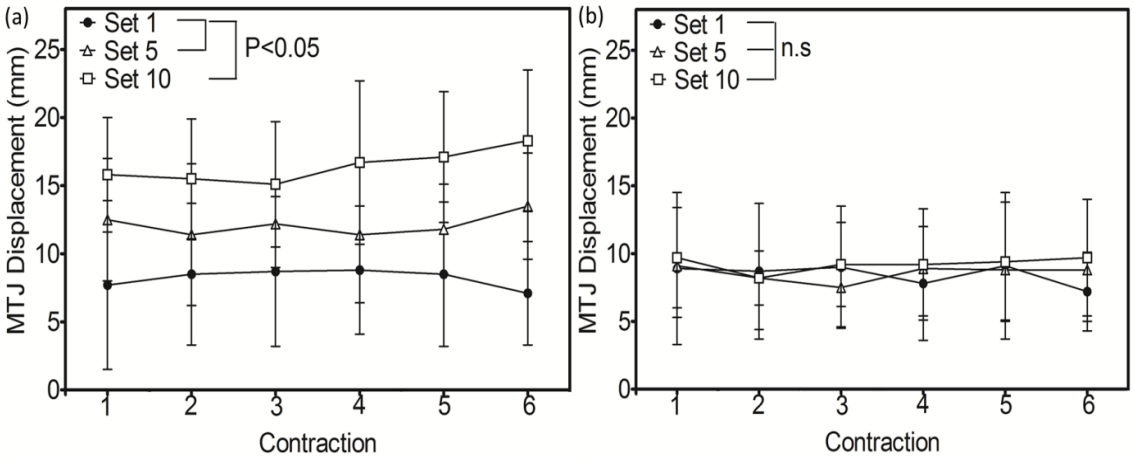

Figure 26. Changes (mean ± SD) in the magnitude of biceps brachii MTJ displacement during eccentric contractions 1 – 6 in the 1st (Set 1), 5th (Set 5) and 10th sets (Set 10) for the first (a) and second (b) bouts. A significant ($P<0.05$) effect of set was found for the first bout, but not (n.s) the second bout.

Figure 27 shows the changes in the magnitude of MTJ displacement over 10 sets (average of each set) during ECC1 and ECC2. No significant difference between bouts was evident for the change in the set 1 (ECC1: 8.2 ± 4.7 mm, ECC2: 8.5 ± 4.0 mm). Displacement in ECC1 significantly increased over sets, but this was not the case for ECC2, and a significant difference between bouts was evident from set 2 onwards. In ECC1, the MTJ displacement doubled from set 1 to set 10 (16.4 ± 4.7 mm); however, there were no significant changes in ECC2 from set 1 to set 10 (9.3 ± 3.1 mm).

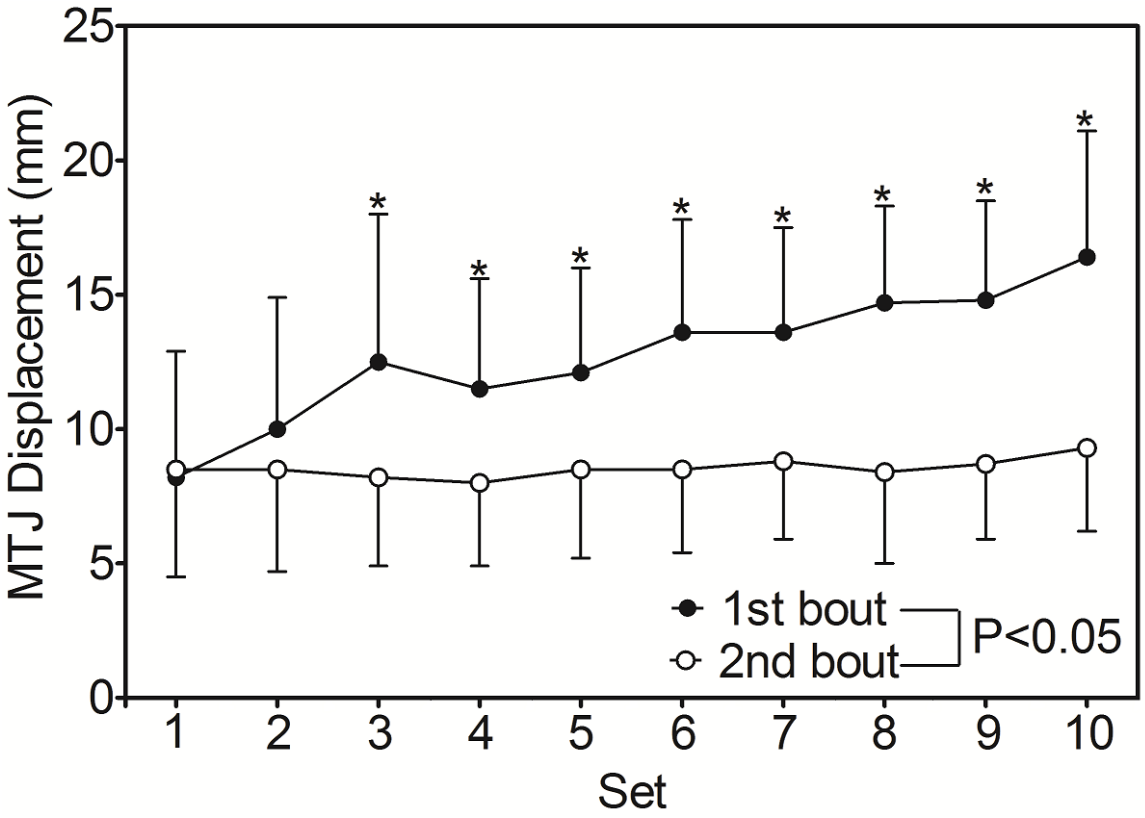

Figure 27. Changes (mean ± SD) in the magnitude of biceps brachii MTJ displacement in a set (average of 6 contractions) over 10 sets for the first and second bouts. A significant ($P<0.05$) interaction effect is shown. * indicates a significant different between bouts.

5.3.4 Correlation between Change in MTJ Displacement and Muscle Damage Markers

A significant correlation was found between the change in MTJ displacement during the first exercise bout and the decrease in MVC torque at 1 day post-exercise (Figure 28a) and the MTJ displacement and the magnitude of change in peak ultrasound echo intensity (Figure 28b); however, no significant correlation was found between the MTJ displacement and changes in other variables. Similarly, a significant correlation between the change in MTJ displacement and the magnitude of change in MVC torque at 1 days post-exercise (Figure 28c) as well as the MTJ displacement and peak ultrasound echo intensity (Figure 28d) were evident for the second bout. However, a significant relationship was not observed for the other markers (ROM: r=0.149, muscle thickness: r=0.110, CK: r=0.260, muscle soreness: r=0.497).

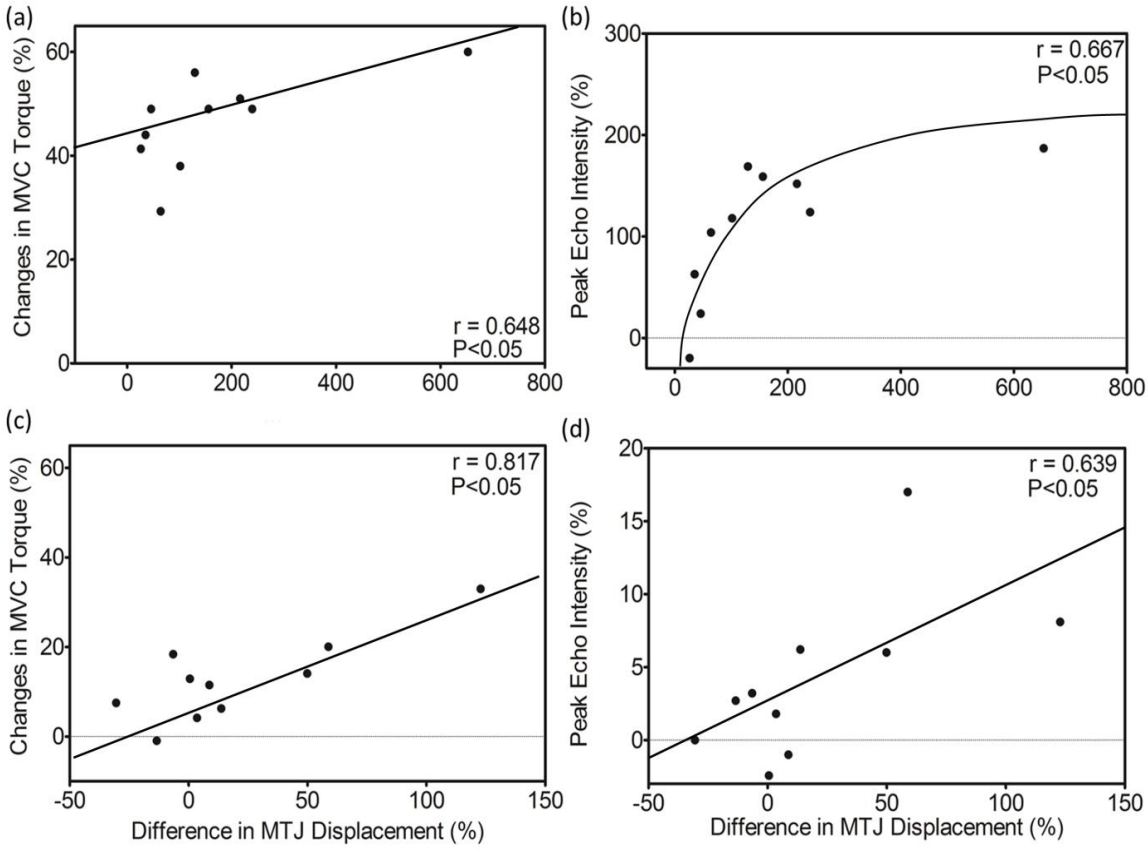

Figure 28. Correlation between the difference in myotendinous junction displacement between the 1st and 10th sets and changes in maximal voluntary isometric contraction (MVC) torque at 1 day post-exercise (a, c), and peak echo intensity from the baseline (b, d) for the first (upper figures) and the second exercise bout (lower figures). For the regression line, the model (either liner or curvilinear) that showed a greater r-value is shown.

5.4 DISCUSSION

The present study was designed to test the hypothesis that the magnitude of muscle lengthening would be less during a second than a first eccentric exercise bout separated by 4 weeks, and that this reduction in lengthening would be associated with a decrease in indirect muscle damage marker changes. Interestingly, no difference in MTJ displacement (i.e. muscle lengthening) was observed between bouts when comparing only the first set of exercise. However, an important and novel finding of the present study was that, while there was a 50% increase in muscle lengthening from the 1st to the 10th set during the first eccentric exercise bout (Figure 27), muscle lengthening was constant between sets 1 to 10 during the second eccentric exercise bout. Thus, the increase in lengthening seen in the first bout was absent in the second. Importantly, there was minimal evidence of muscle damage (i.e. there was a fast recovery of MVC torque and ROM, a lack of increase in muscle thickness, ultrasound echo intensity and serum CK activity, and minimal DOMS) after the second bout of exercise, despite significant evidence of damage being observed after the first bout (Figure 25), indicating a typical repeated bout effect. The finding of lesser muscle elongation as exercise progressed in the repeated bout supports the hypothesis that the repeated bout results from lesser muscle lengthening being imposed even though muscular force and joint range of motion (i.e. total work) are identical. As evidence of a potential causal link between the minimization of length change and the reduction in muscle damage in the second bout, individuals who showed greater increases in muscle lengthening as exercise progressed also showed greater decreases in MVC torque after exercise and greater increases in ultrasound echo intensity following both exercise bouts (Figure 28). These results suggest that the repeated bout is associated with a reduction in muscle

lengthening during eccentric contractions, and that less damage exists when muscle lengthening does not increase as exercise continues during repetitive eccentric contractions.

As shown in Figure 26, there was no change in the magnitude of muscle lengthening between contractions within each set, although it was significantly different between sets in the first bout of eccentric exercise (Figure 27). It is interesting that muscle lengthening did not increase between contractions within a set, but was greater in the subsequent set after 3 min of passive rest (Figure 26). It may be practically important to determine the cause of this in future research, because the mechanisms influencing the muscle lengthening appear to act distinctly between sets rather than between contractions; the resting phase is therefore an important point of future examination. Although the current study was not designed to examine the mechanisms responsible for the effect, it is possible that changes in intra-muscular pressure (i.e. changes in blood flow), afferent feedback (particularly via type III and IV afferent pathways), and rapid effects of calpain-mediated protein degradation are important. Regardless, it is important to note that a greater decrease in MVC torque and increase in ultrasound echo intensity after the first and second exercise bouts were evident in subjects who showed the greatest increase in muscle lengthening as the sets progressed (Figure 28). This is the best evidence thus far that the magnitude of the repeated bout effect is strongly associated with the propensity for muscle lengthening to increase during eccentric exercise.

Another question to be answered in future research is that of how muscle lengthening is reduced in the second bout of exercise, even when 4 weeks are allowed between bouts. McHugh et al. (19) speculated that increases in extensibility of relaxed muscle (passive stiffness) and active muscle (dynamic stiffness), remodelling of the

intermediate filament system, and increased intramuscular connective tissue following eccentric training are mechanical adaptations that could protect against damage from a repeated bout. However, he also pointed to evidence against the mechanical adaptation theory, such as the fact that muscle with greater passive stiffness may be more susceptible to damage. In the present study, the magnitude of muscle length changes in the first set was the same between bouts, but muscle elongation was much less in the second set beyond (Figure 27). This may suggest that an increase in dynamic stiffness is more likely responsible for the repeated bout effect. Previous studies have shown that gastrocnemius muscle stiffness significantly increased following a bout of 15 minutes (50) or 60 minutes (55) of downhill walking by 21% and 16% respectively. It is possible that such results are underpinned by changes in connective tissue integrity. For example, Lapier et al. (74) examined the intramuscular connective tissue of rat extensor digitorum longus muscles after 3 weeks of immobilization in either a shortened or lengthened position, and found that the intramuscular connective tissue concentration increased under both conditions, and that muscle damage was attenuated in these muscles after electrically stimulated eccentric plantar flexor contractions. This finding indicates that, regardless of how it is induced, changes in connective tissue concentration are associated with a decrease in muscle damage. Crameri et al. (32) found increases in the staining of human vastus lateralis intramuscular connective tissue (tenascin C) after voluntary as well as electrically-stimulated eccentric contractions of the knee extensors. Also, Raastad et al. (118) reported that tenascin-C and N-terminal propeptide of procollagen type III increased in the endomysium after 300 eccentric knee extensor contractions, and Mackey et al. (79) showed that laminin-β 1 and types I and III collagen were elevated after the initial eccentric exercise, and concluded that remodelling and strengthening of extracellular matrix (ECM) played a role in the

protective effect. These findings are suggestive of the possibility that a single bout of eccentric exercise remodels the ECM and/or connective tissues to make the muscle more resilient to eccentric contraction-induced muscle damage. It remains to be seen whether such changes remain prominent at least one month after an initial bout of eccentric exercise and whether the magnitude of these changes is related to the magnitude of the repeated bout effect.

It has also been suggested (82, 102) that changes in motor unit recruitment strategies could influence the extent of damage in a repeated bout. For instance, Dartnall et al. showed that the motor unit synchronisation was increased by 34% at 24 h after a single bout of eccentric contractions (35) and remained elevated by 57% at 7 days after the first bout of eccentric exercise (36). These studies suggest that changes in motor unit synchronisation after the initial bout of eccentric exercise may be associated with the repeated bout effect, possibly by altering fibre stress or inter-fibre shear magnitude. What is not known is whether such neural adaptations remain for periods greater than a month, although it is well established that central adaptations may be maintained for months after acquisition (e.g. (8)). Future research may thus examine the time course of changes in motor unit synchronisation, and other neural strategies, to determine their possible influence on the repeated bout effect.

Proske and Morgan (117) suggested that increases in sarcomere number in series were associated with the repeated bout effect and this cellular adaptation theory is indirectly supported by a shift of optimum angle toward a longer muscle length. Yu et al. (139) also found that the increases in sarcomere number in parallel myofibrils following downstairs running eccentric exercise. In the present study, the elbow joint angle to produce the largest isometric torque (optimum angle) was not assessed; however, Chen et al. (24) used a similar eccentric exercise model of the elbow flexors to

that of the present study, and reported 4° shift toward a longer muscle length remaining at 2-3 weeks after the first maximal eccentric exercise bout. They also found in the study that the repeated bout effect was conferred by submaximal (40%-80%) eccentric exercise without any shift of the optimum angle after submaximal eccentric exercise, and stated that the shift of the optimum angle did not appear to be directly related to the mechanisms underpinning the repeated bout effect. It seems that longitudinal addition of sarcomeres fits well to explain the less muscle fibre lengthening in the second eccentric exercise bout, but it is not known whether sarcomere number in series increase in biceps brachii muscle fibres between the first and second eccentric exercise bouts in the present study, thus warrants further study needs to examine this speculation.

In conclusion, the present study showed that the magnitude of biceps brachii muscle lengthening (MTJ displacement) during maximal eccentric contractions increased over 10 sets during the first eccentric elbow flexors bout but did not increase during a second bout performed 4 weeks later. Muscle damage markers showed a typical repeated bout effect, including a faster recovery of muscle function, minimal change in ultrasound echo intensity, attenuated DOMS and a lack of increase in serum CK activity. Notably, individuals who displayed the greatest increase in lengthening over the 10 sets in both the first and second bouts also showed the greatest loss in isometric force and ultrasound echo intensity (i.e. muscle damage). It seems possible that the lesser elongation of the muscle during the second eccentric exercise imposed less mechanical strain on the muscle and muscle fibres, inducing less damage. This may thus be one factor influencing the repeated bout effect; however, the mechanisms that might underpin the resistance to lengthening after the first bout are not known and need to be elucidated in future research.

This chapter demonstrated that the magnitude of muscle lengthening during eccentric contractions is associated with the magnitude of muscle damage following exercise.

CHAPTER SIX

6. OVERALL DISCUSSION AND CONCLUSION

6.1 Discussion

The purposes of this thesis project were to investigate delayed onset muscle soreness (DOMS) after elbow flexor eccentric exercise using several different pain assessments and to test the hypothesis that connective tissue damage-inflammation would be more associated with DOMS than muscle fibre damage-inflammation. This chapter summarises the main findings and provides an integrated discussion of the four studies included in this thesis.

From the Studies 1-2 (Chapters 2 and 3), it was demonstrated that VAS increased 1 to 4 days after exercise and peaked 2 days post-exercise, while PPT decreased most at 1 day post-exercise and did not return to the baseline for 4 days following exercise. No significant difference among the three sites was found for VAS and PPT in Study 1 (5, 9 and 13 cm above the elbow crease) and also in Study 2 (3, 9 and 15 cm above the elbow crease). The magnitude of change in VAS did not significantly correlate with that of PPT in both Study 1 and 2. Study 2 demonstrated that palpation induced greater pain than static pressure, and longitudinal and transverse palpations induced greater pain than circular palpation. In the PPT assessments, PPT was lower at medial regions before exercise, but the pain sensitive regions shifted to the central and distal regions of the biceps brachii at 1-3 days post-exercise. The studies also showed that VAS correlated with CR-10, but not with PPT. These results suggest how to palpate muscle affects the pain level, and the central and distal regions should be included in the DOMS

assessment for both VAS or CR-10 and PPT. The results from both studies indicated that VAS and PPT represented different aspects of DOMS.

Study 3 (Chapter 4) investigated changes in electrical pain threshold (EPT) after eccentric exercise to test the hypothesis that fascia would become more sensitive than muscle. Ten young men performed two eccentric exercise bouts (ECC1, ECC2) consisting of 10 sets of 6 maximal isokinetic eccentric contractions of the elbow flexors with the same arm separated by 4 weeks. EPT was assessed for the biceps brachii fascia (BBF), muscle and brachialis fascia (BF) 1 day before, immediately after, and 1, 2 and 4 days after exercise. EPT decreased after both exercise bouts and the largest decreases were evident at 2 days post-exercise. The decreases in EPT after ECC1 were greater for both BBF and BF than muscle. These results suggest that fascia become more sensitive than muscle to electrical stimulation after eccentric exercise.

Study 4 (Chapter 5) investigated biceps brachii myotendinous junction (MTJ) displacement during maximal eccentric elbow flexor contractions to test the hypothesis that reduced muscle lengthening would be seen during the second (less damaging) exercise bout than the first. The magnitude of MTJ displacement (average of 6 contractions) increased from set 1 to set 10 during ECC1, but no significant change over sets was evident during ECC2. Changes in maximal voluntary isometric contraction strength, range of motion, muscle thickness, ultrasound echo intensity, serum creatine kinase activity and muscle soreness (visual analogue scale) were smaller following ECC2 than ECC1, showing less muscle damage in the repeated bout. These results suggest that a lack of change in muscle lengthening as exercise progresses in a repeated bout of eccentric contractions may be an important factor in the attenuation of muscle damage and DOMS.

Based on the above findings, the following recommendations for the DOMS assessments for eccentric elbow flexor exercise are made as discussed in Chapter 3. 1) Pain level should be assessed using VAS or CR-10 with standardised stimuli such as palpation, stretching, muscle contractions, and movements. 2) It is better to include PPT assessments in order to obtain information regarding pain thresholds, since pain ratings using a scale and pain threshold are different. 3) CR-10 can be used instead of VAS to rate pain level; however, VAS would be better. 4) It is better to include multiple sites (e.g. 3, 9, 15 cm above the elbow crease) covering the distal and central muscle regions for VAS and/or PPT assessments to account for region-specific differences in pain. 5) The muscle should be palpated in either a longitudinal or transverse direction, rather than circular, and this should be standardised before the commencement of testing. 6) EPT could provide internal location of pain, thus it could be added to the DOMS assessments.

Regarding the mechanisms underpinning DOMS, the studies above provided some evidence supporting the hypothesis that DOMS is associated with damage and inflammation to connective tissues surrounding muscle fibres (i.e. endomysium) and/or muscle bundles (i.e. the perimysium or fascia). The PPT 50-grid method (Study 2) showed that more pain-sensitive regions were located close to the distal myotendinous junction after elbow flexor eccentric exercise. Study 3 demonstrated using EPT that the biceps brachii fascia and brachialis fascia became more sensitive to electrical stimulation than the biceps brachii muscle following eccentric exercise. Gibson et al. (15) showed that fascia rather than muscle tissue in the tibialis anterior muscle became more sensitive to hypertonic saline injection when DOMS existed (Figure 10). Ito et al. (70) used EPT and reported that EPT was significantly lower in the fascia compared with the muscle and skin of the forearm 2 days after eccentric exercise of the middle

finger. Moreover, Study 4 showed that biceps brachii MTJ displacement was constant between sets 1 to 10 during the second eccentric exercise bout that induced little DOMS, while there was a 50% increase in the displacement from the 1st to the 10th set during the first eccentric exercise bout that induced greater DOMS. Furthermore, individuals who showed greater MTJ displacement from set 1 to set 10 had greater decreases in MVC torque and greater increases in ultrasound echo intensity after both exercise bouts, and a tendency of greater muscle soreness after the second bout. These findings together with existing evidence suggest that greater mechanical stress to the biceps brachii MTJ could induce greater DOMS to the region close to the MTJ.

The molecular mechanisms of DOMS have been explored recently, and it has been reported that nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) are key molecules sensitising Aδ and C afferent fibres (nociceptors), inducing mechanical hyperalgesia. However, it has not been clarified how eccentric contractions induce bradykinin which is the molecule responsible for NGF and GDNF production. The studies in the thesis suggest that mechanical strain to the connective tissue surrounding muscle fibres and/or fascia induces bradykinin release from the damaged tissue or blood vessels close to it. Further studies are necessary to draw a whole picture of DOMS mechanisms.

These studies which comprise the present thesis have contributed to a body of knowledge in regarding to using different pain measurement method to establish standardised pain assessment protocols, investigating the changes in pain sensitivity between biceps brachii fascia, muscle and brachialis fascia following eccentric exercise, and the changes in MTJ displacement during two bouts of eccentric contractions. This research has provided evidence that a standardised pain assessment protocols are

necessary for DOMS assessments, DOMS is more associated with connective tissue than muscle damage, and greater muscle lengthening during eccentric contractions is associated with the magnitude of muscle damage following eccentric exercise, and these finding provide information that could prove useful in future study design for DOMS assessments.

6.2. Conclusion

This thesis established a pain assessment protocol for DOMS induced after elbow flexor eccentric exercise which is the most frequently used model of exercise-induced muscle damage. The thesis also examined a new pain assessment method, electrical pain threshold, and found that fascia became more sensitive to electrical current than muscle. It also found that the magnitude of biceps brachii MTJ displacement determined the magnitude of muscle damage including DOMS. It appears that DOMS is associated with damage and inflammation to connective tissues surrounding the muscle fibres (i.e. the endomysium) and/or muscle bundles (i.e. the perimysium or fascia), especially close to the distal myotendinous junction.

6.3. Future Research Direction

To further elucidate the mechanisms underpinning DOMS, the following studies are necessary. 1) To investigate how endomysium and perimysium (fascia) are damaged and inflamed during and/or after eccentric contractions. 2) To clarify pain receptors at the endomysium and perimysium, and how pain is induced by the stimulation of the tissue. 3) To examine whether connective tissue produces NGF and GDNF. 4) To investigate why MTJ displacement becomes less in the second eccentric exercise bout, and how exactly this is associated with the magnitude of DOMS. 5) To understand better what DOMS actually indicates, and whether it is a warning signal. I would like to continue investigating these areas of study in my research career.

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APPENDEX I – ETHICS APPROVAL

AUSTRALIA ECU EDITH COWAN UNIVERSITY

JOONDALUP CAMPUS

270 Joondalup Drive,
Joondalup
Western Australia 6027
Telephone 134 328
Facsimile (08) 9300 1257

ABN 54 361 485 361

CRICOS IPC 00279B

22 September 2010

Mr Wing Yin Lau
12 Ardara Bend
BUTLER WA 6036

Dear Ms Lau

I am pleased to write on behalf of the Research Students and Scholarships Committee who have approved your PhD research proposal: **Mechanisms of Delayed Onset Muscle Soreness: Connective Tissue Damage-Inflammation Theory.**

I also wish to confirm that your proposal complies with the provisions contained in the University's policy for the conduct of ethical research, and your application for ethics has been approved. Your ethics approval number is **5320** and the period of approval is: **21 September 2010 to 31 July 2012.**

Approval is given for your supervisory team to consist of:

Principal Supervisor:	Professor Ken Nosaka – ECU
CO Supervisor:	Associate Professor Anthony Blazevich - ECU
CO Supervisor:	Dr Mike Newton - ECU

The examination requirements on completion are laid down in *Part VI of The University (Admissions, Enrolment and Academic progress) Rules for Courses Requiring the Submission of Theses* available at: http://ww.ecu.edu.au/GPPS/legal_legis/uni_rules.html

Additional information and documentation relating to the examination process can be found at the Graduate Research School website: http://research.ecu.edu.au/grs/

Please note: the Research Students and Scholarship Committee has resolved to restrict doctoral theses to a maximum of 100,000 words with a provision that under special circumstances a candidate may seek approval from the Faculty Research and Higher Degrees Committee for an extension to the word length. (RSSC 99/24).

I would like to take this opportunity to offer you our best wishes for your research and the development of your thesis.

Yours sincerely

Patricia Brown
Senior Student Progress Officer
Research Assessments – SSC

Principal Supervisor:	Professor Ken Nosaka – ECU
CO Supervisor:	Associate Professor Anthony Blazevich - ECU
CO Supervisor:	Dr Mike Newton - ECU
HDR	Kristina SFREDDO

From: Research Ethics [research.ethics@ecu.edu.au]
Sent: Wednesday, 22 August 2012 10:03
To: Wing LAU
Cc: Ken NOSAKA; Tony BLAZEVICH; Michael NEWTON
Subject: Project 5320 LAU Annual ethics report request

Dear Wing Yin

Project Number: 5320 LAU
Project Name: Mechanisms of Delayed Onset Muscle Soreness: Connective Tissue Damage-Inflammation Theory

Chief Investigator: Wing Yin LAU
Supervisors:
- Ken Nosaka
- Anthony Blazevich
- Mike Newton

Ethics approval for your research project was granted from 21 September 2010 to 31 July 2013.

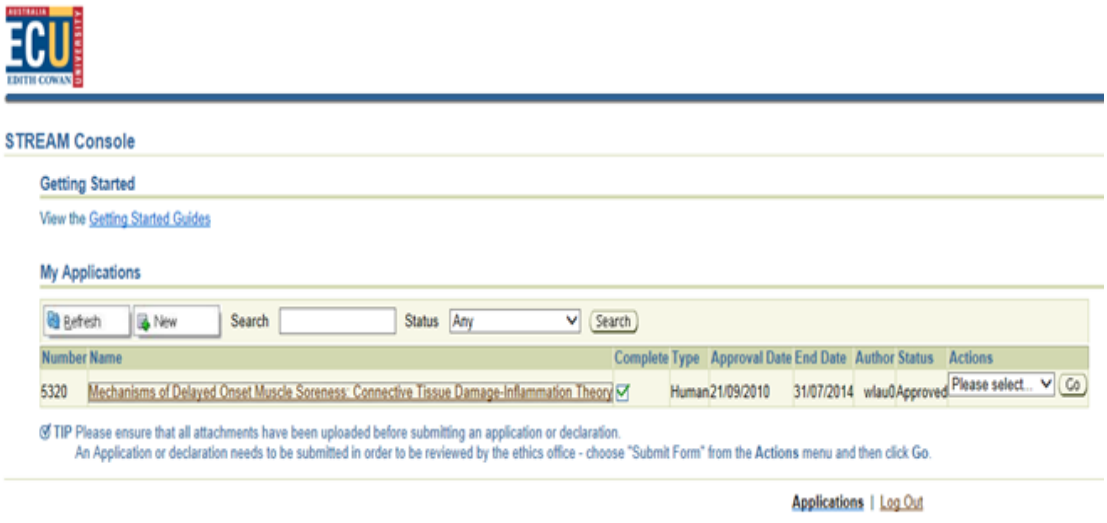
The *National Statement on Ethical Conduct in Human Research* requires that all approved projects are subject to monitoring conditions. This includes completion of an annual report (for projects longer than one year) and completion of a final report at the end of the project.

An **ANNUAL REPORT** is due on **21 September 2012**.

A copy of the ethics report form can be found on the Ethics Website

Please complete the ethics report form and return the signed form to the Research Ethics Office.

For further information, please contact the Research Ethics Office, email: research.ethics@ecu.edu.au



APPENDIX II – INFORMATION LETTER FOR STUDY (1, 2 AND 4)

Edith Cowan University
School of Exercise, Biomedical and Health Sciences

Information Letter

Mechanisms of Delayed Onset Muscle Soreness:

Connective Tissue Damage-Inflammation Theory

(To investigate the difference in muscle pain between two types of elbow flexor exercise)

Investigator: Wing Yin Lau MSc (PhD. Candidate)
Principle Supervisor: Professor Ken Nosaka
Co-Supervisor: Associate Professor Anthony Blazevich
Co-Supervisor: Dr. Mike Newton

School of Exercise, Biomedical and Health Sciences
Edith Cowan University
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Wing Yin Lau (Phone: 6304-5073 Email: wlau0@our.ecu.edu.au)
Ken Nosaka (Phone: 6304-5655 Email: k.nosaka@ecu.edu.au)
Anthony Blazevich (Phone: 6304-5472 Email: a.blazevich@ecu.edu.au)
Mike Newton (Phone: 6304-5961 Email: m.newton@ecu.edu.au)

Thank you for expressing interest in this research.
The purpose of this information sheet is to provide you with an overview of the study that you may participate in as a subject.

Purpose of the Study
This proposed study attempts to investigate the difference in muscle pain between two types of elbow flexor exercise (supinated versus neutral forearm positions) in changes of several pain measures including pressure pain, muscle soreness, blood markers of connective tissue/muscle damage and ultrasound following eccentric exercise from the exercise arm.

Description of the Study

If you agree to participate in the study, you will be asked to come to the laboratory (Joondalup campus, Building 19.150) on 17 separate occasions, consisting of 1 familiarisation day and 2 blocks of 8 days separated by 3 – 4 weeks. One of your arms will perform eccentric exercise when your palm is facing upward (in a supinated wrist position), and the other arm will perform the exercise when you palm is facing to the side (in a neutral wrist position).Your initial laboratory visit will be used to familiarise you with the procedures, exercise apparatus and the testing that will be employed in the study.

For the block of 8 days, your baseline measurements shown below will be taken on the first day. On the next day, you will be asked to perform an eccentric exercise of one arm that has been known to induce delayed onset muscle soreness and strength loss, and the measurements will be taken 1 day prior, before exercise, and immediately after, 1 – 5 and 7 days post exercise. Blood samples will be taken immediately before, 1 – 5 and 7 days after exercise. The sessions will take approximately 3 hours for the exercise day, and 1-1.5 hour for the other days.

Exercise

You will be asked to perform exercise consisting of 10 sets of 6 maximal voluntary eccentric actions of the elbow flexors against the lever arm of an isokinetic dynamometer (Cybex 6000) moving at a constant velocity of 90° per second. You will be positioned on a seated arm preacher curl bench, and the elbow will be aligned with the axis of rotation of the dynamometer. One of your arms will perform the exercise with a supinated forearm position (palm facing upward), and the other arm will perform the same exercise in a neutral forearm position which is separated by 4 weeks after the first arm exercise. You will be asked to resist maximally against the elbow extending motion of the dynamometer from an elbow-flexed position. The rest between sets is 3 minutes.

Measurements

The following measurements will be taken from the exercised arm.

1. Maximal static (isometric) strength: You will be asked to flex your elbow maximally at an elbow joint angle of 90° and 160° on the dynamometer three times with a 30 seconds rest between efforts.
2. Range of motion (ROM): You will be standing with arms relaxed by your side and you will be asked to bring your palm in an upward motion, as far back towards your shoulder as possible without moving your elbow. Elbow joint angle measurements will be taken when your arms are relaxed with palms facing forward and when arms are flexed and extended maximally without moving the elbow. To measure the elbow joint angle, a semi-permanent ink pen will be used to mark 4 dots on your skin to achieve a consistent measurement.

3. Pressure pain threshold (PPT): A device (algometer) to detect pressure will be used to measure pain in the exercised arm by the investigator with a polythene sheet marked with a grid of intercepts 2 cm apart with 32 sites (4 x 8). You will be asked to report the investigator when you start to feel pain in your exercised arm. After PPT measurement, the investigator will locate a trigger point (TrP) of your exercise arm by a flat palpation technique. Then, the investigator will apply the algomter at the TrP of your biceps, and you will report to the investigator when you start to feel pain in your exercised arm.
4. Muscle soreness: The level of pain will be quantified using a 100-mm scale in which 0 indicates no pain and 100 represents a worst pain imaginable on VAS scale; and 0 indicates nothing and 10 represents maximal pain on CR-10 scale. You will be asked to place a mark of the level of perceived pain on the 100 mm line (VAS) and CR-10 scales while the investigator applies pressure to and palpates your exercise arm with the investigator's index and middle fingers and with an algometer (a device of PPT measure). Soreness resulting from pressure and palpation of the exercised muscles (four sites on the upper arm: 3 cm above elbow crease, at 9 cm mid-belly of the biceps brachii, 15 cm above elbow crease and the trigger point of the biceps brachii) will be assessed.
5. Ultrasound images: Using an ultrasound machine, B-mode ultrasound images will be obtained from the elbow joint and the mid-belly of the biceps brachii at the same sites as the muscle soreness measurements. The investigator will place a probe on the distal myotendinous junction (3 cm above elbow crease) during exercise, and also will place on the four marked sites (3, 9, 15 cm from the elbow joint and the trigger point) on the upper arm to obtain images after exercise. From the ultrasound image, the brightness of the echo-signal (echo intensity), muscle thickness and tendon movement (aponeurosis elongation) will also be analysed by using a computer software program thereafter.
6. Blood sampling and analysis: Approximately 8 ml of blood will be drawn by a standard venipuncture technique from the antecubital vein. Blood samples will be taken before, 1 – 5 and 7 days after exercise. Blood samples will be used for analyses of blood markers of muscle damage/inflammation and connective damage/inflammation.

Risk and Ethical Considerations

1. You may experience some degree of muscle soreness and decreases in muscle strength and ROM in some days following the exercise, which may affect daily activities (e.g carrying heavy items), therefore care must be taken. You may also experience swelling of the upper arm and forearm. These symptoms are often seen after unaccustomed eccentric exercise, but will disappear in a week or so. This is unlikely, but if muscle soreness does not disappear or other symptoms do not settle after a week, you should contact researcher directly or by phone or e-mail whatever convenient. The researcher will provide a letter to explain the study that you had participated to a doctor, and ask you to see a doctor (e.g.

General practitioner on the campus or your family doctor). If necessary, the researcher can take you a doctor or come to see a doctor with you. Please let the researcher inform any cost involved in the doctor's appointment and medications required.

2. You will experience some transient discomfort when blood is drawn by a standard venipuncture technique from your vein in the elbow joint. After blood sampling, though rare, it is possible that your wound from the venipuncture technique might get inflected; however, band-aids are always provided for you to minimize the risk of infection.

Requirements and Benefits

1. You will be asked to report to the laboratory as explained above. You will be requested not to perform any unaccustomed exercises or sporting activities, not to take any anti-inflammatory drugs or nutritional supplements, and not to alter your diet and lifestyle (sleeping habits etc) during the experimental periods.
2. You will understand the research topic and methods used in this study. We are happy to provide some information associated with this project upon your request.
3. To compensate for the travel expense, time spent for the study and possible discomfort after the exercise, $50 will be given after completion of the requirements.

Medical Questionnaire

As the study involves an exercise protocol, it is required that you are healthy at the time of testing. For this reason, you will be asked to complete a medical questionnaire prior to the commencement of testing.

Confidentiality of Information

All personal information and test results will remain confidential and will not be used for any purpose other than the current study. No data analysis will include your name or information that may identify you specifically as a subject.

Withdrawing Consent to Participate

You will be free to withdraw from the particular experiment at any stage for any reason without prejudice.

Questions and/or further information

If you have any questions or require more information about the research project, please do not hesitate to contact Wing Yin Lau, Mobile: 0423938266, Email: wlau0@our.ecu.edu.au

Independent Contact Person

If you have any concerns about this research, or would just like to speak to an independent person, you may contact Kim Gifkins (Research Ethics Officer), Human Research Ethics

Committee. Tel: (08) 6304 2170, E-mail: research.ethics@ecu.edu.au

Approval by the Human Research Ethics Committee:
This research project has been approved by the ECU Human Research Ethics Committee. Attached is the letter of approval for your information.

APPENDIX III – INFORMATION LETTER FOR STUDY (3)

Edith Cowan University
School of Exercise, Biomedical and Health Sciences

Information Letter

Mechanisms of Delayed Onset Muscle Soreness:

Connective Tissue Damage-Inflammation Theory

(To investigate the changes in Muscle Soreness and Other Muscle and Connective Tissue Damage Markers following Isokinetic and Isotonic Eccentric Exercise of the Elbow Flexors)

Investigator: Wing Yin Lau MSc (PhD. Candidate)
Principle Supervisor: Professor Ken Nosaka
Co-Supervisor: Associate Professor Anthony Blazevich
Co-Supervisor: Dr. Mike Newton

School of Exercise and Health Sciences
Edith Cowan University
270 Joondalup Drive, Joondalup WA 6027

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Ken Nosaka (Phone: 6304-5655 Email: k.nosaka@ecu.edu.au)
Anthony Blazevich (Phone: 6304-5472 Email: a.blazevich@ecu.edu.au)
Mike Newton (Phone: 6304-5961 Email: m.newton@ecu.edu.au)

Thank you for expressing interest in this research. The purpose of this information sheet is to provide you with an overview of the study that you may participate in as a subject. Please read the information carefully, and please feel free to ask for further explanation if you have any questions.

Purpose of the Study

This proposed study attempts to investigate the changes in several pain measures including pressure pain, muscle soreness, pain assessment upon vibration stimulation and electrical pain threshold (EPT), and other muscle/connective tissue damage markers (maximal voluntary contraction torque, muscle stiffness, ultrasound, blood markers) following the first and second bouts of eccentric exercise of the elbow flexors in two different types of muscle contractions (isokinetic versus isotonic) performed during the exercise.

Eligibility

You will be eligible for this study if you are aged between 18 to 45 years old, and you have no musculoskeletal injuries of the upper extremities. You will be screened with a generic medical questionnaire consisting of several questions about your health and physical conditions. Once you are found to be eligible for the study, you will be invited to participate as a subject in this study.

Description of the Study

If you agree to participate in the study, you will be asked to come to the laboratory (Joondalup Campus, Building 19.150) on 36 separate occasions, consisting of 1 familiarisation day, 2 different days separated by 24 hrs before eccentric exercise, and at 2 days after eccentric exercise for reliability measurement (Part 1) and 4 blocks of 8 days of exercise and measurement period separated by 2 weeks between arms for different exercise and 4 weeks for the same arm of repeated exercise; two bouts with the right arm and another two bouts with the left arm (Part 2). One of your arms will perform "isokinetic" eccentric exercise, and the other arm will perform "isotonic" eccentric exercise when you palm is facing upward (in a supinated wrist position). Both arms will perform the same eccentric exercise 4 weeks later. Your initial laboratory visit will be used to familiarise you with the procedures, exercise apparatus and the testing that will be employed in the study. In part 1, electrical pain threshold (EPT), pressure pain threshold (PPT) and visual analogue scale (VAS) will be used to assess the perceived pain sensation in your muscle on two different days separated by 24 hrs before eccentric exercise, and at 2 days after eccentric exercise when muscle soreness is peaked. For each day, two measurements will be taken, and the measurements will be repeated 1 hour later. A small sample of blood will be taken from your finger before the first EPT measure and 2 days after exercise.

For the block of 8 days (Part 2), your baseline measurements indicated below will be taken before exercise, then you will be asked to perform an eccentric exercise of one arm that has been known to induce delayed onset muscle soreness and strength loss, and all the measurements will be taken 1 days prior, before exercise, and immediately after, 1 – 5 and 7 days after the exercise. The measurement will be repeated 1, 2, 3, 4, 5 and 7 days after the exercise. Blood samples will be taken immediately before, 1 – 5 and 7 days after the exercise by a standard venipuncture technique from your antecubital vein. EPT will be only taken 1 day prior, immediately before and after, 1, 2, 4, and 7 days after, in the two bouts of the isokinetic exercise only.

The sessions will take approximately 3 hours for the exercise day, and 1.5-2 hours for the other days.

Exercise

You will be asked to perform exercise consisting of 10 sets of 6 maximal voluntary isokinetic

eccentric contractions (isokinetic) or sub-maximal isotonic eccentric contractions (isotonic) of the elbow flexors against the lever arm of an isokinetic dynamometer (Cybex 6000) moving at a constant velocity of 20° per second. You will be positioned on a seated arm preacher curl bench, and the elbow will be aligned with the axis of rotation of the dynamometer. One of your arms will perform "isokinetic" exercise and the other arm will perform "isotonic" exercise in a supinated position (palm upwards) which is separated by 2 weeks after the first arm exercise. Both arms will perform the same eccentric exercise 4 weeks later. You will be asked to resist maximally against the elbow extending motion of the dynamometer from an elbow-flexed position (elbow joint angle: 60° from a full extended angle 0°) to a fully-extended position. The rest between sets is 3 minutes.

Measurements

The following measurements will be taken from the exercised arm.

1. Maximal (isometric and isokinetic) strength: You will be asked to flex your elbow maximally at an elbow joint angle of 90° and 60° on the dynamometer two times with a 30 seconds rest between efforts. Then, you will be asked to flex your elbow maximally at two different velocities $30°\cdot s^{-1}$, $210°\cdot s^{-1}$ with the same positioning as the isometric assessment and a 60° range of motion from an elbow extended to a flexed position.
2. Range of motion (ROM): You will be standing with arms relaxed by your side and you will be asked to bring your palm in an upward motion, as far back towards your shoulder as possible without moving your elbow. Elbow joint angle measurements will be taken when your arms are relaxed with palms facing forward and when arms are flexed and extended maximally without moving the elbow. To measure the elbow joint angle a semi-permanent ink pen will be used to mark 4 dots on your skin to achieve a consistent measurement.
3. Upper arm circumference: You will be standing with arms relaxed by your side, and the investigator will use a tension tape to measure the circumference of your exercise arm on two sites: the distal myotendinous junction (approximately 2 cm above elbow crease) and 9 cm above the elbow crease. To measure the arm circumference, a semi-permanent ink pen will be used to mark two dots on your skin to achieve a consistent measurement.
4. Pressure pain threshold (PPT): A device (algometer) to detect pressure will be used to measure pain in the exercised arm in the following sites: the distal myotendinous junction (approximately 2 cm above elbow crease), 9 cm above the elbow crease, and the trigger point by the investigator. You will be asked to report to the investigator when you start to feel pain in your exercised arm. After PPT measurement, the investigator will locate the trigger point of your exercise arm using a flat palpation technique. Then, the investigator will apply the algometer at the trigger point (TrP) of your biceps, and you will report to the investigator when you start to feel pain in your exercised arm.

5. Muscle soreness: The level of pain will be quantified using a 100-mm scale in which 0 indicates no pain and 100 represents the worst pain imaginable on a VAS scale; and 0 indicates nothing and 10 represents maximal pain on a CR-10 scale. You will be asked to place a mark the level of perceived pain on the 100 mm line (VAS) and CR-10 scales while the investigator applies pressure to and palpates your exercise arm with the investigator's index and middle fingers and with a pressure cuff and solid ball. Soreness resulting from pressure and palpation of the exercised muscles (three sites on the upper arm: approximately 2 cm above elbow crease, 9 cm above the elbow crease, and the trigger point of the biceps brachii) will be assessed.
6. Pain assessment upon vibration stimulation: A vibration machine will be used to induce pain in your exercised arm. You will be asked to mark the level of perceived pain on the VAS and CR-10 scale when the investigator applies the vibration machine on the myotendinous junction of the biceps with different vibration frequencies (20, 50, 80 Hz).
7. Electrical pain threshold (EPT): Please see the EPT information letter for further information.
8. Elastography (elastography images): Using an ultrasound machine, elastography images will be obtained from the mid-belly of the biceps brachii at the same sites as the muscle soreness measurements. The investigator will place a probe with a slight compression on the three marked sites (approximately 2 cm above elbow crease, 9 cm above the elbow crease, and the trigger point of the biceps brachii) on the upper arm to obtain images.
9. Ultrasound images: Using an ultrasound machine, B-mode ultrasound images will be obtained from the elbow joint and the mid-belly of the biceps brachii at the same sites as the muscle soreness measurements. The investigator will place a probe on the distal myotendinous junction (2 cm above elbow crease) during exercise, and also will place on the three marked sites (2 cm, 9 cm and the trigger point) on the upper arm to obtain images after exercise. From the ultrasound image, the brightness of the echo-signal (echo intensity), muscle thickness and tendon movement (aponeurosis elongation) will also be analysed by using a computer software program thereafter.
10. Plasma creatine kinase (CK) activity: A 30μL blood sample will be extracted from your finger by a capillary tube, and the presence of CK protein activity will be measured (Part 1).
11. Blood sampling and analysis: Approximately 8 ml of blood will be drawn by a standard venipuncture technique from the antecubital vein. Blood samples will be taken before, 1 – 5 and 7 days after exercise. Blood samples will be used for analyses of blood markers of muscle damage/inflammation and connective damage/inflammation (Part 2).

Risk and Ethical Considerations

1. You may experience some degree of muscle soreness and decreases in muscle strength and ROM in some days following the exercise. You may also experience swelling of the

upper arm and forearm. These symptoms are often seen after unaccustomed eccentric exercise, but will disappear in a week or so. This is unlikely, but if muscle soreness does not disappear or other symptoms do not settle after a week, you should contact researcher directly or by phone or e-mail whatever convenient. The researcher will provide a letter to explain the study that you had participated to a doctor, and ask you to see a doctor (e.g. General practitioner on the campus or your family doctor). If necessary, the researcher can take you a doctor or come to see a doctor with you. Please let the researcher inform any cost involved in the doctor's appointment and medications required.

2. You will experience some transient discomfort when blood is drawn by a standard venipuncture technique from your vein in the elbow joint. After blood sampling, though rate, it is possible that your wound from the venipuncture technique might get inflected; however, band-aids are always provided for you to minimize the risk of infection.
3. You will experience some transient discomfort when an EPT needle is inserted into your muscle to access perceived pain sensation inside your muscle. After EPT insertion, though rare, it is possible that your wound from the EPT needle might get inflected; however, band-aids are always provided for you to minimize the risk of infection. Please see EPT information letter for further information.

Requirements and Benefits

1. You will be asked to report to the laboratory as explained above. You will be requested not to perform any unaccustomed exercises or sporting activities, not to take any anti-inflammatory drugs or nutritional supplements, and not to alter your diet and lifestyle (sleeping habits etc) during the experimental periods.
2. Your participation is really appreciated, which helps us to understand the changes in muscle pain following elbow flexor exercise.
3. You will understand the research topic and methods used in this study. We are happy to provide some information associated with this project upon your request.

Medical Questionnaire

As the study involves an exercise protocol, it is required that you are healthy at the time of testing. For this reason, you will be asked to complete a medical questionnaire prior to the commencement of testing. Answering 'Yes' to a question will not always disqualify you from participation in the study,; however, you may be asked to consult your doctor for clearance prior to participation. The investigators will cover the cost of doing this if required.

Confidentiality of Information

All information provided by you will be treated with full confidentiality. You contact information will only be accessible to the chief researcher during the period of the study. The information and data gathered from you during the study will be used to answer the research

question of the study. People who will have access to the raw information for this study are only limited to the researcher and the supervisors. Data collected will be stored in a password-protected computer and is only available to the researchers. Hardcopy data (data sheet) will only be kept in the researcher's office and locked in a specific drawer/filing cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

Result of the Research Study

The results of this study are intended for completion of a Doctor of Philosophy (Sports Science) thesis and will be presented at conferences/seminars and published in peer-reviewed journals as articles, as an online article or part of a book section and reports. Published results will not contain information that can be used to identify participants unless specific consent for this has been obtained. A copy of published results may be obtained by the participants upon requests.

Withdrawing Consent to Participate

You will be free to withdraw from the particular experiment at any stage for any reason without prejudice. It should be noted however that all of the data collected to the point of withdraw will be maintained as a part of the clinical trial documentation, and subject to the confidentiality provision mentioned above.

Questions and/or further information

If you have any questions or require more information about the research project, please do not hesitate to contact Wing Yin Lau

Office 19.128, School of Exercise, Biomedical, and Health Sciences, Edith Cowan University 270 Joondalup Drive, Joondalup, WA 6027, Australia.

Mobile: 0423938266, Email: wlau0@our.ecu.edu.au

Independent Contact Person

If you have any concerns about this research, or would just like to speak to an independent person, you may contact Kim Gifkins (Research Ethics Officer), Human Research Ethics Committee

Building 1, Block 'B', Level 3, Room 333, Edith Cowan University, 270 Joondalup Drive, Joondalup, WA 6027, Australia, E-mail: research.ethics@ecu.edu.au

Approval by the Human Research Ethics Committee:

This research project has been approved by the ECU Human Research Ethics Committee. Attached is the letter of approval for your information.

APPENDIX IV – EPT INFORMATION LETTER FOR STUDY 3

Edith Cowan University
School of Exercise, Biomedical and Health Sciences

Electronic Pain Threshold (EPT) – Information Letter

Electrical pain threshold (EPT) is a technique to detect pain sensation and evaluate the sensitivity in the deep muscle and/or in connective tissues by using low intensity electrical current stimuli. This technique has been used for many years in scientific research to detect the deep pain sensation in human tissue. Thus, this technique could be used to assess the origin of muscle pain after eccentric exercise of the elbow flexors and could be able to detect muscle pain is induced by which region inside the muscle.

Figure 1. Example of a thin insulated needle electrode

EPT is measured by a pulse algometer that consists of a thin insulated needle electrode (Figure 1) and a constant current stimulator (Figure 2). The size of the steel needle is extremely thin (180µm in diameter) and is safe to insert into the tissue. In the experiment, the investigator will carefully insert this thin needle into your biceps in the following order: the distal myotendinous junction (approximately 2 cm above elbow crease), 9 cm above the elbow crease, and the trigger point (Figure 3); and the pain threshold (sensation) of the skin, fascia and muscle will be assessed respectively. When the investigator carefully inserts the needle into your skin, the intensity of the stimulus current will be carefully increased by the investigator from zero at a constant rate until you feel the first pain, which you will indicate by pressing a button of the algometer, and you will be asked to mark the pain on the VAS and CR-10 scales Then, the investigator will advance the needle to the deeper tissues carefully with the ultrasound system and apply the same technique to assess the pain sensation by EPT of other regions (e.g superficial fascia, biceps brachii, deep fascia and brachialis). After completing the EPT measurement of the skin, superficial fascia, biceps brachii,

Figure 2. Current stimulator

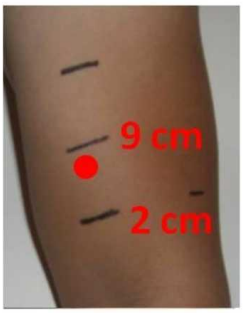

Figure 3. The location of the EPT (needle) insertion

deep fascia and brachialis of one site, the needle will be carefully removed and inserted into another site. Subsequently, the investigator will re-insert the needle into the distal myotendinous junction of your arm with a standardised intensity of current stimulus at (0.5 mA), and you will be asked to mark the pain on the VAS and CR-10 scales. The location of the needle will be carefully monitored by the investigator and the ultrasound image technique. After the needle insertion is completed, the investigator will provide a band-aid for you to cover the site.

The risks associated with this electrical pain threshold measurement procedure are almost nil, and there is no wound management required. In order to reduce the chance of infection, the investigator will apply alcohol wrap to clean the skin surface before the EPT needle is inserted into your muscle, and a new EPT needle will be replaced by the investigator before each insertion and also band-aids will be provided to cover the needle insertion site. Since the EPT needle used in this study is extremely thin (only 180μm in diameter), the pain from the needle insertion is very minimal. Furthermore, the location of needle insertion will only take place at the muscle and connective tissues that are without any veins/arteries or nerves; therefore, the needle insertion in this study will not cause any injury or damage to any veins/arteries or nerves. It is important to note that this technique is safe and has been used in previous studies to detect the pain sensation in the deep tissues, and the measurement procedure is only performed by an appropriately trained researcher who is familiar with all the equipment and procedures.

APPENDIX V – INFORMED CONSENT DOCUMENT

Edith Cowan University
School of Exercise, Biomedical and Health Sciences

Informed Consent Document

Project title: Mechanisms of Delayed Onset Muscle Soreness: Connective Tissue Damage-Inflammation Theory (To investigate the changes in Muscle Soreness and Other Muscle and Connective Tissue Damage Markers following Isokinetic and Isotonic Eccentric Exercise of the Elbow Flexors)

Researchers and Contact details
Investigator: Wing Yin Lau MSc (PhD. Candidate)
Tel: (61) 8 6304 5073, Email: wlau0@our.ecu.edu.au
Supervisor: Professor Ken Nosaka
Tel: (61) 8 6304 5655, Email: k.nosaka@ecu.edu.au
Co-Supervisor: Associate Professor Anthony Blazevich
Tel: 6304-5472, Email: a.blazevich@ecu.edu.au
Co-Supervisor: Dr. Mike Newton
Tel: 6304-5961 Email: m.newton@ecu.edu.au

School of Exercise and Health Sciences
Edith Cowan University
270 Joondalup Drive Joondalup, WA 6027, AUSTRALIA

Statement indicating consent to participate
I confirm the following:

- I have been provided with the "Information Letter" and "Electrical Pain Threshold Information Letter" explaining the research study
- I have read and understood the information provided and the procedures of the study
- I have been given an opportunity to ask questions and I have had any questions answered to my satisfaction
- I am aware that if I have any additional questions, I can contact the research team
- I understand that participation in the research project will involve:
  - 1 familiarisation day, 3 different days for reliability measurement (Part 1) and 4 blocks of 8 day visits to the laboratory (Part 2) in a total of 36 separate occasions
  - Four exercise bouts separated by 2 weeks for different exercises and 4 weeks for repeated bout
  - Repeated visits to the Exercise Physiology Laboratory on 8 consecutive days, starting from 1 day before exercise, exercise day and 1 to 5 days post-exercise and 7 days after exercise.
  - Possible muscle soreness and loss of strength for up to 7 days after exercise
  - Measurements of muscle strength, range of motion, upper arm circumferences, pressure pain threshold, muscle soreness, vibration stimulation
  - Electrical pain threshold will be measured on 1 day prior, immediately before and after, 1, 2, 4, and 7 day after exercise, and only measured in the two bouts of the isokinetic exercise
  - Ultrasound images (muscle thickness, echo intensity, aponeurosis elongation) of the upper arm
  - Elastography (ultrasound images) of the upper arm
  - Plasma blood samples will be taken before the first EPT measure and 2 days after (Part 1)
  - Venous blood samples will be taken before, 1 to 5 and 7 days after exercise (Part 2)
- I understand that my information provided will be kept confidential, and that my identity will not be disclosed without consent
- I understand that the information provided will only be used for the purposes of this research project, and I understand how the information is to be used
- I understand that I am free to withdraw from further participation at any time, without explanation or penalty
- I freely agree to participate in the project

Signed ……
Name…… **Date**……

APPENDIX VI –MEDICAL SCREENING QUESTIONNAIRE

Edith Cowan University
School of Exercise, Biomedical and Health Sciences

Pre-exercise (medical) screening questionnaire

The following questionnaire is designed to establish the background of your medical history, and identify any injury and or illness that may influence your testing and performance.

Please answer all questions as accurately as possible and if you are unsure about anything please ask for clarification. All information provided is strictly confidential. If you answer yes to any non-exercise related question that may contraindicate you from completing this study. A clearance from a qualified medical practitioner will be required prior to commencement of any exercising or testing.

Personal Details

Name: _____ Subject Code: _____

Date of Birth (D/M/Y): _____ Gender: Female Male

Medical History

Have you ever had, or do you currently have any of the following?

		If YES, please provide details
High or abnormal blood pressure	Y N	_____
High cholesterol	Y N	_____
Rheumatic fever	Y N	_____
Heart abnormalities	Y N	_____
Heart attack/disease	Y N	_____
Asthma	Y N	_____
Diabetes	Y N	_____
Epilepsy	Y N	_____

Recurring back pain	Y N	_____
Recurring neck pain	Y N	_____
Severe allergies	Y N	_____
Any infectious diseases	Y N	_____
Any neurological disorders	Y N	_____
Any neuromuscular disorders	Y N	_____
Are you currently on any medications?	Y N	_____
Have you had the flu in the last two weeks?	Y N	_____
Have recently injured yourself?	Y N	_____
Do you have any recurring muscle or joint injuries?	Y N	_____
Have you had any elbow or shoulder problems in the past?	Y N	_____
Have you participated in resistance training in the last six months?	Y N	_____
Have you ever experienced wheezing while breathing or wheezing with exercise or asthma?	Y N	_____
Have you ever had significant periods of dizziness or disorientation after performing maximal exercise?	Y N	_____
Do you have any injuries or medical conditions that you believe might be problematic for maximal exercise performance?	Y N	_____

If YES, how many standard drinks per week?

Do you consume tea or coffee?
If YES, how many cups per day?

Declaration

I acknowledge that the information provided on this form, is to the best of my knowledge, a true and accurate indication of my current state of health.

Participant
Name: _____
Date (DD/MM/YYYY): _____
Signature: _____

Practitioner (only if applicable)
I, Dr _____have read the medical questionnaire, and information letter /consent document have been provided to my patient Mr _____, and I clear him medically for involvement in exercise testing.

Date (DD/MM/YYYY): _____
Signature: _____

APPENDIX VII – PUBLICATION FOR STUDY 1

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healthcare

RESEARCH ARTICLE

Visual Analog Scale and Pressure Pain Threshold for Delayed Onset Muscle Soreness Assessment

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ABSTRACT

Objectives: To investigate the relationship between two assessments to quantify delayed onset muscle soreness [DOMS]: visual analog scale [VAS] and pressure pain threshold [PPT].

Methods: Thirty-one healthy young men [25.8 ± 5.5 years] performed 10 sets of six maximal eccentric contractions of the elbow flexors with their non-dominant arm. Before and one to four days after the exercise, muscle pain perceived upon palpation of the biceps brachii at three sites [5, 9 and 13 cm above the elbow crease] was assessed by VAS with a 100 mm line [0 = no pain, 100 = extremely painful], and PPT of the same sites was determined by an algometer. Changes in VAS and PPT over time were compared amongst three sites by a two-way repeated measures analysis of variance, and the relationship between VAS and PPT was analyzed using a Pearson product-moment correlation.

Results: The VAS increased one to four days after exercise and peaked two days post-exercise, while the PPT decreased most one day post-exercise and remained below baseline for four days following exercise [$p < 0.05$]. No significant difference among the three sites was found for VAS [$p = 0.62$] or PPT [$p = 0.45$]. The magnitude of change in VAS did not significantly correlate with that of PPT [$r = -0.20$, $p = 0.28$].

Conclusion: These results suggest that the level of muscle pain is not region-specific, at least among the three sites investigated in the study, and VAS and PPT provide different information about DOMS, indicating that VAS and PPT represent different aspects of pain.

KEYWORDS: Algometer, eccentric exercise, elbow flexors, muscle damage, palpation

INTRODUCTION

We often experience muscle pain in the next days following exercise or daily activities, and this type of pain is referred to as delayed onset muscle soreness [DOMS] (1). DOMS is characterized by the sensation of a dull, aching pain, usually felt during movement or palpation of the affected muscles, develops within 24 hours after performing exercise and peaks one to three days post-exercise (1,2). The underlying mechanisms of DOMS have not been fully understood, but it has been documented that damage to contractile proteins, intermediate filaments and/or connective tissue surrounding muscle fibers, and subsequent inflammatory process are associated with it (1,3). DOMS is considered a mechanical hyperalgesia, which is characterized by an increased sensitivity of nociceptors [type III and IV afferents] to a stimulus (2) and/or allodynia, in which pain is induced by a stimulus that does not normally provoke pain (4,5).

To quantify the level of muscle soreness is a challenge due to the subjective nature of pain (6). Different pain scales such as a visual analog scale [VAS] (7), verbal rating scale (8), numerical rating scale (9) and descriptor differential scale (10) have been used in previous studies to assess DOMS. Among them, the VAS is most often used for DOMS assessment (7,11). It consists of a certain length of line [e.g. 100 mm] in which one end of the line indicates no pain and the other end indicates worst

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APPENDIX VIII – ABSTRACT FOR CONFERENCE (STUDY 2)

CHANGES IN FASCIA AND MUSCLE PAIN THRESHOLD AFTER ECCENTRIC CONTRACTIONS

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Delayed onset muscle soreness (DOMS) is a symptom of muscle damage and develops several hours after eccentric exercise, but its underpinning mechanisms are not fully understood. Some studies have documented that the extent of DOMS is not associated with the extent of muscle fibre damage, but with damage and inflammation of connective tissue surrounding muscle fibres and fascicles. If this is the case, pain sensation changes in the muscle are different between superficial or deep fascia and muscle. The present study used electrical pain threshold (EPT) to assess changes in pain sensitivity at superficial fascia, muscle and deep fascia after eccentric exercise.

Ten men (24 ± 2.5 y) performed two bouts of eccentric exercise of the elbow flexors consisting of 10 sets of 6 maximal eccentric contractions with the same arm separated by 4 weeks. Maximal voluntary isometric contraction torque (MVC), range of motion (ROM), muscle soreness assessed by a visual analogue scale (VAS) and pressure pain threshold (PPT) were measured before, immediately after and 1 – 5 days after exercise. A pulse algometer was used to assess EPT by inserting a needle electrode into the mid-belly of biceps brachii with current intensity being increased gradually to quantify the pain threshold of the superficial fascia, muscle and deep fascia, respectively at 1 day before, immediately after, and 1, 2 and 4 days after exercise. Changes in the variables were compared between bouts by a two-way ANOVA.

Changes in MVC, ROM, VAS and PPT were smaller ($P<0.05$) after ECC2 than ECC1, showing a typical repeated bout effect. EPT of superficial fascia, muscle and deep fascia decreased significantly after both bouts, but the magnitude of EPT decreased was significantly greater after ECC1 than ECC2. EPT decreased more for superficial facia and deep facia than muscle, and remained below the baseline for 4 days after ECC1.

These results suggest that muscle and fascia became more sensitive to electrical stimulation after eccentric exercise when DOMS exists; however, the greater decreases in EPT of superficial and deep fascia than muscle suggest that connective tissues are more associated with DOMS than muscle fibres.

WINNER

Aspire Academy Young Investigator Award – Sports Science

CHANGES IN FASCIA AND MUSCLE PAIN THRESHOLD AFTER ECCENTRIC CONTRACTIONS

with lead author

Wing Yin Lau

Winthrop Professor Daniel Green
Conference Co-Chairman

Professor Jeff Coombes
Conference Co-Chairman

APPENDIX IX – ABSTRACT FOR CONFERENCE (STUDY 4)

DIFFERENCE IN APONEUROSIS ELONGATION DURING ECCENTRIC CONTRACTIONS BETWEEN THE FIRST AND SECOND EXERCISE BOUTS OF THE ELBOW FLEXORS

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Introduction
Unaccustomed eccentric exercise induces muscle damage characterised by delayed onset of muscle soreness (DOMS) and loss of muscle strength, but a repeated bout of the same exercise within several weeks results in less muscle damage, which is known as the repeated bout effect (1). It has been documented that neural, mechanical and cellular adaptations are associated with the repeated bout effect, but the underlying mechanisms have not been fully understood (2). The present study tested the hypothesis that muscle-tendon behaviours would be different between bouts such that the elongation of the aponeurosis of biceps brachii during eccentric contractions would be smaller during the second than the first eccentric exercise bout.

Methods
Ten untrained men (21-39 y) performed two exercise bouts consisting of 10 sets of 6 maximal isokinetic ($60°·s^{-1}$) eccentric contractions of the elbow flexors with the non-dominant arm separated by 4 weeks. During each eccentric contraction, the elbow joint angle was forcibly extended from a flexed (120°) to a fully extended position (180°) under maximal activation. The movements of the biceps brachii aponeurosis were recorded by B-mode ultrasonography (Aloka SSD-α10, Japan), and the movement distance of the end point of the aponeurosis origin from the beginning to the end of each contraction was calculated. Maximal voluntary isometric contraction strength (MVC), range of motion (ROM) and muscle soreness (VAS) were measured before, immediately after, 1-7 days following exercise. Changes in the movement distance together with the torque from the first to the last set, and changes in MVC strength, ROM and VAS after exercise were compared between first and second bouts by a two-way repeated measures ANOVA.

Results
Compared with the first bout, changes in MVC, ROM and VAS were smaller ($P<0.05$) following the second bout, showing the typical repeated bout effect. During eccentric contractions, the aponeurosis origin was extended, and the average distance of the aponeurosis elongation increased ($P<0.05$) from the 1st set (8.2 ± 4.7 mm) to the 10th set (16.4 ± 4.7 mm) for the first bout; however, the increases were smaller ($P<0.05$) for the second bout (1st set: 8.4 ± 3.9 mm, 10th set: 9.3 ± 3.1 mm).

Discussion
These results suggest that the reduced magnitude of muscle damage in the second exercise bout compared with the first bout is associated with the smaller length changes in the aponerousis in the second bout. It is possible that muscle fibre length changes are smaller in the repeated bout than the initial bout, which reduced the strain to the muscle fibres, resulting in less muscle damage.

Reference
1. Nosaka K, Newton M. *J Strength Cond Res*.16:117-122, 2002
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