Muscle damage and the repeated bout effect in male bodybuilders following unaccustomed resistance exercise

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Muscle Damage and The Repeated Bout Effect in Male Bodybuilders Following Unaccustomed Resistance Exercise

This thesis is presented in partial fulfilment of the degree of Master of Science (Exercise & Sports Science) via Research

Kennedy Blowfield

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School of Medical & Health Sciences
2016
Declaration

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

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Date: 12/12/2016
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ABSTRACT

This study investigated muscle damage of bodybuilders who performed an unaccustomed intense resistive exercise bout. Ten male bodybuilders (age: 23 ± 2 y, height: 177.6 ± 6.3 cm, body mass: 85.7 ± 8.1 kg) with a minimum of 4-years resistance training experience performed 17 exercises targeting the pectoral muscles. Eight of the 10 bodybuilders repeated the same exercises two weeks later. Muscle function (bench press throw, maximal isokinetic elbow extension and flexion concentric torque: MVC torque), muscle soreness using a visual analogue scale (VAS: 100-mm), and plasma creatine kinase (CK) activity were assessed before, immediately after and 24, 48 and 72 hours after exercise. Bench press throw peak force decreased immediately (23 ± 20%) and 24 hours after the first exercise bout (9 ± 15%), but, returned to baseline (930 ± 129 N) by 48 hours post-exercise (P<0.05). MVC torque also decreased but, returned to baseline by 48 hours post-exercise. Muscle soreness peaked 24 hours post-exercise (57 ± 22 mm). No significant changes in plasma CK activity were evident after the exercise at any corresponding time point. When compared between the first and second bouts, changes in bench press throw parameters and MVC torque were similar, but muscle soreness was less (P<0.05) after the second (47 ± 26 mm) than the first bout (68 ± 27 mm). Similarly, the magnitude of volume performed was significantly greater in the second bout (11,433 ± 674 kg) as opposed to the first (10,384 ± 670 kg) due to the repetitions to volition failure (P<0.001). It is concluded that bodybuilders still experience minor loss of muscle function lasting for 2 days after unaccustomed exercise targeting the chest muscles, but muscle soreness was relatively severe, and no repeated bout effect was evident for muscle function measures, but minor repeated bout effect was evident for muscle soreness. This may be a due to potential limitations of continual adaptations.
CHAPTER 1 - INTRODUCTION

Muscle damage is induced by unaccustomed exercise, predominantly in which emphasises eccentric (lengthening) contractions. Muscle damage can still arise from other forms of contractions such as isometric and concentric, however the magnitude of muscle damage is greatest following unaccustomed eccentric exercise and, is characterised by a prolonged reduction in muscle force generating ability and delayed onset muscle soreness (DOMS) (McHugh 2003; Nosaka and Aoki 2011; Pyne 1993). Other common indirect markers of skeletal muscle damage include, increases in intramuscular proteins such as, creatine kinase (CK) in the blood, localized muscle swelling, increased muscle stiffness and abnormalities in B-mode ultrasonography and/or magnetic resonance imaging (Belcastro et al. 1998; Clarkson and Hubal 2002). Changes in these markers of muscle damage are attenuated when the same or similar exercise is repeated within several weeks, and this is referred to as the repeated bout effect (Clarkson and Hubal 2002; Ebbeling and Clarkson 1989; Nosaka 2011; Nosaka and Aoki 2011). Whilst the repeated bout effect is well documented, the mechanistic processes underpinning this phenomenon have not yet been fully elucidated, however it is generally believed that it is related to a combination of neural, mechanical and cellular adaptations proposed by McHugh et al., (1999) and McHugh, (2003) (McHugh 2003; McHugh et al. 1999) (McHugh 2003; McHugh et al. 1999) (McHugh 2003; McHugh et al. 1999) (McHugh 2003; McHugh et al. 1999) (McHugh 2003; McHugh et al. 1999).

Similar to the repeated bout effect, previous studies have reported that the magnitude of muscle damage induced by maximal eccentric contractions is reduced by prior exposure to low-intensity eccentric contractions (Chen et al. 2012a; Chen et al. 2010) or maximal isometric contractions at a long or extended muscle length (Chen et al. 2012b; Chen et al. 2013). Since these contractions are often performed during resistance training, it can be presumed that individuals who are resistance trained (i.e. perform regular resistance exercise) are less susceptible to eccentric exercise-induced muscle damage. Over 400 articles on exercise-
induced muscle damage have been published, however, only a small number of studies have investigated muscle damage characteristics and the repeated bout effect of resistance-trained individuals. To the best of the author’s knowledge, only nine studies (Bloomer et al. 2007; Falvo et al. 2009; Falvo et al. 2007; Howatson et al. 2007; Howatson and van Someren 2007; Meneghel et al. 2014; Newton et al. 2008; Soares et al. 2015; Staron et al. 1991) have reported resistance-trained individuals’ responses to resistance exercise potentially inducing skeletal muscle damage, and 4 of them (Bloomer et al. 2007; Falvo et al. 2009; Falvo et al. 2007; Howatson et al. 2007; Meneghel et al. 2014) have included the investigation of the repeated bout effect.

After critically evaluating the scientific literature, only Newton et al., (2008) performed a comprehensive comparison of the impact of training status on the response to eccentric-exercise induced muscle damage. Specifically, Newton et al. (2008) compared resistance-trained (average of 8 y resistance training experience) and untrained men for changes in maximal voluntary isometric and concentric contraction (MVC) torque of the elbow flexors, range of motion of the elbow joint (ROM), upper arm circumference, plasma CK activity and muscle soreness after 60 maximal voluntary eccentric contractions of the elbow flexors. The authors reported that changes in all measures except muscle soreness (peak muscle soreness was similar between groups) were significantly smaller for the trained than untrained group. Additionally, MVC torque of the trained group returned to baseline by 3 days after exercise, whereas the untrained expressed ~40% reduction in MVC torque at the same corresponding time point. Using resistance-trained men, with an average of 5 years’ experience, Bloomer et al. (2007) reported decreases in isometric bench press strength (immediately: -36% and 1 day post-exercise: -24%), dynamic bench press force (immediately: -10% and 1 day post exercise: -10%) and peak velocity in the bench press throw (immediately: -10% and 1 day post-exercise: -3%) after a bench press exercise (10 sets of 10 repetitions at 70% of bench press one repetition maximum [1RM]), but all of these values returned to baseline by 2 days post-exercise. One
possible explanation as to why the time course of strength recovery after exercise was different between the Newton et al. (2008) and the Bloomer et al. (2007) studies might have been the exercises performed and/or muscles innervated. Newton et al. (2008) utilized a single joint exercise (i.e. biceps curl) for their exercise intervention (biceps emphasis), whilst Bloomer et al. (2007) used a multi-joint exercise (i.e. bench press, pectoral emphasis). The impact of exercise selection or exercise type on the time course of strength recovery after eccentric exercise in resistance-trained men (6 years resistance training experience) was recently investigated by Soares et al. (2015). The researchers observed that MVC strength returned to baseline 1-day after the performance of a multi-joint exercise (i.e. seated row), whereas the return to baseline of a single joint exercise (i.e. biceps curls) was extended to 2-days post-exercise. Based upon these findings it appears that resistance-trained individuals have the ability to recover from strenuous exercise within 2 – 3 days. However, to the author’s knowledge, no previous studies have examined the impact on the time course of MVC recovery of a multifaceted training session which incorporates both single and multi-joint resistance exercise typically used in contemporary training practices.

Whilst examining the scientific literature there seems to be conflicting findings regarding the magnitude of training status and the repeated bout effect in the resistance-trained populations. Howatson et al. (2007a) investigated 16-resistance trained males to identify if 10 maximal eccentric contractions and 45 maximal eccentric contractions conferred protection of a secondary bout of 45 maximal eccentric contractions performed 2-weeks later. The investigators observed protection from both 10 maximal and 45 maximal eccentric contractions performed 2-weeks later for MVC, ROM, soreness CK and median frequency (P<0.01). The authors observed the repeated bout effect. However, Falvo et al. (2007, 2009) compared an initial and secondary bout of 100 eccentric contractions of the bench press at 70% concentric one repetition maximal (1RM) separated by 2 weeks on 15 and 11 resistance trained males, and observed no significant differences in the changes in any muscle function measures between
bouts, but -40% reduction in peak muscle soreness after the second bout when compared with the first bout. In contrast, Meneghel et al. (2014) used 8 resistance-trained men with more than 2-years of resistance training experience and showed that changes in 1RM strength, serum CK activity and muscle soreness after the second bout were -44%, -52% and -55% smaller, respectively when compared with the initial bout. The authors stated that the repeated bout effect was present in resistance trained men, and that the intensity (70% eccentric [ECC] 1RM) of the initial bout played a major role in observing the repeated bout effect in resistance-trained individuals as compared to the studies by Falvo et al. (2007, 2009) who used lower intensity eccentric contractions (70% concentric [CON] 1RM).

It should be noted that to the best of the author’s knowledge there are no known studies that have investigated the occurrence of muscle damage and the repeated bout effect in bodybuilders. Since, bodybuilders regularly engage in large volumes of exercise designed to maximise muscle hypertrophy it is possible that specific muscular adaptations may have occurred to this population from their regular style of training, much like strength athletes and endurance athletes develop specific intramuscular traits and characteristics, it is plausible to assume that muscle damage characteristics in bodybuilders may differ from other resistance trained population. Thus, the study is warranted to systematically examine the impact of an intense exercise session on indirect markers of muscle damage (e.g. DOMS, loss of muscle function), and the repeated bout effects. There is anecdotal evidence to suggest bodybuilders experience DOMS, however the actual time course of muscle function recovery after unaccustomed resistance training is largely unknown. Thus, clarification is required to deduce whether or not bodybuilders experience soreness and muscle damage and the time course of recovery.

Therefore, the aim of the present study was to investigate the extent of muscle damage that occurs to natural competitive male bodybuilders after one bout of unaccustomed resistance exercise targeting the pectoral muscles. The secondary aim was to compare between the first
and second bouts for changes in indirect markers of muscle damage in the bodybuilders. It was hypothesised that changes in markers of muscle damage would be small in bodybuilders and that the muscle function of the bodybuilders would recover back to baseline within 3 days after exercise, but DOMS may persist for 3 days’ post-exercise. In the repeated bout, recovery of muscle function was expected to be faster and muscle soreness would be smaller after the second than the first bout, thus the repeated bout effect would be present in male bodybuilders.
CHAPTER 2 - METHODS

Participants

The sample size was estimated using G*Power 3.1 by setting $\alpha = 0.05$, $\beta = 0.8$ and the effect size as 1 for possible changes in peak force in the bench press throw. The present study incorporated a similar power analyses to that of a previous study (Meneghel et al. 2014). It showed at least 8 participants were required, thus 10 natural male competitive bodybuilders (age: $23 \pm 2$ y, height: $177.6 \pm 6.3$ cm, body mass: $85.7 \pm 8.1$ kg) with a minimum of 4-years (range: 4 - 9 years, average: $6 \pm 2$ years) bodybuilding training experience were recruited for this study. All of them had participated in a natural bodybuilding competition within 6 months prior to the participation of the present study, and claimed that they had never consumed any substances that are prohibited for bodybuilding by the World Anti-Doping Association (WADA). A local natural bodybuilding association confirmed their participations in the competition organised by the association.

Ethical approval was obtained from the Edith Cowan University Human Research Ethics Committee prior to the commencement of the study (please see the Appendix B). Participants retained the right to withdraw from the study for any reason without prejudice. They were informed of the experimental protocol before commencement and signed the informed consent form, then completed the health and medical questionnaire (please see the Appendix C - F). They were instructed to continue their normal dietary habits during the study period, and to record a 24-hour food diary (see Appendix G) 1 day prior to the first exercise session, and this was used to replicate their nutrition intake before the second exercise bout. The participants reported to the laboratory at the same time of the day for each session to avoid circadian or diurnal influences.
Exclusion Criteria

All bodybuilders were required to reframe from directly targeting the pectoral muscle group for the duration of the study. It was also clearly stipulated that any analgesics were not to be consumed and no banned substances in accordance to WADA and ASADA were to be consumed during the study.

Experimental procedure

To investigate changes in indirect markers of muscle damage (as detailed below) after an exercise session, 10 bodybuilders performed an intensive exercise session consisting of a large volume of resistance exercise targeting the pectoral muscles. The chest muscles were chosen because many of the previous studies in which muscle damage of resistance-trained individuals were examined used a chest muscle exercise (Bloomer et al. 2007; Falvo et al. 2009; Falvo et al. 2007; Meneghel et al. 2013). Eight of the 10 bodybuilders repeated the same exercise session 2 weeks later to examine whether any adaptive or protective effect was conferred from the initial session. Participants reported to the laboratory either 5 (single bout) or 9 times (repeated bouts). All of the bodybuilders participated in one familiarisation session, one exercise session, and 3-days follow-up measurement sessions. The participants who performed in the repeated bout, had one other exercise session and another 3-day follow-up measurement sessions (see Figure 1).

On the first visit (familiarisation session), the bodybuilders were screened using a health and medical questionnaire, in which an informed consent form was read and signed. The bodybuilders then performed a one repetition maximum (1RM) bench press test based on the protocol by Baechle & Earle (2008). The 1RM bench press protocol was performed with the individuals instructed to warmup with a light resistance which easily allowed for 5 – 10 repetitions, followed by a 1-minute recovery. Another warmup set was performed with an increase of load between 5 – 10% for 3 – 5 repetitions, which was followed by a 2-minute
recovery. A conservative near maximal load was then selected to perform for 2 – 3 repetitions by adding another 5 – 10% resistance, followed by a 3-minute recovery. Load was then increased by 5 – 10% and the individuals were instructed to perform the set for 1 repetition, followed by a 3-minute recovery. This process continued until a true 1RM was achieved (Baechle and Earle 2008). Following the acquisition of the 1RM, a 30-minute recovery was allocated, which was followed by a 12 – 15 RM dumbbell straight arm flat flye test using the protocol by Baechle & Earle (2012). This test uses the individuals experience in resistance training and instructs the individual to select weights they perceive they can perform for 12 – 15 receptions. Once the load has been selected it is to be multiplied by 0.6 to determine the warmup load. A warm up trial is conducted followed by gradual increments in load until a 12 – 15 repetition max is achieved using correct technique and a 2 minute recovery between sets (Baechle and Earle 2012). A regression equation was used were necessary to determine predicted 1RM (P1RM) loads (Baechle and Earle 2008).

After the testing criterion were completed the participants were familiarised with all measurements and dependent variables. On the second visit (training session) 2 weeks later, a series of exercises (see Table 1) was performed by all bodybuilders. The following measurements were taken before, and 24, 48 and 72 hours after the exercise in the order of plasma CK activity, muscle soreness, flexibility assessments of the chest muscles by external rotation and abduction of the shoulder and elbow joint range of motion, isokinetic elbow extensor and flexor concentric torque and bench press throw. At immediately post-exercise (within 30 minutes after exercise), bench press throw, isokinetic elbow extensor and flexor concentric torque, flexibility tests and muscle soreness were measured in this order, and no serum CK measurement was taken immediately after exercise (see Figure 1).
Figure 1: Schematic illustration of testing procedure. Familiarisation session is performed 2-weeks prior to initial testing session. Testing session is performed in the order of creatine kinase (CK), muscle soreness (MS), flexibility assessments (Flex), isokinetic dynamometer (IKD) and bench press throw (BPT) pre, 24h-post, 48h-post and 72h-post exercise. Testing in the order of; bench press throw, isokinetic dynamometer, flexibility assessment and muscle soreness is done immediately (Imm) after exercise only. Testing and training repeated 2-weeks late for the repeated bout group only.
**Exercise protocol**

A series of exercises were selected from textbooks and articles emphasising the use of the pectorals or chest muscles with barbells and dumbbells (Baechle and Earle 2008; Baechle and Earle 2012; Hackett et al. 2013; Medicine 2013). A total of 17 exercises were included in one exercise session, however the training session was divided into two sections (Table 1). The first section (section 1) consisted of 5 exercises with each exercise performed for 3 – 4 sets of 10 – 12 repetitions at an intensity of 56 – 70% 1RM of bench press or 83% of predicted dumbbell pressing load (Saeterbakken et al. 2011) and dumbbell flye exercise load was determined using the model outlined by Baechle & Earle (2012). All exercises were performed with a 60-s rest for multi-joint exercises (i.e., barbell bench press, incline bench press) and a 30-s rest for the single joint exercises (i.e. dumbbell flat bench press, dumbbell flat flye, dumbbell incline flat flye) between sets. The second section (section 2) consisted of 3 groups of 4 exercises (12 exercises in total), and the intensity of the exercise was set at 40 – 67% 1RM or load determined by protocols based on Saeterbakken et al. (2011) and Baechle & Earle (2012). All exercises in section 2 were performed only once to volitional failure and each exercise within each group of section 2 was performed consecutively without any recovery, but a 2-min recovery was granted between groups of exercises (see Table 1). The load for dumbbell pressing exercises (i.e. dumbbell flat bench press, incline dumbbell bench press etc.) was determined based off 83% of the coinciding barbell exercise for that section (e.g. dumbbell flat bench press load was determined based off 83% of the barbell flat bench press load etc.) this was in accordance with the literature (Saeterbakken et al. 2011). Saeterbakken et al. (2011) reported that dumbbell pressing load was 17% less than barbell flat pressing load; thus the load performed for dumbbell pressing exercises is 83% of which is used for the same or similar barbell pressing exercise. For all exercises, the bodybuilders were instructed to move the load at a consistent cadence during the differing phases of each repetition, i.e. in the eccentric phase the bodybuilders moved the load at a cadence of 2 seconds, 1 second at both isometric phases (at the top and bottom of the
exercise) and as fast as possible in the concentric phase, generally deemed to be approximately 1 second. This was performed for all exercises, thus, eccentric contractions were emphasised. The exercise session was deemed unaccustomed for all participants as no bodybuilders had ever performed the combination of the two sections (section 1 and section 2 in Table 1) in one day and in one session, although they had experienced a similar protocol to section 1 or section 2 separately. The total volume of the session was deemed higher than all of the bodybuilders regular sessions, deemed by each participant. Thus, based off previous resistance trained muscle damage research increasing the intensity of the eccentric contractions making the session unaccustomed, the current study investigated an increased volume to the bodybuilders making the volume of the session unaccustomed.

Since the bodybuilders normally take supplements during their training, standardised supplementation were given before, during and after exercise. This was to ensure no significant difference between supplementation were consumed between individuals. This assured standardisation. Prior to exercise, each participant consumed 14 g of branch chain amino acids with 1g of dextrose monohydrate per kg of body mass (BM). During exercise, 14g of branch chain amino acids with 0.5g of dextrose per kg of BM was consumed by each participant. After exercise ~20g of protein was consumed by each participant with 1g of dextrose monohydrate per kg of BM (Slater and Phillips 2011) and the products of Bulk Nutrients Australia (Tasmania, Australia) was used for the supplementation.
Dependent variables

Bench press throw

The present study used bench press throw to examine the effect of the exercise on muscle function of the chest muscles by assessing peak velocity, peak force and peak power, in accordance with previous studies (Bloomer et al. 2007; Falvo et al. 2009; Falvo et al. 2007; Meneghel et al. 2014). The bench press throw was performed on a flat bench positioned on top of a custom made steel frame placed over a force plate (400 Series Performance Force Plate;
Fitness Technology, Australia) as shown in Figure 2. The bar was rested on adjustable height mounts either side of the Smith machine and was positioned 2-cm above each participant’s chest line for the assurance of safety. A linear position cable-extension transducer (Model PT5A; Celesco, Chatsworth, CA) interfaced with the Ballistic Measurement System (BMS) (Fitness Technology, Adelaide, SA, Australia) computer software allowed for displacement and velocity measures as shown in Figure 2. A magnetic particle brake (Fitness Technology, Adelaide, SA, Australia) was attached directly to the Smith machine bar to stop the eccentric component of the exercise, thus allowing the bar to move freely in the vertical plane concentrically (Figure 2).

The integration of the measurement devices (force plate, linear position cable-extension transducer) and the software (BMS) allowed for automated and real-time calculations of peak velocity, peak force and peak power as demonstrated in Figure 2, by the inbuilt analysis tool of the BMS software. To assess the true magnitude of peak velocity, peak force and peak power, manual adjustments were made to the set points to isolate the beginning and end of bar velocity. The initial set point was positioned at the beginning of bar velocity (the initial point of a directive speed) and the second set point was positioned at the end of the bar’s positive (+) velocity (Figure 2). These set points isolated the measurements which allowed for accurate determinations of peak velocity, peak force and calculated peak power (refer to Figure 3).

A total of 3 maximal bench press throws with a 40-kg weight was performed with a 60-s recovery between trials (Drinkwater et al. 2007). Each participant was asked to grip the bar so that the forearms were perpendicular to the floor (~73 cm apart), and the hand position was measured and recorded to be standardised across all time points. Each participant was instructed to throw the barbell for maximal height from the rested (static) position without any counter movement. The countdown was instructed and individuals were encouraged to push from the adjustable height mounts on the countdown of “3, 2, 1” and “push”. The force of each trial was recorded from the force plate sampling at 600 Hz for all variables, using a Butterworth filter.
with frequency cut offs to provide visual smoothing of the signal at the highest possible frequency (displacement = 60 Hz, velocity = 20 Hz, acceleration = 16 Hz, force 50 Hz) as to minimise distortion of the signal by external or environmental factors such as background noise, to ensure only signals of interest were collected by the BMS software. Three trials were taken at each time point and the peaks of each measure was averaged to determined mean peak force, mean peak power and mean peak velocity.

**Figure 2:** Set-up of bench press throw. A force plate, positioning mount for the bench, adjustable height mounts, linear position cable extension transducer and magnetic particle brake, and a laptop computer with a ballistic measurement system software are indicated in the photo to deduce peak velocity, peak force and peak power.
Figure 3: Example of raw data from the Ballistic Measurement System (BMS) for a bench press throw trial (A), from which a force x time relationship was obtained (B), followed by power (C). A built in analysis software of BMS calculated peak velocity, peak force and peak power (D). In this particular example; peak velocity, peak force and peak power were 1.118 m/s, 680.5 N and 669 W respectively. It should be noted that the above data was obtained 24h after exercise.
**Maximal voluntary isokinetic elbow extension and flexion concentric torque**

A Biodex 3.0 isokinetic dynamometer (System 4 Pro, Shirley, NY, USA) was used to measure peak torque of the elbow extensors and flexors at two differing velocities; 30°/s and 180°/s. As shown in Figure 3, the power head of the Biodex dynamometer was rotated to 30° to allow abduction of the humerus, and the armrest was used to support the upper arm to make sure there was no restriction of movement (Deighan et al. 2003). The elbow joint was aligned to the axis of rotation of the dynamometer, and two chest straps and a hip strap were used to secure the participant in place. Each participant was instructed to grip the handle bar in a supinated forearm position and “push” through the elbow extension phase and “pull” through the elbow flexion phase as maximally and fast as possible for three repetitions of each velocity using the right arm. The measurement at 30°/s was performed first followed by that at 180°/s with a 60-second rest between the two velocities. Torque-angle curves during elbow extension and flexion of the right arm were recorded by the Biodex 3.0 inbuilt computer software. The highest peak torque of elbow extension and flexion, for the three repetitions was used for further analysis.

**Figure 4:** Measurement of isokinetic elbow concentric torque of the elbow extensors and elbow flexors.
Flexibility of the shoulder and elbow joint

A handheld goniometer was used to measure resting angle (RANG) and stretched angle (SANG) around the shoulder joint to assess the flexibility of the pectoral muscle and an assessment of triceps flexibility via a measurement of elbow joint range of motion (ROM) through maximal extension and maximal flexion (Reese and Bandy 2002). Each measurement was taken three times, and the average of the three measures was used for further analysis.

For RANG and SANG, each participant laid supine with the legs extended and lumbar spine flat on a massage table. The chest and shoulder muscles were relaxed, and the arm was supinated and abducted to 90° (Reese and Bandy 2002), the angle of the axis of rotation was aligned with the acromion process and the stationary arm perpendicular to the table was measured as RANG (Figure 5A). In SANG, each participant was instructed to maximally stretch the arm and chest muscles to maximally increase the angle of the shoulder joint from the RANG position (Figure 5B). The difference between RANG and SANG were also calculated. The landmarks were clearly indicated on the skin using a semi-permanent ink pen, and the same marks were used to assess the change over time.

The ROM of the elbow joint was assessed also according to the methods described by Reese & Bandy (2002). As shown in Figure 6, each participant sat on the edge of a massage table and was instructed to supinate their right arm and extend it as vertical as possible (this measure was considered to be full extension of the elbow joint). The goniometer was aligned with the axis on the lateral epicondyle of the elbow, the stationary arm directed towards the acromion process and the moving arm directed towards the radial styloid. Each participant was then instructed to flex their elbow as much as possible whilst maintaining the position of the elbow vertically, and the elbow joint angle was measured using the same criteria as elbow extension, the change of movement of the elbow from extension to flexion was considered the range of motion of the elbow joint and was used to assess triceps flexibility. Each assessment
of RANG, SANG and ROM were assessed 3 times to reduce the significance of error, all three trials were averaged and the mean score of each assessment was used for statistical analysis.

**Muscle soreness**

Figure 5: RANG assessment of pectoral flexibility at the shoulder joint with location markers on the acromion process and lateral epicondyle of the elbow (A). SANG assessment of pectoral flexibility at the shoulder joint with location markers on the acromion process and lateral epicondyle of the elbow (B).

Muscle soreness was assessed with palpation of four separate locations; clavicular region and sternal region of the pectoralis major, lateral head of triceps brachii and the medial

Figure 6: Elbow joint ROM in the elbow extended position, aligning goniometer with the acromion process, lateral epicondyle of the shoulder and radial styloid of the wrist (A). Elbow joint ROM in the elbow flexed position, aligning goniometer with the acromion process, lateral epicondyle of the shoulder and radial styloid of the wrist (B).
head of biceps brachii as shown in Figures 7, 8. Chest muscle soreness was also assessed when throwing a 40-kg barbell maximally during the bench press throw. A visual analogue scale (VAS) consisting of a 100-mm line with “0” representing “no pain” and “100” representing “the worst muscle pain experienced” was used to quantify the perceived level of pain upon palpation and movement (bench press throw). Each participant was asked to draw a vertical line on the VAS to indicate the level of perceived pain (Newton et al. 2008). The same investigator applied the palpations using the index and middle fingers moving in a circular clockwise motion (Newton et al. 2008), the pressure of palpation was deemed to be consistent across all time points as previous pilot data showed consistency amongst individuals. Palpation locations were clearly marked with a semi-permanent marker pen as shown in figures 7, 8. The positions were located by palpating and marking the intertubercular sulcus, the most medial region of the clavicle and the most distal region of the manubrium in which half way points of the marked positions measured halfway between the intertubercular sulcus and the marked positions to ascertain approximated regions for palpation of the clavicular head of the pectoralis major and the sternal head of the pectoralis major (Field and Hutchinson 2006). The triceps brachii and biceps brachii palpation landmarks were ascertained by determining the midline distance between the acromion process and the radial styloid (upper-arm circumference measurement) where the belly of the biceps brachii was marked in line with the midline distance as well as the lateral head of the triceps brachii, these were the positions of muscle palpation (Figure 8).
Plasma CK activity

A sterile lancet was used to puncture the index finger to collect 30-μL of blood using a pipette, and the blood was loaded on to a CK test strip (Reflotron CK, Inverness Medical, Cheshire, UK) and assayed by a Reflotron spectrophotometer (Boehringer-Maheim, Pode, Czech Republic). The measurement was duplicated between days, and the average of the two values was used for further analysis. According to the information provided with the

Figure 7: Palpation landmarks for the clavicular portion of pectoralis major (A) and sternal portion of the (B).

Figure 8: Palpation landmarks for the lateral head of the triceps brachii (A) and biceps brachii (B).
The manufacture of the CK test strip, the “normal” reference range for CK using this method was 24 – 195 IU/L at 37°C.

Test-retest reliability

The test-retest reliability of each variable was assessed using pre-exercise values taken during the familiarisation session and immediately before the exercise session, and expressed as coefficient of variation (CV = SD/mean x 100) and typical error. As shown in Table 2, most of the CV values were low (<5%) except plasma CK activity (15.4%).

Table 2. Coefficient of variation (CV) and typical error (TE) of each dependent variable based on the two baseline measures taken 5 days and immediately before the first exercise session.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CV %</th>
<th>TE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bench press throw</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Force (N)</td>
<td>1.9</td>
<td>28.3</td>
</tr>
<tr>
<td>Peak Velocity (m/s)</td>
<td>1.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Peak Power (W)</td>
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<td>58.7</td>
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<tr>
<td><strong>Isokinetic torque</strong></td>
<td></td>
<td></td>
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<tr>
<td>Elbow Extension Peak Torque at 30°/s (N/m)</td>
<td>5.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Elbow Flexion Peak Torque at 30°/s (N/m)</td>
<td>2.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Elbow Extension Peak Torque at 180°/s (N/m)</td>
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<td>3.1</td>
</tr>
<tr>
<td>Elbow Flexion Peak Torque at 180°/s (N/m)</td>
<td>6.9</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>Flexibility Assessments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoulder – RANG (deg)</td>
<td>1.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Shoulder – SANG (deg)</td>
<td>1.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Elbow – ROM (deg)</td>
<td>1.8</td>
<td>3.7</td>
</tr>
<tr>
<td><strong>Muscle Soreness (VAS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpation - Sternal region (mm)</td>
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<td>1.1</td>
</tr>
<tr>
<td>Palpation - Clavicular region (mm)</td>
<td>9.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Palpation - Triceps brachii lateral head (mm)</td>
<td>13.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Palpation - Biceps brachii medial head (mm)</td>
<td>7.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Bench throw – Chest muscles (mm)</td>
<td>5.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Plasma creatine kinase activity (IU/L)</td>
<td>15.4</td>
<td>151.6</td>
</tr>
</tbody>
</table>
**Statistical analyses**

A one-way repeated measures analysis of variance (ANOVA) was used to analyse changes in each dependent variable over time after the initial exercise bout. When the ANOVA showed a significant time effect ($P<0.05$), independent t-tests with Bonferroni correction were applied as a post hoc test to locate a significant difference from the pre-exercise value. A two-way repeated measures ANOVA was used to compare changes in the dependent variables over time between the initial and second exercise bouts. When the two-way ANOVA showed a significant ($P<0.05$) interaction effect, a series of Bonferroni *post-hoc* tests were followed to compare between bouts for each time point. Statistical significance was set at $P<0.05$ for all analyses. All analyses were performed on statistical software SPSS 22.0 (SPSS, Inc., Chicago, IL). The results are presented mean ± SD.
CHAPTER 3 – RESULTS

Exercise

All participants were able to complete the 12 exercises as instructed, but the total volume varied among them. The average total volume performed during the first five exercises was 6779 ± 867 kg and that of the subsequent 12 exercises performed to volitional failure was 3439 ± 1418 kg. The total volume of the entire exercise session was 10,218 ± 1337 kg.

Bench press throw

Significant changes were observed for peak force (Figure 9A), peak velocity (Figure 9B) and peak power (Figure 9C) following the exercise. Peak force decreased by -23 ± 11% (p=0.002) from before to immediately after exercise, and remained lower than the baseline by -9 ± 7% (p=0.031) at 1-day post-exercise, but returned to the baseline by 2 days post-exercise. The time-course changes of peak velocity and peak power was similar to that of peak force; however, the magnitude of the decrease immediately after exercise was greater for peak velocity (-46 ± 11%) and peak power (-61 ± 14%) than peak force. At 1-day post-exercise peak velocity and peak power was -6 ± 9% and -12 ± 14% lower than the baseline, respectively. The magnitude of decrease in peak velocity and peak power was significantly (P=0.001) greater than that of peak force, and a significant (P=0.004) difference was also evident between peak velocity and peak power (P<0.05).
Elbow extensor concentric torque

As shown in Figure 9A, peak torque for the elbow extensors at 30°/s decreased immediately after exercise by -21 ± 10% (p=0.002), and remained below the baseline at 1 day (-16 ± 10%, P=0.01) and 2 days (-13 ± 9%, P=0.026) after exercise. Peak torque of the elbow extensors at 180°/s decreased immediately after exercise by -14 ± 13% (P=0.028) and 1-day by -16 ± 9% (P = 0.002) (Figure 9B). The elbow extensor torque at both velocities returned to the baseline by 3 days post-exercise. No significant changes in elbow flexor torque were evident after exercise for both velocities.

Figure 9: Changes (mean ± SD of 10 participants) in peak force (A), peak velocity (B) and peak power (C) in the barbell bench throw, before (pre), immediately after (0), and 24, 48 and 72 hours after the initial resistance exercise bout. * shows a significance (P<0.05) from the “pre” value.
Shoulder RANG showed no significant changes over time from the baseline (161 ± 9°). Similarly, SANG also showed no significant changes after exercise from the baseline levels (150 ± 7°). The difference between the measures of RANG and SANG (12 ± 2°) did not change over time.

Elbow extension and flexion angles were 10.8 ± 4.4° and 110.7 ± 7.8° respectively at the baseline. No significant changes in elbow joint angles were observed following the exercise bout for the extension (P=0.435) and flexion (P=0.052).

**Figure 10:** Changes (mean ± SD of 10 participants) in peak elbow extension torque at 30°/s (A) and 180°/s (B) during isokinetic concentric before (pre), immediately after (0), and 24, 48 and 72 hours after the initial resistance exercise bout. * shows a significance (P<0.05) from the “pre” value.

**Flexibility**

Shoulder RANG showed no significant changes over time from the baseline (161 ± 9°). Similarly, SANG also showed no significant changes after exercise from the baseline levels (150 ± 7°). The difference between the measures of RANG and SANG (12 ± 2°) did not change over time.

Elbow extension and flexion angles were 10.8 ± 4.4° and 110.7 ± 7.8° respectively at the baseline. No significant changes in elbow joint angles were observed following the exercise bout for the extension (P=0.435) and flexion (P=0.052).
Muscle soreness

Baseline muscle soreness values for the clavicular and sternal regions were 13 ± 11 mm and 18 ± 14 mm, respectively. The level of muscle soreness increased significantly after the exercise, and peaked at 1-day post-exercise (57 ± 22 mm; P=0.001), and remained elevated at 2 days (55 ± 20 mm; P<0.001) and 3 days (31 ± 17 mm; P=0.005) after exercise for the clavicular region (Figure 10A). Similar changes in muscle soreness were observed for the sternal region, peaking 1 day (66 ± 15 mm; P<0.001) and 2-days (61 ± 20 mm; P=0.001) after exercise, however no significance difference from the baseline was observed at 3-days post-exercise (Figure 10B). No significant changes in VAS values of the lateral head of the triceps brachii and medial head of the biceps brachii were evident (P>0.05). Muscle soreness of the chest muscles whilst performing the bench press throw increased significantly from the baseline (12 ± 8 mm) to 1 day (44 ± 20 mm; P=0.004) and 2 days (31 ± 12 mm; P=0.048) after exercise (Figure 10C).

Figure 11: Changes (average ± SD of 10 participants) in the perception of muscle pain on a visual analogue scale (0 – 100mm) when palpating clavicular region (A) and sternal region of the pectoralis major (B) clavicular region of the pectoralis major, and dynamic soreness of the chest muscles during the barbell bench throw (C), before (pre), immediately after (0), and 24, 48 and 72 hours after the initial resistance exercise bout. * shows a significance (P<0.05) from the “pre” value.
**Plasma CK Activity**

Baseline plasma CK activity was 468 ± 227 IU/L. No significant changes in plasma CK activity were found after exercise from the baseline (P=0.211).

**Repeated bout effect**

Total volume increased significantly from the first (10,384 ± 670 kg) to the second bout (11,433 ± 674 kg) by 10 ± 1% (P<0.001). This was mainly due to a significant increase of 27 ± 6% (P<0.001) in the repetitions in section 2 where exercises were performed to volitional failure (bout 1: 3634 ± 1382 kg; bout 2: 4606 ± 1461 kg) as shown in Figure 11.

Table 3 compares between the first and second bouts for changes in the dependent variables over time. No significant differences in the changes were observed for bench press throw parameters between bouts (P>0.05). Isokinetic elbow extensor concentric torque at 30°/s showed a significant time x bout interaction effect (P=0.036) and a significant reduction in the magnitude of muscle soreness after the second in comparison to the first bout for the palpation of the clavicular region (P=0.037) and sternal region (P=0.02) of the pectoral muscles, but soreness of the chest muscles during the bench press throw did not show significant difference between bouts (P=0.313). No significant differences between bouts were found for any other variable.

![Figure 12: Volume (mean ± SD of 8 participants) of sets X repetitions X load. * shows significant (P<0.05) difference from the first bout. Black = Bout 1; Greyscale = Bout 2](image)
Table 3. Comparison between the first and second bouts of exercise for changes (mean ± SD of 8 bodybuilders) in the barbell bench throw peak force, peak velocity and peak power, peak torque of isokinetic concentric torque of the elbow extensors at 30°/s and 180°/s, visual analogue scale (VAS) for muscle soreness upon palpation of clavicular and sternal region of the pectoralis muscle, and plasma CK activity, before (pre), immediately after (0 h) and 24, 48 and 72 hours after the first (1st) and second (2nd) exercise bouts. One the right column, F and P values based on the interaction effect (bout x time) from two-way ANOVA are shown.

<table>
<thead>
<tr>
<th>Measures</th>
<th>n=8</th>
<th>Pre</th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak force (N)</td>
<td></td>
<td>1st 913.6 ± 140.6</td>
<td>690.3 ± 101.7</td>
<td># 832.2 ± 160.6</td>
<td># 851.3 ± 133.3</td>
<td>890.7 ± 159.1</td>
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<td>P= 0.246</td>
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<tr>
<td></td>
<td></td>
<td>2nd 884.0 ± 151.2</td>
<td>688.9 ± 72.7</td>
<td># 854.0 ± 140.9</td>
<td># 894.4 ± 146.2</td>
<td>911.6 ± 163.5</td>
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<td></td>
</tr>
<tr>
<td>Peak velocity (m/s)</td>
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<td>1st 1.6 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td># 1.5 ± 0.2</td>
<td># 1.6 ± 0.2</td>
<td># 1.6 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>F= 0.986</td>
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<tr>
<td></td>
<td></td>
<td>2nd 1.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td># 1.6 ± 0.2</td>
<td># 1.6 ± 0.2</td>
<td># 1.7 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>P= 0.986</td>
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<td>Peak power (W)</td>
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<td>458.5 ± 184.8</td>
<td># 1066.9 ± 276.2</td>
<td># 1119.3 ± 277.9</td>
<td>1173.5 ± 338.1</td>
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<td>P= 0.154</td>
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<td>2nd 1102.7 ± 272.5</td>
<td>444.1 ± 117.8</td>
<td># 1069.2 ± 279.4</td>
<td># 1129.4 ± 294.4</td>
<td>1152.6 ± 328.5</td>
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<td>Peak torque at 30°/s (Nm)</td>
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<td>1st 62.6 ± 24.8</td>
<td>50.0 ± 20.1</td>
<td># 53.7 ± 21.0</td>
<td># 55.3 ± 22.0</td>
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<td>P= 0.036</td>
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<td></td>
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<td>2nd 57.4 ± 22.3</td>
<td>50.3 ± 18.4</td>
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<td># 56.7 ± 21.0</td>
<td>59.3 ± 21.6</td>
<td>F= 3.583</td>
<td>P= 0.036</td>
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<tr>
<td>Peak Torque at 180°/s (Nm)</td>
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<td># 38.5 ± 10.8</td>
<td># 42.3 ± 10.7</td>
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<td>40.1 ± 15.5</td>
<td># 42.6 ± 15.9</td>
<td># 42.8 ± 16.7</td>
<td>44.8 ± 16.2</td>
<td>F= 2.091</td>
<td>P= 0.148</td>
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<tr>
<td>Shoulder joint ROM (°)</td>
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<td>1st 13.8 ± 6.9</td>
<td>11.0 ± 4.9</td>
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<td># 13.1 ± 6.4</td>
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<tr>
<td></td>
<td></td>
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<td># 14.0 ± 6.1</td>
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<td>F= 1.238</td>
<td>P= 0.321</td>
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<td>Elbow joint ROM (°)</td>
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<td>F= 2.096</td>
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<td></td>
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<td>2nd 111.0 ± 38.1</td>
<td>96.8 ± 38.8</td>
<td># 110.1 ± 38</td>
<td>111.3 ± 38</td>
<td>111.8 ± 38.1</td>
<td>F= 2.096</td>
<td>P= 0.185</td>
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<td>VAS upon palpation of clavicular region (mm)</td>
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<td>16.6 ± 14.3</td>
<td>58.0 ± 29.6</td>
<td>60.0 ± 26.8</td>
<td>34.8 ± 18.8</td>
<td>F= 3.790</td>
<td>P= 0.037</td>
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<tr>
<td></td>
<td></td>
<td>2nd 13.3 ± 11.7</td>
<td>10.4 ± 8.6</td>
<td>51.6 ± 22.6</td>
<td>35.9 ± 21.2</td>
<td>20.1 ± 15.9</td>
<td>F= 3.790</td>
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<tr>
<td>VAS upon palpation of sternal region (mm)</td>
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<td>1st 19.1 ± 15.7</td>
<td>15.9 ± 10.0</td>
<td>68.3 ± 27.1</td>
<td>66.8 ± 27.6</td>
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<td></td>
<td></td>
<td>2nd 10.5 ± 8.8</td>
<td>12.5 ± 12.3</td>
<td>47.1 ± 25.5</td>
<td>36.3 ± 20.6</td>
<td>19.4 ± 17.9</td>
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<td>VAS of chest muscles in bench throw (mm)</td>
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<td>1st 11.9 ± 9.6</td>
<td>27.9 ± 24.1</td>
<td>44.8 ± 22.9</td>
<td>31.8 ± 13.9</td>
<td>25.7 ± 15.9</td>
<td>F= 1.159</td>
<td>P= 0.348</td>
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<tr>
<td></td>
<td></td>
<td>2nd 14.4 ± 10.4</td>
<td>20.2 ± 17.4</td>
<td>36.7 ± 15.7</td>
<td>26.4 ± 16.5</td>
<td>15.1 ± 9.9</td>
<td>F= 1.159</td>
<td>P= 0.348</td>
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<tr>
<td>Plasma CK activity (IU/L)</td>
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<td>1st 517.6 ± 268.5</td>
<td>732.0 ± 359.3</td>
<td>661.1 ± 368.0</td>
<td>654.5 ± 410.8</td>
<td>F= 3.967</td>
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<td>2nd 474.9 ± 346.8</td>
<td>584.6 ± 326.6</td>
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<td>398.6 ± 331.0</td>
<td>F= 3.967</td>
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*: Significantly (P<0.05) different from the value of the first bout, #: Significantly (P<0.05) different from the pre-exercise value
CHAPTER 4 – DISCUSSION

It was hypothesised that changes in indirect markers of muscle damage would be minimal in bodybuilders such that decreases in muscle function would be minuscule, but the magnitude of muscle soreness would persist for several days following exercise. It was also considered that the rate of recovery would be greater for bout 2 as opposed to bout 1, thus the repeated bout effect would be present in bodybuilders.

Muscle damage and the repeated bout effect in bodybuilders

Exercise induced muscle damage is best indicated by a prolonged loss of muscle function after exercise (Ebbeling and Clarkson 1989; Warren et al. 1999). However, it has been well documented that when the same exercise is repeated within several weeks, recovery of muscle function is faster, the development of muscle soreness is smaller, and increases in blood markers of muscle damage are blunted after the second bout when compared with the initial bout (McHugh 2003; McHugh and Tetro 2003; Meneghel et al. 2013; Nosaka et al. 2001). This phenomenon is referred to as the repeated bout effect.

In the initial investigation, the pectoral muscles averaged ~300 repetitions per subject utilising concentric-isometric-eccentric contractions of the chest muscles using 17 exercises. Falvo et al. (2007, 2009) used an eccentric bench press protocol of 100 ECC only contractions at 70% CON-1RM for both bouts. They reported no repeated bout effect. Meneghel et al. (2014) used a similar protocol but, the volume was smaller, and the intensity was greater (36 ECC only contractions at 70% ECC-1RM) and reported a large attenuation of changes in bench press 1RM strength, DOMS and CK activity after the second bout. They concluded that the repeated bout effect existed in this resistance-trained population. Similarly, Howatson et al. (2007a) reported that the repeated bout effect existed in resistance trained individuals following 10 maximal ECC and 45 maximal ECC contractions of the elbow flexors followed by 45 maximal
ECC contractions of the elbow flexors performed 2-weeks later. The author reported that both 10 maximal ECC contractions and 45 maximal ECC contractions confer protection again 45 maximal ECC contractions of the elbow flexors performed 2-weeks later.

Unlike other studies, the present study utilised more than one contraction type to test the effects of ‘traditional’ like training on the magnitude of muscle damage and the development of muscle soreness. When concentric and eccentric phases are equated, the average number of contractions performed in the 17 exercises was ~600+ (excluding the isometric contractions performed at an extended and shortened muscle length). All participants had performed each exercise in their training previously, but none of them had previously experienced performing 17 exercises in a single session. Thus, the training session used in the present study was deemed unaccustomed.

When comparing the changes in the dependent variables after the initial bout between the first 10 bodybuilders and that of the 8 bodybuilders who also performed the second bout, the changes were similar (8x8 [repeated bout], 10x8 [initial compared to repeated]. Thus, it seems reasonable to assume that the data of the repeated bout effect represents all bodybuilders used in the study.

Significant increases for total volume performed was increased from the first to the second bout in the present study (Figure 12). This was due to the ability to perform more repetitions to volitional failure (e.g. 21% increase in the repetitions for the dumbbell flat flye and 15% increase for the dumbbell flat bench press), it is important to note that although the repetitions increased, the intensity remained constant. Propositions for the ability to perform an increased amount of work has been previously reported and said to be due to neural adaptations such as improved motor unit synchronisation, increased motor unit firing, increased usage of synergistic muscles and/or increased slow twitch muscle fibre recruitment (Clarkson and Hubal 2002; Nosaka and Aoki 2011). The greater amount of volume in the second bout would likely result in a greater exercise stimulus to induce muscle damage than the first bout. However, no
significant differences were observed for changes in the bench press throw (see Table 3). In fact, significant attenuation of muscle soreness was observed after the second bout when compared with the first bout. Muscle soreness developed 1-day post-exercise and remained elevated for 3 days in the initial bout, but the magnitude of muscle soreness was significantly attenuated following the second bout (see Table 3). Previous studies (Bloomer et al. 2007; Falvo et al. 2009; Falvo et al. 2007) have reported similar magnitudes of soreness (mild to severe) in resistance-trained individuals after eccentric exercise of the chest muscles. However, Newton et al. (2008) reported no significant differences in the degree of muscle soreness developed after eccentric exercise of the elbow flexors between resistance-trained and untrained men, regardless of the large difference in other indirect markers of muscle damage. They speculated that the poor correlation of soreness and damage may have attributed to these dissimilarities (Meneghel et al. 2014; Newton et al. 2008; Warren et al. 1999). The results of the present study suggest that the repeated bout effect was evident for the bodybuilders at least for muscle soreness (see Table 3).

Although previous studies have reported large (>-20%) decreases in MVC strength lasting for more than 3 days after eccentric exercise in non-trained individuals (Jamurtas et al. 2005; Nosaka and Newton 2002) the present study (see Table 3) reported that peak force, peak velocity and peak power decreased significantly immediately after (-23 – 60%) and 1 day after exercise (-6 – 12%), but returned to the baseline by 2 days post-exercise (see Table 3). The reduction in muscle function in the bouts were similar to those reported in previous studies in which resistance-trained individuals were used (Bloomer et al. 2007; Falvo et al. 2009; Falvo et al. 2007; Meneghel et al. 2014). However, the elusion as to why there remains a prolonged loss of function is still largely debated. It is clear from previous research that the prolonged decrement in muscle function, in particular the impairment of muscle force generating ability is linked to damage to or in the muscle fibres. But, it seems elusive as to where the damage is located. Some papers suggest that the prolonged reduction in muscle force generating capacity
comes from damage to the myofibrils contractile filaments and/or structural proteins (McHugh 2003) whereas other research suggests damage to the sarcolemma (Crameri et al. 2007) and/or damage to the excitation contraction (E-C) coupling (Ingalls et al. 1998) system known as E-C uncoupling or E-C coupling failure. It may be that there is a combination of these three sites for potential damage to onset. However, in the present paper it seems likely that the unaccustomed exercise session caused damage to the E-C coupling system. Ingalls et al. (1998) reported that 57 – 75% of maximal isometric tetanic force decreases seen at 0 – 5 days after eccentric exercise is associated with the E-C coupling failure, but the main cause of the force loss if seen at 5 days or later is due to muscle fibre damage, based on an animal study. It is also plausible that the damage in the present investigation is caused by a combination of myofibril damage or sarcomere disruptions and E-C coupling failure. Allen (2001) reported that when sarcomeres are elongated excessively (eccentric contractions) the myofilaments reinterdigitate when the sarcomeres return to a normal or resting length. Thus, it is possible that the repetitive eccentric contraction causes a reduction or inability for the myofilaments to reinterdigitate causing damage to the cytoskeletal filaments damaging a multitude of sarcomeres (Allen 2001). Thus, the results of the present study may in fact be related to the inability to reinterdigitate following multiple and repetitive eccentric contractions of the pectorals, resulting in weakened sarcomeres and a shift in the fore-length relationship, changes to the E-C coupling process leading to reductions of Ca\(^{2+}\) secretion and force production (Allen 2001).

Although the present study reported a fast recovery in bench press throw parameters, there seemed to be a delay in recovery for the isokinetic elbow extensor concentric torque at 30°/s. The elbow extensor concentric contraction torque recovered back to baseline by 3 days’ post-exercise, which was one day longer than other measures including the torque measure at 180°/s. Newton et al., (2008) reported similar results for the elbow flexors, showing that maximal concentric contraction torque at 30°/s in trained individuals returned to baseline by 2 days post-exercise, but at 210°/s returned to the baseline at 1 day post-exercise. However, no clear
explanation was given for this reason in the study. It should be noted that MVC torque was greater for 30°/s than 180°/s. This may explain the longer recovery time taken for 30°/s than 180°/s. Similarly, Soares et al., (2015) reported a greater decrease in MVC strength for single joint than multi-joint exercise. It should be noted that most of the exercises used in the present study were multi-joint exercises.

The present study did not find any significant changes in ROM around the shoulder and elbow joints after exercise for either bouts. Previous studies have reported decreases in ROM around the elbow joint (Jamurtas et al. 2005; Newton et al. 2008; Nosaka and Newton 2002) or knee joint (Jamurtas et al. 2005) after eccentric exercise performed by non-resistance-trained individuals. Nosaka et al. (1991) reported that following 70 maximal eccentric contractions of the elbow flexors in untrained men, RANG decreased by -20 – -30% 1-5 days after the first bout, but only a half of the magnitude (-10 – -15%) after the second bout. Newton et al. (2008) reported a small decrease in ROM only at immediately (-6°) and 1 day (-4°) after maximal eccentric exercise of the elbow flexors performed by resistance-trained individuals. But, the present study did not, this may be due to differences in muscles used in the previous studies as opposed to the present study. It seems likely that ROM was a less sensitive marker of muscle damage, especially for the bodybuilders.

In the present study, no significant increases in plasma CK activity were found. Newton et al. (2008) also reported no significant increases in plasma CK activity following maximal eccentric exercise of the elbow flexors performed by resistance-trained individuals. It has been documented that the activation of phospholipase A² by increased intracellular Ca²⁺ concentration leads to sarcolemma damage, resulting in increased plasma membrane permeability and leakage of muscle specific proteins such as CK to the extracellular matrix and blood stream (Allen et al. 2016; Armstrong et al. 1991; Friden and Lieber 1992). Thus, it appears that muscle fibre damage was protected by the regular resistance training in bodybuilders. However, as pointed out above, it should be noted that DOMS experienced by
the bodybuilders was not necessarily small. It is becoming clear that muscle soreness does not necessarily correspond to the magnitude of muscle damage, but may be due to damage and/or inflammation of the fascia or other connective tissue (Gibson et al. 2009; Lau et al. 2015). It is possible that the magnitude of protective effect conferred by regular resistance training is different between muscle fibres and/or connective tissue surrounding muscle fibres and fascicles, and the protective effect may be greater for muscle fibres than connective tissue.

The present study highlights that the damage occurred may be from non-contractile elements evidenced by low CK changes but, increased DOMS. It is plausible to assume that there may be disruption or damage to non-contractile elements. Future research should endeavour to elucidate if connective tissue damage is a leading contributor to strength loss in bodybuilders and/or resistance trained individuals. Similar studies have looked at the investigation in untrained individuals identifying a significant increase in urinary hydroxyproline and hydroxylysine indirectly associated with collagenous breakdown of connective tissue following eccentric exercise (Brown et al. 1997). Future researchers should test if increases of hydroxyproline and/or hydroxylysine increases following a similar training protocol as the present study in bodybuilders or highly resistance trained individuals.

Taken together, muscle damage characteristics of bodybuilders are similar to those of other resistance trained populations reported in the previous studies (Falvo et al. 2009; Falvo et al. 2007; Howatson et al. 2007; Meneghel et al. 2014). However, it appears that the recovery of muscle function was faster in bodybuilders when compared with other resistance-trained populations previously researched. It is important to note that mild to severe DOMS still develops in bodybuilders, although no increases in intramuscular marker of muscle damage (CK activity) were evident for the bodybuilders. Thus, bodybuilders are said to experience less muscle damage and moderate to severe levels of DOMS after a strenuous bout of unaccustomed resistance exercise.
Future research direction

Future research should endeavour to investigate if further damaging exercise consisting of mainly eccentric contractions was performed by bodybuilders would they experience greater muscle damage than that shown in the present study? And, whether other muscles would also show similar symptoms of muscle damage after strenuous exercise bouts to that shown by the chest muscles? It is also interesting to ascertain if the common bodybuilder saying “no pain, no gain” is true. It may be that bodybuilders can train harder and more frequently than other resistance trained populations without the onset of negative physiological occurrences, however, this is still yet to be examined. In the present study, only indirect markers of muscle damage were used, but it is necessary to include direct markers of muscle damage such as histological observations and the investigation of connective tissue damage in the future studies to elucidate the mechanisms underpinning the muscle damage and connective tissue damage and the repeated bout effect of resistance trained individuals and bodybuilders.

CONCLUSION

The purpose of this investigation was to confirm the anecdotal claim that bodybuilders regularly experience muscle soreness and to identify the time course of changes in indirect markers of muscle damage after the initial and secondary bout of the strenuous resistance exercise session. The main findings of the present studies were (i) although a large volume of unaccustomed resistance exercise was performed by bodybuilders the susceptibility of indirect markers of muscle fibre damage was minimal, but the extent of muscle soreness was relatively large, and (ii) the magnitude of the repeated bout effect on muscle function was minimal for the bodybuilders, but muscle soreness was attenuated in the repeated bout. It appears that the muscle damage and the repeated bout characteristics of the bodybuilders exists and are similar to those of other resistance-trained individuals, but the magnitude of the repeated bout effect is limited to muscle soreness.
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APPENDICES

APPENDIX A
Exercises

Figure 12. Incline Barbell Bench Press Exercise

Figure 13. Flat Barbell Bench Press Exercise
Figure 14.  Wide Grip Barbell Bench Press Exercise

Figure 15.  Incline Dumbbell Bench Press Exercise
Figure 16. Flat Neutral Bench Press Exercise

Figure 17. Flat Dumbbell Bench Press Exercise
Figure 18. Dumbbell Flat Flye Exercise

Figure 19. Dumbbell Incline Flye Exercise
APPENDIX B:
PRESENTATION ABSTRACTS

EUROPEAN COLLEGE OF SPORT SCIENCE (ECSS) –
AUSTRIA (VIENNA)

MUSCLE DAMAGE OF BODYBUILDERS AFTER AN UNACUSTOMED STRENUS
OUS RESISTANCE TRAINING SESSION

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1: ECU (Australia)

Introduction
Muscle damage is induced by unaccustomed exercise consisting of eccentric contractions, and
is best indicated by a prolonged loss of muscle function [1]. Delayed onset muscle soreness
(DOMS) is considered to be a symptom of muscle damage, but its magnitude does not
necessarily correspond with the magnitude of muscle damage [2]. Anecdotally, bodybuilders
experience DOMS after resistance training, however no previous studies have reported muscle
damage of bodybuilders. Newton et al. [3] reported faster recovery of muscle function after
unaccustomed elbow flexor eccentric exercise for resistance-trained than untrained men, but
found similar magnitudes of DOMS between groups. Thus, the present study tested the
hypothesis that reductions in muscle function and increases in plasma creatine kinase (CK)
activity following an unaccustomed training session would be small, but DOMS would still be
developed in bodybuilders.

Methods
Ten male bodybuilders (age: 23 ± 2 y, height: 176 ± 4 cm, body mass: 85 ± 8 kg) with a
minimum of 3-y resistance training experience were recruited for the study. They performed a
large volume of exercises targeting the pectoral muscles, consisting of 5 exercises at 8-12
repetitions with a moderate load followed by 12 exercises to volitional failure. Muscle function
measures in barbell bench throw (e.g. peak force, peak velocity), isokinetic maximal elbow
extension torque and muscle pain during palpation of the clavicular and sternal portion of the
chest using a visual analogue scale (VAS: 100-mm) were taken before, immediately after, and
1-3 days after exercise.

Results
Significant changes in all variables were observed after exercise (P<0.05). For example, peak force in barbell bench throw decreased immediately (23 ± 20%) and 1 day after exercise (9 ± 15%) but returned to baseline (930 ± 129 N) by 2 days post-exercise. Additionally, other muscle function measures showed similar changes. DOMS developed after exercise and peaked 1-day post-exercise (e.g. 57 ± 22 mm for the clavicular portion of the chest). No significance changes in plasma CK activity were observed after exercise.

Discussion
These results were similar to that shown by the previous study of resistance-trained men [3]. Despite small decreases in muscle function 1-day post-exercise, DOMS showed higher values than the levels that the participants usually experienced after their regular training sessions. It appears that regular resistance training had conferred a protective effect against muscle fibre damage, but bodybuilders are still susceptible to DOMS, which may be associated with connective tissue damage and inflammation [2].

**Keywords:** Delayed Onset Muscle Soreness, Barbell Bench Throw, Creatine Kinase

**References**

Title: MUSCLE DAMAGE IN BODYBUILDERS AFTER AN INITIAL AND SECONDARY BOUTS OF UNNACUSTOMED INTENSE RESISTANCE EXERCISE SESSIONS

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Abstract body: Muscle damage is characterised by prolonged reductions in muscle function and delayed onset muscle soreness (DOMS). It has been reported that resistance-trained individuals are less susceptible to eccentric exercise-induced muscle damage, and develop minor repeated bout effects. However, no previous studies have examined muscle damage characteristics of bodybuilders. The present study compared changes in muscle function measures, muscle soreness and plasma creatine kinase (CK) activity after an initial and secondary bout of an unaccustomed intense resistance exercise session performed by eight male bodybuilders (23 ± 2 y, 175 ± 4 cm, 84 ± 8 kg) with 4 - 9 years of bodybuilding training experience. They performed 17 exercises targeting the pectoral muscles consisting of 5 exercises for 3 - 4 sets and 8 - 12 repetitions with a moderate load of 45 - 67% of their one repetition maximum (1RM) or predicted 1RM (P-1RM) and 12 exercises at 30 - 67% of 1RM or P-1RM performed to volitional failure. None of them had experienced this kind of exercise session previously. The same exercise was repeated 2 weeks later. Changes in bench press throw (peak force, velocity, power), muscle soreness (100-mm visual analogue scale) and plasma CK activity were assessed before, immediately after, 24, 48 and 72 hours following each bout. Bench press throw peak force decreased (P<0.05) 24 ± 28%, 9 ± 14%, 7 ± 5%, immediately, 1 day and 2 days after exercise, respectively from the baseline similarly between bouts, and this was also the case for peak velocity and power. Plasma CK activity did not change. Muscle soreness developed after both bouts, but the magnitude was greater (P<0.05) after the first (peak: 60 ± 27 mm) than second bout (peak: 52 ± 23 mm). It is concluded that bodybuilders still experience muscle damage especially muscle soreness, and the repeated bout effect is small.
Dear Ken

**Project Number: 12094 BLOWFIELD**  
**Project Name: Muscle Damage and Adaptation in Natural Male Competitive Bodybuilders**

**Student Number: 10220157**

The ECU Human Research Ethics Committee (HREC) has reviewed your application and has granted ethics approval for your research project. In granting approval, the HREC has determined that the research project meets the requirements of the *National Statement on Ethical Conduct in Human Research*.

The approval period is from 7 January 2015 to 21 December 2015.

The Research Assessments Team has been informed and they will issue formal notification of approval. Please note that the submission and approval of your research proposal is a separate process to obtaining ethics approval and that no recruitment of participants and/or data collection can commence until formal notification of both ethics approval and approval of your research proposal has been received.

All research projects are approved subject to general conditions of approval. Please see the attached document for details of these conditions, which include monitoring requirements, changes to the project and extension of ethics approval.

Please feel free to contact me if you require any further information.

Kind regards

Rowe

Rowe Oakes

Ethics Support Officer, Office of Research & Innovation, Edith Cowan University, 270 Joondalup Drive, Joondalup, WA 6027

Tel: +61 08 6304 2943 | Fax: +61 08 6304 5044 | CRICOS IPC 00279B
APPENDIX D:
INFORMED CONSENT FORM

Consent Form

Muscle Damage and Adaptations in Natural Male Competitive Bodybuilders

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0416053946

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The individual has been provided a copy of the information letter, explaining the research study and has read and understood the information provided. The participant has been given the opportunity to ask questions and has had any questions answered to their satisfaction. The participant is aware that if they have any additional questions or concerns they can contact the research team, and understands the participation in the research project will involve:

- 2 training sessions of a combined protocol of bodybuilding training plus high intense single set training separated by a 2 week period
- 3 days of follow up testing following each training session
- Testing involving height, weight, strength tests, power tests, blood analysis, muscle soreness and flexibility testing
- No direct training of the pectorals muscle group during the duration of the study
- No use of any illegal performing enhancing substances
- Normal nutritional practices are to be kept constant throughout the duration of the study

By signing this consent form, you are not waiving your legal rights or releasing the investigator(s) or involved institution(s) from their legal and professional responsibilities.

I have read the information presented in the information letter about the study being conducted by Ken Blowfield at the School of Exercise and Health Sciences, Edith Cowan University. I have had the opportunity to ask any questions related to this study to receive satisfactory answers to my questions, and any additional details I wanted. I am aware that I may withdraw from the study at any time without penalty by advising the researchers of this decision.

This project has been reviewed by, and received ethical clearance through the Human Research Ethics Committee ECU. I was informed that if I have any comments or concerns resulting from my participation in this study, I may contact the Research Ethics Support Officer:
Phone: (08) 6304 5122.
Email: research.ethics@ecu.edu.au

I have full knowledge of all foregoing, I agree, of my own free will to participate in this study.

_________________________  ___________________________  ___________________________
(Print Name)  (Signature of Participant)  (Date)
APPENDIX E:
INFORMATION LETTER

INFORMATION LETTER
Muscle Damage and Adaptations in Natural Male Competitive Bodybuilders

Chief Investigator:
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Supervising Researchers:
Prof. Ken Nosaka Contact: (08) 6304 5655 Email: k.nosaka@ecu.edu.au
Dr. G. Gregory Haff Contact: (08) 6304 5416 Email: g.haff@ecu.edu.au

Thank you for expressing your interest in this specific research project aimed at identifying the muscle damage and adaptations that occurs to natural competitive bodybuilders. The reason for the following information is to fully inform you of the purpose and nature of this project which has been approved by the ECU Human Research Ethics Committee. Please read all the information carefully and do not hesitate to contact the Chief Investigator if anything is unclear or requires further explanation.

BACKGROUND:
Exercise induced skeletal muscle damage is seen most commonly in individuals performing unaccustomed eccentric (lengthening) contractions that is most commonly characterised by a large reduction in force generating ability as well as strength loss, reduction in flexibility and the onset of delayed muscle soreness (DOMS).

Muscle damage is more profound when the exercise performed is unaccustomed to the individual; however an interesting phenomenon occurs after the initial bout of unaccustomed eccentric exercise has concluded. It has been shown that when the exercise is repeated again within a 6-month period, a notable reduction in muscle damage and soreness is observed; this is known as the repeated bout effect, whereby a protective mechanism or adaptation has occurred. Similar mechanisms have been observed whereby a comparable adaptation occurs known as the protective effect following submaximal contractions against maximal contractions.

Muscle damage has been extensively studied in untrained populations; however minimal studies have been conducted in highly trained individuals and no studies have used bodybuilders as a highly trained population. Therefore lies a fundamental gap within the literature, PURPOSE: the purpose of this study is to add clarification to the repeated bout effect and muscle damage literature performed on a highly trained population, and to also assess muscle damage which occurs to bodybuilders following a bout of unaccustomed combination training and a repeated bout of the same protocol.

SIGNIFICANCE: This study will determine the effects that occur to natural male competitive bodybuilders following an extreme bodybuilding training protocol. It will also clarify the protective adaptations that may occur following a repeated bout of the combined training. This will provide a better understanding of the repeated bout effect in resistance-trained individuals and work progressively to close the fundamental gap apparent within the literature.

AIM:
The aims of this study are twofold:

(1) To examine the extent of muscle damage that occurs to natural competitive bodybuilders after an unaccustomed training bout which is the Bodybuilding Training (BT) and the High Intense Single Set Training (HISST), and

(2) To compare between the first and second bout of a combination of BT and HISST for changes in muscle damage markers to investigate whether the repeated bout effect exists. From these studies, it is expected to clarify muscle damage and adaptation (the repeated bout effect) profile of natural competitive bodybuilders.
METHODS:
Sixteen natural competitive bodybuilding males, aged between 18 – 30 years old will be recruited for this study. All participants will have no current or existing injuries that may affect their ability from completing the study. All individuals will be required to have a minimal bodybuilding training experience of 3 years, and yield a minimum of one state level entrance show. The experiment will be performed using the Strength and Conditioning Laboratory (JO 19.149) and the Exercise Physiology Research Laboratory (JO 19.150), where the necessary equipment will be set up. The participants who volunteer for the study will be asked to report to the Strength Laboratory on 9 occasions for between 45min – 2 hours. It will be a requirement not to perform any resistance training directly targeting the chest muscles for the duration of the study and avoid training the triceps and shoulder 24 hours before and 24 hours following the training session.

- **Warm-up protocol:** The warm-up protocol is performed before each training session and before the performance measures on 1-3 days after each training session. The warm-up will consist of dynamic stretching: 10 arm swings side to side, 10 arm swings up, down and back and 2 warm-up sets of the barbell bench press.

- **One Repetition Max (1RM) and Ten Repetition Max (10RM) Test:** 1RM and 10RM testing is performed during the familiarisation session following the warm-up. A Barbell Bench Press will be performed to obtain the 1RM. A Dumbbell Flat Flye will be performed to obtain the 10RM, separated by a 15min recovery. These results will be used to determine the training load.

- **Bodybuilding Training (BT):** The bodybuilding training protocol (BT) will be incorporating multiple exercises, based on the training principles most commonly used in the bodybuilding community and recommendations from several articles and textbooks. In total 6 exercises will be used, focusing on multiple joint exercises initially and concluding using single joint movements. Repetitions of between 6-12 will be utilised and sets of 3-4 will be encompassed. Multi-joint exercises will be set at a recovery of 60 seconds whereas the single joint exercises will be set at a recovery of 30 seconds, with no more than a 120-second recovery between exercises. The cadence of movement will be based off standard movement patterns using a ratio of 2:1:2:1 (eccentric, isometric, concentric, isometric). Total sets will equate to 21 and total repetitions will approximate to 221.

- **High Intense Single Set Training (HISST):** The HISST protocol will include 4 circuits of between 4-8 exercises per circuit. Each circuit is to be performed once and each exercise is to volitional failure with a slow cadence during both the eccentric and concentric phase with a ratio of approximately 3:1:2 (eccentric, isometric, concentric) with the load being lifted approximately between 6-15 repetitions with a 120-second recovery between circuits. Total exercises performed will equate to 19, total repetitions will vary from athlete to athlete.

- **Supplementation:** Supplementation will be standardised for the pre-workout drink, Intra-workout drink, and post-workout drink endorsed by Bulk Nutrients Australia. The pre-workout drink and intra-workout drink will be BCAA’s recovery formulated with Dextrose Monohydrate (sugar – monosaccharide). All formulations are to be consumed by the end of the training session. Once training has finalised the consumption of the post workout formula will be administered consisting of whey protein isolate (WPI) and Dextrose Monohydrate which will be consumed following all testing protocols. Any participant with allergens to any of these products will be excluded from the study.
TESTING:

- **Isometric bench press:** This test requires the participant to assume a bench press position, creating a 90° angle with the elbows. The participant will push vertically as hard as possibly until peak force has been obtained. This exercise will be performed 3x separated by a 60 second recovery and a test of muscle soreness following each set.

- **Barbell bench throw:** The barbell bench throw will be performed in a Smith machine on a flat bench. The participant will begin by assuming a bench press position, from there they will unhook the pre-determined 40 kg load (20kg bar + 2 x 10kg plates) and perform the movement by drawing the bar to the nipple line and then explosively throwing the bar as high as possible. The participant will catch the bar during the descent, gradually reduce the speed of the moving bar, bring it back up to the extended position and repeat the movement twice. This test will be performed 3x separated by a 60 second recovery.

- **Isokinetic elbow extensor concentric contraction torque:** The isokinetic elbow extensor concentric torque will be performed using a Biodex 3.0 isokinetic dynamometer; this will determine peak torque and optimal angle. This will involve performing an elbow extension exercise set at a constant cadence.

- **Muscle soreness:** The soreness of the muscle will be obtained through the use of a visual analogue scale whereby the participant will rate the soreness of their chest following palpation of the targeted area. During the isometric bench press measure, the participant will be asked to rate the level of pain experienced after each set. Muscle soreness will also be assessed during maximal voluntary muscle stretching. Participants will sit on a chair and be asked to abduct their arms to a 90° angle to the body, with the arms internally rotated and asked to stretch back until no more pain is tolerable and the level of pain will be measured according to the VAS.

- **Flexibility:** Flexibility of the chest and triceps brachii will be assessed by the use of a goniometer. This will be performed to determine muscle flexibility following intensive exercise training.

- **Plasma CK Activity:** Using capillary tubes approximately 30 μL of blood will be collected from the your index finger with the use of a sterile lancet. Using a pipette the blood will be immediately transferred to a CK test strip and assayed by a Reflotron to determine the levels of creatine kinase in your bloodstream.
ETHICAL APPROVAL:
This project has been reviewed by, and received ethical clearance through the Human Research Ethics Committee ECU.

BENEFITS OF PARTICIPATION:
You will receive information regarding muscle damage and adaptations that occur to resistance-trained athletes, as well as the ability to ask questions regarding common modalities of bodybuilding training. We will also be able to show you your data and explain the analysis process of what that data represents. Each participant will also receive a Bulk Nutrients supplement bag, which will include product information and sample products.

POTENTIAL RISKS AND DISCOMFORTS:
As you will be asked to perform maximal muscle contractions and very extreme types of training, with training to volitional failure during the experiment there is a small risk of musculoskeletal injury. This risk will be further minimised through the use of appropriate warm-up practices, familiarisation session and supervision by a qualified researcher. We will consistently remind you; to tell us immediately if you experience any pain during the procedures. Another potential risk and/or discomfort, which is classified as a minor risk, is the use of the lancets to obtain a creatine kinase measure, however these lancets can only pierce skin and no notable risks are associated with a minor prick. There are no other notable risks involved with the any of the other testing procedures. If you have any questions about the risks to you, please contact or ask us immediately.

PARTICIPATION RIGHTS:
Participation in this study is completely voluntary. You retain the right to withdraw from the study or refuse measurements at any time, and without the need to give reason for your decision. There will be no consequences or prejudice for your withdrawal or refusal of single measurements. Reporting of the study findings will be done with complete confidentiality and your identity will not be disclosed to anyone outside of the study at any time. You have the right to receive information regarding your own data/results at any time during the study from a member of the research team.
DATA COLLECTION/STORAGE:

The head investigator (Ken Blowfield at ECU) and the supervisory team (Professor Ken Nosaka, Dr. G. Gregory Haff) will store the data securely so that personal information cannot be accessed by those external to the study. Data collection sheets (paper version) and back-up copies on hard disk drives will be safeguarded by storage in a locked cabinet and/or protected by the above researchers on a password-protected computer.

USE OF RESULTS:

The results of this study will be published in a peer-reviewed scientific journal, and will also be presented in conferences in the future.

QUESTIONS:

If you have any questions or require further information about the research project, please contact Chief Researcher: Ken Blowfield on 0416 653 94, email ken.blowfield@ecu.edu.au or supervising researchers Prof. Ken Nosaka at (08) 6304 5655 or Dr. G. Gregory Haff at (08) 6304 5416. If you have any concerns of complaints about the research project and wish to talk to an independent person, you may contact the Edith Cowan University Research Ethics Officer at (08) 6304 2170 or Email: research.ethics@ecu.edu.au

Lead Investigator:
Ken Blowfield (SEHS)
Faculty of Health, Engineering and Science
Exercise & Sports Science, ECU
ken.blowfield@ecu.edu.au
6304 5073
0416053946

Supervisor’s:
Prof. Ken Nosaka (SEHS)
Faculty of Health, Engineering and Science
Exercise & Sports Science, ECU
k.nosaka@ecu.edu.au
6304 5655
Dr. G. Gregory Haff (SEHS)
Faculty of Health, Engineering and Science
Exercise & Sports Science, ECU
g.haff@ecu.edu.au
6304 5416

If you have concerns regarding the study or would like to speak to an independently person, you may contact the research ethics officer at the following:
Telephone: 6304 2170
Email: research.ethics@ecu.edu.au

Thank-you for your interest in this study, and taking the time to read this information booklet.
Yours In Fitness and Health,

Ken Blowfield, MSc*, Ex&Sprts Sc., Adv. Dip Nutri., AWC/SPC., AWFM, ESSAM
Prof. Ken Nosaka
Dr. G. Gregory Haff
APPENDIX F:
MEDICAL QUESTIONNAIRE

Name: ___________________________ Age: _____ yr  Weight: ___ kg  Height: ___ cm

Emergency Contact – Name: ___________________  Number: ____________________

Heart Rate (bpm): ______________  Blood Pressure (S/D): __________________________

Briefly describe the type and amount of exercise you perform:

Type: ___________________________________________________________

Sets: __________________________  Reps: __________________________

Amount: _______________________________________________________

Do you smoke? YES NO
Have you smoked in the past? YES NO

Have you ever been diagnosed with –

being overweight? YES NO
diabetes? YES NO
asthma? YES NO
any bleeding disorders? YES NO

Have you ever used any illegal performance enhancing drugs/substances? E.g. Androgen anabolic steroids, peptides, illegal stimulants?

YES NO

If YES please give details

_____________________________________________________________

Have you ever been told by your GP that you are at risk of developing cardiovascular disease? YES NO

If YES please give details

_____________________________________________________________
Have you ever had rheumatic fever?  

YES  NO

If YES please give details

Have you ever experienced heat exhaustion or heat stroke?  

YES  NO

If YES please give details

Is there anything that you are aware of that may limit your capacity to exercise? (e.g., Chronic back pain and/or other joint pain, severe headaches?)  

YES  NO

If YES please give details

Are you currently on any prescribed or non-prescribed medications?  

YES  NO

If YES please give details

Have you suffered from any viral infections, chronic tiredness or donated blood in the past two months?  

YES  NO

If YES please give details
Do you have any skeletal, muscular or connective tissue damage that may affect your ability to participate in this experiment (upper or lower body)?

YES NO

If YES please give details

Do you have any other complaint or any other reason that you know of which you think may prevent you from participating in and completing this experiment?

YES NO

If YES please give details

I, (full name), declare that the above answers are true and correct to the best of my knowledge and that I will, if required, reveal to the research investigators all circumstances known to me concerning my health and fitness that are relevant for the participation of which I am applying. I acknowledge that the provision of incorrect information or the omission of any information regarding my health and fitness may result in the cancellation of my participation or my dismissal from any position in the research investigation.

Print Name  Signed  Date

_________________________  ___________________  __________
APPENDIX G:

24H FOOD RECALL

24-Hour Food Record

This food record booklet is provided for you to record what you have eaten and drank for the last 24 hours. Please be as accurate as possible and record all the foods and drinks consumed to the nearest known measurement.

If you have any queries when completing this food record, please do not hesitate to ask questions.
Instruction to Complete the Food Record

Food records allow detailed description of all food and beverages consumed over a period of 24 hours. The food record provides a way to keep a usually highly fluctuating variable constant. Data from the food records will be analysed statistically. It is very important that as much detail regarding the food and drinks you consumed, including the meal you consumed for, the amount you consumed, the place of consumption and preparation is written down.

- Record everything you eat and drink, including snacks
- Complete the Meal, time, and place prepared and consumed columns for each meal or snack. Please write in name of restaurant if you know it.
- You do not have to use the whole booklet, just record what you eat and drink to the best of your recalling ability.
- Please be honest and true
- Please write clearly

How to Record Each Food

- Describe each food and beverages in detail, as best you can. See example below

<table>
<thead>
<tr>
<th>INCLUDE</th>
<th>FOR EXAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>How prepared</td>
<td>Fried, grilled, stir fried, roast, barbeque, pâm fry</td>
</tr>
<tr>
<td>Added fat</td>
<td>Cook with oil butter</td>
</tr>
<tr>
<td>Brand name and type</td>
<td>Wheatbelt Bakehouse Magnificent Multigrain with added Chia and quinoa</td>
</tr>
<tr>
<td>Portion size</td>
<td>1/2 cup, 2 slices, 1 tbsp</td>
</tr>
</tbody>
</table>

- Describe each ingredient in a mixed dish in the food record, or use Recipes section on page 34. See example below

**Spaghetti Bolognese**

11/2 cups cooked spaghetti, 125g lean beef mince, 1/4 large onion, sliced, 1 clove garlic, 100g crushed tomato, sliced mushrooms, 2 tbsp salt reduced tomato paste

**Pizza**

3/4 cup flour, 1 tbsp vegetable oil, 40g cheese, 38g tomato, 20g mushroom, capsicum and
- Ideally the known weight and amount of food consumed would be ideal, however if you do not know this please estimate the amount of each ingredient to the best of your ability. Demonstration on how to weigh food and drinks with the scale will be provided. Weights of food can be calculated by weighing total and subtracting utensil weight.

**Accuracy and detail is essential. This includes obtaining information such as:**
- Dairy foods – varying fat contents, calcium enriched
- Bread – type, e.g. white, multigrain, wholemeal, fruit/raisin, sour dough
- Method of preparing food = e.g. boiled, baked, pan fried, stir fried, steamed, roast
- Amount and type of fat used in cooking – olive, canola, sesame, butter
- Use of fortified foods – e.g. calcium enriched, fortified cereals with nuts and seeds
- Brand names whatever possible – for bread, cereals

**An Example:**

**Food Record:**

<table>
<thead>
<tr>
<th>Day: _<em><strong><strong><strong><strong>1</strong></strong></strong></strong></em></th>
<th>Date: <strong>21 (DD)</strong>/ <strong>8 (MM)</strong>/ <strong>2013 (YYYY)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale and measuring tools used?</td>
<td>□ Yes □ No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>Place prepared and consumed</th>
<th>Food and Beverages (Please describe Brand name, cooking method used, type i.e. low fat, and any additional information)</th>
<th>Amount and unit of consumption, (e.g. Tbsp, Tsp, Oz., Pieces, Slice, Cup, gram, handful, glass, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>8am</td>
<td>H</td>
<td>Homemade breakfast cereal: 1 bowl</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Milk, reduced fat, Harvey Fresh</td>
<td>200ml</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Corn flakes, Freedom Foods</td>
<td>1/4 cup</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wheat germ powder</td>
<td>2 tsp</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Almonds</td>
<td>6 nuts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pistachio</td>
<td>4 nuts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dried cranberry</td>
<td>5 berries</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>White chocolate chips</td>
<td>8 chips</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oat, minute, McKenzie’s</td>
<td>3/4 cup</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>All microwaved</td>
<td></td>
</tr>
</tbody>
</table>

3
Food Record:

Day: _________________________  Date: ___ / ___ / ___

Scale and measuring tools used?  [ ] Yes  [ ] No

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>Place prepared and consumed</th>
<th>Food and Beverages (Please describe Brand name, cooking method used, type i.e. low fat, and any additional information)</th>
<th>Amount and unit of consumption, (e.g. Tbsp, Tsp, Oz., Pieces, Slice, Cup, gram, handful, glass, etc.)</th>
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</thead>
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</table>


**Food Record - Recipes:**

This section is for you to record the details of the recipes you use. It is not necessary to write in this section as long as you write down the ingredients and preparation method of the recipe, and the amount you have eaten in the food record section. If you choose to record here, please record the number of servings made, and the number of servings you ate.

Example: _pasta and tuna salad_

# of servings (1 cup each) made: __6__  # of servings (1 cup each) you have: __1__

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>PREPARATION</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaroni or pasta tube</td>
<td>Boiled, cooked</td>
<td>375g</td>
</tr>
<tr>
<td>(penne)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>Hard boiled, peeled, cut into quarters</td>
<td>2</td>
</tr>
<tr>
<td>Large tomatoes</td>
<td>Cut, raw</td>
<td>2</td>
</tr>
<tr>
<td>Can tuna in water</td>
<td>Drained, mashed</td>
<td>185g per can</td>
</tr>
<tr>
<td>Green capsicum</td>
<td>Diced, raw</td>
<td>1</td>
</tr>
<tr>
<td>Olive oil</td>
<td></td>
<td>2 tsp</td>
</tr>
<tr>
<td>Lemon juice</td>
<td></td>
<td>2 tsp</td>
</tr>
</tbody>
</table>

Recipe 1 ______________________

Day and date you cooked this recipe _________, ____/____/____

Number of servings made: _____  Number of servings you have: _____

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>PREPARATION</th>
<th>AMOUNT</th>
</tr>
</thead>
</table>