2011

The influence of set-repetition configuration in eccentric exercise on muscle damage and repeated bout effect

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MASTERS THESIS

THE INFLUENCE OF SET-REPETITION CONFIGURATION IN ECCENTRIC EXERCISE ON MUSCLE DAMAGE AND REPEATED BOUT EFFECT

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This thesis is presented for the award of Master of Science (Sports Science)

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Date of Submission: 31st of March 2011

USE OF THESIS

ABSTRACT

It is well known that maximal eccentric exercise induces muscle damage, especially when it is performed for the first time. However, muscle damage is attenuated in subsequent bouts of the same or similar exercise, which is known as the repeated bout effect. One of the factors affecting the magnitude of muscle damage and the repeated bout effect is the number of eccentric contractions; however, it is unknown if different set-repetition configurations with the same number of eccentric contractions would result in different magnitudes of muscle damage and adaptation. This study investigated changes in muscle strength, range of motion (ROM), muscle cross sectional area (CSA), muscle soreness and plasma creatine kinase (CK) activity following an initial bout of maximal eccentric contractions with the same total number of contractions but different set-repetition configurations (e.g. 3 sets of 10 reps vs. 10 sets of 3 reps) and a second bout (20 sets of 3 repetitions) separated by four weeks. Since the present study was the first to use the ultrasound extended field of view (EFOV) technique to quantify bicep brachii CSA for an indication of muscle swelling, the reliability and validity of the technique were assessed in a separate study using 6 men (27.5 ± 1.9 y). In the main study, 10 non-resistance trained men (26.1 ± 4.1 y) performed two bouts of eccentric exercise of the elbow flexors for each arm (4 bouts in total). One arm performed 3 sets of 10 maximal eccentric contractions (3x10) followed 4 weeks later by 20 sets of 3 maximal eccentric contractions (20x3). The contralateral arm performed 10 sets of 3 maximal eccentric contractions (10x3) followed 4 weeks later by 20x3. The order of the exercise (3x10, 10x3) and the use of arm (dominant, non-dominant) were counterbalanced amongst subjects. Changes in the criterion measures over time and peak torque were compared among the initial bouts (3x10 vs. 10x3) and the repeated bouts (20x3 vs. 20x3) by a factorial repeated-measures analysis of variance (ANOVA) with two factors. Significance level was set at $P<0.05$ for all statistical analyses. In the study to investigate the validity against magnetic resonance imaging (MRI) and test-retest reliability, the results showed that EFOV was valid to measure biceps brachii CSA and could detect approximately 1% change in the CSA reliably. In the main study, the torque produced over 30 eccentric contractions was similar between 3x10 and 10x3, and the changes in torque during both 20x3 exercises were similar between arms. Maximal voluntary contraction strength, ROM, biceps brachii CSA, muscle soreness and plasma CK activity changed significantly after the first bouts without significant differences between 3x10 and 10x3, and changes in the measures following 20x3 were similar between arms. No significant differences in the changes of the criterion measures were evident between bouts. These results showed that the set-repetition configuration had little effect on muscle damage, which was likely to be due to similar peak torques produced during exercise between the 3x10 and 10x3 bouts. The repeated bout effect was similar between arms, suggesting that the set-repetition configuration in the first bouts did not affect the second bout. The similar changes in criterion measures between the first and second bouts showed that the repeated bout effect was attenuated by increasing the number of contractions. It is concluded that the number of contractions rather than the set-repetition configuration affects the magnitude of muscle damage and repeated bout effect.

DECLARATION

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

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ACKNOWLEDGEMENTS

This project would not have been completed if not for the help of a number of people, which I would like to take this opportunity to thank these people.

First of all, I would like to express my deepest gratitude and appreciation to my parents, grandparents and Charlene for their endless support and sacrifices they have made so that I could pursue my dream of studying overseas, which I could never have achieved without your love and encouragement. I love all of you and would like to dedicate this most fulfilling accomplishment of my life thus far to each one of you.

Secondly, I wish to thank Prof. Ken Nosaka and Dr. Michael Newton for providing me a remarkable learning environment that certainly had broadened my horizons. Ken always has an answer to any questions I raised and he never fails to impress me with his knowledge on eccentric exercise and muscle damage whereas Mike has always offered views from another perspective. I sincerely thank you, Ken and Mike, for your patience and willingness to impart knowledge. I really appreciate your assistance and efforts in aiding me to complete my Masters degree.

Thirdly, I would like to thank Prof. Trevor Chen and A Prof. Valmor Tricoli for their time and effort in reviewing my thesis, which most definitely has made it a better one.

Lastly, to those who helped me one way or the other, especially the laboratory technicians, Nadija, Jack and fellow Sport Science post graduates (2009-2010), thank you all for always being ever ready to lend me a helping hand, be it laboratory related, volunteering as subjects or leisure activities, you are all an awesome bunch! I will always remember the numerous coffee breaks to Aroma and Cafe Six and the stress-relieving walks around the campus.

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CHAPTER 1

INTRODUCTION

1.1 Background

Strenuous unaccustomed eccentric exercise induces muscle damage that is characterised by a prolonged impairment of muscle function, delayed onset muscle soreness (DOMS), muscle swelling and increases in muscle proteins in the circulation (Clarkson & Sayers, 1999). Previous studies (Clarkson & Tremblay, 1988; Howatson, Van Someren, & Hortobagyi, 2007; Nosaka, Sakamoto, Newton, & Sacco, 2001) have reported that the magnitude of muscle damage is affected by the number of eccentric contractions. Clarkson and Tremblay (1988) showed significantly greater muscle strength loss, decreases in range of motion (ROM), increases in muscle soreness and serum creatine kinase (CK) activity following 70 maximal eccentric contractions when compared with 24 maximal eccentric contractions of the elbow flexors. Similarly, Howatson et al. (2007) reported that changes in ROM, muscle soreness and serum CK activity were significantly greater following 45 than 10 maximal eccentric contractions of the elbow flexors, and the decreases in isometric muscle strength were four times greater after 45 than 10 maximal eccentric contractions.

When eccentric contractions are performed in training, they are generally configured as sets and repetitions. For example, 30 repetitions can be performed in different configurations such as 3 sets of 10 repetitions (3x10), 6 sets of 5 repetitions (6x5), or 10 sets of 3 repetitions (10x3). Even if the number of total eccentric contractions is the same, it is possible that the magnitude of muscle damage is different among the different configurations. However, it is not known if altering the configurations with the same total number of repetitions (e.g. 3x10 vs. 10x3) would result in a different magnitude of muscle damage. It is known that the greater the forces generated during eccentric contractions, the greater the magnitude of muscle damage (Chen & Nosaka, 2006). Assuming that the decreases in force are smaller for a smaller number of repetitions (e.g. 3 maximal eccentric contractions) in a set than a greater number of repetitions (e.g. 10 maximal eccentric contractions) in a set, it could be due to greater mechanical stress imposed on the exercised muscle in performing a small number of repetitions with a large number of sets (e.g. 10 sets of 3 contractions; 10x3) compared with performing a large number of repetitions with a small number of sets (e.g. 3 sets of 10 contractions; 3x10). If so, it seems that the magnitude of muscle damage induced by eccentric exercise would be greater for 10x3 than 3x10.

It is well documented that when a bout of eccentric exercise is repeated after the initial exercise bout within several weeks, impairment of muscle function, DOMS, muscle swelling and increases of muscle proteins in circulation are attenuated, and this protective adaptation is termed as the repeated bout effect (Howatson et al., 2007; McHugh, 2003). Several studies have investigated the effect of the number of eccentric contractions on the repeated bout effect (Chen & Nosaka, 2006; Howatson et al., 2007; Nosaka et al., 2001). Nosaka et al. (2001) examined whether 2 or 6 maximal eccentric contractions would induce protective effect against 24 maximal eccentric contractions performed 2 weeks later. They showed that recovery of muscle strength and ROM was enhanced, and increases in upper arm circumference, muscle soreness, and plasma CK activity and myoglobin (Mb) concentration were reduced following 24 maximal eccentric contractions when 2 or 6 maximal eccentric contractions were performed prior to 24 maximal eccentric contractions, but such effects were greater for 6 than 2 maximal eccentric contractions (Nosaka et al., 2001). Howatson et al. (2007) compared the protective effect conferred by 10 and 45 maximal eccentric contractions against 45 maximal eccentric contractions of the elbow flexors performed 2 weeks later. They found no significant differences in the magnitude of the protective effect between 10 and 45 maximal eccentric contractions, and concluded that 10 maximal eccentric contractions conferred the same protective effect as 45 maximal eccentric contractions (Howatson et al., 2007). No previous study has investigated the effect of set-repetition configuration on the repeated bout effect. It may be that a greater change in the set-repetition configuration from the first eccentric exercise bout (e.g. 3x10) to the second bout (e.g. 20 sets of 3 maximal eccentric contractions, 20x3) would confer less protective effect when compared with the condition that the number of sets is increased from the first (e.g. 10x3) to the second bout (e.g. 20x3) without changing the number of repetitions.

In order to evaluate the effect of eccentric exercise on muscle swelling, which has been accepted as one of the indications of muscle damage (Chleboun, Howell, Conatser, & Giesey, 1998; Clarkson, Nosaka, & Braun, 1992; Foley, Jayaraman, Prior, Pivarnik, & Meyer, 1999), previous studies have measured limb circumference using a tape measure (e.g. Paddon-Jones & Abernethy, 2001) and/or muscle thickness using B-mode ultrasound images after eccentric exercise (e.g. Nosaka & Newton, 2002b). However, to evaluate muscle swelling more accurately, it is better to measure muscle CSA. Ultrasound extended-field-of-view (EFOV) is a relatively new technique and can form a panoramic image over an area larger than what the traditional ultrasound window can contain. With this technique, as the ultrasound transducer is moved along the region of interest, new imaging frames are combined with previous frames (Weng et al., 1997).

Noorkoiv et al. (2010) assessed the reliability and validity of EFOV against CT for quadriceps muscle CSA at 10, 20, 30, 40 and 50% between the central point of the patella and the medial aspect of the anterior superior iliac spine. They reported that the differences in CSA between EFOV and CT were 0.6-4.3% depending on the sites (greater differences for smaller CSAs), intra- and inter-experimenter reliability ranged 0.6-2.7%. They concluded that EFOV was a valid and reliable tool for assessing quadriceps muscle CSA, and noted that EFOV could provide even more accurate estimates of anatomical CSA of muscles than CT.

Ahtiainen et al. (2010) used the EFOV technique to assess vastus lateralis muscle CSA changes after 21-weeks of resistance training and compared those with MRI, reporting high correlation (R=0.997) between the two measures. They stated that the EFOV technique could provide information on muscle CSA in response to training, immobilisation or sarcopenia.

It seems reasonable to assume that the EFOV technique can be used to assess CSA of other muscles, but to the best of our knowledge, only the knee extensors have been examined. Noorkoiv et al. (2010) stated that it was difficult to keep the whole transducer sensor area in contact with the skin without deviating from the original plane or without applying pressure on the tissue where the surface was curvy. Since the surface of the elbow flexors is curvier than the knee extensors, it was assumed that obtaining good EFOV images might be challenging. Thus, if EFOV can be used to reliably measure CSA of the elbow flexors, it is possible to assess muscle swelling more precisely.

1.2 Purposes of the study

The purposes of this study were 1) to evaluate the EFOV technique for the measurement of CSA of elbow flexor muscles in the upper arm for its validity against MRI measures and intra-experimenter reliability (STUDY 1), 2) to investigate if 10x3 would induce greater muscle damage than 3x10, and 3) to examine whether a greater difference in the set-repetition configuration from the first bout to the second bout (3x10 for the first bout, 20x3 for the second bout) would attenuate the repeated bout effect in comparison to the condition that only the number of sets is increased in the second bout (10x3 for the first bout, 20x3 for the second bout) (STUDY 2).

1.3 Significance of the study

No previous studies have examined the validity and reliability of EFOV ultrasound techniques to assess biceps brachii CSA. The present study will determine whether the EFOV technique is valid and reliable for biceps brachii CSA measures, and whether the changes in the CSA correspond to the changes in upper arm circumference measures that are often used in previous studies (Chleboun et al., 1998; Nosaka, Newton, & Sacco, 2002) to assess muscle swelling after eccentric exercise of the elbow flexors.

Numerous studies have investigated the importance of eccentric contractions over concentric and isometric contractions in inducing muscle damage (Clarkson et al., 1992; Gibala et al., 2000; Hortobagyi et al., 1996; Nosaka & Clarkson, 1996) and muscle hypertrophy (Barroso et al., 2010; Farthing & Chilibeck, 2003; Higbie, Cureton, Warren, & Prior, 1996). However, no study has investigated how different set and repetition configurations with identical number of contractions could affect the magnitude of muscle damage. Therefore, this study will be the first to investigate if different configurations with the same total number of contractions have different magnitudes of muscle damage.

Several studies have shown that after performing a bout of unaccustomed exercise, a protective effect known as the repeated bout effect would be conferred on subsequent bouts of similar exercise (Chen & Nosaka, 2006; Howatson et al., 2007; Nosaka et al., 2001). However, no study has examined how much would the magnitude of the repeated bout effect be affected when different set-repetition configurations were performed in the first and second bouts. Therefore, this study is the first to examine how much difference would a greater change in set-repetition configuration administered in the second bout (3x10 to 20x3) compared to only an increase in the set configuration (10x3 to 20x3) affect the magnitude of the repeated bout effect.

1.4 Research Questions

1. Is the EFOV technique valid when compared with the gold standard of CSA measurement and reliable when repeated on several occasions? <STUDY 1>

2. Does a bout of 10 sets of 3 repetitions (10x3) induce greater muscle damage compared with a bout of 3 sets of 10 repetitions (3x10)? <STUDY 2>

3. Does a greater difference in the set-repetition configuration from the first bout to the second bout (3x10 for the first bout to 20x3 for the second bout) attenuate the repeated bout effect in comparison to the condition that only the number of sets is increased in the second bout (10x3 for the first bout to 20x3 for the second bout)? <STUDY 2>

1.5 Hypotheses

1. Yes, the EFOV technique will be valid when compared with MRI and reliable when repeated on several occasions by the same researcher.

2. Yes, 10x3 will induce greater muscle damage compared with 3x10.

3. Yes, a greater change in set-repetition configurations (3x10 to 20x3) will result in the attenuation of the repeated bout effect compared with only increasing the number of sets (10x3 to 20x3).

CHAPTER 2

METHODS

2.1 STUDY 1

2.1.1 Subjects

Six healthy men (age = 27.5 ± 1.9 yr, height = 170.0 ± 10.0 cm, weight = 74.0 ± 12.5 kg) were recruited for the study. Subjects were screened with a medical questionnaire to affirm that they had not suffered from any musculoskeletal injury of the upper extremities. Resistance-trained individuals were excluded from the study. Subjects were requested to abstain from consuming any anti-inflammatory drugs, nutritional supplements, and/or any therapeutic and prophylactic interventions, and to avoid performing any unaccustomed or vigorous physical activities during the experimental period. Potential risks and requirements of this study were outlined in an information letter and a written consent was obtained from the subjects before their participation in the study. The study was approved by the Human Research Ethics committee at Edith Cowan University.

2.1.2 Study design

2.1.2.1 Extended-field-of-view imaging

Prior to data collection, the investigator practiced the EFOV technique on several individuals on many occasions until the image was deemed to be acceptable "(i.e., whole biceps brachii muscle could be seen with a complete border of the humerus bone)". It was found that the entire brachialis muscle image by this technique was not possible to obtain because the investigator was unable to move the ultrasound probe around the upper arm of a subject due to a limited range of rotation of the wrist joint. However, it was possible to trace the whole biceps brachii muscle (see Figure 1 as examples). Thus, decision was made to focus on the CSA of the biceps brachii for the present study.

Two regions, 0 cm and 3 cm below mid-point of upper arm were marked on both arms. The length of the upper arm was determined to be from the acromion process of the clavicle to the lateral epicondyle of the humerus (Figure 2A). These two regions were chosen because the largest biceps brachii CSA generally coincides within the 0-cm and 3-cm region. Subjects were seated in a comfortable position with arms resting at 90º of shoulder flexion and 0º elbow angle (i.e., straight arm) on a padded arm chair. Pressure was applied minimally but consistently, avoiding compression of the muscle and transmission gel was applied to aid in acoustic coupling. EFOV scans were obtained using an Aloka SSD–α10 (Aloka Co. Ltd., Tokyo, Japan) with a 7.5 MHz 4.0 cm probe (UST-5412, Aloka Co. Ltd, Japan) by moving the probe along the marked lines axially from the medial aspect to the lateral aspect of the upper arm in a continuous single view (Figure 2B). Scanning velocity was controlled to allow clear EFOV images and care was taken to avoid exerting too much pressure on the skin surface. Three scans were taken from each region, and the biceps brachii muscle was traced to calculate its CSA using a computer software program (Image J, version 0.0, National Institute of Health, USA).

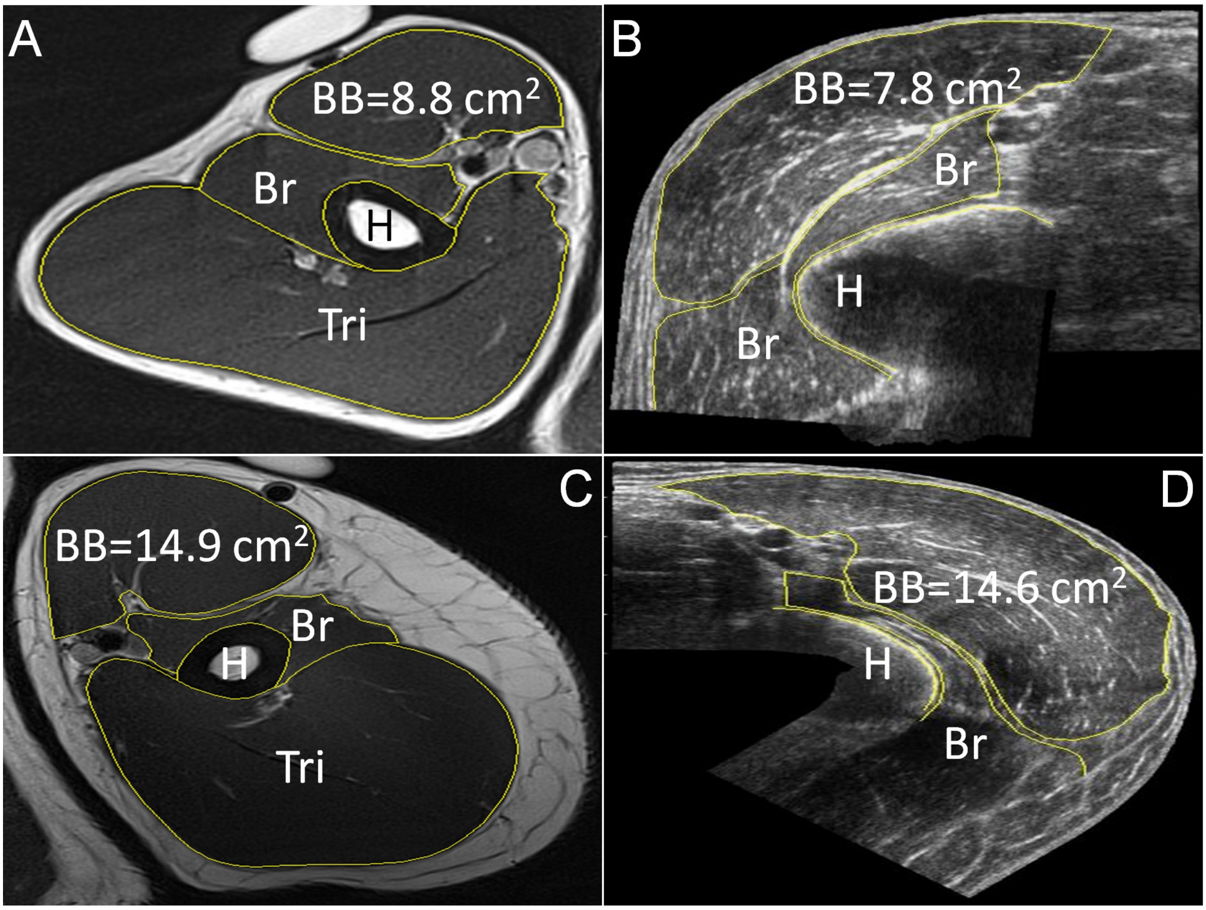

Figure 1: Corresponding axial plane images of the elbow flexors measured at 3 cm below the mid-point of the upper arm by MRI (A, C) and EFOV (B, D). Images A and B were from the right arm of one subject, and images C and D were from the left arm of another subject. Major anatomical landmarks are identified; BB – biceps brachii, Br – brachialis, H – humerus, Tri –triceps brachii.

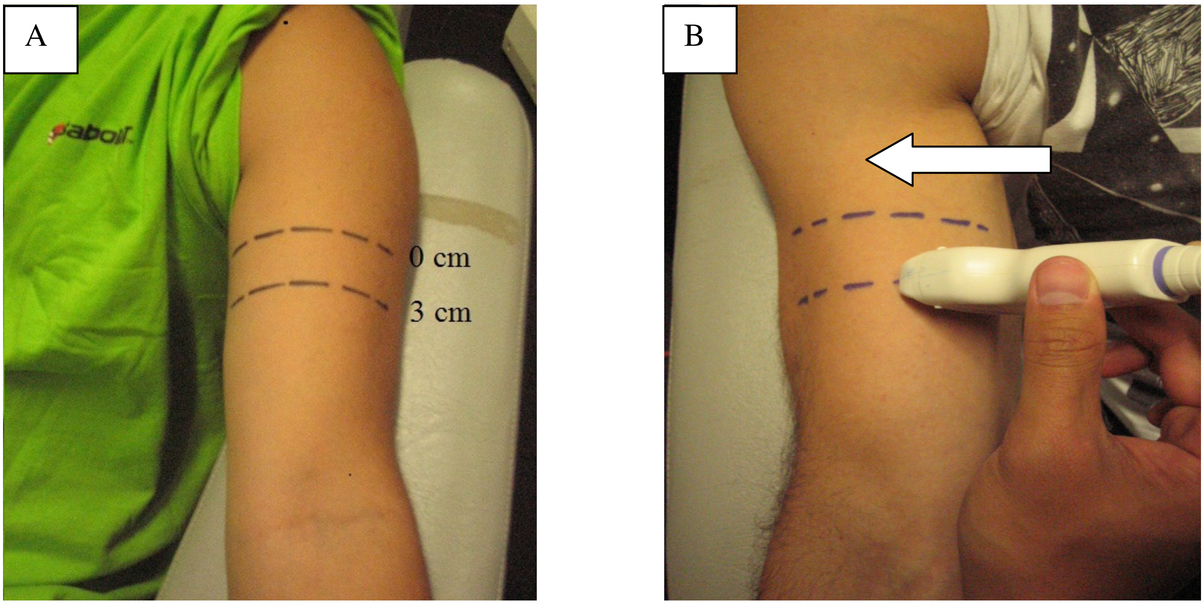

Figure 2: Ultrasound scanning procedure for extended field of view images of the upper arm at two different sites (mid-point of the upper arm: 0 cm, 3 cm below the mid-point: 3 cm) <A>. The arrow indicates the direction of the transducer movement for scanning and the dotted lines indicate the scanning distance <B>.

2.1.2.2 Magnetic resonance (MR) imaging

Multiple MR T1-weighted images were taken on the same day as the EFOV imaging using a Siemens Magnetom Espree 1.5T (Siemens AG, Erlangen, Germany). Subjects were laid down with a magnetic coil wrapped around the body and the arm of interest. After the first scan, the magnetic coil was wrapped around the body and the contralateral arm. Using fish oil pills to demarcate the corresponding sites at 3 cm below the mid-point of the upper arm that was used for the EFOV scanning, MR images (Figures 1A and 1C) were taken with a scan slice thickness of 4 mm and an inter-slice gap of 0 mm (contiguous images). The biceps brachii muscle CSA was measured using the same software as that used for the EFOV images.

2.1.2.3 Changes in biceps brachii CSA following eccentric exercise

Ten healthy men (age = 26.1 ± 4.1 yr, height =173.1 ± 6.1 cm, weight = 72.4 ± 9.1 kg) performed 3 sets of 10 maximal eccentric contractions of the elbow flexors on an isokinetic dynamometer (Cybex 6000, Lumex Inc. Runkonkoma, USA) with a HUMAC system (CSMI Medical Solutions, Massachusetts, USA) installed in a computer (Lenovo Think Center, IBM, New York, USA). Each subject was seated on a preacher curl bench that secured shoulder flexion angle at 45º and performed the eccentric exercise on an isokinetic dynamometer set to allow arm movements from full extension to full flexion while recording the force generation at the wrist during the contraction. During each eccentric contraction, the elbow joint was extended under maximal resistance from an elbow flexed (90º) to a full-extended position (0º) at an angular velocity of $30°\cdot s^{-1}$. Between each contraction, a 9-s rest was provided during which the elbow joint was passively returned to the flexed position at a velocity of $10°\cdot s^{-1}$. A 228-s passive rest interval occurred between sets. The procedures for obtaining EFOV images were the same as those described above. The EFOV images were collected before, immediately after (within 10 min post-exercise) and 1 to 4 days following eccentric exercise to obtain biceps brachii CSA at the 0 and 3 cm regions. Two EFOV scans were taken from each site and the average of the two measures was used for further analysis.

2.1.2.4 Upper arm circumference

Upper arm circumference was measured twice for each site by a constant tension tape measure (Gulick Anthropometric Tape, North Coast Medical, USA) when the subjects were standing with arms relaxed at their side in a neutral position (palms facing the thighs), and measurements were taken at the same sites as those used for the CSA measures. The average of the two measures was used for further analysis. The measurements of CSA and upper arm circumference were taken before, immediately after, and 1-4 days following exercise.

2.1.3 Statistical analyses

Four subjects were used for the validity test to compare the CSA obtained by EFOV and MR images. The MR images were taken approximately 2 hours after the EFOV images. Both EFOV and MR images were taken from both arms, resulting in a sample size of 8 for validity analysis using a Pearson product-moment correlation coefficient and a paired t-test. For the reliability assessment, six subjects underwent two sessions of EFOV scans separated by one hour. For each EFOV session, three scans were taken from the 0 cm and 3 cm regions from each arm and the two closest CSA results were used for further analysis. The intra-tester reliability of the EFOV technique was assessed in two ways; using the same scan image and tracing it twice (between-traces) and scanning two images from the same site (between-scans). The test-retest reliability was also assessed using the images taken from the same site one hour apart. Coefficient of variation (CV) and Intra-class correlation (ICC) were used to determine the between-trace, between-scan and between-time reliability. ICC values within the range of 0.8 – 1.0 were considered as "good" reliability (Zwinderman & Cleophas, 2009).

Changes in the CSA and upper arm circumference following eccentric exercise 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 applied to compare the values between time points. The correlation between the relative changes in CSA and upper arm circumference from pre-exercise values was analysed by a Pearson product-moment correlation coefficient using the data from all time points (i.e., immediately post, 1-4 days post). Significance level was set at $P<0.05$. The results are shown in mean ± SD, unless otherwise stated.

2.2 STUDY 2

2.2.1 Subjects

Ten healthy men (mean ± SD age: 26.1 ± 4.1 y, height: 173.1 ± 6.1 cm, body weight: 72.4 ± 9.1 kg) participated in this study. Subjects were screened with a medical questionnaire to affirm that they did not have any musculoskeletal injury of the upper extremities and other contraindications that would affect their participation in the study. Resistance-trained individuals were excluded from the study. Subjects were requested to abstain from consuming any anti-inflammatory drugs, nutritional supplements, and/or any therapeutic and prophylactic interventions, and to avoid performing any unaccustomed or vigorous physical activities during the experimental period. Potential risks and requirements of this study were explained in an information letter and a written consent was obtained from the subjects before their participation in the study. This study was approved by the Human Research Ethics committee at Edith Cowan University.

2.2.2 Study design

All subjects performed two bouts of eccentric exercise for each arm separated by two weeks, with 4 weeks between bouts for the same arm (Table 1). One arm performed 3 sets of 10 maximal eccentric contractions (3x10) of the elbow flexors in the first week (Week 1) and the contralateral arm performed 10 sets of 3 maximal eccentric contractions of the elbow flexors (10x3) two weeks later (Week 3). The second exercise bout consisted of 20 sets of 3 maximal eccentric contractions (20x3) for both arms, and was performed four weeks after the first exercise bout for each arm (Week 5 for the arm that was used first, Week 7 for the contralateral arm). The order of the first exercise bout (3x10, 10x3) and the use of arms (dominant and non-dominant) were randomised and counter-balanced amongst subjects. The dependent variables consisted of muscle strength, ROM, biceps brachii cross sectional area using B-mode ultrasound, muscle soreness by visual analogue scale (VAS) and pressure pain threshold of the elbow flexors, and plasma CK activity. These measures were taken before, immediately after and 1 – 4 days following each exercise bout except plasma CK activity, which was measured before, 2 and 4 days following each exercise bout. Changes in these variables over time were compared between 3x10 and 10x3 bouts, between arms following the second bout (20x3), and between the first and second bouts for each arm.

Table 1: Study design. Each subject performed four eccentric exercise bouts over the 7 week period. One arm (Arm 1) performed 3 sets of 10 eccentric contractions (3x10) or 10 sets of 3 eccentric contractions (10x3) for the first bout (Week 1) and 20 sets of 3 eccentric contractions (20x3) for the second bout 4 weeks later (Week 5). The contralateral arm (Arm 2) performed 10 sets of 3 eccentric contractions (10x3) or 3 sets of 10 eccentric contractions (3x10) for the first bout (Week 3) and 20 sets of 3 eccentric contractions (20x3) for the second bout 4 weeks later (Week 7). The first bout exercise (3x10 or 10x3) was different between arms (if Arm 1 performed 3x10 at Week 1, Arm 2 performed 10x3 at Week 3), and the choice of dominant or non-dominant arm was randomised and counterbalanced among the subjects. For convenience, the condition that the arm performed 3x10 for the first bout is referred to as Group A, and the condition that the arm performed 10x3 for the first bout is referred to as Groups B.

Week	1	2	3	4	5	6	7
Arm 1	3x10 or10x3	-	-	-	20x3	-	-
Arm 2	-	-	10x3 or 3x10	-	-	-	20x3

2.2.3 Familiarisation session

Subjects performed a familiarisation session at least one week before the first exercise bout. In the session, subjects performed maximal isometric contractions at 60º flexion (0º is an extended elbow joint angle) and maximal concentric contractions at $210º\cdot s^{-1}$ twice for each measure using an isokinetic dynamometer described below. No eccentric contraction was performed; however the subjects were briefed on eccentric exercise protocols. Other measurements such as plasma CK activity, muscle soreness, ultrasound measures were also demonstrated.

2.2.4 Exercise protocol

Each subject was seated on a preacher curl bench, securing the shoulder flexion angle at 45º and performed eccentric contractions of the elbow flexors while keeping the forearm in a supinated position, using an isokinetic dynamometer (Cybex 6000, Lumex Inc. Runkonkoma, USA) operated by a HUMAC system (CSMI Medical Solutions, Massachusetts, USA) installed in a computer (Lenovo Think Center, IBM, New York, USA) (Figure 3). Subjects were asked to maximally resist the lengthening motion of the dynamometer and verbal encouragement was provided to the subjects during each eccentric contraction. In each eccentric contraction, the elbow joint was forcibly extended under maximal resistance from a flexed (90º) to a fully extended position (0º) at an angular velocity of $30°\cdot s^{-1}$. After each contraction, the elbow joint was passively returned to the flexed position at a velocity of $10º\cdot s^{-1}$, allowing approximately a 9-s rest between contractions. Torque signals were recorded via a data acquisition system (Powerlab16, ADInstruments, Castle Hill, Australia) at a sampling rate of 2000 Hz, and real-time visual feedback of torque was displayed on a computer monitor. The peak torque value of each eccentric contraction was recorded and used for subsequent analysis. The rest time between sets for 3x10, 10x3 and 20x3 were 228 s, 60 s and 60 s respectively, so that the entire exercise duration including the rest time between repetitions and sets was similar between 3x10 and 10x3.

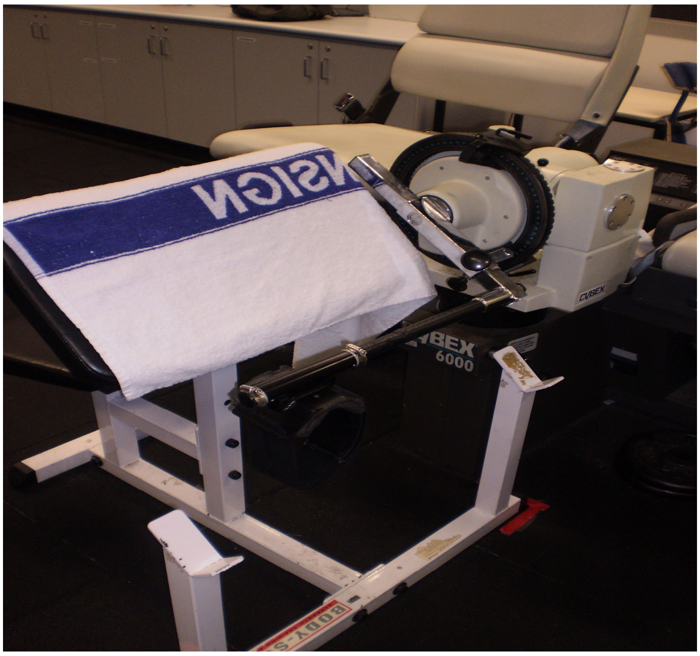

Figure 3: Cybex isokinetic dynamometer set-up with a preacher curl bench

2.2.5 Criterion measures

2.2.5.1 Muscle strength

Each subject was seated in a preacher curl bench in the same position as that of the exercise, and performed isometric and concentric contractions of the elbow flexors on the isokinetic dynamometer while keeping forearm supinated. Maximal voluntary isometric contraction torque (MVC-ISO) was measured at 90º and 60º (where 0º represents a fully extended elbow joint angle) in this order. The duration of each isometric contraction was 3 s with a 30-s rest between contractions at the same angle, and a 60-s rest between the different angles. After a 3-min rest, maximal isokinetic concentric contraction torque (MVC-CON) was measured at two different velocities ($30º·s^{-1}$ and $210º·s^{-1}$) in this order for a range of motion from 0º (extended) to 90º (flexed) with a 30-s rest between contractions at the same velocity and a 60-s of rest between the different velocities. Subjects were verbally encouraged to give their maximal effort during the muscle strength tests. The average value of the two measurements for each test was used for subsequent analysis.

2.2.5.2 Range of motion (ROM)

ROM of the exercised arm was determined by measuring flexed (FANG) and extended (EANG) elbow joint angles with a Lafayette Gollehon goniometer (Model 01135, Lafayette Instrument Co, Inc., Indianapolis, USA). Subjects were asked to fully flex the elbow joint without raising the elbow for the FANG measurements and to fully extend the elbow joint as much as possible for the EANG measurements. Reference points (styloid process of radius, lateral epicondyle of humerus and deltoid insertion) were marked with a semi-permanent marker. FANG and EANG were measured twice and ROM was obtained by subtracting the mean FANG from the mean EANG.

2.2.5.3 Biceps brachii cross sectional area (CSA) by ultrasonography

Two regions (0 cm and 3 cm below mid-point of upper arm respectively) were marked out on the exercise arm based on the length of the upper arm that was determined by the acromion process of the clavicle to the lateral epicondyle of the humerus. Each subject was seated with his exercised arm resting at 90º shoulder flexion angle and 0º elbow flexion on a padded arm chair. Extended-field-of-view (EFOV) scans were taken using an Aloka SSD–α10 (Aloka Co. Ltd., Tokyo, Japan) with a 7.5 MHz 4-cm probe (UST-5412, Aloka Co. Ltd, Japan) by moving the probe along the marked lines axially from the medial aspect to the lateral aspect of the upper arm in a continuous single view. For the probe movement, pressure was applied minimally but consistently, avoiding compression of the muscle, and transmission gel was used to aid in acoustic coupling. Three scans were taken from each region, and the biceps brachii muscle was traced to calculate its CSA using a computer software program (Image J, version 0.0, National Institute of Health, USA). The average of the three scans was used for further analysis.

2.2.5.4 Muscle soreness

A VAS incorporating a 100-mm line, anchoring 0 for no soreness and 100 for maximally imaginable soreness, was used to assess muscle soreness. Subjects were asked to rate the soreness felt upon palpation by the investigator. The palpation was applied to 5 sites including the mid-point of the upper arm, 30 and 60 % of the distance from the elbow crease to mid-point of the upper arm, brachioradialis (5cm below the lateral epicondyle) and brachialis (half way between mid-point of the upper arm and lateral epicondyle). The mid-point of the upper arm was determined as a half way between the anatomical landmarks (i.e., epicondyle and acromion) based on Deighan et al. (2006). Each site was palpated twice, slowly in circular movements 5 times by the same investigator using his index and middle fingers. The investigator paid attention to standardise the palpation pressure between measurements and among subjects.

2.2.5.5 Pressure pain threshold

Pressure pain threshold (PPT) was assessed for the same sites as those mentioned in muscle soreness section by an electronic algometer (Somedic AB, Sweden) with a probe area of 1.0 cm^2 (Figure 4). The head of the probe was placed perpendicular to each of the 5 sites in the same order as mentioned in muscle soreness section. Force was gradually applied at 50 $kPa \cdot s^{-1}$ until the subject reported his first feeling of pain at the site, and the value was recorded. Two measurements were taken for each site with a 30-s interval between them. The average value of the two measurements was used for further analysis.

Figure 4: An algometer to assess PPT

2.2.5.6 Plasma CK activity

Blood samples were collected before, 2 and 4 days after eccentric exercise, as it is known that CK activity generally peaks 3 – 5 days after performing maximal eccentric contractions of the elbow flexors (Nosaka & Clarkson, 1996). A finger tip of the non-exercised arm of the subjects was pricked with a spring-loaded lancet and 30 μl of blood was collected into a capillary tube. The blood were loaded onto a CK test strip (Reflotron CK, Inverness Medical, Cheshire, UK) and measured by a Reflotron (Boehringer Mannheim GmbH, W. Germany). The normal CK reference range was 24 – 195 $IU \cdot L^{-1}$ according to the information provided by the company of the CK strip.

Figure 5: Reflotron to assess plasma CK activity

2.2.6 Statistical analyses

Changes in peak torque during the eccentric exercise and the criterion measures over time were compared between 3x10 and 10x3, between arms for the 20x3 bout, and between the first and second bouts for each arm by a two-way repeated measure of analysis of variance (ANOVA). When a significant interaction effect was returned, a Tukey's post hoc test was applied to compare the values for each time point. Significance level was set at $P<0.05$ and the results are reported as mean ± standard error of measurement (SEM) unless otherwise stated.

CHAPTER 3

RESULTS

3.1 STUDY 1

3.1.1 Validity

The average (± SD) CSA obtained from the MR and EFOV images, and the absolute differences between the two techniques are shown in Table 2. The absolute difference in CSA between the two techniques was small (0.4 ± 0.3 cm^2) but significant (P=0.004), and the CSA measured by MRI was greater at both 0 cm (5.1 ± 4.1 %) and 3 cm (4.5 ± 5 %) sites compared with the EFOV values. Pearson product-moment correlation coefficient indicated that CSA measured by EFOV was highly correlated with that by MRI (r=0.99).

Table 2. Biceps brachii cross sectional area obtained from magnetic resonance image (MRI) and ultrasound extended field of view image (EFOV), and the absolute difference between the two measures for the mid-point of the right (R) and left (L) arms of 4 subjects and their mean (± SD) values. At the bottom of the table, the results of a paired t-test (P) and a Pearson product-moment correlation coefficient (r) between the two measures are shown.

Subject-Arm	MRI (cm^2)	EFOV (cm^2)	Absolute Difference (cm^2)
1-R	14.4	14.1	0.3
1-L	14.9	14.6	0.3
2-R	7.4	6.6	0.8
2-L	7.1	6.7	0.4
3-R	21.7	21.5	0.2
3-L	21.3	21.2	0.1
4-R	7.9	7.5	0.4
4-L	8.8	7.8	1.0
Mean (± SD)	12.9 (± 6.1)	12.5 (± 6.3)	0.4 (± 0.3)
P	0.004		
r	0.999		

3.1.2 Reliability

Table 3 shows that the between-trace reliability was high as indicated by low CV and high ICC R-values for both 0 cm and 3 cm regions. This was also the case for the between-scan reliability (Table 4) and the test-retest reliability as shown in Table 5.

Table 3. Biceps brachii cross sectional area (CSA) of 6 subjects (mean ± SD) measured by the ultrasound extended field of view images taken at the mid-point of the upper arm (0 cm) and 3 cm below mid-point of the upper arm (3 cm) from right and left arms of the subjects, giving a sample size of 12 for each site. Using the same image, the biceps brachii was traced on two separate occasions (Trace 1 and Trace 2) by the investigator. Coefficient of variation (CV) and Intra-class correlation (ICC) based on the two traces are shown.

	CSA (cm^2)		CV	ICC
Site	Trace 1	Trace 2	%	R
0 cm	11.5 ± 2.9	11.5 ± 2.9	0.08	1
3 cm	12.5 ± 3.2	12.5 ± 3.2	0.07	1

Table 4. Biceps brachii cross sectional area (CSA) of 6 subjects (mean ± SD) measured by the ultrasound extended field of view images taken at the mid-point of the upper arm (0 cm) and 3 cm below mid-point of the upper arm (3 cm) from right and left arms of the subjects, giving a sample size of 12 for each site. The images were taken from the same site twice (Scan 1 and Scan 2) and the biceps brachii for each image was traced to measure the CSA by the same investigator. Coefficient of variation (CV) and Intra-class correlation (ICC) based on the two scans are shown.

	CSA (cm^2)		CV	ICC
Site	Scan 1	Scan 2	%	R
0 cm	11.5 ± 2.8	11.5 ± 2.8	0.1	1
3 cm	12.5 ± 3.1	12.5 ± 3.1	0.1	1

Table 5. Biceps brachii cross sectional area (CSA) of 6 subjects (mean ± SD) measured by the ultrasound extended field of view images taken at the mid-point of the upper arm (0 cm) and 3 cm below mid-point of the upper arm (3 cm) from right and left arms of the subjects, giving a sample size of 12 for each site. The images were taken from the same site at two different occasions separated by a one-hour interval (Time 1 and Time 2) and the biceps brachii for each image was traced to measure the CSA by the investigator. Coefficient of variation (CV) and Intra-class correlation (ICC) based on the two time points are shown.

	CSA (cm^2)		CV	ICC
Site	Time 1	Time 2	%	R
0 cm	11.6 ± 2.6	11.6 ± 2.7	0.7	0.99
3 cm	12.4 ± 3.3	12.3 ± 3.3	0.6	0.99

3.1.3 Changes in biceps brachii CSA and upper arm circumference following eccentric exercise

Changes in the average CSA obtained from the 0 cm and 3 cm sites before, immediately after and 1-4 days following eccentric exercise are shown in Figure 6A. Both sites showed significant increases in CSA compared with the baseline values. The largest increase in CSA was found at 1 day post-exercise (12.6 ± 7.5 %) for the 0-cm site, and immediately post-exercise (9.2 ± 5.9 %) for the 3-cm site. The largest increase in circumference was observed immediately after exercise for the 0-cm site (1.3 ± 1.3 %) and day 2 after for the 3-cm site (1.9 ± 1.3 %), and remained greater than the baseline values for 4 days post-exercise (Figure 6B).

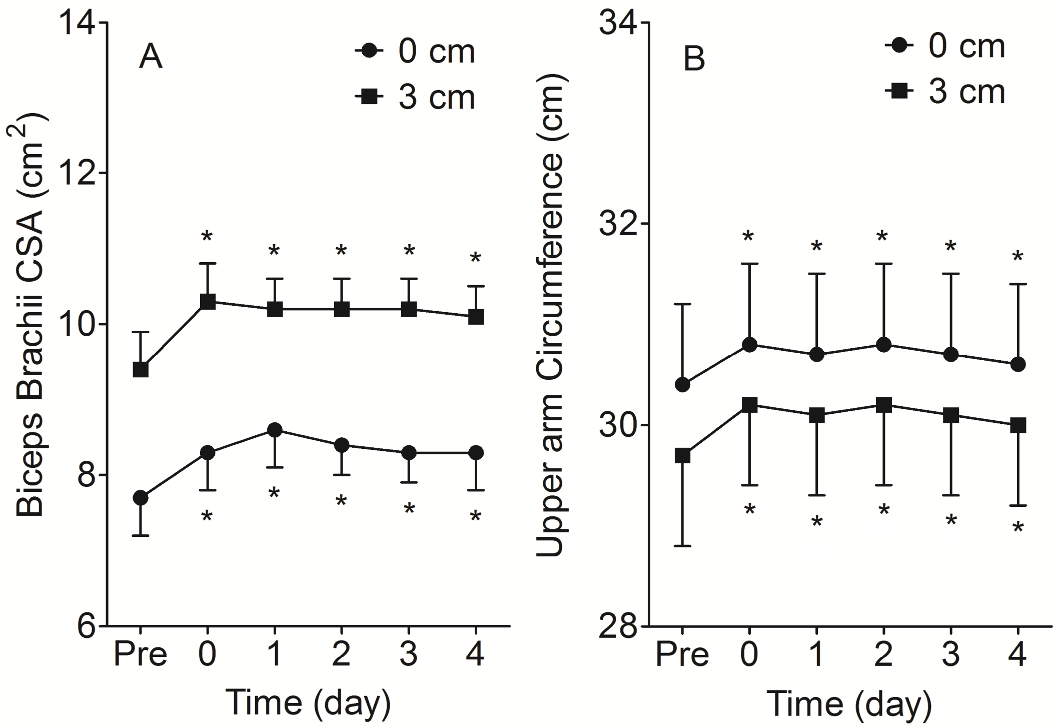

Figure 6: Changes (mean ± SEM, n=10) in biceps brachii cross-sectional area (A) and upper arm circumference (B) at the mid-point (0 cm) and 3 cm below the mid-point (3 cm) before (pre), immediately after (0) and 1-4 days following 3 sets of 10 maximal eccentric contractions of the elbow flexors. * indicates a significant ($P<0.05$) difference from the pre-values.

3.1.4 Correlation between biceps brachii CSA and upper arm circumference changes

As shown in Figure 7, the relative changes in CSA and upper arm circumference from baseline were significantly ($P=0.000$) but poorly correlated ($r=0.428$). This was also the case for the analysis for each site separately (0 cm: $r=0.43$, 3 cm: $r=0.50$, $P<0.001$). An increase in CSA did not necessarily correspond to an increase in upper arm circumference, and vice versa.

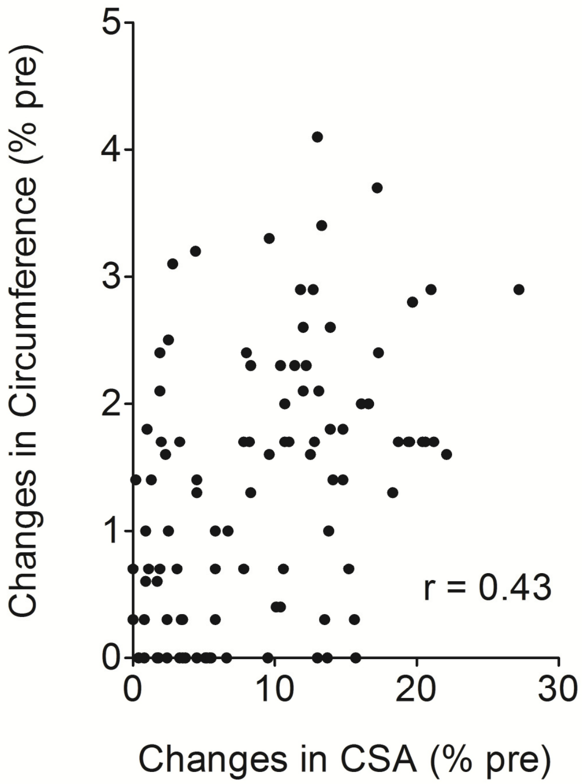

Figure 7: Correlation between biceps brachii cross-sectional area (CSA) and upper arm circumference changes following eccentric contractions of the elbow flexors (0 and 3 cm regions, immediately post and 1-4 days post exercise, 10 subjects: 2 x 5 x 10 = 100 pair samples).

3.2. STUDY 2

3.2.1 Eccentric exercise performance

Figure 8 shows the changes in peak torque over 30 eccentric contractions for the 3x10 and 10x3 bouts, and over 20 sets (average of 3 eccentric contractions) for each arm in the 20x3 bout. No significant difference in the peak torque changes was observed between 3x10 and 10x3, and between arms for the 20x3 bout. When comparing the magnitude of decrease in peak torque from the first three to the last three contractions of 3x10 or 10x3 and

20x3 bouts, the average peak torque of the first three contractions was 50.4 ± 1.6 Nm for the 10x3/3x10 and 48.4 ± 1.5 Nm for 20x3, and the average peak torque of the last three contractions was 36.3 ± 1.0 for 10x3/3x10 and 32.7 ± 0.9 Nm for 20x3. The decreases in peak torque were significantly smaller for 20x3 (31.7 ± 2.2 %) compared with 3x10/10x3 bout (35.9 ± 1.7%).

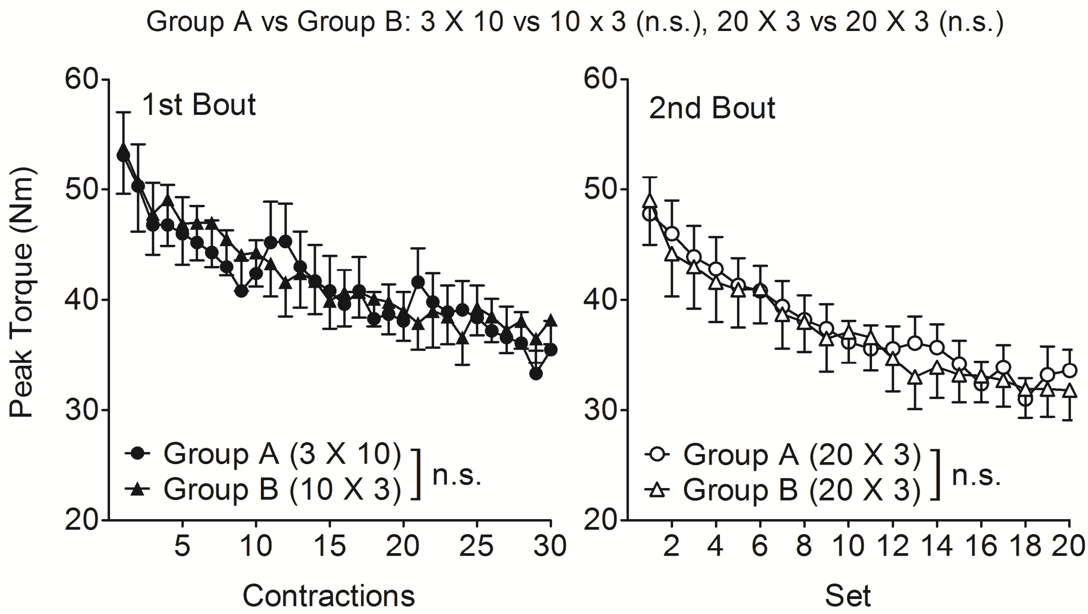

Figure 8: Changes (mean ± SEM) in peak torque over 30 eccentric contractions (3 sets of 10 repetitions: 3x10 – Group A, 10 sets of 3 repetitions: 10x3 – Group B) in the first bout and over 60 eccentric contractions (20 sets of 3 repetitions: 20x3) in the second bout (2nd bout) for each group (Group A and Group B). n.s. indicates not significantly different between groups.

3.2.2 Muscle Strength

Baseline values were not significantly different among the four exercise bouts; however, significantly different values were evident amongst MVC-ISO at 60° (49.3 ± 1.8 Nm), 90° (57.7 ± 2.0 Nm), MVC-CON at 30°/s (39.5 ± 1.6 Nm) and 210°/s (48.0 ± 1.5 Nm). Figure 9 shows the changes in MVC-ISO at 60° and 90° before, immediately after and 1-4 days following eccentric exercise. Figure 10 shows the changes in MVC-CON at 30°/s and 210°/s before, immediately after and 1-4 days following eccentric exercise. For MVC-ISO at 90°, muscle strength decreased significantly immediately after exercise by 38.2 ± 2.1% and gradually recovered to the baseline by 3 days post-exercise. No significant difference in the changes was observed between 3x10 and 10x3, between arms for the 20x3 bouts, and between the first and second bouts for each arm. This was also the case for other muscle strength measures, although the magnitude of changes in MVC was different amongst the measures.

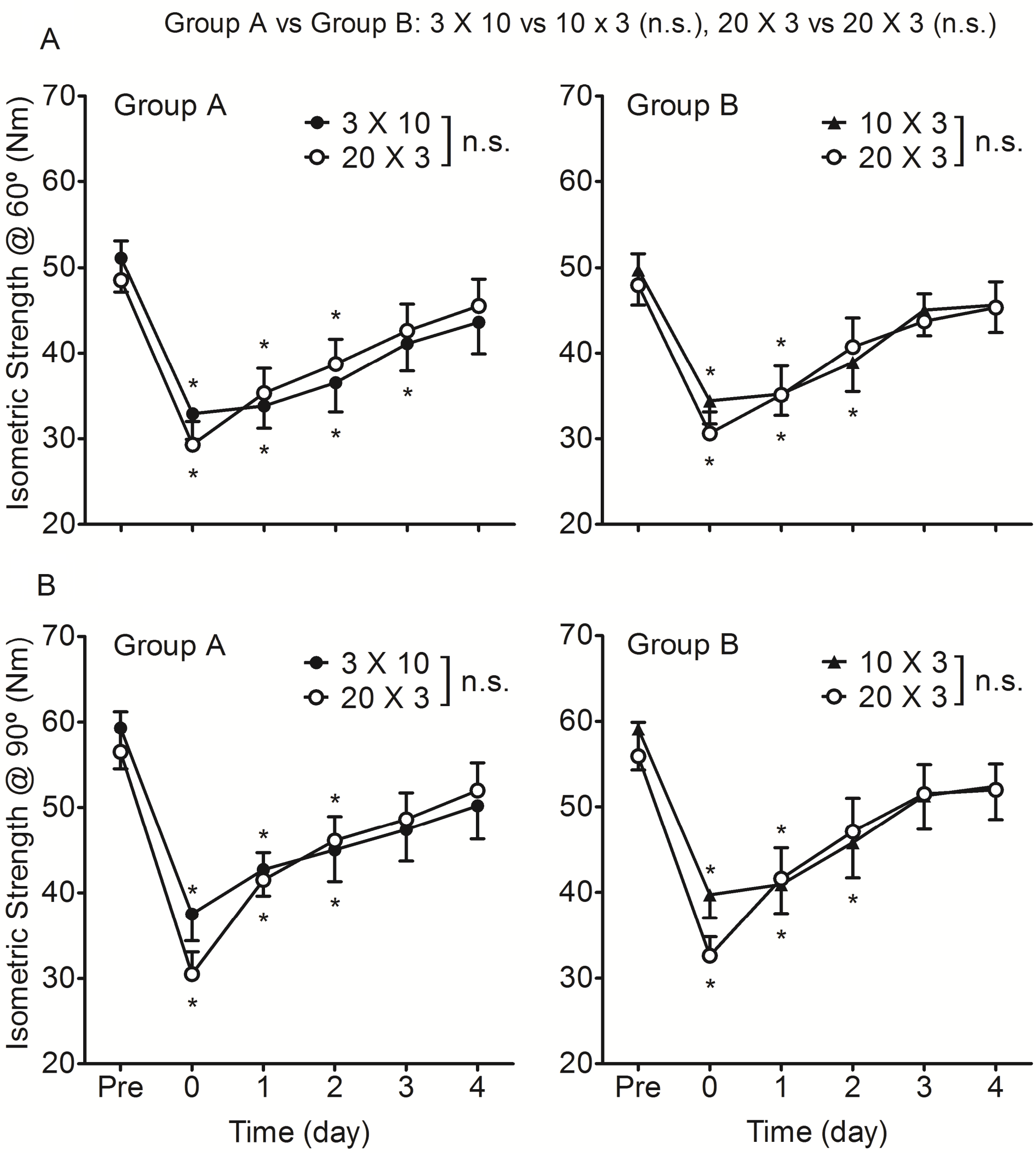

Figure 9: Changes (mean ± SEM) in maximal voluntary isometric contraction strength at 60° (A) and 90° (B) before (Pre), immediately after (0), and 1 – 4 days following 3 sets of 10 maximal eccentric contractions (3x10, Groups A), 10 sets of 3 maximal eccentric contractions (10x3, Group B), and 20 sets of 3 maximal eccentric contractions (20x3, Groups A & B). The results of comparison between groups are shown on the top of the graphs. n.s. indicates not significantly different and * indicates significantly (P<0.05) different from the pre-value.

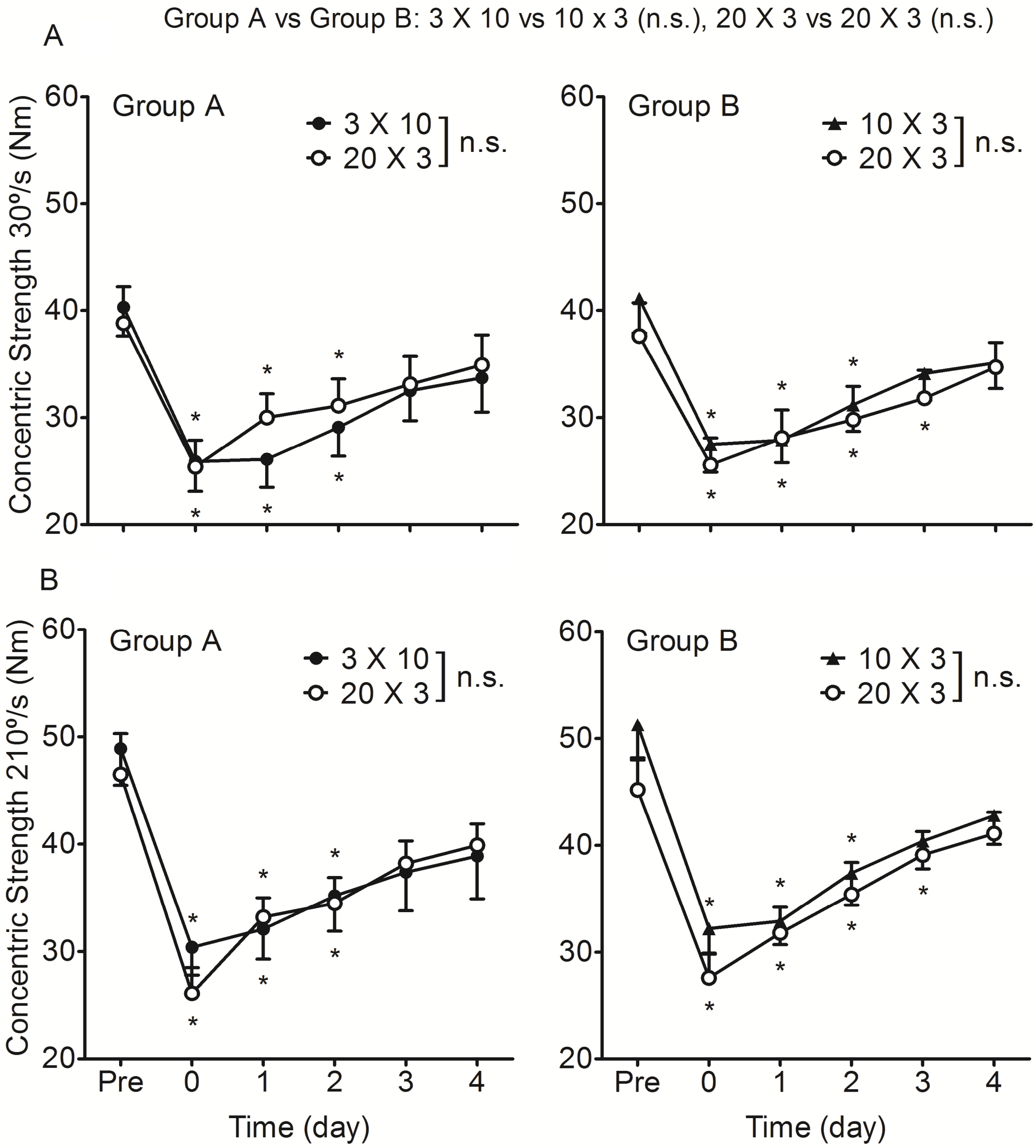

Figure 10: Changes (mean ± SEM) in maximal voluntary isokinetic concentric contraction strength at 30°/s (A) and 210°/s (B) before (Pre), immediately after (0), and 1 – 4 days following 3 sets of 10 maximal eccentric contractions (3x10, Groups A), 10 sets of 3 maximal eccentric contractions (10x3, Group B), and 20 sets of 3 maximal eccentric contractions (20x3, Groups A & B). The results of comparison between groups are shown on the top of the graphs. n.s. indicates not significantly different and * indicates significantly (P<0.05) different from the pre-value.

3.2.3 ROM

As shown in Figure 11, ROM was similar for both arms before each exercise bout (average: 130.9 ± 0.9°). ROM decreased significantly immediately after eccentric exercise and did not recover to baseline by 4 days post-exercise. No significant difference in the changes was observed between 3x10 and 10x3. However, significant differences were observed when comparing between arms for the 20x3 bout, between 3x10 and 20x3, and between 10x3 and 20x3. When comparing between arms for the changes following 20x3, the magnitude of decrease in ROM was significantly greater for the arm that performed 3x10 for the first bout compared to the arm that performed 10x3 for the first bout. The magnitude of decrease in ROM immediately after to 2 days following 3x10 was significantly smaller than that after 20x3, but the magnitude of decrease in ROM immediately after to 3 days following 10x3 was significantly greater than that after 20x3.

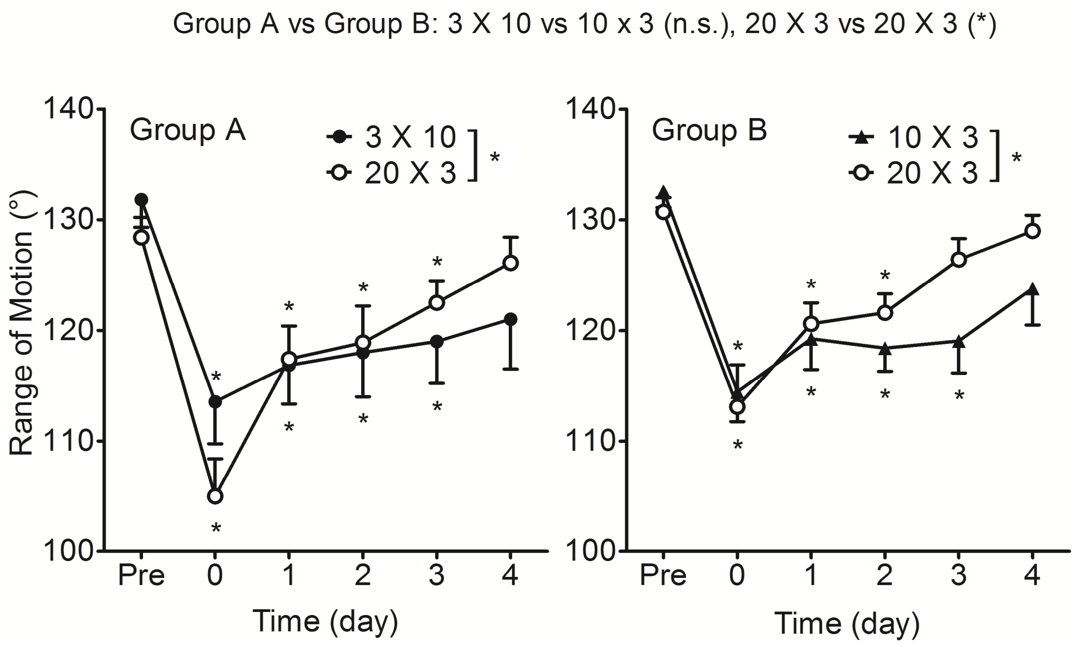

Figure 11: Changes (mean ± SEM) in range of motion (ROM) before (Pre), immediately after (0), 1 – 4 days following 3 sets of 10 maximal eccentric contractions (3x10, Groups A), 10 sets of 3 maximal eccentric contractions (10x3, Group B), and 20 sets of 3 maximal eccentric contractions (20x3, Groups A & B). The results of comparison between groups are shown on the top of the graphs. n.s. indicates not significant different between bouts, * indicates significance (P<0.05) difference from pre-values.

3.2.4 Biceps brachii CSA

Biceps brachii CSA was greater for the 3 cm region (9.6 ± 0.3 cm^2) when compared with the 0 cm region (7.7 ± 0.4 cm^2); however, the changes in CSA were similar between the two regions. Figure 12 shows the biceps brachii CSA at the 3cm region. The CSA increased significantly immediately after exercise and remained elevated for 4 days post-exercise. No significant difference in the CSA changes was observed between 3x10 and 10x3, between arms for the 20x3 bouts, and between the first and second bouts for each arm.

Group A vs Group B: 3 X 10 vs 10 x 3 (n.s.), 20 X 3 vs 20 X 3 (n.s.)

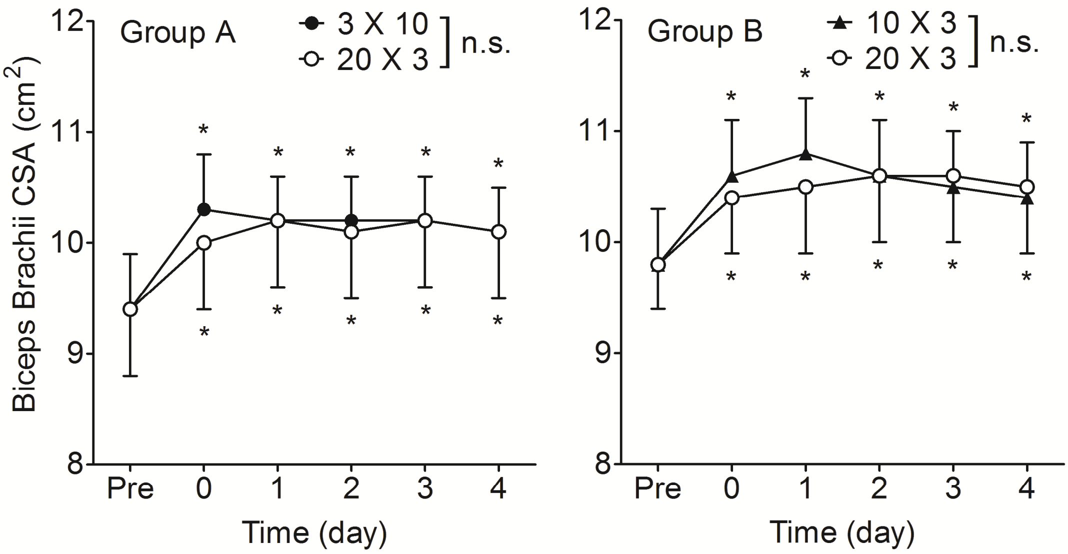

Figure 12: Changes in (mean ± SEM) biceps brachii cross-sectional area (CSA) at 3 cm below mid-point of upper arm before (Pre), immediately after (0), 1 – 4 days following 3 sets of 10 maximal eccentric contractions (3x10, Groups A), 10 sets of 3 maximal eccentric contractions (10x3, Group B), and 20 sets of 3 maximal eccentric contractions (20x3, Groups A & B). The results of comparison between groups are shown on the top of the graphs. n.s. indicates not significantly different between bouts and * indicates significantly ($P<0.05$) different from pre-values.

3.2.5 Muscle soreness and PPT

The palpation measures from the five different sites were summed (i.e., maximal value of 500 mm) to represent muscle soreness of whole elbow flexors (Figure 13). The soreness rating increased on day 1 and peaked on day 2 after exercise. No significant difference was observed between 3x10 and 10x3, between arms for the 20x3 bouts, and between the first and second bouts for each arm.

All PPT measures were summed in the same manner as the muscle soreness VAS. The baseline PPT values were not significantly different amongst the four exercise bouts (average between 1345.1 ± 124.1 kPa and 1491.7 ± 231.8 kPa). PPT decreased immediately after exercise, with a largest decrease was observed on day 1 post-exercise (average between 1070.6 ± 130.5 kPa and 1212.2 ± 136.0 kPa), and gradually return to the baseline in the next three days; however, it was still 5.2 % – 16.2 % lower than the baseline at 4 days post-exercise. No significant difference was observed between 3x10 and 10x3, between arms for the 20x3 bouts, and between the first and second bouts for each arm.

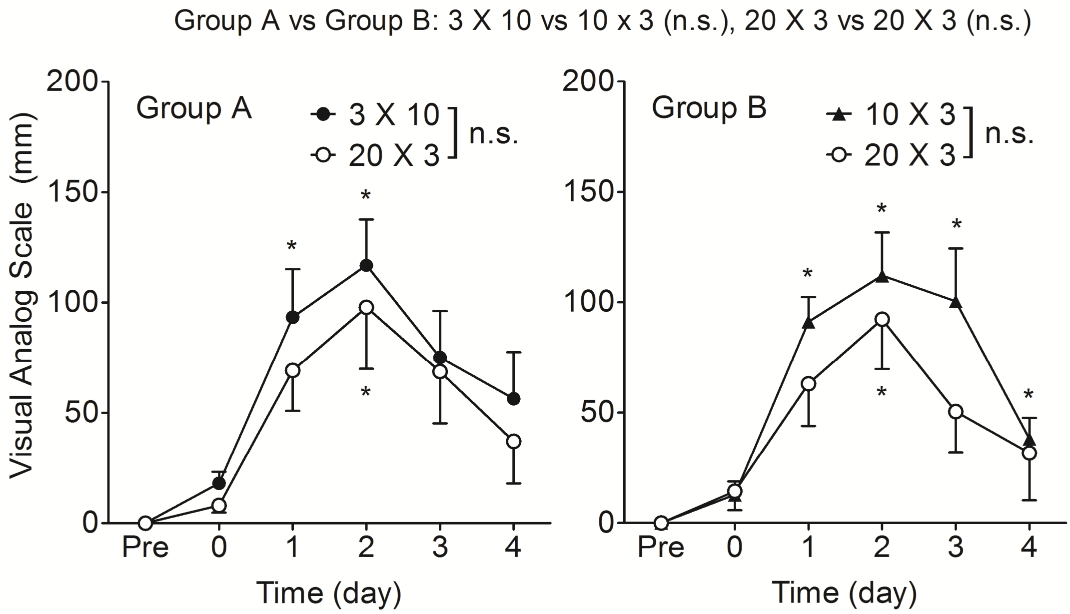

Figure 13: Changes in (mean ± SEM) muscle soreness upon palpation (sum of the five different sites: 500 mm is the potential maximal value) assessed by visual analogue scale before (Pre), immediately after (0), 1 – 4 days following 3 sets of 10 maximal eccentric contractions (3x10, Groups A), 10 sets of 3 maximal eccentric contractions (10x3, Group B), and 20 sets of 3 maximal eccentric contractions (20x3, Groups A & B). The results of comparison between groups are shown on the top of the graphs. n.s. indicates not significant different between bouts and * indicates significantly (P<0.05) different from pre-values.

3.2.6 Plasma CK activity

No significant difference in the changes in plasma CK activity were observed between 3x10 and 10x3, between arms for the 20x3 bouts, and between the first and second bouts for each arm. Plasma CK activity increased significantly at 4 days post-exercise (average of 4 bouts: 181.7 ± 19.5 IU·L^{-1}) from the baseline (average of 4 bouts: 134.9 ± 7.3 IU·L^{-1}), but the magnitude of decrease was small (Figure 14).

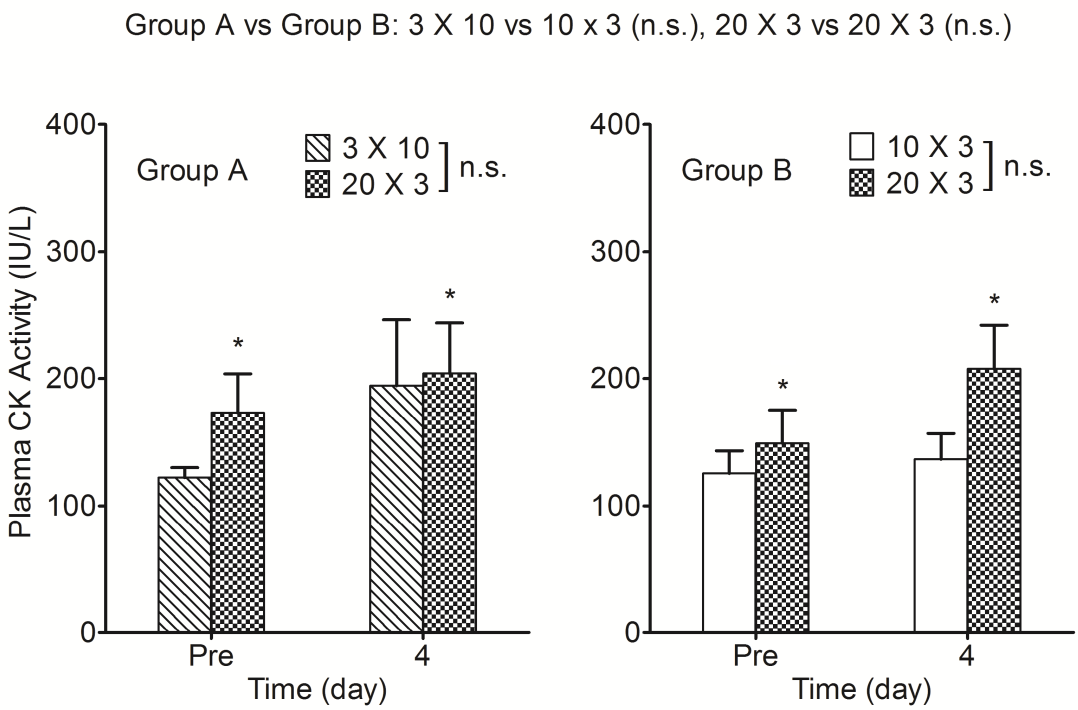

Figure 14: Changes in (mean ± SEM) serum CK activity levels before (Pre) and day 4 following 3 sets of 10 maximal eccentric contractions (3x10, Groups A), 10 sets of 3 maximal eccentric contractions (10x3, Group B), and 20 sets of 3 maximal eccentric contractions (20x3, Groups A & B). The results of comparison between groups are shown on the top of the graphs. n.s. indicates not significant different between bouts and * indicates significantly (P<0.05) different from pre-values.

CHAPTER 4

DISCUSSION

The present study tested three hypotheses that 1) EFOV would be valid and reliable to measure bicep brachii CSA, 2) 10x3 would induce greater magnitude of muscle damage than 3x10, and 3) the magnitude of the repeated bout effect would be smaller for the condition that 20x3 was performed after 3x10 compared with 20x3 performed after 10x3 (Figures 9-14).The results of this study supported the hypothesis that 1) the EFOV technique was valid and reliable for measuring biceps brachii CSA at the mid-arm regions (Tables 2-5). However, in contrast to the hypotheses, the results showed 2) no significant differences in the changes in peak torque during eccentric exercise and criterion measures following exercise between 3x10 and 10x3 (Figure 8), and 3) no significant differences in the changes in criterion measures after 20x3 between arms (Figures 9-14); suggesting no effect of the different set-repetition configuration on muscle damage and the repeated bout effect. The results also revealed that the changes in the criterion measures were similar between the first and second bouts, regardless of the difference in the set-repetition configuration in the first exercise bout.

4.1 STUDY 1

The results of this study showed that the EFOV technique was valid and reliable for measuring biceps brachii CSA at the mid-arm regions. Although the difference was small, the CSA obtained from the EFOV was approximately 1 % smaller than that assessed by MRI (Table 1). When the EFOV technique was applied to assess changes in biceps brachii CSA following eccentric exercise of the elbow flexors, increases in the CSA were found; however, the changes in CSA were poorly correlated with the changes in upper arm circumference.

It should be noted that the EFOV technique was unable to capture the whole brachialis muscle and measure its CSA in the present study. In order to obtain the whole brachialis muscle image, the transducer has to be moved from the medial to lateral aspect of the upper arm, which we found was not possible. In the present study, the transducer (3.6 cm in width) did not allow us to obtain images from subjects with small upper arms, because the transducer would produce overlap between successive images and was unable to glide through the cross section of the arm without deviating from the scanning plane. A smaller transducer (e.g. 2 cm) may be able to overcome this shortcoming; however such transducer is not currently available. This is a limitation for using the EFOV technique to assess entire elbow flexor muscle CSA, since most studies utilising MRI or CT techniques to assess CSA of the upper arm include brachialis or the anterior compartment instead of focusing on biceps brachii muscle (Deighan et al., 2006; Housh et al., 1992; Kanehisa et al., 1994; Vikne et al., 2006). However, identifying the biceps brachii CSA may be useful. The average biceps brachii CSA value measured at 3 cm (~12.5 cm^2) in the present study was similar to that reported by Kanehisa et al. (1994) who used a specially designed ultrasonic system. McCall et al. (1996) also found similar biceps brachii CSA (11.8 ± 2.7 cm^2) to that of the present study using MRI for men prior to commencing a resistance training program.

The present study found that the biceps brachii CSA assessed by EFOV was smaller than by MRI (Table 1). Noorkoiv et al. (2010) compared the CSA of quadriceps muscles at 10, 20, 30, 40 and 50 % of the length between the centre of the patella and the medial aspect of the anterior superior iliac spine using EFOV and CT, and found that EFOV showed smaller CSA when compared to CT technique at the 10 and 20 % regions. They stated that the reason for the difference between the techniques was due to the different measuring planes used such that the CT measures perpendicular to the measurement table while the EFOV measures perpendicular to the muscle. Ahtiainen et al. (2010) reported that EFOV technique systematically underestimated the CSA of vastus lateralis muscle compared with MRI, as MRI measures in the vertical axis and perpendicularly to the measurement table but EFOV measures perpendicular to the skin. In the present study, it was noted that subjects with smaller biceps brachii CSA had curvier surfaces. In these instances, the EFOV technique was probably more appropriate as the biceps brachii had to be scanned at a slight angle perpendicular to the skin in contrast to the vertically scanned MR images. This could possibly explain why subjects with smaller biceps brachii CSAs had larger absolute CSA differences with the MRI technique (Table 1). Thus, in consideration of the suggestion by Noorkoiv et al. (2010) and the curved nature of the upper arm in the present study, EFOV would be considered as a more accurate estimate of CSA.

The present study showed that the biceps brachii CSA assessed by the EFOV technique was reliable between-traces, between-scans, and between-measures (Tables 2-4). However, it is important to note that measurement errors could easily occur when scanning EFOV images and tracing a biceps brachii muscle on an EFOV image. In the present study, the investigator practiced EFOV scanning technique many times before actually taking images for the present study. It is also important to know how an EFOV image shows a target muscle to be examined. As shown in Figure 2, the shape of muscles shown in the EFOV images is not the same as that shown in the MR images. EFOV uses image registration between sequentially acquired images for motion estimation and constructs a large panoramic image in real time (Weng et al., 1997). The images obtained using EFOV are not as detailed as those taken using MRI or CT, showing only elbow flexor muscle compartment compared to the full cross-sectional images of the upper arm, showing the entire elbow flexors and extensors muscle compartments in MRI and CT slices. Tracing a muscle accurately also requires practice, however, once the technique is established, the error involved in the tracing procedure is small as shown in Table 2. Once scanning technique is established, variation in the CSA between different images from the same site is also minimal as shown in Table 3. The present study showed that the test-retest reliability is high for the measurements taken 1 hour apart, with a CV of only 0.6-0.7 %. Thus, this technique could detect as small as a 1 % change in biceps brachii CSA.

As shown in Figure 6, the biceps brachii CSA increased approximately 10 % immediately after to 4 days following eccentric exercise of the elbow flexors. This is the first study reporting biceps brachii CSA changes using EFOV technique after eccentric exercise. It has been well documented that muscle swelling is induced by eccentric exercise of the elbow flexors, however, previous studies used upper arm circumference measures (Chleboun et al., 1998; Nosaka & Clarkson, 1996; Nosaka & Newton, 2002a) and/or elbow flexors muscle thickness measures by B-mode ultrasound (Nosaka & Newton, 2002a; Nosaka et al., 2002) to quantify the magnitude of swelling. For example, Nosaka et al. (2002) found that upper arm circumference increased by 8.3 mm immediately after 24 repetitions of maximal eccentric exercise, with further increments of up to 14 mm four days after performing the exercise. In contrast, upper arm circumference in the present study showed 4-5 mm (1.3-1.7 %) increase immediately after eccentric exercise, but no continuous increase was recorded as circumference recovered to baseline values at day 4 following exercise. It should be noted that CSA was greater at 3 cm compared with 0 cm site, but it was the opposite for the upper arm circumference. The greater upper arm circumference at the 0 cm site is likely due to the greater inclusion of the triceps brachii. The similar time course in the changes suggests that the cause of the increase is the same, but it should be noted that the magnitude of change was much greater for the CSA (average: 9.1 %) than upper arm circumference (average: 1.1 %).

This may be partially due to the difference in the area change versus length change such that a 10 % change in length could result in a 21 % change in area (e.g. $5.5^2\pi/5^2\pi=1.21$). It is important to note that the changes in CSA and upper arm circumference were poorly correlated. As shown in Figure 7, there are many cases showing no increases in upper arm circumference, even when increases in CSA were evident. It could be that the circumference measure could not detect possible swelling as accurately as CSA. Although limb circumference is generally used to estimate muscle swelling, it cannot discriminate swelling in different muscle compartments within the same limb (Howell et al., 1993; Nosaka & Clarkson, 1996). Imaging of the muscle compartment by MRI, CT or ultrasound could provide a more direct way of assessing muscle swelling (Chleboun et al., 1998). Further study is necessary to investigate the relationship between limb circumference and CSA.

It is concluded that the EFOV technique is a valid and reliable method to measure biceps brachii CSA, and it is possible to detect 1 % CSA change, thus it can be employed in studies examining changes in CSA over time. However, ample practise with scanning and tracing techniques are necessary to obtain accurate results. Muscle swelling after eccentric exercise of the elbow flexors was detected by increases in biceps brachii CSA using EFOV, however CSA changes were not highly correlated with the upper circumference changes. Therefore, to quantify muscle swelling after eccentric exercise it may be better to measure CSA than circumference, as the circumference measure has a greater possible margin of errors and the magnitude of change in upper arm circumference is smaller than that of CSA.

4.2 STUDY 2

The present study tested two hypotheses that 1) 10x3 would induce greater magnitude of muscle damage than 3x10, and 2) the magnitude of the repeated bout effect would be smaller for the condition that 20x3 was performed after 3x10 compared with 20x3 performed after 10x3 (Figure 8). Contrary to the hypotheses, the results showed that 1) no significant differences in the changes in peak torque during eccentric exercise and criterion measures following exercise between 3x10 and 10x3, and 2) no significant difference in the changes in criterion measures after 20x3 between arms (Figure 9-14); suggesting no effect of the different set-repetition configuration on muscle damage and the repeated bout effect. The results also revealed that the changes in the criterion measures were similar between the first and second bouts, regardless of the difference in the set-repetition configuration in the first exercise bout.

All criterion measures returned to baseline before performing the second exercise bout for both arms, showing that four weeks were sufficient for full recovery from the previous eccentric exercise bout. No significant differences in peak torque over 30 eccentric contractions were evident between 3x10 and 10x3 (Figure 1), suggesting that the exercise were performed similarly between 3x10 and 10x3, despite the different set-repetition configurations. The assumption that decreases in force would be smaller for a smaller number of repetitions (i.e., 10x3) compared with a larger number of repetitions in a set (i.e., 3x10) was not supported by the results of the present study. Although the set-repetition configurations were different, the rest period between contractions in a set was the same (9 s) for 3x10 and 10x3. Although the rest period between sets was longer for 3x10 (228s) compared with 10x3 (60 s), it appears that the difference in the duration had limited effect on the torque production, probably because no additional effect of rest between sets was provided by increasing the rest time from 60 s to 228 s.

Changes in any of the muscle strength measures were not significantly different between 3x10 and 10x3 (Figures 9 – 10). This indicates that both bouts of 30 maximal eccentric contractions resulted in similar magnitude muscle damage. Previous studies (Chen et al., 2009; Chen & Hsieh, 2001) reported approximately 40 % decrease in MVC-ISO measured at 90° elbow flexion immediately after performing 3 sets of 10 maximal eccentric contractions for the range of motion from 50° to 180°. The present study also found a comparable decrease in strength loss immediately after 3x10 or 10x3; however, the decrease in MVC-ISO at 4 days post-exercise was smaller for the present study (3x10: 13.8 ± 4.9 %, 10x3: 9.9 ± 3.8 % lower than baseline) compared with previous studies (Chen et al., 2009; Chen & Hsieh, 2001) reporting that MVC-ISO was still 24-37 % lower than baseline. Compared with the previous studies (Chen et al., 2009; Chen & Hsieh, 2001), the decreases in ROM and increases in serum CK activity following 30 maximal eccentric contractions were much smaller in the present study. It is not clear why the 30 maximal eccentric contractions in the present study did not induce as much changes in the criterion measures as those shown in the previous studies, since the eccentric exercise protocols were comparable between the studies. It may be that the subjects recruited in the present study were less susceptible to muscle damage than those recruited in the previous studies (Chen et al., 2009; Chen & Hsieh, 2001), although the present study also recruited "non resistance-trained" men as the previous studies did. It has been shown that "resistance-trained" men show smaller decrease and faster recovery of muscle strength and ROM, and no increases in CK activity in the blood (Newton et al., 2008). A large variability among subjects for their responses to eccentric exercise has been reported, even for "untrained" individuals (Chen, 2006; Chen et al., 2011; Hubal et al., 2005). It might be that the subjects who participated in the present study were more "accustomed" to the eccentric exercise than those in the previous studies.

This is the first study to compare 3x10 and 10x3 for changes in criterion measures with the assumption that a difference in the set-repetition configuration would exist. Contrary to the hypothesis, the present results did not show significant differences in the changes in any of the criterion measures between 3x10 and 10x3. As shown above, peak torque generated over 30 eccentric contractions was similar between 3x10 and 10x3. This seems likely to be a reason for the similar changes in the criterion measures between 3x10 and 10x3. We assumed that less fatigue would be induced when the subjects performed a small number of repetitions in a set (i.e., 10x3) than a large number of repetitions (i.e., 3x10), thus a greater torque production for the former than the later. However, this was not the case for the present study, and no significant difference in the changes of peak torque over 30 contractions was found. The force production during eccentric contractions is the main factor determining the magnitude of muscle damage (Lavender & Nosaka, 2006; Raastad et al., 2010; Smith & Newham, 2007). The similar peak torque output during the exercise between 3x10 and 10x3 (Figure 1) suggests that mechanical stress to the exercised muscles was similar between the two conditions, resulting in a similar magnitude of muscle damage.

When a second bout of 20x3 was repeated 4 weeks after the first bout, no significant differences in most of the criterion measures were found between arms (Figures 9-10 and 12-14) regardless of the difference in the set-repetition configuration in the first bout (3x10 vs. 10 x3). It seems likely that no significant differences in the changes in criterion measures after the first exercise bouts between 3x10 and 10x3 were the reason for no significant differences in the changes in criterion measures after the second bout (20x3) between arms. It is interesting to note that ROM showed a significantly greater decrease immediately after 20x3 for the arm that performed 3x10 for the first bout compared with the arm that performed 10x3 for the first bout (Figure 11). However, the differences were small, and considering the fact that no significant differences between arms were found for other variables, the difference in ROM does not appear to be physiologically significant. It is well documented that when the same or similar eccentric exercise is repeated within a certain period (e.g. 8 weeks), changes in muscle damage markers following the second bout are significantly smaller and recovery is significantly faster compared with the first bout (Lavender & Nosaka, 2008; Nosaka et al., 2005). It should be noted that this was not the case for the present study, and the responses of all criterion measures except ROM were similar between the first (3x10 or 10x3) and second (20x3) bouts. Since the present study did not have an additional group performing 20x3 in the first bout, it is not known how the criterion measures respond following 20x3 when it is performed without the previous exercise bout (i.e., 3x10 or 10x3). Howatson et al. (2007) reported that 10 maximal eccentric contractions of the elbow flexors conferred the same magnitude of protective effect as 45 maximal eccentric contractions against 45 maximal eccentric contractions performed 2 weeks later. If this could have occurred in the present study, we should have seen attenuated changes in the criterion measures following the second bout (60 maximal eccentric contractions; 20x3) compared with the first bout (30 maximal eccentric contractions; 3x10 or 10x3). However, the changes in the dependent variables were similar between the first and second bouts (Figures 2-5). Thus, it appears that the magnitude of repeated bout effect conferred by the first eccentric exercise bout consisting of 30 maximal eccentric contractions against the second eccentric exercise bout consisting of 60 maximal eccentric contractions was not as great as those reported in the previous studies. The reason for this is not clear, but it might be that the relatively small responses to the first bout are associated with the attenuated repeated bout effect as discussed below.

Falvo et al. (2007) investigated the responses of resistance-trained subjects to repeated bouts of a bench press exercise (10 sets of 10 repetitions using a load of 70 % of concentric one repetition maximum) separated by 2 weeks. They reported that muscle soreness was reduced in the second bout; however, no significant differences in the changes in maximal isometric strength, rate of force development and dynamic bench press throw performance were observed between bouts, suggesting that the magnitude of repeated bout effect in resistance-trained individuals is limited. In the following study, Falvo et al. (2009) found similar results to the first study using the same exercise performed by resistance-trained subjects, and also showed that changes in serum CK activity were similar between bouts. Although the present study used non resistance-trained men as subjects, the changes in the criterion measures following the first eccentric exercise bout were relatively smaller compared with those shown in the previous studies as discussed above. Thus, it might be that the responses of the subjects in the present study to the repeated bouts of eccentric exercise were similar to those shown in the studies by Falvo et al. (2009). It is interesting to investigate further how "resistance-trained" individuals respond to the repeated bout protocol that was used in the present study (i.e., 3x10 or 10x3 for the first bout followed by 20x3).

The present study utilised a new method to assess muscle swelling by measuring muscle CSA using B-mode ultrasonography with EFOV imaging. Previous studies reported greater increases in upper arm circumference 3 to 4 days than 1 to 2 days following eccentric exercise of the elbow flexors (Chen & Nosaka, 2006; Chleboun et al., 1998; Nosaka et al., 2001). However, further increases in CSA were not observed 2-4 days post-exercise in the present study. It should be noted that other muscle groups such as brachialis and triceps brachii were included in the circumference measures of the previous studies (Chen & Nosaka, 2006; Chleboun et al., 1998; Nosaka et al., 2001), but the swelling information was solely based on biceps brachii CSA in the present study, because of the difficulty in obtaining whole cross section images of the brachialis and triceps brachii by the EFOV method. Thus, it is possible that muscle swelling information is different between biceps brachii CSA and upper arm circumference measures. As discussed above, the magnitude of muscle damage in the present study was smaller than that of the previous studies (Chen & Nosaka, 2006; Nosaka et al., 2001). Further studies are necessary to assess biceps brachii CSA changes following eccentric exercise that results in greater muscle damage than that of the present study.

In resistance training, the number of repetitions can be classified into 3 ranges, low (1-5), moderate (6-12) and high (15+) (Baechle et al., 2008). Low repetitions with high loads are generally prescribed to increase strength and power, and moderate repetitions with high loads are used to increase muscle size (Baechle et al., 2008; Ratamess et al., 2009; Schoenfeld, 2010). Regarding set-repetition configurations, multiple sets were shown to be more superior in inducing muscle strength and hypertrophy to single-set configurations (Baechle et al., 2008). However, it does not appear that set-repetition configurations with a very large number of sets and a small number of contractions that was used in the present study (e.g. 10x3, 20x3) are seldom used in an actual training. Thus, the alteration of the set-repetition configuration from the first to the second bout in the present study is considered as an extreme case. Since the extreme case did not show any significant effect on muscle damage profile, it does not appear that changing set-repetition configuration is not a way to minimise the repeated bout effect, if muscle damage should be maximised in training.

Muscle damage is anecdotally documented to be necessary for muscle hypertrophy. Several studies have reported the superiority of eccentric training to concentric training for muscle hypertrophy (Farthing & Chilibeck, 2003; Hortobagyi et al., 2000; Vikne et al., 2006). For example, Farthing and Chilibeck (2003) demonstrated that eccentric training of elbow flexors at fast ($180^{\circ}\cdot s^{-1}$) and slow ($30^{\circ}\cdot s^{-1}$) velocity resulted in significant increases in muscle thickness (13 % and 7.8 %, respectively) compared to concentric training at fast ($180^{\circ}\cdot s^{-1}$) and slow ($30^{\circ}\cdot s^{-1}$) velocities (5.3 % and 2.6 %, respectively). Vikne et al. (2006) showed that eccentric training (2-3 times a week over 12 weeks) increased cross sectional area of the elbow flexors (11 %) and their muscle fibre areas (41 %) significantly more than concentric training (3 % and 4 %, respectively). It is widely accepted that higher forces associated with eccentric training could have greater potential to induce muscle hypertrophy compared with other contractions modes (Adams et al., 2004; Farthing & Chilibeck, 2003). Martineau and Gardiner (2001) demonstrated that the increases in mitogen-activated protein kinase (MAPK), and p54 c-Jun NH2-terminal kinase and p44 extracellular regulated kinase phosphorylation were greater after eccentric contractions compared with isometric and concentric contractions. The rate of protein synthesis mediated by activations of protein kinase B (Akt), mammalian target of rapamycin (mTOR), and p70 S6 kinase ($p70^{s6k}$), and the Akt/mTOR/$p70^{s6k}$ pathway are known to be involved in exercise-induced muscle hypertrophy (Atherton et al., 2005; Bolster et al., 2003). Eliasson et al. (2006) reported that maximal eccentric contractions of the knee extensors activated $p70^{s6k}$ in the vastus lateralis, but maximal concentric contractions did not, suggesting that maximal eccentric contractions were more effective than maximal concentric contractions in stimulating protein synthesis. Thus, it is possible that muscle damage induced by maximal eccentric contractions could also contribute to the greater muscle hypertrophy induced by eccentric training compared with concentric training. It is also known that satellite cell activation and proliferation are necessary for muscle hypertrophy (Charge & Rudnicki, 2004), and muscle damage activates satellite cells (Crameri et al., 2004; Toth et al., 2011). However, these do not necessary mean that muscle damage is necessary for muscle hypertrophy (Nosaka et al., 2003). It is important to note that although muscle damage is attenuated in progressive training, muscle hypertrophy is being induced. There is no doubt that eccentric contractions could stimulate muscle hypertrophic responses, but it is necessary to distinguish the superiority of eccentric contractions on muscle hypertrophy from muscle damage. Further research is required to investigate whether muscle damage is necessary for muscle hypertrophy.

In conclusion, the present study showed that manipulating the set-repetition configuration of a single bout of maximal eccentric exercise of the elbow flexors (3x10 and 10x3) did not affect the changes in indirect muscle damage markers and protective effect. However, most of the criterion measures except ROM showed no significant differences between the first and second bouts, implying that the repeated bout effect was attenuated by increasing the number of maximal eccentric contractions from the first to the second bout.

Future studies should investigate whether altering the set-repetition configuration over a longer period of time (e.g. > 8 weeks) would result in greater increases in muscle function and size compared with a monotonous training scheme (repeating the same exercise over a period of time), how it is possible to induce greater muscle damage by attenuating the repeated bout effect, and whether such strategy if any could produce better outcomes in resistance training.

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Appendix A

Extended-Field-Of-View Advertisement

SCHOOL OF EXERCISE, BIOMEDICAL AND HEALTH SCIENCES
Exercise and Sports Science

AUSTRALIA ECU EDITH COWAN UNIVERSITY

Free ultrasound scans!!!

- Are you interested in knowing more about ultrasound?
- Do you know how your **MUSCLES** looks like underneath your skin?
- Are you:- Male aged 18 – 35 years?
  Generally inactive?
  Free from muscles and skeletal injuries of your upper body?
  Not currently undertaking any form of nutrition supplementations?

IF YES!!!

You are invited to participate in an ultrasound study investigating the validity and user reliability of a new ultrasound measure (Extended-field-of-view) of muscle cross-sectional area.

Contact: Roy 0411738793 (r.chan@ecu.edu.au)

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Appendix B

Eccentric exercise Advertisement

SCHOOL OF EXERCISE, BIOMEDICAL AND HEALTH SCIENCES
Exercise and Sports Science

AUSTRALIA ECU EDITH COWAN UNIVERSITY

MUSCLE HYPERTROPHY

- Are you interested in **FREE RESISTANCE EXERCISE** to build **MUSCLES**?
- Do you believe that **MUSCLE SORENESS** is a result of **MUSCLE DAMAGE**?
- Do you believe that **MUSCLE SORENESS** allows your **MUSCLES** to grow **BIGGER**?
- Are you:- Male aged 18 – 35 years?
  Generally inactive?
  Free from muscles and skeletal injuries of your upper body?
  Not currently undertaking any form of nutrition supplementations?

IF YES!!!

You are invited to participate in a study comparing between two different configurations (3 x 10, 20 x 3 vs. 10 x 3, 20 x 3) for changes in markers of muscle damage and muscle size.

Contact: Roy 0411738793 (r.chan@ecu.edu.au)

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Appendix C

Extended Field Of View Information Letter

Information Letter to Participants

(Reliability Study)

Thank you very much for indicating your interest in participating in this study. The purpose of this document is to explain the study that you are going to participate. Please read carefully and understand the information below, and do not hesitate to ask any questions.

Project Title

Reliability of Extended-Field-Of-View Ultrasonographic Measurement of Elbow Flexor Muscle Cross Sectional Area

Researchers

This research project is being undertaken as part of the requirements of a Masters by Research (Sports Science) at Edith Cowan University (ECU).

MSc Student: Roy Chan (r.chan@ecu.edu.au) 6304 5152

Supervisor: Prof. Ken Nosaka (k.nosaka@ecu.edu.au) 6304 5655

Co-Supervisor: Dr. Mike Newton (m.newton@ecu.edu.au) 6304 5961

Further details on supervisors and School of Exercise, Biomedical and Health Sciences are available at http://www.sebhs.ecu.edu.au .

Purpose of the study

This study will investigate whether a new ultrasonographic measurement of elbow flexor muscle cross-sectional area (CSA) is accurate when it is repeated by the same researcher within a day and week.

Eligibility

You will be eligible for this study if your age is between 18 and 35, and you have no musculoskeletal injuries of the upper extremities, and no metal objects implanted within your body (e.g. pacemakers, braces, cochlear implants). 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.

Requirements

You will be asked to come to the Exercise Physiology Laboratory (JO 19.150) for an ultrasound session. Each session will last approximately 60 minutes. Covered shoes and a sleeveless top are required for all sessions.

Measurements

Height and weight will be measured prior to testing on the first testing occasion. The first session will include an introduction session where the following will be conducted:

- Length of upper arm (determined from acromion of the scapula to lateral epicondyle of the humerus).
- Marking out of the upper arm for ultrasound measures using a semi-permanent ink marker on three sites:
    - Midpoint of upper arm
    - 3cm and 6cm below midpoint of upper arm

In the session, the CSA of the bicep brachii will be measured twice by an ultrasound machine on the marked sites of both arms, which is achieved by manually moving the probe along the lines of the marked sites thereby creating a continuous single view, which is called extended-field-of-view. You will be seated on a preacher curl bench with your non-dominant arm fully extended on the cushion of the bench. Transmission gel will be applied on the probe and the marked sites to aid in obtaining clearer images. Light pressure will be applied to the muscles.

Risks

- Ultrasound scans are relatively safe; however some discomfort may be felt by keeping the same position during the session.

Benefits

- You will be able to experience a new method of ultrasound measurement for muscle cross sectional area and understand how ultrasonography is being utilised in a study
- You will be able to find out about the cross-sectional area of your arms

Confidentiality of Information

All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by 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 this 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. Hard copy data (paper etc) 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.

Results of the Research Study

The results of this study are intended for completion of a Masters by Research thesis and may be presented in conferences/seminars and published in peer-reviewed journal(s), as magazine 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 can be obtained from the investigator upon request.

Voluntary Participation

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose not to participate. Your decision if you do not want to participate or continue to participate will not disadvantage you or involve any penalty.

Withdrawing Consent to Participate

You are free to withdraw your consent to further involvement in this research project at any time. You also have the right to withdraw any personal information that has been collected during the research with your withdrawal.

Questions and/or Further Information

If you have any questions or require any further information about the research project, please do not hesitate to contact:

Roy Chan
Office 19.127, School of Exercise, Biomedical, and Health Sciences, Edith Cowan University
100 Joondalup Drive, Joondalup, WA 6027 Australia.
Mobile: 0411 738 793
Email: r.chan@ecu.edu.au

Independent Contact Person

If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:

Kim Gifkins (Research Ethics Officer)

Building 1, Block 'B', Level 3, Room 333, Edith Cowan University, 100 Joondalup Drive, JOONDALUP WA 6027

Phone: (+61 8) 6304 2170

Email: research.ethics@ecu.edu.au

Website: http://www.ecu.edu.au/GPPS/ethics

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.

Informed Consent Form

Project: Reliability of Extended-Field-Of-View Ultrasonographic Measurement of Elbow Flexor Muscle Cross Sectional Area

- You do not have any problems participating in the study
- You will report to the laboratory 1 day
- Your arms will be marked on 3 sites stated above and two readings will be obtained from the ultrasound assessment of each site

I have read the information sheet and the consent form. I agree to participate in this study with the above title and give my consent freely. I understand that the study will be carried out as described in the information sheet, a copy of which I have retained. I realise that whether or not I decide to participate is my decision. I also realize that I can withdraw from the study at any time and that I do not have to give any reasons for withdrawing. I have had all questions answered to my satisfaction.

Name: ______________________________________ Date: ____________________

Signature: __________________________________

Appendix D

Eccentric exercise Information letter

Information Letter to Participants

Thank you very much for indicating your interest in participating in this study. The purpose of this document is to explain the study that you are going to participate. Please read carefully and understand the information below, and do not hesitate to ask any questions.

Project Title

Comparison of two different set-repetition configurations (3 sets x 10 reps, 20 sets of 3 reps vs. 10 sets x 3 reps, 20 set of 3 reps) for changes in repeated bout effect and markers of muscle damage

Researchers

This research project is being undertaken as part of the requirements of a Masters by Research (Sports Science) at Edith Cowan University (ECU).

MSc. Student: Roy Chan (r.chan@ecu.edu.au) 6304 5152

Supervisor: Prof. Ken Nosaka (k.nosaka@ecu.edu.au) 6304 5655

Co-Supervisor: Dr. Mike Newton (m.newton@ecu.edu.au) 6304 5961

Further details on supervisors and School of Exercise, Biomedical and Health Sciences are available at http://www.sebhs.ecu.edu.au .

Purpose of the study

We are interested in examining potential difference in muscle damage between two resistance exercise protocols consisting of the same number of total eccentric (lengthening) contractions but different set and repetition relationship (e.g. 3 sets of 10 repetitions, 20 sets of 3 repetitions versus 10 sets of 3 repetitions, 20 sets of 3 repetitions).

Eligibility

You will be eligible for this study if your age is between 18 and 50, 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.

Requirements

You will be required to complete one familiarisation session at least one week before the first exercise session. You will be asked to come to the Exercise Physiology Laboratory (JO 19.150) on 20 days (4 blocks of 5 days) over 7 weeks (1 week break between blocks). For each block, you will be asked to perform an arm exercise on the first day followed by 4 consecutive measurement days. Each exercise session will last approximately 60 minutes and each measurement session will take approximately 45 minutes. Covered shoes and a sleeveless top are required for all sessions. You will be requested not to perform any strenuous exercise apart from the exercise performed in the study, and maintain similar diet throughout the three weeks.

Familiarisation session

Height and weight will be measured during the familiarisation session. Your arm strength will be measured by maximally flexing your elbow on a dynamometer whilst your elbow joint angle is set at 60º, where 0º refers to the arm being completely extended. All of the other measurements and the exercise that you will be performing will be demonstrated.

Exercise

You will perform either 3 sets of 10 maximal eccentric or 10 sets of 3 maximal eccentric (lengthening) contractions of the elbow flexors (upper arm) as the Bout 1 and the latter as Bout 2. 20 sets of 3 maximal eccentric (lengthening) contractions of the elbow flexors will be subsequently performed on each arm as Bout 3 and Bout 4 with a week rest in between each exercise bout. The researcher will determine which exercise will be performed first and which arm will be used for the first exercise. Each exercise bout will be separated by two weeks. In each eccentric contraction, your elbow joint will be forcibly extended by the dynamometer under maximal resistance from an elbow flexed (90º) to a full-extended position (0º) at a slow velocity (angular velocity of $30°\cdot s^{-1}$). After each contraction, the elbow joint will be passively returned to the flexed position slowly (at a velocity of $10°\cdot sec^{-1}$) allowing approximately 9 seconds rest between contractions. The rest times between sets are 228 seconds for 3 sets of 10 repetitions and 60 seconds for 10 sets of 3 repetitions.

Measurements

Immediately before and after exercise, and 1-4 days following exercise, the following measurements will be taken from the exercised arm.

- Maximal shortening (concentric) strength: You will be asked to flex your bicep maximally at an elbow joint angle torque of 30°/s and 210°/s on the dynamometer twice with a 30 seconds rest between efforts.
- Maximal static (isometric) strength: You will be asked to flex your elbow maximally at an elbow joint angle of 60° and 90° on the dynamometer twice with a 30 seconds rest between efforts.
- Muscle soreness: You will be asked to rate the level of muscle soreness felt upon flexing and extending the elbow joint throughout a full range of motion by yourself and upon palpation by the researcher on a 100-mm line, anchoring 0 for no soreness and 100 for maximal soreness, on 2 sites including the mid point of the upper arm and 3cm below the mid point of the upper arm.
- Pain pressure threshold (PPT): A device (algometer) to detect pressure will be used to measure pain in the exercised arm by the researcher on the same sites mentioned in muscle soreness. You will report to the researcher when you start to feel pain in your exercised arm.
- Range of motion (ROM): You will be standing with arms relaxed by your side with palms facing forward 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 maximally without moving the elbow.
- Upper arm circumference: You will be standing with arms relaxed by your side in a neutral position (palms facing the thigh). The upper arm circumference will be measured using a constant tension tape at 2 different sites (midpoint of the upper arm and 3cm below the midpoint of the upper arm) marked by a semi-permanent ink marker.
- Ultrasound: Ultrasound images will be taken from your arm to assess muscle thickness on the exercised arm at the same 2 sites mentioned in upper arm circumference. From the ultrasound image, the brightness of the echo-signal (echo intensity) will also be analysed by using a computer software program thereafter.
- Blood: A small amount of blood sample (30 μl) will be taken from your finger before exercise, and 2 and 4 days following exercise to measure blood marker of muscle damage.

Risks

- You will experience muscle soreness and discomfort for several days after exercise; however, this is normal after performing eccentric exercise, and they will disappear within a week.
- You will experience weakness in the exercised arm, which may affect daily activities (e.g. carrying heavy items, washing your face, driving) and may lead to injuries (e.g. dropping heavy items onto your foot as you are unable to handle the weight), so care must be taken and you should be aware that your arm is weaker than normal for a while before your arm completely recovers, which may take up to a week.
- After blood sampling, though rare, but it is minutely possible that your wound from the finger prick might get infected however band-aids are always provided for you to minimise the risk of infection.
- You might encounter a minor possibility of infection should your finger comes into contact with contaminated items however band aids should always be applied if you need to handle contaminated items.

Benefits

- You will be able to improve your knowledge about resistance training
- You will be able to know how strong your arm is
- You will be able to understand the various markers of muscle damage and comprehend how they are being utilised in research
- You will have better understanding on laboratory research procedures and techniques
- The background information about the study will be explained to you upon request
- The results of the study will be provided to you upon request

Confidentiality of Information

All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by 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 this 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. Hard copy data (paper etc) 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.

Results of the Research Study

The results of this study are intended for completion of a Masters by Research thesis and may be presented in conferences/seminars and published in peer-reviewed journal(s), as magazine 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 request.

Voluntary Participation

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose not to participate. Your decision if you do not want to participate or continue to participate will not disadvantage you or involve any penalty.

Withdrawing Consent to Participate

You are free to withdraw your consent to further involvement in this research project at any time. You also have the right to withdraw any personal information that has been collected during the research with your withdrawal.

Questions and/or Further Information

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

Roy Chan

Office 19.127, School of Exercise, Biomedical, and Health Sciences, Edith Cowan University

270 Joondalup Drive, Joondalup, WA 6027 Australia.

Mobile: 0411 738 793

Email: r.chan@ecu.edu.au

Independent Contact Person

If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:

Kim Gifkins (Research Ethics Officer)

Building 1, Block 'B', Level 3, Room 333, Edith Cowan University, 100 Joondalup Drive, JOONDALUP WA 6027

Phone: (+61 8) 6304 2170

Email: research.ethics@ecu.edu.au

Website: http://www.ecu.edu.au/GPPS/ethics

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.

Informed Consent Form

Project: Comparison of 2 different set-repetition configurations (3 sets x 10 reps, 20 sets x 3 reps vs. 10 sets x 3reps, 20 sets x 3 reps) for changes in repeated bout effects and markers of muscle damage

Please make sure;

- You do not have any problems participating in the study
- You will attend a familiarisation session before commencement of the study
- You will report to the laboratory 20 days in total over 7 weeks, 4 blocks of 5 consecutive days with one week between blocks
- You will perform maximal exercise of both arms twice using a different arm for each occasion
- Several measurements will be taken before, immediately after, and 1-4 days following exercise
- Blood sample will be taken from your finger before and 2 and 4 days after exercise
- You will experience muscle soreness, weakness, and discomfort for several days after exercise

I have read the information sheet and understood the points in the informed consent form. I agree to participate in this study with the above title and give my consent freely. I understand that the study will be carried out as described in the information sheet, a copy of which I have retained. I realise that whether or not I decide to participate is my decision. I also realize that I can withdraw from the study at any time and that I do not have to give any reasons for withdrawing. I have had all questions answered to my satisfaction.

Name: ____________________________________ Date: ___________________

Signature: _________________________________

Appendix E

Informed Consent

Informed Consent Form

This consent form confirms that subjects have not overlooked any important information in the Medical Checklist, Information Letters and that they fully understood the expectations of this study. This consent form also confirms that all information provided by the subjects is genuine.

1. Are you aware that, although very rare, maximal exercise can result in fainting, severe exhaustion or cardiac events leading to death?	YES / NO
2. Are you aware that the fatigue caused by the exercise can impair your ability to perform tasks such as driving for a short while after the cessation of exercise?	YES / NO
3. Have you been given the opportunity to experience maximal exercise testing techniques?	YES / NO
4. Are you aware that your finger will be pricked and a small sample of blood will be collected for analysis?	YES / NO
5. Are all information provided by you genuine and/or to the best of your knowledge?	YES / NO

Name of Participant: ____

Signature of Participant: _________________ Date: _________________________

Name of Witness: ____

Signature of Witness: ___________________ Date: _________________________

Appendix F

Medical Questionnaire

Medical Questionnaire

The following questionnaire is designed to establish a 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 any thing 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 a testing or training session, a clearance from a qualified medical practitioner may be required prior to participation.

Personal Details

Name:____

Date of Birth (DD/MM/YYYY):_________________ 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	______________________
Asthma	Y	N	______________________
Diabetes	Y	N	______________________
Epilepsy	Y	N	______________________
Recurring back pain	Y	N	______________________
			If YES, please provide details
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 taking any medications? Y N________________________

Have you had flu in the last two weeks? Y N________________________

Have you recently injured yourself? Y N________________________

Do you have any recurring muscle
or joint injuries? Y N________________________

Is there any other condition not previously
mentioned which may affect your ability to perform exercise?
Y N________________________

Lifestyle Habits (some questions may not relate to minors)

Do you exercise regularly? Y N
If YES, what do you do?

____

____

How many hours per week?

____

Do you smoke tobacco? Y N
If YES, how much per day?

____

Do you consume alcohol? Y N
If YES, how much per week?

____

Do you consume tea or coffee? Y N
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/ consent form provided to my patient Mr/Miss/ Ms____________________________________, and clear him/ her medically for involvement in exercise testing.

Date (DD/MM/YYYY):________________________

Signature:____________________________________

Appendix G

Visual Analogue Scale

Visual Analogue Scale

Subject:______________ **Bout:_____________** **Exercised arm: L R**

Date:______________ **Time:____________**

Using the visual analog scale provided, please report the extent of your muscle soreness while your upper arm and forearm are being palpated, and while the elbow joint is being extended and flexed by the investigator. The zero on the visual analog scale represents "no pain" while the 100 at the other end of the line represents "unbearably painful". The 100 should be associated with the worst pain that you have ever experienced.

Pre D1 D2 D3 D4 D5 D6 D7

Upper arm Palpation

Palpation 1 (Mid) 0____100

Palpation 2 (30%) 0____100

Palpation 3 (60%) 0____100

Palpation 4 (Br) 0____100

Palpation 5 (B) 0____100

Palpation 1 (Mid) 0____100

Palpation 2 (30%) 0____100

Palpation 3 (60%) 0____100

Palpation 4 (Br) 0____100

Palpation 5 (B) 0____100

Flexion and Extension

Extension 0____100

Flexion 0____100

Extension 0____100

Flexion 0____100

Upper arm Pressure Pain Threshold

Palpation 1 (Mid) __________

Palpation 2 (30%) __________

Palpation 3 (60%) __________

Palpation 4 (Br) __________

Palpation 5 (B) __________

Palpation 1 (Mid) __________

Palpation 2 (30%) __________

Palpation 3 (60%) __________

Palpation 4 (Br) __________

Palpation 5 (B) __________

Appendix H

Ethics Approval

Dear Roy

Project Number: 4537

Project Name: Influence of Eccentric Training Variation on Muscle Damage and Adaptation of the Elbow Flexors

Ethics approval for your research project was granted from 10 December 2009 to 31 December 2010.

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 completion of the project.

A **FINAL REPORT** is due on **31 December 2010**.

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 Ms Kim GIFKINS.

Note that ethics approval is required for both the collection and use (analysis) of data. If the project is still continuing, please complete the form and apply for an extension of ethics approval.