

**The influence of the repeated bout effect on skeletal muscle power production,
damage-induced strength loss and fatigue-induced strength loss**

by

Adam Hess

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Human Health and Nutritional Sciences

Guelph, Ontario, Canada

© Adam Hess, September, 2018

ABSTRACT

THE INFLUENCE OF THE REPEATED BOUT EFFECT ON SKELETAL MUSCLE POWER PRODUCTION, DAMAGE-INDUCED STRENGTH LOSS AND FATIGUE-INDUCED STRENGTH LOSS

Adam Hess

University of Guelph, 2018

Advisor:

Geoffrey A. Power

High intensity unaccustomed eccentric contractions result in fatigue and damage, as well as prolonged strength deficits. The relative contribution of fatigue and damage is difficult to tease apart. The repeated bout effect (RBE) is established in its protection of damage and strength loss following a subsequent bout of eccentric contractions but it is unknown whether it protects power production, a more functional measure of dynamic muscle performance. This thesis aims to investigate the influence of the RBE on power production and to determine if the protection from damage will allow for the assessment of fatigue induced strength loss during and following a subsequent bout of eccentric contractions. I found that the RBE protects the rates of torque and velocity development ultimately protecting the production of peak power. Furthermore, the magnitude of protection against damage permitted the assessment of fatigue only related neuromuscular impairments following high intensity unaccustomed eccentric contractions.

ACKNOWLEDGEMENTS

Geoff, Jeeeeef , Gpow, gp – Thank you for realizing very early that I need to complete my tasks in a logically sequential order, thank you for pushing me through the times when I couldn't see all the pieces yet and would have tinkered for months and gotten stuck. Thank you for being an always available, on-time, morning person; I'm sure this is why we work so well together. Thank you for the all-encompassing Friday meetings talking about bikes (even though I didn't care), talking about soccer (even though you didn't care), talking about the new beers we've been trying, talking really fast because you've had a lot of coffee already and of course talking about the freshest science on Twitter. I greatly appreciate the opportunities you provided me and am excited to see how the lab develops in the years to come.

Jackey, Matt, Nicole – For making yourselves available every morning while I was testing, for adjusting schedules and coming in on weekends. This study was ambitious and time consuming and would not have succeeded without you guys. I would like to not only thank you for your help in my study but also for the laughs and fun we had while in the lab, there were far too many to list here.

Participants –Thank you, thank you, thank you. Only you guys know how thankful I am that you were willing to endure this study.

Mom and Dad – Thank you for everything. There are not enough words to express how thankful I am that you've always supported me.

Morgan – You push me to be better at everything I do because that's the only way to do anything. Thank you for the game breaks when I needed them and for pushing me through this last stretch.

TABLE OF CONTENTS

Abstract	ii
Acknowledgements	iii
Table of Contents.....	iv
List of Figures.....	vii
List of Terms	x
List of Appendices	xii
1 Brief Introduction.....	1
2 Review of the Literature.....	3
2.1 Skeletal Muscle Organization and Contraction.....	3
2.1.1 Eccentric Contractions and Damage.....	7
2.2 Skeletal Muscle Fatigue.....	11
2.2.1 Central Fatigue	12
2.2.2 Peripheral Fatigue.....	13
2.3 The Repeated Bout Effect.....	15
2.3.1 Contributors.....	16
2.3.2 Mechanisms.....	17
2.4 Purpose and Hypotheses	22
3 Methods	23
3.1 Ethical Approval.....	23
3.2 Participants and Time Points.....	23
3.3 Experimental Set-up	24
3.3.1 Musculocutaneous Nerve Stimulation	25

3.3.2	Maximal Voluntary Contraction and Percutaneous Muscle Stimulation.....	26
3.3.3	Serum Creatine Kinase.....	27
3.4	Experimental Protocol	29
3.4.1	Baseline Parameters.....	29
3.4.2	Eccentric Protocol.....	30
3.4.3	Recovery Protocol.....	30
3.5	Data and Statistical Analysis.....	32
3.5.1	Isometric Contractions (MVC, VA and LFF assessment)	32
3.5.2	Electromyography.....	32
3.5.3	Dynamic Contractions.....	33
3.5.4	Statistical Analysis.....	33
4	Results	34
4.1	Creatine Kinase and Soreness	34
4.2	Strength Loss.....	36
4.3	Peak Power and Time Dependent Measures.....	37
4.3.1	Peak Power	37
4.3.2	Rate of Velocity Development	37
4.3.3	Rate of Torque Development	37
4.4	Unconstrained Shortening.....	41
4.4.1	Unloaded Rate of Velocity Development	41
4.4.2	Maximal Shortening Velocity	41
4.5	Electrical Stimulation	44
4.5.1	Voluntary Activation, Doublet Peak Torque and Low Frequency Fatigue	44
4.6	Electromyography	48

4.6.1	Biceps Brachii and Triceps Brachii	48
5	Discussion.....	50
5.1	Damage Induced Strength Loss.....	51
5.2	Fatigue Induced Strength Loss.....	52
5.3	Peak Power.....	55
6	Conclusion	57
6.1	Limitations	57
6.2	Future Directions.....	58
6.3	Conclusion	59
	References	61
7	Appendices	70
7.1	Research Ethics Certification.....	70
7.2	Liquid Creatine Kinase Datasheet and Protocol	72

LIST OF FIGURES

Figure 1: The process of muscle contraction begins with an action potential as it propagates along the sarcolemma. 1) Action potential as it propagates down the T-tubule structure. 2) Ca^{2+} release from the sarcoplasmic reticulum. 3) Exposure of myosin binding site. 4) Power stroke from myosin head causing the actin filaments to slide closer which causes myofibrillar shortening. 5) Full contraction of the muscle as a result of many shortened muscle fibers. Adapted from (Betts et al., 2017).

Figure 2: The force-length relationship. The length of individual sarcomeres influences the overlap of actin and myosin filaments, this influences the force that can be produced (Betts et al., 2017).

Figure 3: The force-velocity relationship. An isometric contraction (dashed line) produces optimal force as there is maximal cross-bridge cycling. When contracting concentrically (positive velocity) the force produced decreases as velocity increases. When contracting eccentrically (negative velocity) forces exceed that which is seen in isometric or concentric contractions.

Figure 4: The phosphocreatine pathway which utilizes creatine kinase (CK) to transfer a phosphate group from phosphocreatine (PCr) to a cytosolic adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) to provide energy for muscle activity. Adapted from (Baird et al., 2012).

Figure 5: Mechanisms underpinning the repeated bout effect. Adapted from (Hyldahl et al., 2017).

Figure 6: Experimental set-up with subject seated and secured in the dynamometer. Dynamic arm range of motion depicted by dashed red line with solid red lines depicting top (50°), isometric (110°) and bottom (140°) positions. Focused picture provides an example of electrode placement on a participants BB. Green electrodes are the customized percutaneous stimulation electrodes and positioned between them the surface EMG electrodes.

Figure 7: Schematic representation depicting the order of procedures during the experimental protocol. H1 and H2 are venipunctures occurring on baseline day and follow-up days, respectively. A is the ITT-LFF assessment where black triangles represent twitches, white triangles are doublets, black bar is MVC, white bolt is 10Hz stimulation and black bolt is 50Hz stimulation. B is practice dynamic movements. C represents a torque-frequency curve. D dynamic contractions for assessment of peak power. E represents the eccentric protocol with the ITT-LFF occurring after each set. F represents the recovery time points from 0min-7 days post.

Figure 8: Serum CK activity during ECC1 (black) and ECC2 (red) was significantly greater than baseline during ECC1 at 72Hr and 96Hr ($p<0.05$)** and ECC2 did not differ significantly from baseline at any time point ($p>0.05$). There was an effect of bout at time points 72Hr and 96Hr ($p<0.05$)† . Mean \pm SE.

Figure 9: Self-reported soreness for ECC1 (black) and ECC2 (red). ECC1 showed a significant deviation from baseline at time points: post, 24Hr, 48Hr and 72Hr ($p<0.05$)** and an effect of time ($p<0.05$)*. ECC2 did not deviate significantly from baseline at any time point ($p>0.05$). An effect

of bout was seen between ECC1 and ECC2 at time points: post, 48Hr, 72Hr and 96Hr ($p<0.05$) †. Mean \pm SE.

Figure 10: MVC torque for ECC1 (black) and ECC2 (red). ECC1 is reduced below baseline for all time points and ECC2 is only reduced up to 30min ($p<0.05$)**. A significant effect of time exists up to 96Hr ($p<0.05$)* and an effect of bout from 0min-7D ($p<0.05$)†. Mean \pm SE.

Figure 11: Peak power for ECC1 (black) and ECC2 (red). ECC1 remained significantly below baseline for all time points and ECC2 only up to 30min ($p<0.05$)**. An effect of time was seen up to 96Hr ($p<0.05$)*. An effect of bout was seen for all time points ($p<0.05$)†. Mean \pm SE.

Figure 12: Rate of velocity development for ECC1 (black) and ECC2 (red). ECC1 remained below baseline at all time points and ECC2 remained below baseline up to time 20min ($p<0.05$)**. There was an effect of bout and an effect of time ($p<0.05$) †,*. Mean \pm SE.

Figure 13: Rate of torque development for ECC1 (black) and ECC2 (red). ECC1 remained below baseline at all time points and ECC2 remained below baseline up to 30min post ($p<0.05$)**. There was an effect of bout from time point 24Hr to 7D ($p<0.05$)†. Mean \pm SE.

Figure 14: Unloaded rate of velocity development for ECC1 (black) and ECC2 (red). ECC1 remained below baseline at all time points and ECC2 remained below baseline up to 30min post ($p<0.05$)**. There was an effect of bout and time ($p<0.05$) †,*. Mean \pm SE.

Figure 15: Maximal unconstrained shortening velocity for ECC1 (black) and ECC2 (red). ECC1 remained below baseline for all time points except 72Hr and 7D ($p<0.05$)**. ECC2 was only below baseline at 2 time points: 15min and 20min ($p<0.05$)**. There was an effect of bout observed ($p<0.05$)†. Mean \pm SE.

Figure 16: Voluntary activation for ECC1 (black) and ECC2 (red). ECC1 was lower than baseline from S3 to 20min and ECC2 was lower than baseline from S2 to 0min ($p<0.05$)**. There is a difference between ECC1 and ECC2 up to 30min post ($p<0.001$). Mean \pm SE.

Figure 17: Doublet peak torque for ECC1 (black) and ECC2 (red). ECC1 remained lower than baseline for all time points and ECC2 remained lower than baseline from S3 to 24Hr post ($p<0.05$)**. There is a difference between ECC1 and ECC2 ($p<0.001$). Mean \pm SE.

Figure 18: 10Hz, 50Hz and 10:50Hz torque for ECC1 (black) and ECC2 (red). A) 10Hz stimulation torque remained lower than baseline for both ECC1 and ECC2 from time points 0min to 30min ($p<0.05$)**. B) 50Hz stimulation torque remained lower than baseline for ECC1 from time points S4 to 30min and for ECC2 from S2 to 30min ($p<0.05$)**. There is a difference between ECC1 and ECC2 torque ($p<0.05$). C) 10:50Hz torque for ECC1 remained below baseline from 5min to 30min and ECC2 from 0min to 30min ($p<0.05$)**. There was no effect of bout ($p>0.05$). Mean \pm SE.

Figure 19: A: RMS EMG of the BB for ECC1 (black) and ECC2 (red). EMG was lower than baseline for ECC1 from time points S3 to 20min and ECC2 from S2 to 30min ($p<0.05$)**. There

was no difference between bouts ($p>0.05$). B: RMS EMG of the TB (antagonist coactivation) did not differ from baseline at any time point and no effect of bout was seen ($p>0.05$). Mean \pm SE.

LIST OF TERMS

ADP – Adenosine diphosphate.

ATP – Adenosine triphosphate.

ANOVA – Analysis of variance.

BB – Biceps Brachii.

Ca²⁺ – Calcium ion.

CK – Creatine kinase.

DISL – The magnitude of strength loss between baseline and the 24Hr time point. The long lasting (>24Hr) nature of this strength loss is referring to its damage related mechanisms.

Doublet – The product of two electrical stimuli delivered at a high frequency (100Hz) to the nerve or muscle which produces a mechanical response. Current is increased until response is maximized.

EC Coupling – Excitation contraction coupling.

ECC1 – The 6 day protocol including the first eccentric exercise protocol and time points 24Hr, 48Hr, 72Hr, 96Hr and 7D.

ECC2 - The 6 day protocol including the second eccentric exercise protocol and time points 24Hr, 48Hr, 72Hr, 96Hr and 7D.

EMG – Electromyography.

FISL – The magnitude of strength loss between the peak minimum and 24Hr time point. The short term (<30min) nature of this strength loss is referring to its fatigue related mechanisms.

ITT – Interpolated twitch technique.

LFF – Low frequency fatigue.

M_{max} – The compound muscle action potential which signifies activation of all motor units via peripheral nerve stimulation. The wave form is recorded through surface electromyography.

MVC – Maximal voluntary isometric contraction. The maximum torque produced during an isometric contraction.

Peak Power – The resistive load (%MVC) at which an individual produces the greatest power.

Power – The product of torque and velocity which provides a functional measure of contraction.

RBE – Repeated bout effect.

ROM – Range of motion.

RTD – Rate of torque development. The maximum slope of the torque-time curve produced during isometric and dynamic contractions.

RVD – Rate of velocity development (acceleration). The maximum slope of the velocity-time curve produced during dynamic, loaded contractions.

TB – Triceps Brachii.

Twitch – The product of a single electrical stimulus delivered to the nerve or muscle which produces a mechanical response of the muscle. Current is increased until response is maximized.

ULRVD – Unloaded rate of velocity development. The maximum slope of the velocity-time curve produced during an unloaded isokinetic shortening contraction.

VA – Voluntary activation.

LIST OF APPENDICES

Appendix 7.1 Research Ethics Certification

Appendix 7.2 Liquid Creatine Kinase Datasheet and Protocol

1 Brief Introduction

An eccentric contraction is when skeletal muscle is active while lengthening (Betts et al., 2017). Unique to eccentric contractions is the coexistence of damage (Fridén et al., 1983; Hough, 1900) and fatigue (Abbott and Aubert, 1951; Hough, 1900). Through differing mechanisms, damage and fatigue ultimately manifest in similar short term deficits in muscle strength, power and velocity (Choi and Widrick, 2009; Pasquet et al., 2000; Power et al., 2010). The duration of these deficits also varies whether the root cause is damage (long lasting >24Hr) or fatigue (short lasting <30min) (Enoka and Duchateau, 2008; Nosaka et al., 1991). Rate of torque development (RTD) and rate of velocity development (RVD) are crucial time-dependent measures required to achieve peak power; however, both are negatively impacted by damage and fatigue (Power et al., 2010, 2013; Wallace et al., 2016). The concomitant existence of damage and fatigue poses an interesting problem; how can we determine deficits owing to fatigue and damage following repeated eccentric contractions?

The time following an initial bout of unaccustomed eccentric contractions provides an opportunity for muscle to recover and adapt (Clarkson and Tremblay, 1988). When a second bout of eccentric exercise is performed there is an attenuation of muscle damage, this is known as the repeated bout effect (RBE) (Nosaka et al., 1991). The RBE is well established in its attenuation of overall strength loss and muscle damage (Nosaka and Aoki, 2011). However, its influence on fatigue induced strength loss (FISL) and damage induced strength loss (DISL) as they pertain to muscular power is not well understood.

I used the RBE as a unique paradigm to isolate the effects of fatigue and damage following identical high intensity eccentric exercise protocols of the elbow flexors, performed 4 weeks apart. Furthermore, by normalizing isotonic loads (used to calculate power during shortening contractions) to daily maximal torque values the muscular weakness associated with damage was corrected for. Correcting for weakness provided insight to whether the RBE protects time dependent factors (RTD, RVD) which drive power production

The aims of this thesis were threefold:

- 1) To investigate the influence of the repeated bout effect on power-loss following repetitive lengthening contractions.
- 2) To investigate the influence of the repeated bout effect on damage induced strength loss.
- 3) To investigate the influence of the repeated bout effect on fatigue induced strength loss.

I hypothesized that there would be:

- 1) A maintenance of power production resulting from a protection of RTD and RVD owing to the repeated bout effect.
- 2) A protection of muscle damage, as measured by torque deficits and serum creatine kinase activity, thereby reducing the magnitude of DISL.
- 3) A protection from damage which will isolate the effects of fatigue following a subsequent bout of eccentric contractions, thus allowing the assessment of fatigue only related deficits in neuromuscular function (i.e., strength, power) following high intensity eccentric exercise.

2 Review of the Literature

2.1 Skeletal Muscle Organization and Contraction

Skeletal muscle is a hierarchical structure, whole muscles are comprised of smaller compartments of muscle fascicles, fibers, myofibrils and sarcomeres that are sheathed in fibrous connective tissue (Jones et al., 2004). Sarcomeres are the smallest contractile unit of muscle and a crystalline arrangement of sarcomeres forms a myofibril (Korthuis, 2011). Within the sarcomere, actin and myosin filaments are interdigitated and during contraction, the actin filaments are drawn closer together by the attachment and power stroke of myosin heads (Betts et al., 2017). Contractions are initiated by an action potential which, when distal to the neuromuscular junction, propagates along the sarcolemma. Action potentials continue propagating down a T-tubule structure, triggering receptors which release Ca^{2+} (Betts et al., 2017). The influx of Ca^{2+} to the myofibril binds troponin which changes conformation and removes tropomyosin from the actin filament exposing a site for myosin heads to bind. When actin and myosin are bound to each other, the active state of cross-bridge cycling causes rotation of myosin filaments and the power stroke of their heads (Betts et al., 2017). The shortening of the sarcomere is what ultimately results in a complete muscle contraction (**figure 1**).

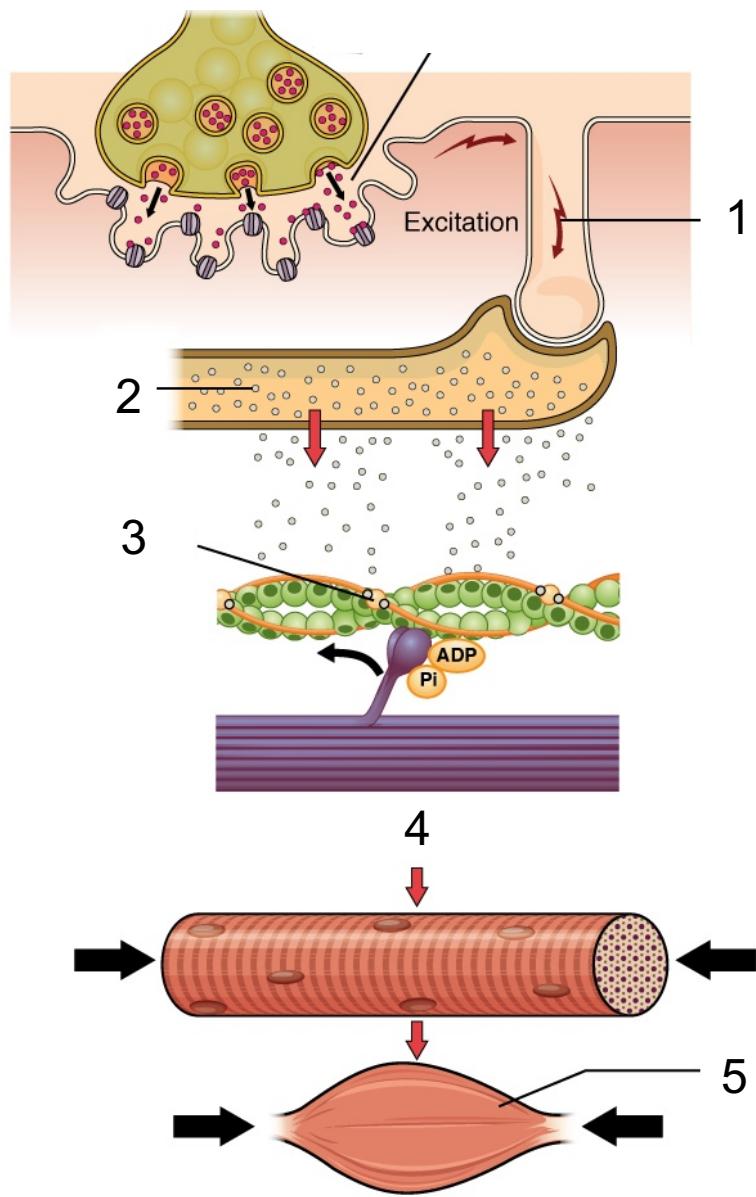


Figure 1: The process of muscle contraction begins with an action potential as it propagates along the sarcolemma. 1) Action potential as it propagates down the T-tubule structure. 2) Ca^{2+} release from the sarcoplasmic reticulum. 3) Exposure of myosin binding site. 4) Power stroke from myosin head causing the actin filaments to slide closer which causes myofibrillar shortening. 5) Full contraction of the muscle as a result of many shortened muscle fibers. Adapted from (Betts et al., 2017).

Contractions can be classified into three distinct orders; 1) Isometric: a static contraction in which the active muscle is not accompanied by any joint rotation. 2) Concentric: a contraction in which there is joint rotation and the active muscle is shortened. 3) Eccentric: a contraction in which there is joint rotation and the active muscle is lengthened. There are several determinants which govern the force produced by each contraction type.

Firstly, the overlap of actin and myosin filaments is what dictates the force-length relationship (Gordon et al., 1966) (**figure 2**). The absolute length of a sarcomere, and consequently the muscle as a whole, inherently affects the force that can be produced and, as such, the force produced by a muscle changes as it proceeds through range of motion. Overlap of these filaments is directly related to the number of actin and myosin cross-bridges that can be formed, if the positioning of the filaments in relation to one another is unfavorably stretched or shortened, select myosin heads are not positioned to attach, thereby reducing the force (Pollack, 1983). The velocity with which a muscle contracts also governs the magnitude of force produced, this is the force-velocity relationship. As seen in **figure 3**, increased shortening velocity is associated with lower force production owing to the decreased probability of forming actin-myosin bonds associated with high velocity contractions (Katz, 1939). Power is a measure of dynamic muscular function and consists of the trade-off between torque and velocity (Sargeant, 2007). As dictated by the force-velocity relationship, there must be a velocity and external load at which optimal power is generated from the muscle, this is referred to as peak power.

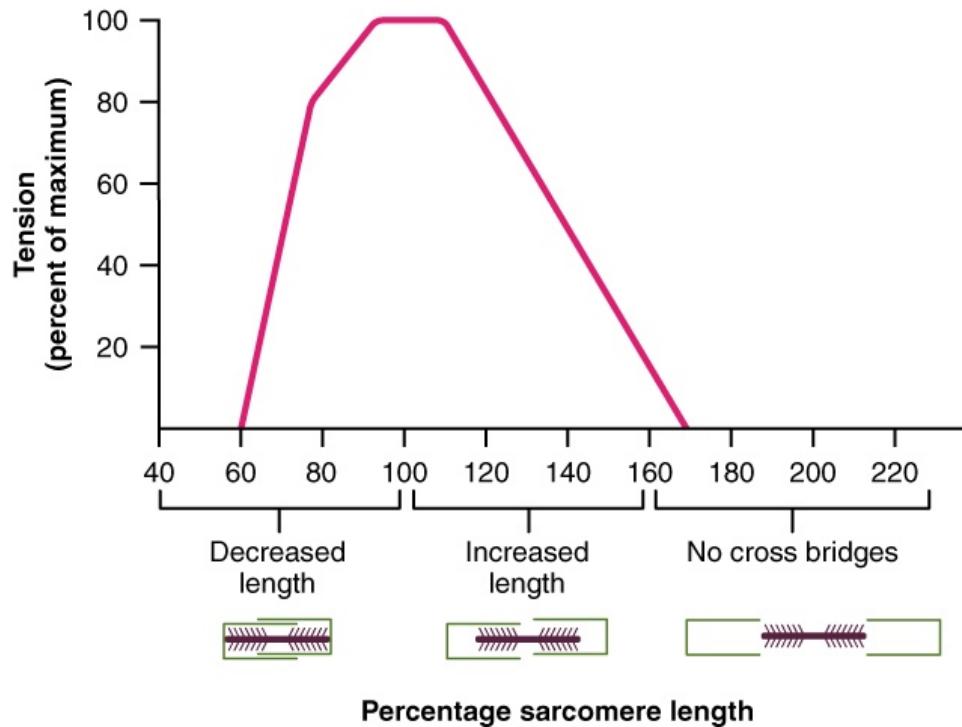


Figure 2: The force-length relationship. The length of individual sarcomeres influences the overlap of actin and myosin filaments, this influences the force that can be produced (Betts et al., 2017).

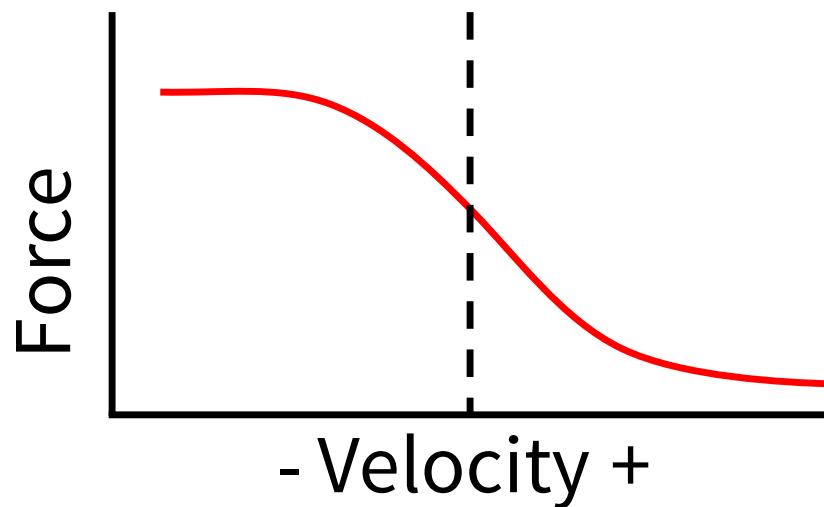


Figure 3: The force-velocity relationship. An isometric contraction (dashed line) produces optimal force as there is maximal cross-bridge cycling. When contracting concentrically (positive velocity) the force produced decreases as velocity increases. When contracting eccentrically (negative velocity) forces exceed that which is seen in isometric or concentric contractions.

2.1.1 Eccentric Contractions and Damage

Active lengthening of a muscle occurs when an external load exceeds the force output of the muscle. Actin and myosin bonds are forcibly broken as the muscle lengthens under tension, force production is greater (Huxley, 1957) and neuromuscular activity is decreased (Bigland and Lippold, 1954) when compared to isometric and concentric contractions. The force-velocity relationship dictates that as a muscles shortening velocity increases, the force produced decreases; this is directly associated with the probability of forming cross-bridges (Hill, 1938). Conversely, if a muscle is contracted and forcibly lengthened the magnitude of force increases relative to the maximal isometric contraction (Huxley, 1957). Increased force production during lengthening is apparent through the established force-velocity relationship (Hill, 1938).

The study of eccentric contractions has since elucidated several unique mechanisms that may help explain what occurs when a muscle is lengthened under tension. Marco Linari and colleagues first noted that increased tension associated with active lengthening may trigger the attachment of a secondary myosin head in frog muscle (Linari et al., 2000), and later confirmed this in human tissue (Linari et al., 2015). The addition of a secondary myosin attachment during eccentric contractions aids in explaining the greater magnitude of forces seen during lengthening. Furthermore, the forcible breaking of actin-myosin bonds does not return the myosin head to their fully ‘off’ position but rather, they remain in close proximity to the actin filament and quickly reattach (Huxley, 1998; Linari et al., 2004). As the myosin heads remain close they are not completing a full cross-bridge cycle before reattaching, this reduces the demand for adenosine triphosphate (ATP) during eccentric contractions and subsequently lowers the energetic demands (Curtin and Davies, 1975). This forcible detaching of the actin and myosin filaments disrupts the

sarcomere and causes damage to the passive connective tissues which become heavily relied upon when sarcomeres are stretched too far (Fridén et al., 1983).

Following a single bout of unaccustomed eccentric exercise a cascade of detrimental effects is seen. Most notably, there is a significant reduction in maximal force production immediately following the exercise intervention (Clarkson and Hubal, 2002; Hough, 1900; Power et al., 2010). The long lasting deficits of lengthening contractions was first noted by Theodore Hough in his seminal paper discussing fatigue and soreness as it is associated with eccentric contractions (Hough, 1900). Hough described a short lasting reduction in force which he attributed to fatigue of the muscle and a long lasting soreness paired with prolonged force-loss which he attributed to muscular damage or rupture. Of course without direct evidence, Hough could only speculate on what was occurring at the muscle but he was very accurate in his assumptions.

Direct measures of muscle damage have proven to be few and far between. The gold standard, a muscle biopsy, can show unequivocal evidence of sarcomeric disruption (Fridén et al., 1983), however, a localized biopsy fails to provide a global assessment of the muscle damage and therefore is difficult to extrapolate the data to the muscle as a whole (Peake et al., 2016). Furthermore, the biopsy procedure itself has been shown to cause damage to sarcomeres which introduces uncertainty (Roth et al., 2000). To add to this, certain study designs are not conducive to muscle biopsies. For instance, when a repeated measures design is employed following an exercise intervention the soreness and swelling associated with a biopsy would likely impede the performance of the subjects.

Inability to use the muscle biopsy technique has resulted in the search for adequate indirect measures of muscle damage. Volitional and electrically stimulated maximal strength measurements (Byrne et al., 2004), RTD (Peñailillo et al., 2015), power (Power et al., 2013), shifting of the torque-length relationship (Jones et al., 1997), blood biomarkers (creatine kinase (CK), troponin, inflammatory cytokines) (Chen et al., 2013; Peake et al., 2005; Rebalka and Hawke, 2014) and self-reported soreness (Paleckis et al., 2015) have all been used to infer muscle damage following exercise interventions. It seems that due to the lack of direct measures, a wealth of indirect measures is necessary to support claims of muscle damage.

Commonly used and supported indirect measures of muscle damage are a prolonged decrease in maximal voluntary strength and an increase in CK activity in the blood (Byrne et al., 2004; Warren et al., 1999a). Strength loss, when compared to a baseline, provides insight to maximal muscle function following damaging contractions (Clarkson and Hubal, 2002) and increased CK in the blood suggests myofibrillar disruption occurred (Koch et al., 2014). CK is an enzyme that exists in the muscle as an integral part of the phosphocreatine pathway and acts to transfer a phosphate group from creatine to an adenosine diphosphate (ADP) molecule in order to generate ATP for muscle activity (**figure 4**) (Baird et al., 2012). Following damaging contractions expression of CK in the blood serum is not immediate, it travels through the lymphatic system before it is released in the blood stream and typically spikes 2-4 days following the damaging exercise (Baird et al., 2012; Koch et al., 2014; Nosaka et al., 1991). CK expression is a delayed response and as such it does not correlate with immediate deficits in strength or other performance measures but acts as an independent marker of damage (Baird et al., 2012). Furthermore, increased

CK in the blood is not a quantitative measure of damage but is a reliable qualitative marker that damage to the cell did indeed occur (Baird et al., 2012; Totsuka et al., 2002).

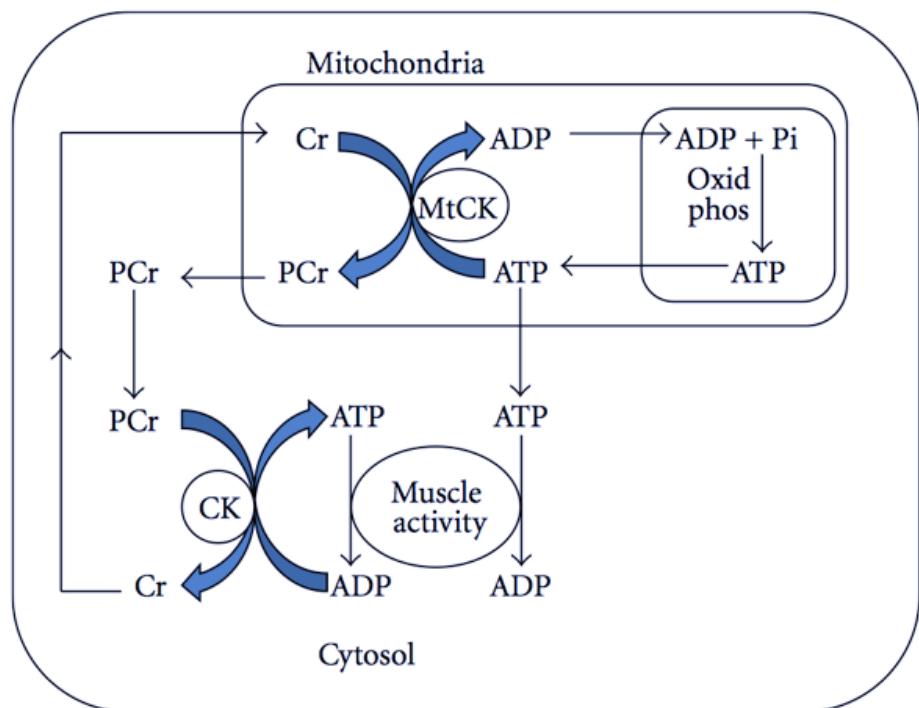


Figure 4: The phosphocreatine pathway which utilizes creatine kinase (CK) to transfer a phosphate group from phosphocreatine (PCr) to a cytosolic adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) to provide energy for muscle activity. Adapted from (Baird et al., 2012).

2.2 Skeletal Muscle Fatigue

As noted above, high intensity lengthening contractions cause a short-term and long-term reduction in force production capacity owing to both muscle fatigue and damage. While muscle damage is long lasting (days-weeks), fatigue recovers relatively rapidly (minutes). Skeletal muscle fatigue is transient and begins from the onset of exercise, persisting throughout the duration of a task until failure or termination (Gandevia, 2001). Fatigue is typically portrayed as a submaximal force which is produced even though a maximal effort is given (Enoka and Duchateau, 2008). Following fatiguing exercise, recovery is relatively quick with strength returning to baseline within ~30-45 minutes (Carroll et al., 2017). However, the magnitude and recovery from fatigue is not always consistent and is influenced by many factors (Bigland-Ritchie et al., 1995).

Fatigue and recovery have been shown to be mode/task dependent through isometric and dynamic fatiguing tasks (Carroll et al., 2017; Cheng and Rice, 2009). As well, a muscle dependency exists which is commonly exhibited when comparing upper and lower limb muscles (Cadigan et al., 2017; Cheng and Rice, 2010; Power et al., 2013). During dynamic tasks, a preferential loss of rate of velocity development (RVD) during fatiguing exercise has a significant negative effect on power production (Lanning et al., 2017). Furthermore, fatigue can be caused through peripheral changes in the muscle or central factors which drive the muscle (Carroll et al., 2017; Gandevia, 2001).

Central and peripheral locations of fatigue are typically determined through various methods of electrical stimulation. Determining the location of fatigue provides a more accurate interpretation of the mechanisms which are failing or contributing to the decline in performance. If a maximal effort is progressively declining, the mechanism(s) at play cannot be determined

without appropriate neuromuscular testing. The following section will discuss central and peripheral fatigue along with common techniques which are used to assess both.

2.2.1 Central Fatigue

It is suggested that central fatigue is a failure to drive/activate a muscle voluntarily (Gandevia, 2001). The mechanisms of central fatigue reside external to the muscle as descending control is affected and the muscle is no longer driven maximally. The application of a maximal electrical stimulus to the motor nerve or muscle itself during a submaximal contraction is seen to increase the torque produced by the muscle. During a maximal effort, if central fatigue is present, a stimulus to the nerve or muscle will activate more motor units, again producing a greater torque and implying there is a volitional failure to excite the entire motor neuron pool (Martin et al., 2004; Merton, 1954; Rutherford et al., 1986). The use of a superimposed stimulus torque and a potentiated resting stimulus torque of the same intensity can be used to calculate a percent voluntary activation of the muscle.

$$\%VA = 1 - \left(\frac{Superimposed\ Stimulus\ Torque}{Potentiated\ Stimulus\ Torque} \right) * 100$$

The initial variation of this was coined the interpolated twitch technique (Merton, 1954) but has since been modified to include high frequency doublets (Duchateau, 2009) as well as high frequency stimuli trains (Bigland-Ritchie et al., 1978). The use of doublets should be commonly

practiced as the doublet stimulus has shown to mitigate variability experienced with single twitches when calculating voluntary activation (Oskouei et al., 2003).

2.2.2 Peripheral Fatigue

Peripheral fatigue is depicted as fatigue induced by alterations to contractile machinery or processes which occur at or distal to the neuromuscular junction (Gandevia, 2001). Peripheral fatigue can be assessed through a multitude of stimulation protocols applied to the peripheral nerve or directly to the muscle (Byrne et al., 2004; Jones, 1996; Martin et al., 2004; Millet et al., 2011).

Low frequency fatigue (LFF) has been heavily associated with reduced Ca^{2+} release from the sarcoplasmic reticulum as a result of disrupted excitation-contraction (EC) coupling and decreased Ca^{2+} sensitivity owing to an increase of hydrogen ion (Allen et al., 2008; Westerblad et al., 1993). LFF is characterized as reduced force/torque output from a muscle when stimulated at low frequencies (Martin et al., 2004). It has been observed that damage associated with eccentric contractions is partially implicated with the onset of low frequency fatigue and may play a role in the disruption of the EC coupling mechanisms following exercise, however, LFF is still present through non-damaging exercise (Chin and Allen, 1996; Jones, 1996). Similar deficits in torque are observed when high frequency stimulations are applied and is implicated with fatigue following damaging exercise, but neither fully explain the reductions in strength (Millet et al., 2003).

Peripheral mechanisms of fatigue can also be assessed by analyzing changes in the Mwave or potentiated twitches and doublets. The use of potentiated doublets has shown to be a sensitive marker of peripheral muscle fatigue and closely related to the decline in MVC strength (Kufel et

al., 2002). Smaller amplitude or elongation of the Mwave is characteristic of the impaired or slowed of propagation of action potentials as they proceed across the sarcolemma (Hedayatpour et al., 2009). Impaired propagation of action potentials along the sarcolemma is generally attributed to Na^+ imbalances across the membrane (Allen et al., 2008).

There are other metabolic contributors to fatigue. The accumulation of inorganic phosphate $[p_i]$ during fatiguing contractions is associated with an inability to form strongly bound cross-bridges. Thus, a corresponding decrease in force production follows an increased intracellular presence of $[p_i]$ (Allen et al., 2008). Furthermore, an accumulation of $[p_i]$ is owing to impairments in the phosphocreatine pathway where intracellular ADP becomes overabundant (Westerblad et al., 1998). The increased cellular ADP is thought to drive the reduction in shortening velocity associated with fatigue (Westerblad et al., 1998).

The combined deficits in force and shortening velocity certainly play an important role in power loss following fatiguing and damaging contractions (Power et al., 2010, 2013; Wallace et al., 2016). However, the relative contribution of fatigue and damage is at times difficult to decipher owing to their similar presentation. Separate assessment of FISL and DISL could be made possible if the damaging effects of eccentric contractions can be mitigated following an identical repeated bout of eccentric exercise.

2.3 The Repeated Bout Effect

Following an initial bout of unaccustomed lengthening contractions muscle will experience prolonged deleterious effects often attributed to muscle damage (Clarkson and Hubal, 2002). Fortunately for individuals experiencing repeated bouts of lengthening contractions, there is an inherent protective mechanism that attenuates the deficits associated with an initial bout (Nosaka and Clarkson, 1995). This protection is commonly known as the repeated bout effect (RBE), and the magnitude of its protection is influenced by several contributors (Chen et al., 2007; Howatson and Someren, 2007; Nosaka et al., 2001; Zourdos et al., 2015). It is well established that a second similar bout of eccentric exercise performed days to weeks following the initial bout will not incur the same magnitude of detriments to performance. Quantification of the RBE is typically seen by comparing changes in baseline variables following two separate eccentric exercise bouts. A ‘protective index’ has been used by the Nosaka group which utilizes the maximal decrease from baseline.

$$\frac{(\Delta \text{ of variable following bout 1} - \Delta \text{ of variable following bout 2})}{(\Delta \text{ of variable following bout 1})} \times 100$$

Commonly measured variables for investigating the RBE often consist of; a muscle biomarker such as CK; performance measurements such as force or torque, velocity, RTD, RVD, and optimal angle of torque production; as well as measures of self-reported muscle soreness, swelling, passive tension/resting angle and EC coupling impairments through electrical

stimulation. It seems, through reviewing literature, inference of a RBE is strongest with the attenuation of blood CK, force-loss, and a measure of self-reported muscular soreness.

2.3.1 Contributors

The magnitude of the initial insult and the relative protection following the second bout is greatly influenced by numerous contributors such as the quantity, velocity, intensity and ROM of contractions as well as the age, sex and muscle group, for a comprehensive review see (Hyldahl et al., 2017). Generally it is seen that stronger and faster eccentric contractions going to longer muscle lengths incur more damage in the initial bout but produce a greater protective effect (Chapman et al., 2008; Chen et al., 2013; Nosaka et al., 2005). Initial studies of the RBE focused on performing the same exercise for both bouts, however, as support for similar bouts was established researchers began to explore the effect of non-similar exercise bouts by manipulating contributors.

When lengthening contractions at velocities of $30^{\circ}/s$ are performed, a protection is seen when a second bout is performed two weeks later and at a greater velocity of $210^{\circ}/s$ (Chapman et al., 2008, 2011). An initial bout of 6 eccentric contractions conferred a protective index on 24 eccentric contractions similar to the magnitude of protection conferred by an initial bout of 24 eccentric contractions (Nosaka et al., 2001). Furthermore, as the relative intensity of the eccentric contractions decrease so does the magnitude of protection when the second bout consists of maximal contractions (Chen et al., 2007). Though the protective index is less when initial bouts are 40% and 60% of maximum, there still remains a significant RBE. As well, it appears the elbow flexors and extensors experience a greater RBE when compared to the knee flexors and extensors (Chen et al., 2010).

All of the contributors to the RBE discussed above are performed on the ipsilateral limb, however, there is research showing that damaging eccentric exercise performed on one limb incurs a RBE on the contralateral limb (Chen et al., 2018; Howatson and Someren, 2007). Therefore, a final and less studied contributor to the RBE is prior exercise of the contralateral limb which can alter the response of the RBE.

2.3.2 Mechanisms

There are several key mechanisms underpinning the RBE, however, their relative contributions remain equivocal (**figure 5**) (Hyldahl et al., 2017). Immediate alterations and protection from a secondary bout suggests there must be a fast acting mechanism (i.e. neural) (Dartnall et al., 2011), reduced mechanical disruption infers there are long lasting structural adaptations (muscle-tendon complex and extracellular matrix remodeling) (Mackey et al., 2011; Proske and Morgan, 2001) and potential changes in the inflammatory response could accelerate the myogenic response (Deyhle et al., 2015). Together these adaptations provide a basis on how muscle damage is attenuated following a second bout of eccentric exercise.

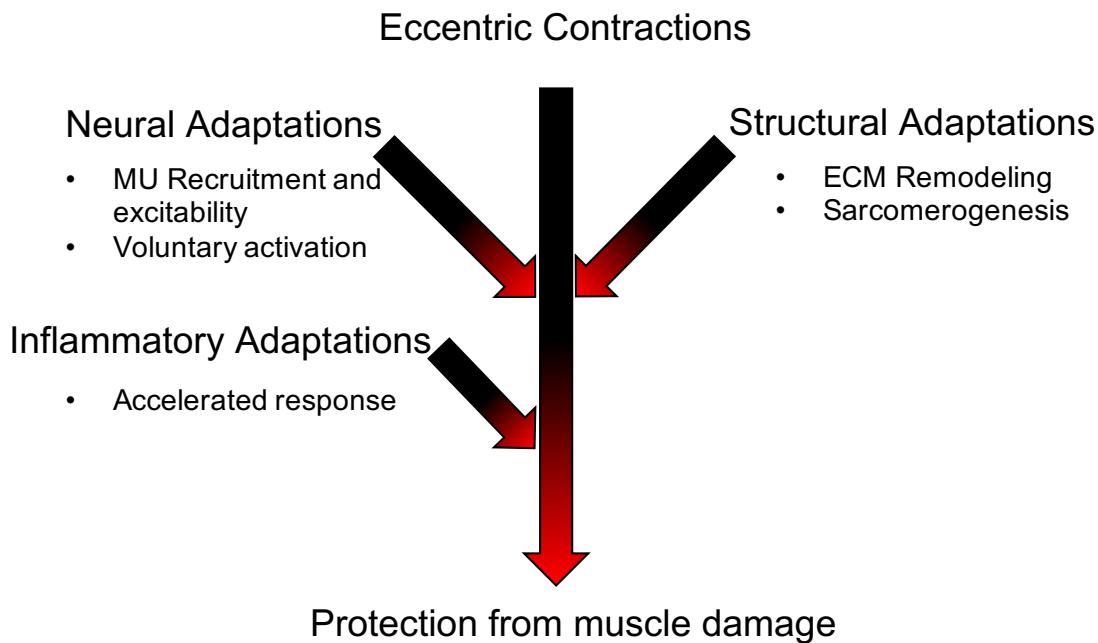


Figure 5: Mechanisms underpinning the repeated bout effect. Adapted from (Hyldahl et al., 2017).

2.3.2.1 Neural Adaptations

Of the proposed mechanisms attempting to explain the RBE, neural changes seem to offer more in the short term compared to others (Hyldahl et al., 2017). When a second bout of eccentric exercise is performed only 3 or 4 days following the first, the muscle is not further damaged, recovery is not stunted and performance is not hindered (Chen, 2003; Mair et al., 1995). Further to this, if the second bout is of a higher intensity it does not damage or stunt recovery (Chen, 2003). Given that structural remodeling of the muscle does not become significantly protective until ~2-3 weeks post initial damage, the protection must be from a quickly adaptive source.

Immediately following damaging eccentric contractions, alterations in central drive occur which manipulate the excitability of the motor neuron pool (McHugh et al., 1999). These changes

are considered to play an important role following damage as there is a reported 30% increase in motor unit synchronization and a lowered motor unit recruitment threshold (Dartnall et al., 2008, 2009). Furthermore, prolonged reductions in voluntary activation following maximal eccentric contractions has been observed and attributed to inhibition of the motor cortex (Prasartwuth et al., 2005) and potentially the inflammatory response following (Goodall et al., 2017). Neural adaptations aide in explaining the quick response of the repeated bout effect, the neuromuscular system efficiently recruits more motor units, preferentially recruits slow-twitch motor units and recruits synergistic muscles following the initial bout (Hortobágyi et al., 1996; Warren et al., 2000). These alterations effectively share the load across fibers and muscles to reduce the damage which had been experienced during the initial bout and are a result of a modulated central drive (Dartnall et al., 2011).

Further support of a neural mechanism exists in studies that show an eccentric training regimen increases the strength of an unexercised contralateral limb by 16-77% and electromyography (EMG) by 54%, nearly double that experienced through concentric training (Hortobágyi et al., 1997; Weir et al., 1995). Hortobágyi et al also show that there is a cross-over effect that must be driven by neural adaptations following lengthening contractions as this does not occur following concentric training (Hortobagyi et al., 1996; Hortobágyi et al., 1997). Similarly, the repeated bout effect influences a non-exercised limb by attenuating deficits when the first and second bout are experienced by contralateral limbs (Howatson and Someren, 2007). The magnitude of protection from the contralateral repeated bout effect is less (40% -60%) than if performed on the ipsilateral limb, however, it would be expected that the protective effect be

lowered as neural adaptations are not thought to be the only mechanisms supporting the RBE (Chen et al., 2018; Howatson and Someren, 2007; Tsuchiya et al., 2018; Xin et al., 2014)

2.3.2.2 Structural (Sarcomerogenesis and ECM Remodeling)

Structural mechanisms supporting the repeated bout effect exist in the remodeling of the extracellular matrix and connective tissue, muscle-tendon unit adaptations and sarcomerogenesis. Intermediate filaments of the sarcomere, such as desmin, act to maintain structural integrity as tension is applied to the muscle (Morgan, 1990). When a muscle is loaded at a longer length, sarcomeres are in a disadvantageous position to resist the lengthening movement. Passive tissues and intermediate filaments become heavily relied upon to absorb the energy and are subsequently damaged (Paulin and Li, 2004). Evidence of mechanical disruption can be found when looking at Z-band streaming at the level of the sarcomere (McHugh, 1999). A decrease in Z-band streaming, when comparing first and second bout biopsies, suggests that an adaptation of the intermediate filaments has occurred. An increase in intramuscular connective tissue can reduce the amount of stress put on the muscle during eccentric loading and help mitigate the loss in force that is associated with muscle injury (Lapier et al., 1995). Following casting, muscle has been shown to remodel and increase the presence of connective tissues, this remodeling is associated with only an 8% loss of force rather than a 40% loss (Lapier et al., 1995).

The strain of a sarcomere over a set length or excursion is a main contributor to myofibrillar damage and following an initial bout it has been proposed that sarcomerogenesis, the addition of sarcomeres in series, reduces the strain experienced per sarcomere as the relative excursion of each is less owing to an overall longer muscle length (Chen et al., 2007; Morgan, 1990). Sarcomerogenesis is significantly increased following eccentrically biased exercise (Butterfield et

al., 2005; Fridén, 2002) and a preferential shift to longer optimal lengths occurs (Gregory et al., 2007; Power et al., 2010).

2.3.2.3 Inflammatory

Mechanical disruption of muscle fibers following eccentric contractions initially triggers a local pro-inflammatory response (Peake et al., 2016). Damaged and fragmented tissue quickly attracts leukocytes such as macrophages and neutrophils which infiltrate the area, initiate the pro-inflammatory response and begin removing the debris (Peake et al., 2005). The initiation of the inflammatory response is due to the release of pro-inflammatory cytokines from the localized macrophages and the inflammation generally plateaus ~48 hours following the initial insult (Buford et al., 2014). Localized neutrophils begin to produce free radicals as they degrade the tissue which initiates a secondary wave of damage and prolongs the detrimental effects of the initial insult (Tidball, 2011). Neutropenic mice showed a 38% decrease in muscle damage following an ischemic-reperfusion study (Kyriakides et al., 1999), this suggests that an attenuated pro-inflammatory response (i.e. a decreased influx of neutrophils) could reduce the magnitude of damage and the recovery time associated with muscle damage.

The implications of an altered inflammatory response on the RBE following muscle damage is understudied and provides equivocal data. Three studies suggest opposing views on the matter. Firstly, a blunted inflammatory response in mice following a repeated bout was observed and suggests that reduced mechanical disruption requires less of an initial inflammatory response to clear debris and therefore less requirement for myogenic responses (Pizza et al., 2002). Secondly, in humans there appears to be an accelerated inflammatory response following a second bout of eccentric exercise (Deyhle et al., 2015). Thirdly, observed in a contralateral RBE study,

attenuated upregulation of pro-inflammatory transcription factors is seen in the contralateral arm which the authors attributed to a potential neural crossover mechanism (Xin et al., 2014).

It is through these mechanisms (neural, structural and inflammatory) that the RBE works to reduce damage and thereby the strength loss experienced following eccentric contractions.

2.4 Purpose and Hypotheses

The purpose of this thesis was to investigate the influence of the repeated bout effect on three components. Firstly, on power loss. Secondly, on DISL to confirm the existence of a RBE in the study. Thirdly, on FISL to determine if the RBE could isolate the effects of fatigue during eccentric contractions.

I hypothesized that there would be:

- 1) A maintenance of power production resulting from a protection of RTD and RVD owing to the repeated bout effect.
- 2) A protection of muscle damage, as measured by torque deficits and serum creatine kinase activity, thereby reducing the magnitude of DISL
- 3) Protection from damage which will isolate the effects of fatigue following a subsequent bout of eccentric contractions, thus allowing us to assess only FISL during the second bout.

3 Methods

3.1 Ethical Approval

Participants were informed on all aspects of the protocol both orally and with a written copy. Written consent was obtained from all participants on the first study day and ongoing verbal consent was obtained on consecutive visits. Participants completed a health questionnaire prior to the study to ensure no existing medical conditions or procedures that could influence the study or put them at risk for injury. This study was approved by the University of Guelph research ethics board for research involving human participants (**Appendix 7.1**). This study conformed to the Declaration of Helsinki.

3.2 Participants and Time Points

Male participants (n=12) were recruited for this study (23.7 ± 2.3 years, 74.7 ± 13.8 kg, 177.4 ± 6.6 cm). Participants were healthy and recreationally active but had not recently strength trained their upper bodies (<6months). Participants were asked not to perform any unaccustomed or eccentrically biased exercise throughout the duration of the study. Two participants were excluded from data analysis. Following the venipuncture at baseline and several other time points one participant was unable to perform maximally at baseline and as such the data provided was inconsistent and unreliable. The second participant was excluded as there was no evidence of the RBE, because this was a necessary measure this participant was also excluded from the study.

Participants completed 12 study visits all on separate days. Visits were split between two separate bouts; ECC1 (6 days) and ECC2 (6 days). The first day of ECC1 and ECC2 were identical and separated by 4 weeks. On these two days, participants completed the baseline parameters, eccentric protocol and recovery protocol which will be explained in detail within this chapter. Follow-up study visits for ECC1 and ECC2 were identical and occurred at time points 24, 48, 72, 96Hr and 7 days. See **figure 7**.

3.3 Experimental Set-up

Participants were seated in a HUMAC NORM dynamometer (CSMi Medical Solutions, Stoughton, MA) for the duration of each study visit. Subjects were secured at the waist and the shoulders with an adjustable 4-point non-elastic harness and the exercised shoulder was secured further with a 5 inch Velcro strap running across the body for the duration of the study except when measuring Mmax potentials. The wrist of the exercised arm was placed in a customized holster to keep the moment arm consistent throughout day to day trials and allow movement of the wrist and all distal joints. The elbow axis of rotation was set in line with the rotating axis of the dynamometer. Full extension of the elbow was considered 180°, ROM of the arm was a 140° – 50° excursion and the isometric angle for all static contractions was 110° (**figure 6**).

EMG was collected from the biceps brachii (BB) and the lateral head of triceps brachii (TB) with a ground electrode placed on a bony prominence. The skin was shaved and rubbed with alcohol prior to placement of silver-silver chloride (Ag-AgCl) recording electrodes (2cm diameter; ConMed, Utica, NY, USA) and placed ~3cm apart centered on the muscle belly while the elbow

was at 110° extension. Two custom aluminum stimulation electrodes (3cm x 4cm) were placed on the distal and proximal aspects of the muscle belly of the BB with conductive gel applied on the skin.

Torque, position and velocity were sampled at 1000 Hz using a 12-bit analog-to-digital converter (PowerLab System 16/35, ADInstruments, Bella Vista, Australia). EMG data was collected at 2000 Hz and band-pass filtered at 10Hz – 1000Hz.

3.3.1 Musculocutaneous Nerve Stimulation

M_{max} potentials were obtained from the BB via peripheral stimulation of the musculocutaneous nerve using a bar electrode coated in conductive gel while participants were seated and the elbow was at 110°. Participants were asked to flex the BB while the musculocutaneous nerve was palpated distal to the axilla just under the medial border of the BB. Peripheral nerve stimulations were completed using a high voltage stimulator (DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK) with a pulse width of 200 µs. Participants were relaxed when peripheral nerve stimulations were delivered, current was increased by 20 mA increments until a plateau of the Mwave peak-peak amplitude was observed. On the two visits when participants completed eccentric protocols the current was increased by 120% for the post and 30min time points.

3.3.2 Maximal Voluntary Contraction and Percutaneous Muscle Stimulation

All maximal voluntary contractions (MVC) were accompanied with loud verbal encouragement and biofeedback in the form of a live torque trace. Participants were instructed to pull as hard and fast as possible while rotating about the elbow and focusing on contracting only elbow flexors. Maximal torque was recorded as the average within a 0.5 second window prior to the superimposed doublet. To assess voluntary activation, the interpolated twitch technique was administered.

$$\%VA = 1 - \left(\frac{Superimposed\ Stimulus\ Torque}{Potentiated\ Stimulus\ Torque} \right) * 100$$

Percutaneous muscle stimulation via custom aluminum electrodes was administered for twitches and doublets as well as for constructing a torque-frequency curve. Doublet (1000 μ s, 100Hz) stimulation current was increased until torque plateaued. The current to establish torque-frequency relationships was determined by increasing the current of a 50Hz stimulation until torque plateaued. The frequency at which torque plateaued during the construction of the torque-frequency curve was consistently 50Hz. The 50Hz stimulation was used throughout the duration of the study as the high frequency stimulation.

3.3.3 Serum Creatine Kinase

Serum CK activity was assessed at baseline and prior to each consecutive visit (24, 48, 72, 96Hr and 7 days post). Blood was procured through venipuncture of the antecubital vein in the non-exercised arm and collected in a serum separator tube. After 30 minutes samples were centrifuged, aliquoted and immediately stored at -80°C. Quantification of serum CK activity was completed using a liquid CK reagent set (Pointe Scientific, Canton, MI) and a kinetic mode spectrophotometer according to the specifications indicated in (**Appendix 7.2**). 11.9 μ L of sample was loaded directly into 238.1 μ L of reagent buffer in a prepared 96 well plate. All samples were tested in duplicate.

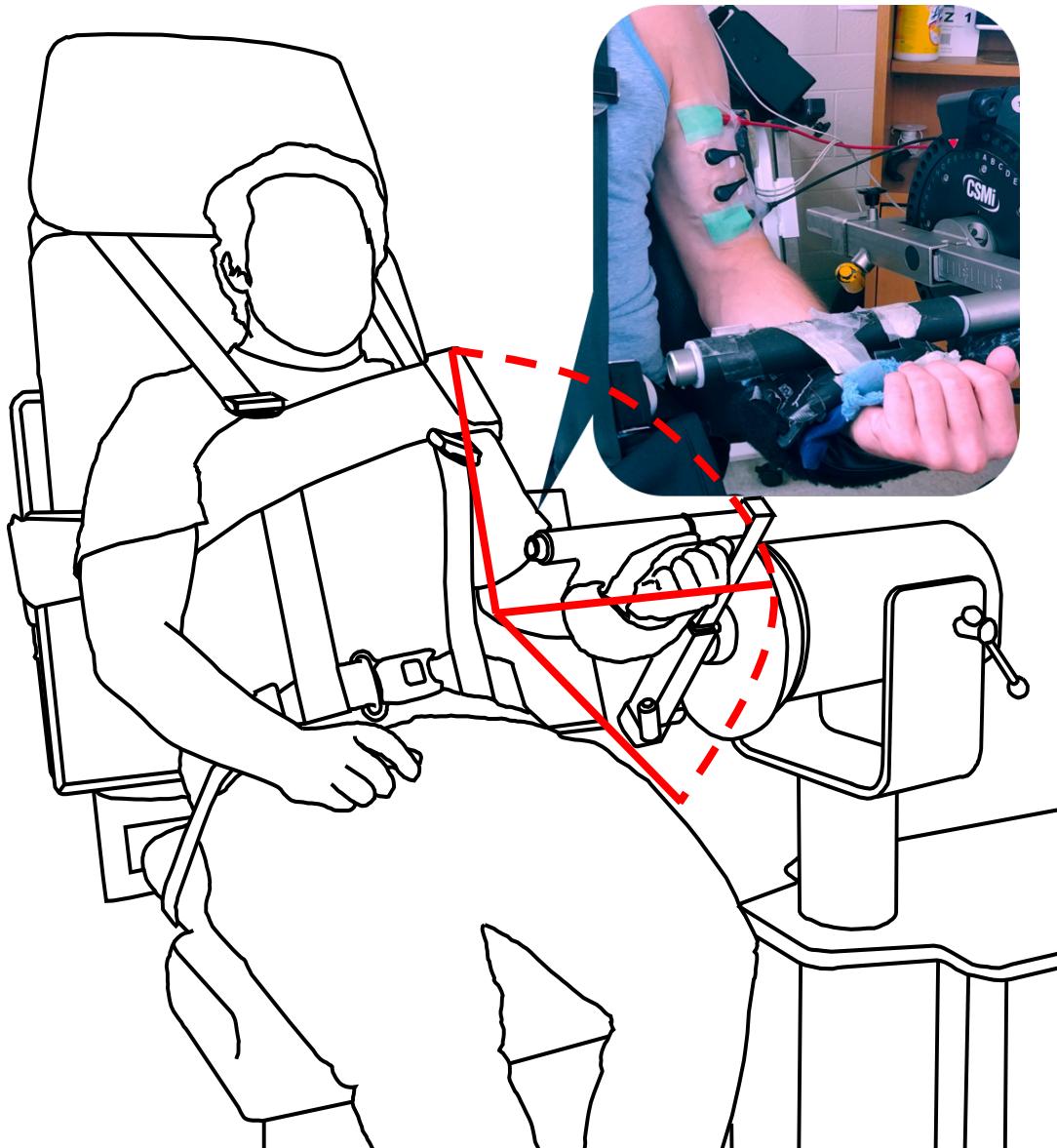


Figure 6: Experimental set-up with subject seated and secured in the dynamometer. Dynamic arm range of motion depicted by dashed red line with solid red lines depicting top (50°), isometric (110°) and bottom (140°) positions. Focused picture provides an example of electrode placement on a participants BB. Green electrodes are the customized percutaneous stimulation electrodes and positioned between them the surface EMG electrodes.

3.4 Experimental Protocol

3.4.1 Baseline Parameters

To normalize antagonist EMG, participants were asked to give a maximal effort while performing a maximal isometric TB extension at 110°. Following this, the BB Mmax was recorded to normalize agonist EMG. Percutaneous pad stimulation twitch current was then increased until a plateau in twitch torque was reached. The ITT was then performed with and MVC to determine maximal torque output on each day, a minimum of two contractions at >95% activation were required to continue, 3 minutes rest was given between all MVC efforts. In a similar manner to the determination of maximum twitch torque, 50Hz stimulation current was increased until a plateau occurred. Following this, an ITT and an assessment of LFF (ITT-LFF) was performed which consisted of a twitch – doublet – MVC (with interpolated doublet) – twitch – doublet – 10Hz – 50Hz stimulation. A force frequency curve was established using 1, 6, 10, 20, 30, 40, 50 and 100 Hz stimulations. Isotonic shortening contractions were performed at varied loads (unloaded, 10, 20, 30, 40, 50, and 60 %MVC), each load was performed twice with 3 minutes rest between loads. Participants were instructed to pull as hard and fast as possible. Load orders were randomized, verbal encouragement and biofeedback in the form of an angular velocity tracing were provided. These isotonic shortening contractions determined the peak power of each participant and were normalized to daily MVC torque.

3.4.2 Eccentric Protocol

Participants performed 5 sets of 30 maximal isokinetic eccentric contractions with an ITT-LFF assessment performed between sets. Eccentric contractions were cyclic and began at 50° flexion and ended at 140° extension. A 1 second isometric contraction at 50° flexion preceded each eccentric contraction to ensure active lengthening through the entire ROM. The dynamometer arm then pulled the resisting arm through a 90° excursion at 180°/s. The participant was instructed to relax at the end range of 140° and the dynamometer would passively bring the elbow back to 50° flexion. Participants were verbally encouraged through each effort and given biofeedback in the form of a live torque recording with an isometric torque reference, this ensured participants were giving maximal efforts through each contraction.

3.4.3 Recovery Protocol

Immediately following the eccentric protocol 3 musculocutaneous nerve stimulations were administered to monitor peak to peak amplitude changes in Mmax, the highest amplitude obtained was accepted. Participant recovery time points were recorded at 0, 2, 5, 10, 15, 20 and 30 minutes. The 0 and 2 minute time points consisted of an ITT-LFF assessment, unloaded shortening contraction and 30% isotonic load. The 5, 10, 15 and 20 minute time points also started with a ITT-LFF assessment but incorporated all varied isotonic loads. Time point 30 incorporated all baseline measures in the order of: Mmax, ITT-LFF assessment, torque-frequency curve and varied load isotonic contractions. Return visits were scheduled for 24, 48, 72, 96Hr and 7 days following

the ECC protocol. At these time points, all baseline parameters were recorded again to track recovery (**figure 7**).

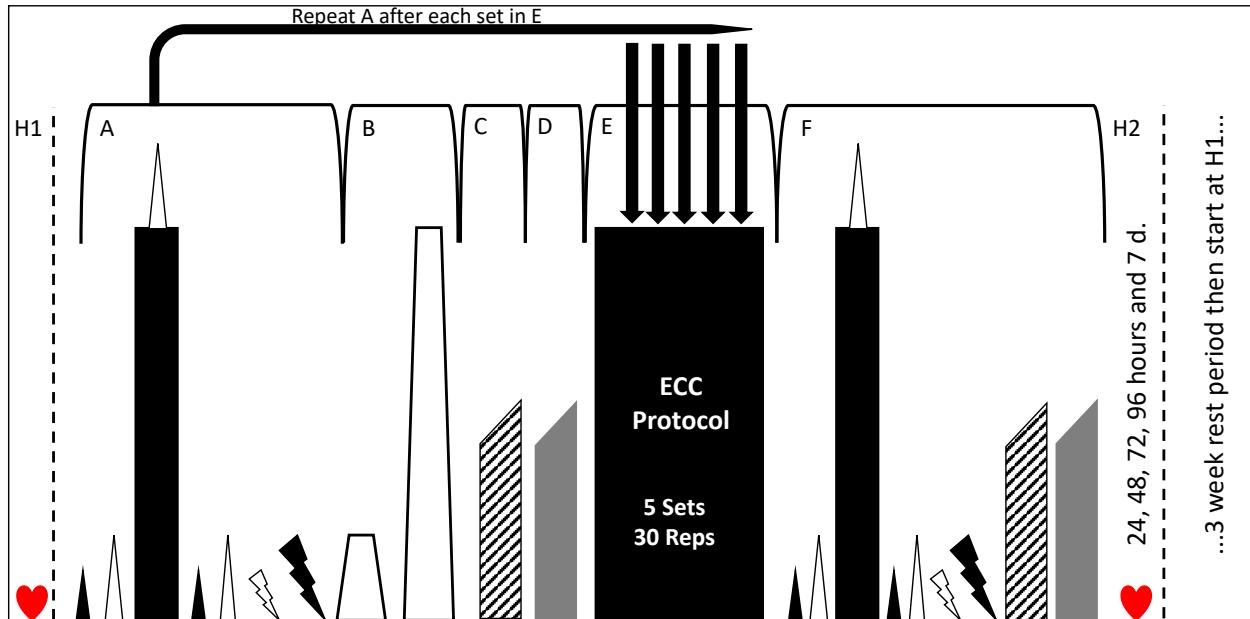


Figure 7: Schematic representation depicting the order of procedures during the experimental protocol. **H1** and **H2** are venipunctures occurring on baseline day and follow up days, respectively. **A** is the ITT-LFF assessment where black triangles represent twitches, white triangles are doublets, black bar is MVC, white bolt is 10Hz stimulation and black bolt is 50Hz stimulation. **B** is practice dynamic movements. **C** represents a torque-frequency curve. **D** dynamic contractions for assessment of peak power. **E** represents the eccentric protocol with the ITT-LFF occurring after each set. **F** represents the recovery time points from 0min-7 days post.

3.5 Data and Statistical Analysis

3.5.1 Isometric Contractions (MVC, VA and LFF assessment)

%VA was assessed during every MVC using the ITT equation below with a superimposed doublet and a potentiated doublet. MVC torque (Nm) was calculated as the average torque across a 0.5s window preceding the superimposed doublet of the ITT and doublet torque (Nm) was calculated as the peak torque of the potentiated doublet. LFF was assessed as the ratio (10Hz:50Hz) of the peak torque produced from a 10Hz stimulation and a 50Hz stimulation.

$$\%VA = 1 - \left(\frac{\text{Superimposed Stimulus Torque}}{\text{Potentiated Stimulus Torque}} \right) * 100$$

3.5.2 Electromyography

Root mean square (RMS) surface EMG of the BB and TB was analyzed during all MVC's. RMS EMG of the BB was normalized to daily Mmax peak-peak amplitude values and was recorded as the average of a 0.5s window preceding the superimposed doublet of each MVC. RMS EMG of the TB was recorded as the average of a 0.5s window preceding the superimposed doublet of each MVC and was normalized to maximal RMS EMG recorded from daily maximal voluntary efforts.

3.5.3 Dynamic Contractions

Peak power, RTD, RVD, unloaded RVD and unloaded shortening velocity was calculated for each dynamic shortening contraction. Initial baseline values were considered maximal and were used for normalization. Power (W) was calculated as the product of torque (Nm) and angular velocity (°s^{-1}) and peak power was considered to be the load (%MVC) at which each participant achieved the greatest power output when completing baseline contractions. RTD (Nms^{-1}) and RVD (°s^{-2}) were calculated as the maximal slope over an iterating 20ms window of the peak slope of the torque-time and velocity-time traces, respectively. Unloaded RVD (°s^{-2}) was calculated as the maximal slope over an iterating 20ms window of the velocity-time trace during unloaded shortening contractions. Unloaded shortening velocity (°s^{-1}) was calculated as the velocity at which peak power was reached during the unloaded trials.

3.5.4 Statistical Analysis

Two-Way repeated measures ANOVA's (analysis of variance) (bout [ECC1, ECC2] x time [BL – 7D]) were used to determine significant effects and interactions, if significance was reached Holm-Sidak post-hoc tests were employed to determine significance at specific time points. If data were non-normally distributed a Friedman's ANOVA was employed paired with a Dunnett's post-hoc test and a Wilcoxon signed rank test to determine significance at specific time points and between bouts. The level of significance for all tests was set to $p < 0.05$. All data in text are reported as mean \pm standard deviation (SD) and in all figures as mean \pm standard error (SE).

4 Results

4.1 Creatine Kinase and Soreness

Serum CK activity during ECC1 was greater than baseline at time points 72Hr ($696 \pm 911\%$) and 96Hr ($944 \pm 1105\%$) whereas during ECC2 no significant deviation from baseline was seen ($p>0.05$). An effect of bout was seen at time points 72 and 96Hr ($p<0.05$) **Figure 8**.

Self-reported soreness as recorded through VAS for ECC1 was significantly greater than baseline for time points: Post, 24, 48 and 72Hr ($p<0.05$). ECC2 did not significantly differ from baseline ($p>0.05$). Soreness for ECC2 was significantly lower than ECC1 at time points: Post, 48, 72 and 96Hr ($p<0.05$). **Figure 9**.

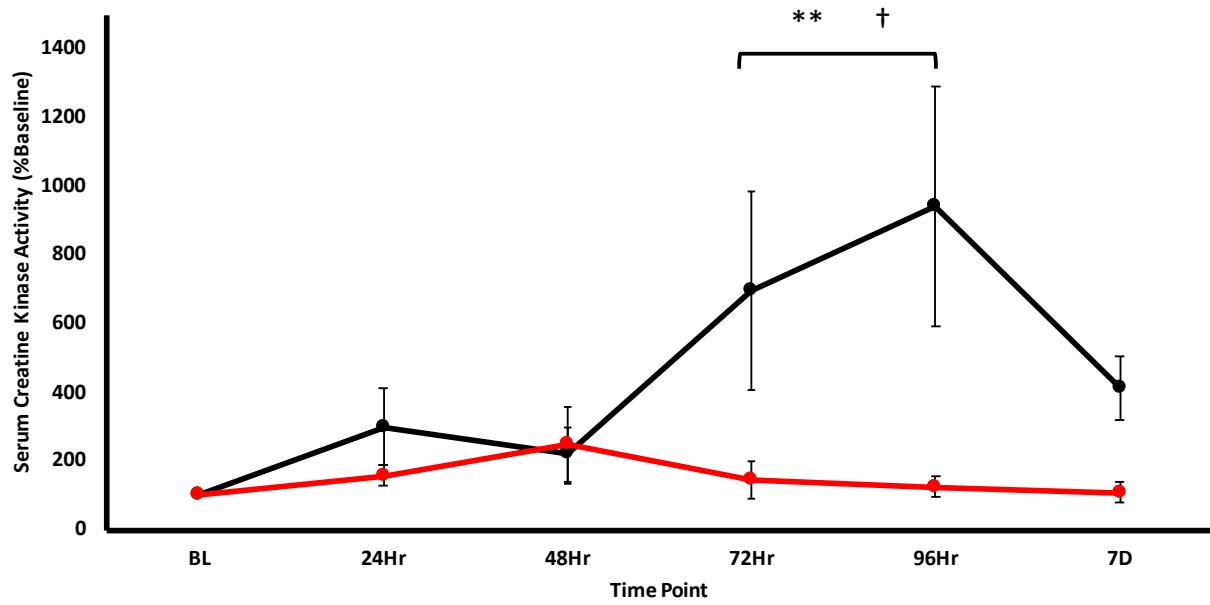


Figure 8: Serum CK activity during ECC1 (black) and ECC2 (red) was significantly greater than baseline during ECC1 at 72Hr and 96Hr ($p<0.05$)** and ECC2 did not differ significantly from baseline at any time point ($p>0.05$). There was an effect of bout at time points 72Hr and 96Hr ($p<0.05$)† . Mean \pm SE.

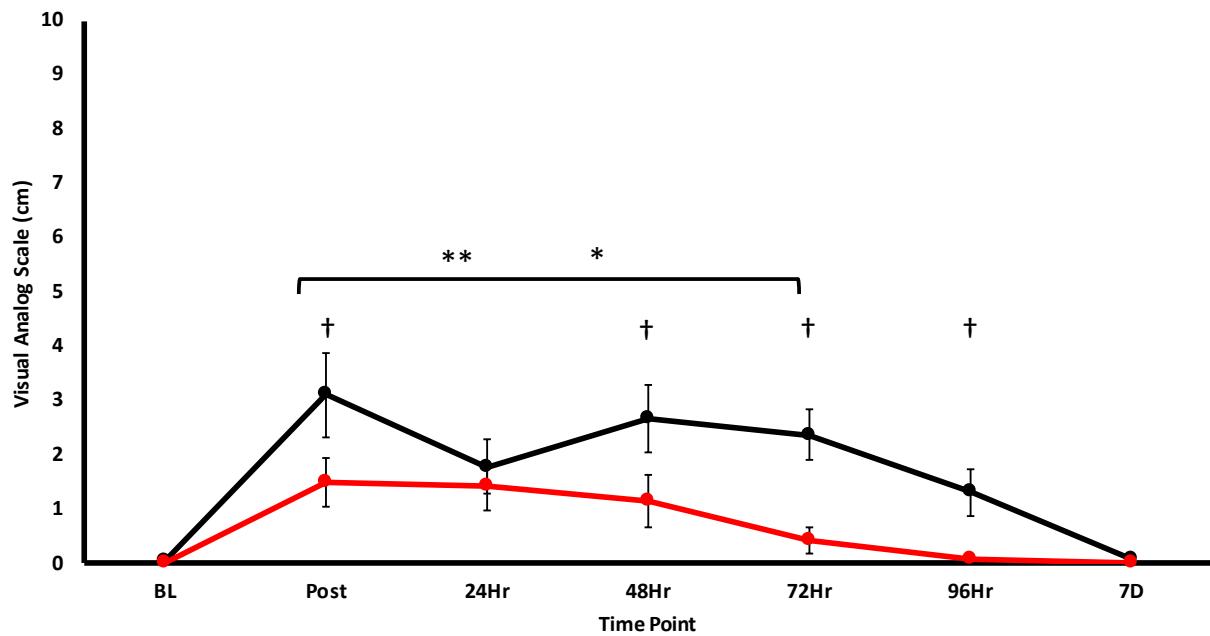


Figure 9: Self-reported soreness for ECC1 (black) and ECC2 (red). ECC1 showed a significant deviation from baseline at time points: post, 24Hr, 48Hr and 72Hr ($p<0.05$)** and an effect of time ($p<0.05$)*. ECC2 did not deviate significantly from baseline at any time point ($p>0.05$). An effect of bout was seen between ECC1 and ECC2 at time points: post, 48Hr, 72Hr and 96Hr ($p<0.05$) †. Mean \pm SE.

4.2 Strength Loss

There was an effect of bout for MVC torque ($p<0.05$), as shown in **figure 10**. Torque decreased precipitously from baseline during the eccentric sets for both ECC1 and ECC2. MVC torque for ECC1 at time point 0min was reduced to $66 \pm 13\%$ whereas for ECC2 it was only reduced to $81 \pm 8\%$. MVC torque during ECC1 did not recover fully by 7days ($88\pm12\%$), however, ECC2 recovered fully by 24Hr ($95\pm3\%$).

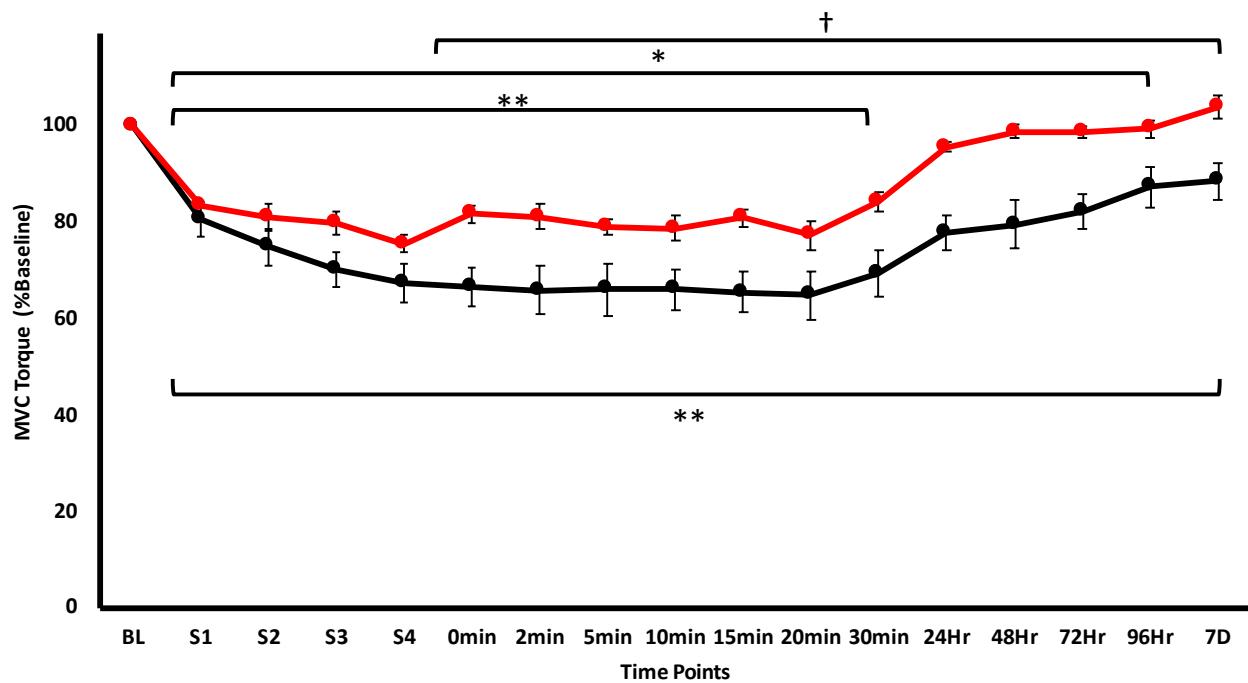


Figure 10: MVC torque for ECC1 (black) and ECC2 (red). ECC1 is reduced below baseline for all time points and ECC2 is only reduced up to 30min ($p<0.05$)**. A significant effect of time exists up to 96Hr ($p<0.05$)* and an effect of bout from 0min-7D ($p<0.05$)†. Mean \pm SE.

4.3 Peak Power and Time Dependent Measures

4.3.1 Peak Power

There was an effect of bout for peak power ($p<0.05$), **figure 11**. ECC1 peak power was reduced to $56 \pm 23\%$ and recovered to $60 \pm 25\%$ at 30min and only to $80 \pm 18\%$ by 7 days post. In contrast, ECC2 peak power was reduced to $72 \pm 14\%$ and recovered to $87 \pm 10\%$ at 30min and $106 \pm 12\%$ by 7 days post.

4.3.2 Rate of Velocity Development

There was an effect of bout and time for RVD ($p<0.05$), **figure 12**. ECC1 remained at $\sim 70\%$ of baseline up to 30min and recovered only to $81 \pm 11\%$ by 7 days ($p<0.05$) whereas ECC2 was only significantly lower than baseline for time points: 5, 10, 15 and 20min ($p<0.05$) and recovered to $84 \pm 12\%$ at 30min and $100 \pm 9\%$ by 7 days post.

4.3.3 Rate of Torque Development

There was an effect of bout and time for RTD for time points 24Hr onwards ($p<0.05$). ECC1 remained significantly lower than baseline for all time points ($p<0.05$) and ECC2 remained lower than baseline for time points: 5, 10, 15, 20 and 30min ($p<0.05$). At time point 7D, ECC1 had recovered to 72% and ECC2 recovered to 97%.

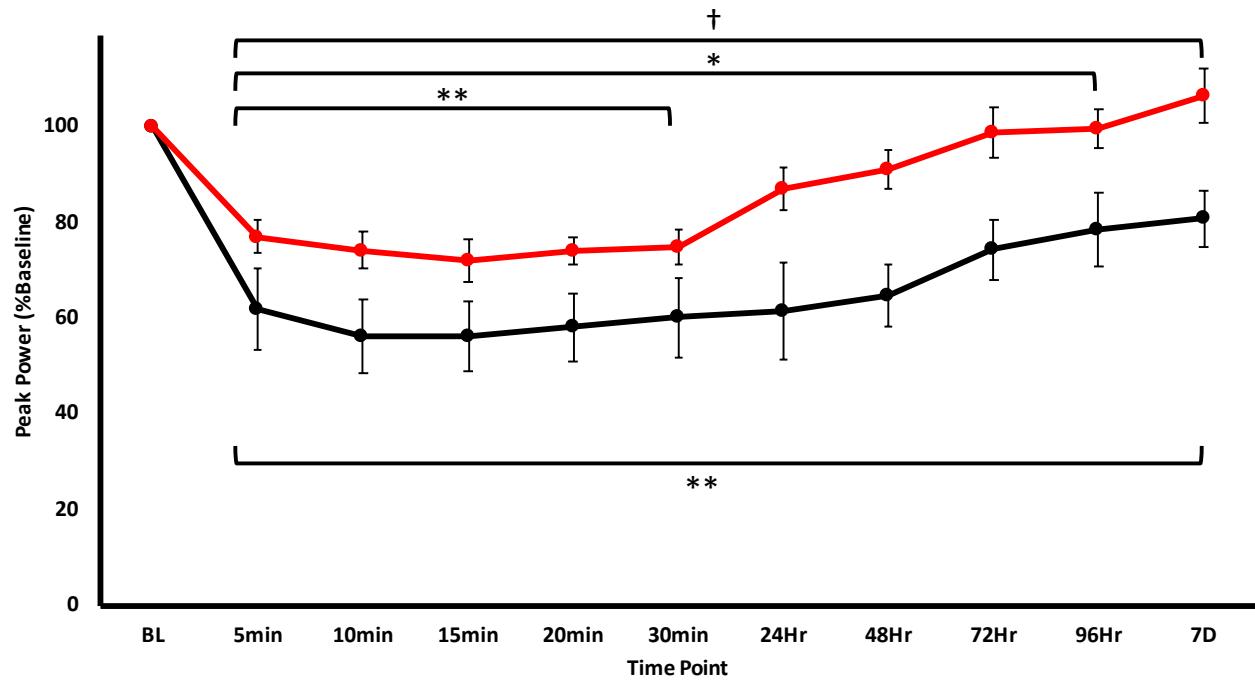


Figure 11: Peak power for ECC1 (black) and ECC2 (red). ECC1 remained significantly below baseline for all time points and ECC2 only up to 30min ($p<0.05$)**. An effect of time was seen up to 96Hr ($p<0.05$)*. An effect of bout was seen for all time points ($p<0.05$) †. Mean \pm SE.

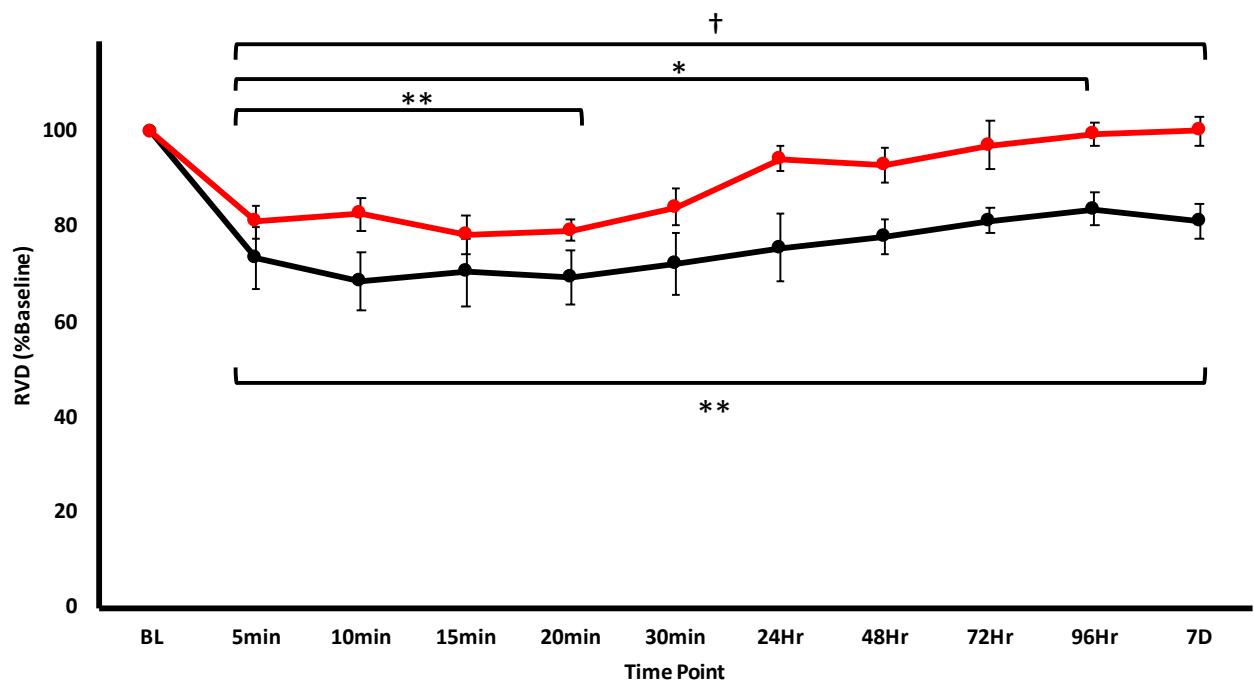


Figure 12: Rate of velocity development for ECC1 (black) and ECC2 (red). ECC1 remained below baseline at all time points and ECC2 remained below baseline up to time 20min ($p<0.05$)**. There was an effect of bout and an effect of time ($p<0.05$) †,*. Mean \pm SE.

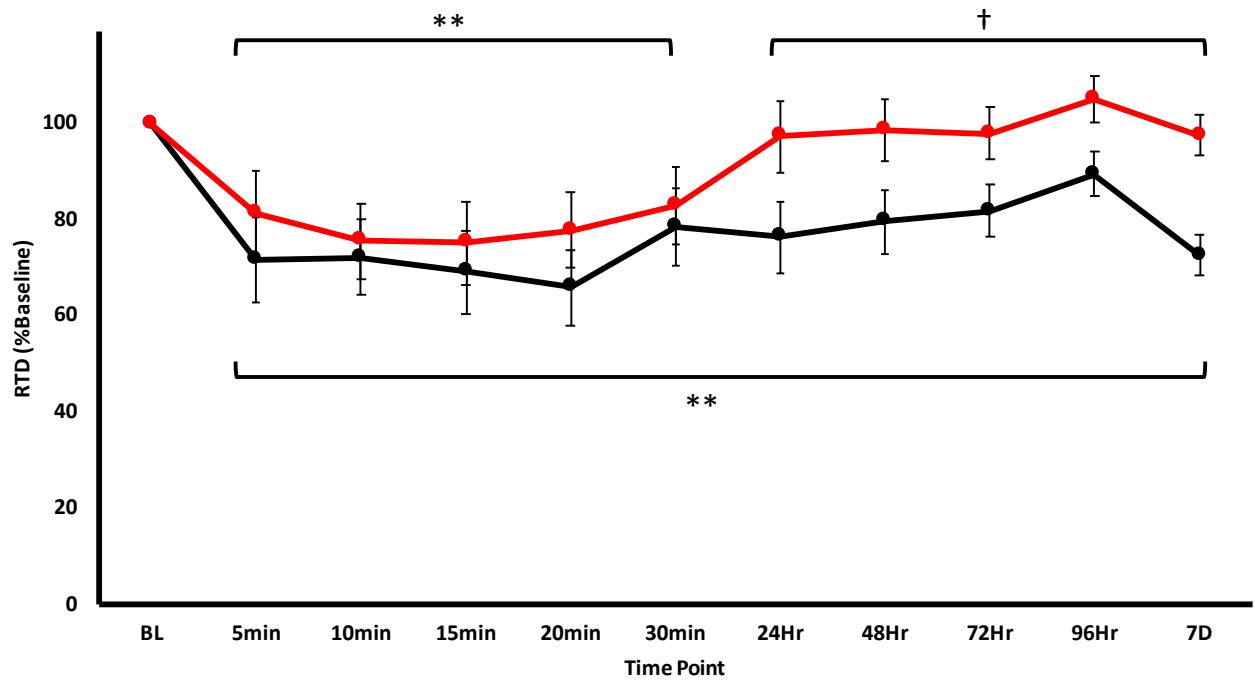


Figure 13: Rate of torque development for ECC1 (black) and ECC2 (red). ECC1 remained below baseline at all time points and ECC2 remained below baseline up to 30min post ($p<0.05$) **. There was an effect of bout from time point 24Hr to 7D ($p<0.05$) †. Mean \pm SE.

4.4 Unconstrained Shortening

4.4.1 Unloaded Rate of Velocity Development

Unloaded rate of velocity development remained lower than baseline for all time points during ECC1 ($p<0.05$), however, for ECC2 it only remained lower than baseline at time points: 5, 10, 15, 20 and 30min ($p<0.05$). There was an effect of bout for time points: 24, 48, 72, 96Hr and 7D ($p<0.05$) and for time points: 10, 15 and 20min ($p<0.05$).

4.4.2 Maximal Shortening Velocity

Unloaded maximal shortening velocity during ECC1 declined and was reduced up to 48Hr post eccentric exercise ($p<0.05$). It appears there was less of a decrease during ECC2 and an effect of bout was seen from 24Hr onwards ($p<0.05$). **Figure 15.**

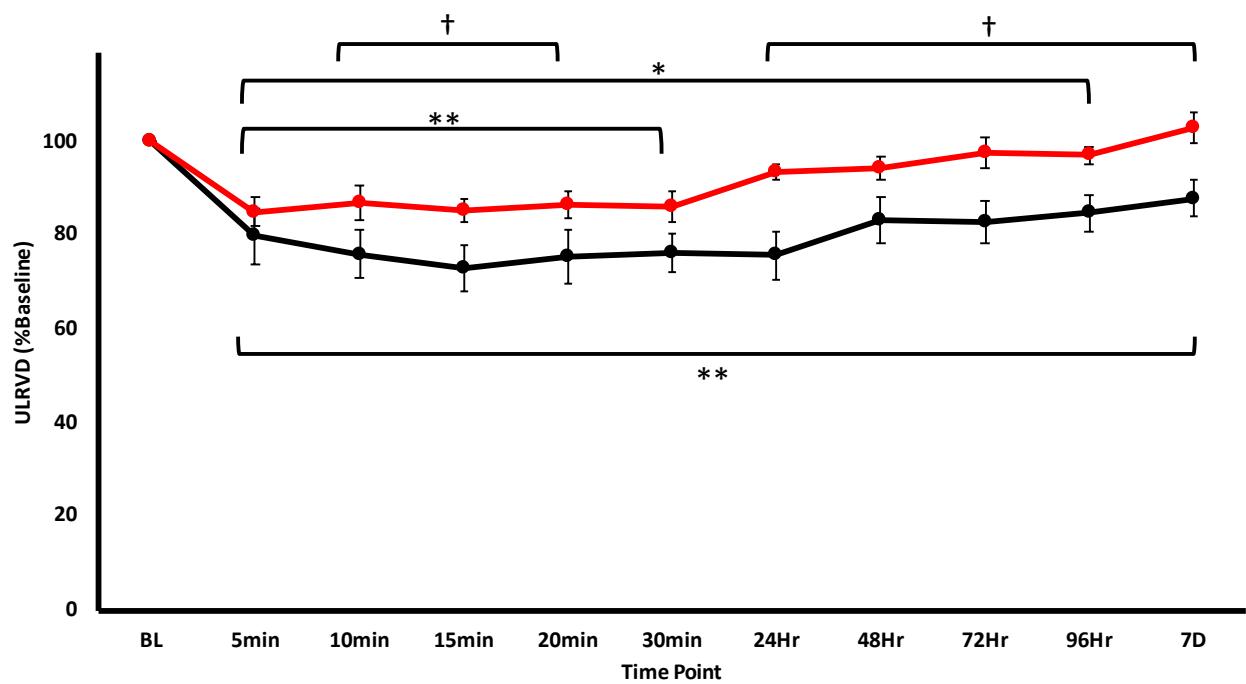


Figure 14: Unloaded rate of velocity development for ECC1 (black) and ECC2 (red). ECC1 remained below baseline at all time points and ECC2 remained below baseline up to 30min post ($p<0.05$)**. There was an effect of bout and time ($p<0.05$) †, *. Mean \pm SE.

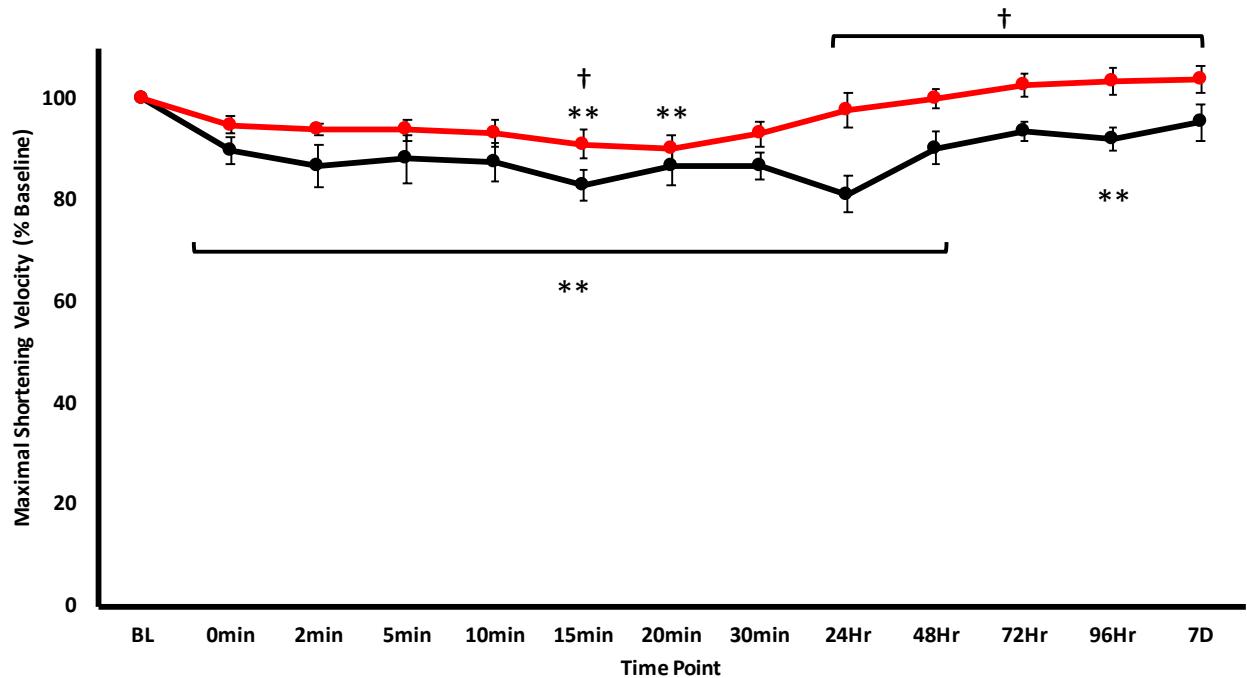


Figure 15: Maximal unconstrained shortening velocity for ECC1 (black) and ECC2 (red). ECC1 remained below baseline for all time points except 72Hr and 7D ($p<0.05$)**. ECC2 was only below baseline at 2 time points: 15min and 20min ($p<0.05$)**. There was an effect of bout observed ($p<0.05$) †. Mean \pm SE.

4.5 Electrical Stimulation

4.5.1 Voluntary Activation, Doublet Peak Torque and Low Frequency Fatigue

Voluntary activation (**figure 16**) declined for both bouts during the eccentric sets. During ECC1, %VA was reduced to $68 \pm 26\%$ following the last eccentric set whereas ECC2 was reduced to $86 \pm 16\%$ at the same time point. %VA did not differ from baseline beyond 0min for ECC2 but remained depressed for ECC1 until 20min post. %VA recovered much quicker during ECC2 as there remained a 10-15% difference between bouts.

Doublet peak torque (**figure 17**) was greatly reduced for both bouts. At the 30min time point, ECC1 and ECC2 doublet torque decreased to $41 \pm 21\%$ and $45 \pm 17\%$ respectively. ECC2 recovered by 48Hrs ($90 \pm 15\%$) whereas ECC1 only recovered to $81 \pm 22\%$ by 7 days post.

Figure 18 depicts the 10:50Hz (C) and is broken down into individual 10Hz (A) and 50Hz (B) torque components. Torque at 10Hz did not differ between bouts ($p>0.05$) and was similarly reduced below baseline between 0 and 30min ($p<0.05$). At 30min ECC1 and ECC2 10Hz torque was reduced to $38 \pm 15\%$ and $39 \pm 18\%$. Both bouts recovered by 24Hr. Torque at 50Hz was reduced through ECC1 and ECC2 up to 30min but there was a difference seen between bouts ($p<0.05$). At 30min ECC1 was reduced to $59 \pm 28\%$ where ECC2 was reduced to $76 \pm 9\%$. An approximately 15% difference remained between ECC1 and ECC2 following the eccentric protocol up to 30min post. Both bouts recovered by 24Hr. The 10:50Hz ratio for both bouts follows similar trends. The 10:50Hz ratio at 30min for ECC1 was reduced to $68 \pm 15\%$ and to $52 \pm 23\%$ for ECC2. There was no difference between bouts for 10:50Hz torque ($p>0.05$).

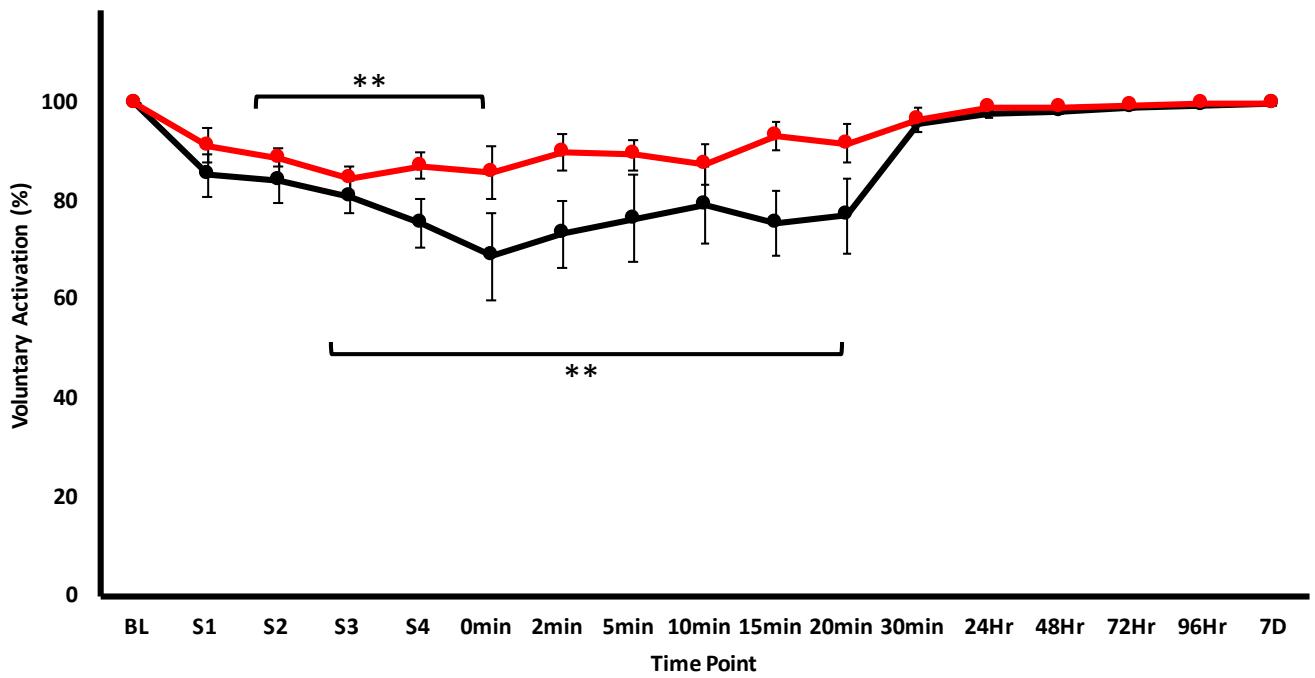


Figure 16: Voluntary activation for ECC1 (black) and ECC2 (red). ECC1 was lower than baseline from S3 to 20min and ECC2 was lower than baseline from S2 to 0min ($p<0.05$)**. There is a difference between ECC1 and ECC2 up to 30min post ($p<0.001$). Mean \pm SE.

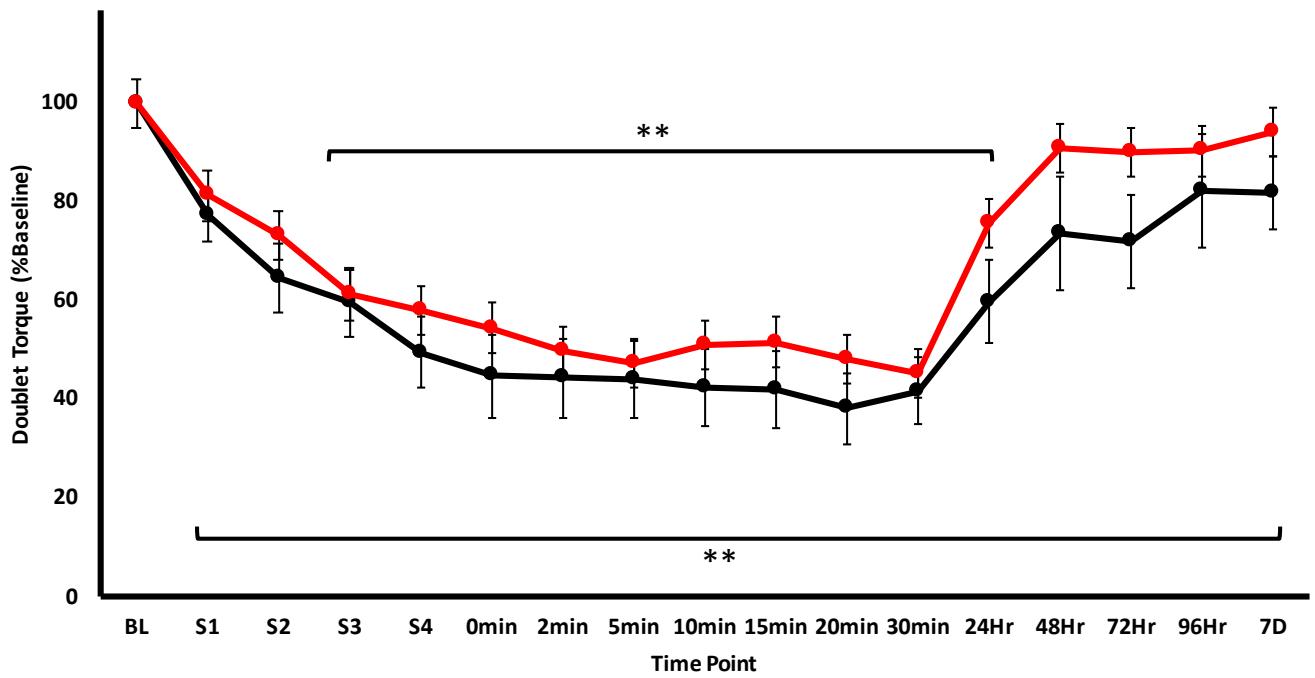


Figure 17: Doublet peak torque for ECC1 (black) and ECC2 (red). ECC1 remained lower than baseline for all time points and ECC2 remained lower than baseline from S3 to 24Hr post ($p<0.05$)**. There is a difference between ECC1 and ECC2 ($p<0.001$). Mean \pm SE.

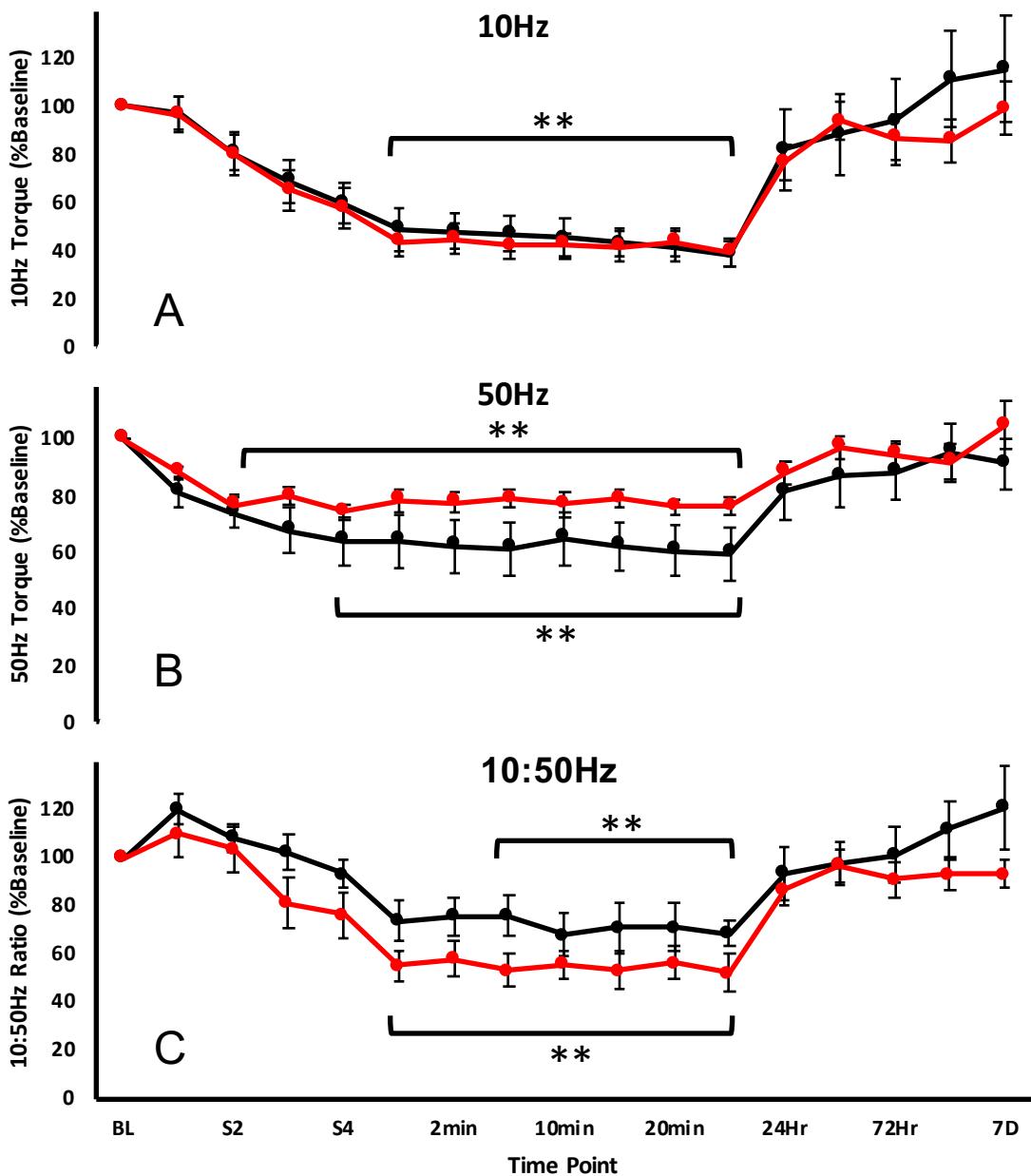


Figure 18: 10Hz, 50Hz and 10:50Hz torque for ECC1 (black) and ECC2 (red). A) 10Hz stimulation torque remained lower than baseline for both ECC1 and ECC2 from time points 0min to 30min ($p<0.05$)**. B) 50Hz stimulation torque remained lower than baseline for ECC1 from time points S4 to 30min and for ECC2 from S2 to 30min ($p<0.05$)**. There is a difference between ECC1 and ECC2 torque ($p<0.05$). C) 10:50Hz torque for ECC1 remained below baseline from 5min to 30min and ECC2 from 0min to 30min ($p<0.05$)**. There was no effect of bout ($p>0.05$). Mean \pm SE.

4.6 Electromyography

4.6.1 Biceps Brachii and Triceps Brachii

RMS EMG of the BB (**Figure 19A**) declined from baseline similarly during ECC1 and ECC2 and remained decreased for the duration of recovery ($p<0.05$). The RMS EMG of the BB recovered to a similar baseline percentage of Mmax by 24Hr and onwards. There was no effect of bout ($p>0.05$). Antagonist coactivation as measured by RMS EMG of TB (**Figure19B**) did not change throughout the duration of the study ($p>0.05$).

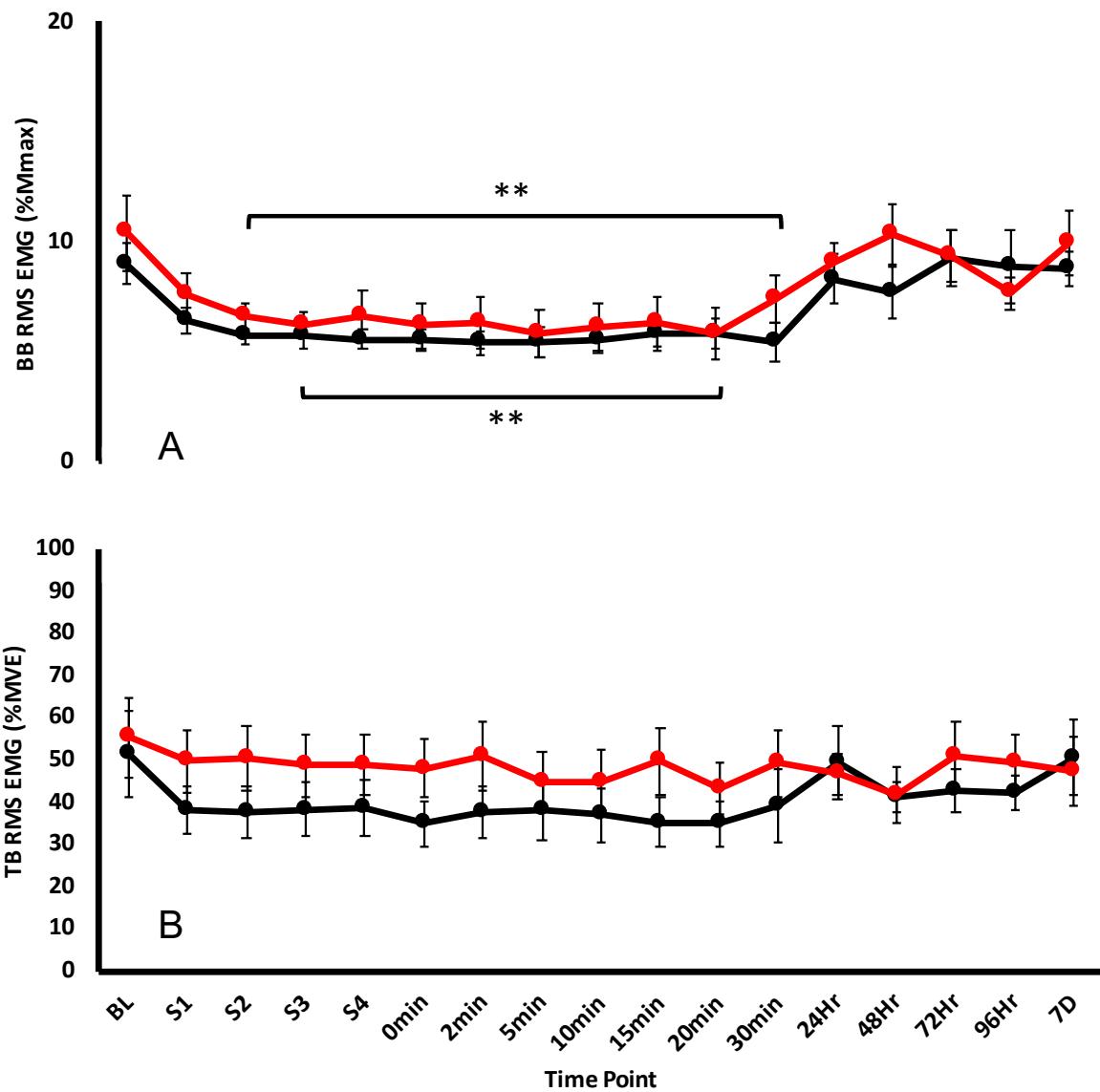


Figure 19: A: RMS EMG of the BB for ECC1 (black) and ECC2 (red). EMG was lower than baseline for ECC1 from time points S3 to 20min and ECC2 from S2 to 30min ($p<0.05$)**. There was no difference between bouts ($p>0.05$). B: RMS EMG of the TB (antagonist coactivation) did not differ from baseline at any time point and no effect of bout was seen ($p>0.05$). Mean \pm SE.

5 Discussion

The main aim of this thesis was to investigate the influence of the repeated bout effect on peak power production. Secondary to this, its influence on damage-induced strength loss (DISL) and fatigue-induced strength loss (FISL) were investigated. I hypothesized that there would be an attenuated power-loss following a second bout of eccentric contractions ultimately owing to the influence of the RBE on RTD and RVD. Furthermore, I hypothesized there would be a decrease in DISL and because of this I would be able to assess FISL during and following high intensity eccentric contractions without the influence of damage. I found that there was indeed a major protection against damage as there were no long lasting ($>24\text{Hr}$) deficits during ECC2 and thereby a significant RBE was observed. A RBE of this magnitude provided a unique view of FISL as the level of fatigue remained similar between bouts and became the main contributor to the short term ($<30\text{min}$) strength loss seen during ECC2. However, the most interesting finding of this thesis is that the protection of the RBE has been expanded to include the time dependent components of power production (RTD, RVD). A novel method of normalizing isotonic loads to daily MVC torque normalized the effects of muscle weakness. Thereby, controlling for weakness allowed for the observation of the time dependent components of power (RTD, RVD), because participants performed isotonic shortening contractions against the same relative load each day. Interestingly, at the same relative load there still remained a prolonged deficit in peak power during ECC1 which was protected during ECC2. It seems that the RBE has a protective influence on RTD and RVD as they recover sooner and to a greater extent during ECC2.

5.1 Damage Induced Strength Loss

As hypothesized, during ECC2, there was a reduction in muscle DISL. One of the most reliable measures of muscle damage, MVC torque (Warren et al., 1999a), showed a protective index of 82% seen at the 24Hr time point. Peak serum CK activity was 97% protected, also soreness was 57% protected at 48Hr and 81% protected at 72Hr. These findings are consistent with other RBE studies (Chen, 2003; Clarkson and Tremblay, 1988; Nosaka and Clarkson, 1995).

As serum CK activity is commonly used to infer myofibrillar disruption, the absence of any increase during ECC2 suggests there was little to no muscle damage. Increased integrin and collagen content following the lengthening contractions could have played a role in structural remodeling and protected from subsequent damage (Hyldahl et al., 2015; Takagi et al., 2016, 2018). Furthermore, as suggested initially by Morgan et al., the addition of sarcomeres in series following eccentric contractions reduces the strain on myofibrils as the absolute stretch experienced per sarcomere is reduced and the length-tension relationship is altered (Gregory et al., 2007; Morgan, 1990). Indeed, there could have been a shift of optimal angle to longer lengths which would have ultimately protected from subsequent damage during ECC2. These structural mechanisms of the RBE certainly could have protected the muscle from further damage by maintaining the integrity of the EC coupling mechanisms which are most commonly implicated with damage induced strength loss (Clarkson and Hubal, 2002; Prasartwuth et al., 2005).

However, it also seems plausible that if minimal damage did occur during ECC2 that an attenuated and accelerated local inflammatory response (Deyhle et al., 2015) quickly removed tissue debris without triggering any soreness. The initiation of an inflammatory response is

contingent on the existence of debris from damage and this inflammatory response is a secondary effect of damage which in turn produces a delayed onset muscle soreness (Peake et al., 2005).

The attenuation of damage and associated long lasting strength loss is not a novel finding. However, the presence of these reductions is necessary to associate the RBE with any further alterations seen.

5.2 Fatigue Induced Strength Loss

The effects of fatigue during high intensity eccentric contractions is difficult to quantify because of the concomitant existence of damage. Some groups compare indices of fatigue between concentric and eccentric actions (Pasquet et al., 2000) while others show that the time course of recovery can separate the relative contributions of damage and fatigue during eccentric actions (Power et al., 2010, 2013). Using the RBE as a novel paradigm to isolate fatigue I was able to assess the fatigue response associated with high intensity eccentric contractions uninfluenced by damage during ECC2.

Reduced cortical drive to the muscle (central fatigue) has been reported for up to 48 hours following high intensity eccentric contractions and these alterations are suggested to be resultant from inhibition to the motor cortex (Prasartwuth et al., 2005) or owing to the detrimental upstream effects of inflammatory cytokines (Goodall et al., 2017). In this study, it was observed during ECC1 that voluntary activation was reduced to 68% immediately following the eccentric protocol but was recovered by 30min. Interestingly, this decrease in voluntary activation was ~52% protected as seen in ECC2 (figure 18) and this is consistent with other groups (Goodall et al., 2017). Furthermore, I observed a similar decline of RMS EMG of the BB between both bouts and

no change in antagonist coactivation. Lowered RMS EMG is indicative of fatigue and typically, similar responses are seen whether fatigue is initiated through concentric or eccentric contractions (Warren et al., 1999b, 2000). This study further confirms that the RBE results in less strength loss originating from central fatigue as shown by the 52% protection of voluntary activation.

Conversely, the similar LFF response observed between bouts as measured by 10:50Hz torque indicates the RBE in this study, did not influence the mechanisms of LFF such as impaired Ca^{2+} release and sensitivity (Jones, 1996; Martin et al., 2004). Deficits in 10:50Hz observed in this study are consistent with others that show LFF following single bouts of eccentric contractions (Martin et al., 2004; Power et al., 2010). Similar LFF responses between bouts is an extremely interesting finding because it is often suggested that LFF is driven by damage to EC coupling mechanisms such as the release of Ca^{2+} from the sarcoplasmic reticulum (Jones, 1996). Furthermore, the similar reductions in doublet twitch torque observed in this study also support the existence of EC coupling impairment (Balnave and Allen, 1995; Prasartwuth et al., 2005). If damage is attenuated via the RBE then it would make sense to see a reduction in LFF following a subsequent bout, however that was not observed in this thesis. This may suggest that damage is less implicated in EC coupling failure and it may be driven more by fatigue related ion imbalances.

The reduction in maximal shortening velocity observed during ECC1 is consistent with an accumulation of cellular ADP (Westerblad et al., 1998). Increased ADP is believed to be a result of impaired phosphocreatine energy buffering which may explain the prolonged reduction seen in this study as there was significant loss of CK during ECC1 (Baird et al., 2012; Westerblad et al., 1998). Interestingly, there was a maintenance of maximal shortening velocity during ECC2 and no increase in serum CK. Given the timeframe of recovery, it seems that maximal shortening

velocity was more impaired by fatigue than damage and the attenuation of central fatigue during ECC2 is likely to be the reason for this maintenance of maximal shortening velocity (Wallace et al., 2016).

It seems that part of the fatigue response seen during ECC1 could have been driven by central factors and a loss of intracellular CK but this is not the case during ECC2 as the RBE appears to attenuate these responses. A greater maintenance of voluntary activation means muscles are able to fully activate when a maximal contraction is required and attempted. Furthermore, reduced serum CK during ECC2 suggests there was a maintenance of intracellular CK which would provide adequate phosphate buffering and production of ATP for the muscle. However, it appears that the contribution of central fatigue and CK loss to the overall level of fatigue may remain minimal as a similar LFF and doublet torque fatigue response was still observed during ECC2 and the near-equilibrium reaction rate of CK would likely be uninfluenced by a loss to the circulation (Walsh et al., 2001). The influence of the RBE may therefore be limited to the protection of central but not peripheral fatigue and this is supported by the RBE's suggested influence on central factors as seen through the contralateral RBE and crossover effect of lengthening contractions (Chen et al., 2018; Hortobágyi et al., 1997).

The relative contributions of fatigue and damage on strength loss were estimated in this study and are visually represented by **figure 10**. During ECC1 I observed ~37% loss of strength up to 30min, 14% of which was caused by fatigue and 23% by damage. During ECC2 I observed ~24% loss of strength up to 30min, 20% of which was caused by fatigue and 4% caused by damage. Perhaps the small difference in fatigue responses between ECC1 and ECC2 is owing to the greater

voluntary activation and maintenance of intracellular CK I observed or there was minimal damage experienced which caused small deficits in strength that were fully recovered by 48Hr.

5.3 Peak Power

The protective adaptation known as the repeated bout effect is almost always discussed in the context of attenuating damage and strength loss following a second bout of eccentric contractions. In the present study, normalizing isotonic loads to daily MVC torque values controlled for the loss of strength and provides an opportunity to investigate components of peak power such as RVD and RTD, within a repeated bout effect paradigm. The influence of RVD and RTD on achieving peak power has been established and it is imperative that torque be generated rapidly to overcome the initial resistance of a load and that following this, the muscle is able to accelerate the load quickly to produce peak power (Lanning et al., 2017; Power et al., 2013; Wallace et al., 2016). Deficits to these time dependent components results in slower, weaker and less powerful movements.

Of particular interest in this study was the fact that there remained a reduction of peak power, RVD and RTD for up to 7 days following ECC1. Even though the relative loads remained constant at each visit there was an approximate 64% protection of peak power seen during ECC2 and a recovery of RVD and RTD by the 24Hr time point. RTD and RVD are highly correlated with generating and achieving peak power (Power et al., 2013; Wallace et al., 2016) and it seems very plausible that muscle damage caused the prolonged (>24Hr) loss of RVD and RTD seen during ECC1 and was a main contributor to the power-loss observed. Conversely, during ECC2 a short term (<30min) fatigue-induced reduction of RVD and RTD seems to have contributed to power loss, however, both RVD and RTD were recovered by 24Hr during ECC2 and assisted in

maintaining peak power beyond the time-frame of fatigue. This suggests that the RBE must influence the time-dependent components of power production such as RVD and RTD and aide in maintaining power production following high intensity eccentric contractions.

6 Conclusion

6.1 Limitations

A limitation in this study exists in the use of indirect markers of muscle damage rather than the gold standard of muscle biopsy, however, the use of MVC torque, CK and soreness is commonplace when inferring the absence or presence of muscle damage (Warren et al., 1999a). While the use of a biopsy to determine muscle damage provides visual evidence, in this study it was not possible to include muscle biopsies because of their potential to negatively impact the performance of participants. Regardless of whether there was muscle damage or not there exists a prolonged muscular weakness following unaccustomed eccentric exercise. This muscular weakness was significantly attenuated following a second bout of eccentric exercise and this repeated bout effect was investigated. Furthermore, indirect measures gathered from surface EMG and electrical stimulations were used to infer fatigue. Unfortunately the placement of surface EMG electrodes can vary slightly from day to day, however the use of indwelling EMG is equally as variable. I discussed many potential mechanisms of fatigue but used the pool of literature to infer my findings. These mechanisms were not directly measured as they cannot be while using human participants. The potential mechanisms surrounding the existence of LFF during both bouts is particularly interesting and could benefit from direct analysis.

Another limitation lies in the assessment of unloaded contraction velocity and unloaded RVD. The isokinetic setting on the dynamometer used for these contractions allowed participants to quickly contract without resistance of the machine, however, there remained the weight of the wrist holster and the weight of the distal limb for the muscles to overcome. These contractions were as near to unloaded as possible given the use of a dynamometer. A wrist holster was

deconstructed and rebuilt with light-weight materials for cushions and straps with the purpose of making our unloaded contractions nearer to unloaded.

Lastly, the absence of a torque-length curve is a major limitation when considering the potential mechanisms of the RBE. It is commonly seen that the optimal angle of torque production shifts to longer lengths following eccentric contractions (Gregory et al., 2007) and this study would have benefitted from the addition of this data.

6.2 Future Directions

Two interesting ideas developed in my mind throughout this study. Firstly, fatiguing fibers prior to lengthening contractions reduces the magnitude of damage and allows for a quicker recovery of peak power as compared to lengthening contractions alone (Choi and Widrick, 2009). Perhaps the influence of prior fatigue would reduce the magnitude of the RBE experience because less damage was experienced. This could help in assessing if damage is a significant trigger for the onset of the RBE. Secondly, there was often a significant recovery of variables between the 30min and 24Hr time points. It would be interesting to investigate further into what drives this rapid recovery before 24Hr as it plateaus and no other 24 hour window experiences such rapid changes.

This thesis showed an attenuation of central fatigue owing to the repeated bout effect and as such the potential implications on corticospinal changes should be investigated further. There is limited research surrounding this, however, there seems to be a growing understanding that the RBE branches into neural adaptations (Goodall et al., 2017; Hyldahl et al., 2017). In addition to

this, there seems to be an assumption in the literature that LFF is always caused by damage, however it was observed in this study that LFF persisted during ECC2 even though damage was mitigated. It would benefit us to understand if LFF is equally influenced by fatigue and damage or if eccentric contractions inherently cause dysfunction of the EC coupling mechanism without the presence of damage.

6.3 Conclusion

The aims of this thesis were to investigate the influence of the repeated bout effect on power-loss, DISL and FISL. As hypothesized, I observed an attenuated loss of power which was driven by a protection of RVD and RTD owing to the influence of the RBE. Furthermore, there was a protection against DISL and this provided the opportunity to investigate FISL without the influence of damage. I observed a 62% contribution of damage and a 38% contribution of fatigue during ECC1 which equated to a ~37% deficit in strength. Conversely, during ECC2 I observed a similar strength loss but a nearly 100% contribution of fatigue which resulted in a ~24% short term reduction in strength. This thesis expands on the body of literature investigating the RBE in several ways. I was able to use the RBE as a novel method to observe fatigue during and after high intensity eccentric contractions without the concomitant existence and interference of damage. The ability to remove the detriments of damage provides future research an opportunity to assess only fatigue related changes as they relate to high intensity eccentric contractions, something which has been difficult to isolate in the past. Furthermore, I was able to demonstrate that the RBE protects critical time dependent components of dynamic muscle function (RVD, RTD) and allows individuals to maintain peak power following a subsequent bout of eccentric contractions. The idea that the RBE

only protects from damage and strength loss is becoming an antiquated theory as there are clearly corticospinal changes occurring (Goodall et al., 2017; Hortobágyi et al., 1997; Hyldahl et al., 2017) as well, this thesis shows there is a maintenance of peak power owing to the protection of RVD and RTD.

References

- Abbott, B.C., and Aubert, X.M. (1951). Changes of energy in a muscle during very slow stretches. *Proceedings of the Royal Society of London. Series B, Biological Sciences* *139*, 104–117.
- Allen, D.G., Lamb, G.D., and Westerblad, H. (2008). Skeletal Muscle Fatigue: Cellular Mechanisms. *Physiological Reviews* *88*, 287–332.
- Baird, M.F., Graham, S.M., Baker, J.S., and Bickerstaff, G.F. (2012). Creatine-Kinase- and Exercise-Related Muscle Damage Implications for Muscle Performance and Recovery. *Journal of Nutrition and Metabolism* *2012*, 1–13.
- Balnave, C.D., and Allen, D.G. (1995). Intracellular calcium and force in single mouse muscle fibres following repeated contractions with stretch. *J. Physiol. (Lond.)* *488 (Pt 1)*, 25–36.
- Betts, J., Desaix, P., Johnson, E., Johnson, J., Korol, O., Kruse, D., Poe, B., Wise, J., Womble, M., and Young, K. (2017). Anatomy and Physiology (OpenStax).
- Bigland, B., and Lippold, O.C. (1954). The relation between force, velocity and integrated electrical activity in human muscles. *The Journal of Physiology* *123*, 214–224.
- Bigland-Ritchie, B., Jones, D.A., Hosking, G.P., and Edwards, R.H. (1978). Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. *Clinical Science and Molecular Medicine* *54*, 609–614.
- Bigland-Ritchie, B., Rice, C.L., Garland, S.J., and Walsh, M.L. (1995). Task-dependent factors in fatigue of human voluntary contractions. *Advances in Experimental Medicine and Biology* *384*, 361–380.
- Buford, T.W., MacNeil, R.G., Clough, L.G., Dirain, M., Sandesara, B., Pahor, M., Manini, T.M., and Leeuwenburgh, C. (2014). Active muscle regeneration following eccentric contraction-induced injury is similar between healthy young and older adults. *Journal of Applied Physiology* *116*, 1481–1490.
- Butterfield, T.A., Leonard, T.R., and Herzog, W. (2005). Differential serial sarcomere number adaptations in knee extensor muscles of rats is contraction type dependent. *J. Appl. Physiol.* *99*, 1352–1358.
- Byrne, C., Twist, C., and Eston, R. (2004). Neuromuscular Function After Exercise-Induced Muscle Damage: Theoretical and Applied Implications. *Sports Medicine* *34*, 49–69.
- Cadigan, E.W.J., Collins, B.W., Philpott, D.T.G., Kippenhuck, G., Brenton, M., and Button, D.C. (2017). Maximal Voluntary Activation of the Elbow Flexors Is under Predicted by

Transcranial Magnetic Stimulation Compared to Motor Point Stimulation Prior to and Following Muscle Fatigue. *Frontiers in Physiology* 8:707.

Carroll, T.J., Taylor, J.L., and Gandevia, S.C. (2017). Recovery of central and peripheral neuromuscular fatigue after exercise. *Journal of Applied Physiology* 122, 1068–1076.

Chapman, D.W., Newton, M., McGuigan, M., and Nosaka, K. (2008). Effect of lengthening contraction velocity on muscle damage of the elbow flexors. *Med Sci Sports Exerc* 40, 926–933.

Chapman, D.W., Newton, M.J., McGuigan, M.R., and Nosaka, K. (2011). Effect of slow-velocity lengthening contractions on muscle damage induced by fast-velocity lengthening contractions. *Journal of Strength and Conditioning Research* 25, 211–219.

Chen, T.C. (2003). Effects of a second bout of maximal eccentric exercise on muscle damage and electromyographic activity. *European Journal of Applied Physiology* 89, 115–121.

Chen, T.C., Nosaka, K., and Sacco, P. (2007). Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. *Journal of Applied Physiology* (Bethesda, Md.: 1985) 102, 992–999.

Chen, T.C., Chen, H.-L., Lin, M.-J., Wu, C.-J., and Nosaka, K. (2010). Potent protective effect conferred by four bouts of low-intensity eccentric exercise. *Med Sci Sports Exerc* 42, 1004–1012.

Chen, T.C., Chen, H.-L., Lin, M.-J., Chen, C.-H., Pearce, A.J., and Nosaka, K. (2013). Effect of two maximal isometric contractions on eccentric exercise-induced muscle damage of the elbow flexors. *European Journal of Applied Physiology* 113, 1545–1554.

Chen, T.C., Lin, M.-J., Chen, H.-L., Yu, H.-I., and Nosaka, K. (2018). Contralateral Repeated Bout Effect of the Knee Flexors. *Medicine and Science in Sports and Exercise* 50, 542–550.

Cheng, A.J., and Rice, C.L. (2009). Isometric torque and shortening velocity following fatigue and recovery of different voluntary tasks in the dorsiflexors. *Applied Physiology, Nutrition, and Metabolism* 34, 866–874.

Cheng, A.J., and Rice, C.L. (2010). Fatigue-induced reductions of torque and shortening velocity are muscle dependent. *Medicine and Science in Sports and Exercise* 42, 1651–1659.

Chin, E.R., and Allen, D.G. (1996). The role of elevations in intracellular [Ca²⁺] in the development of low frequency fatigue in mouse single muscle fibres. *The Journal of Physiology* 491 (Pt 3), 813–824.

Choi, S.J., and Widrick, J.J. (2009). Combined effects of fatigue and eccentric damage on muscle power. *Journal of Applied Physiology* 107, 1156–1164.

- Clarkson, P.M., and Hubal, M.J. (2002). Exercise-Induced Muscle Damage in Humans: American Journal of Physical Medicine & Rehabilitation 81, S52–S69.
- Clarkson, P.M., and Tremblay, I. (1988). Exercise-induced muscle damage, repair, and adaptation in humans. Journal of Applied Physiology 65, 1–6.
- Curtin, N.A., and Davies, R.E. (1975). Very high tension with very little ATP breakdown by active skeletal muscle. Journal of Mechanochemistry & Cell Motility 3, 147–154.
- Dartnall, T.J., Nordstrom, M.A., and Semmler, J.G. (2008). Motor unit synchronization is increased in biceps brachii after exercise-induced damage to elbow flexor muscles. Journal of Neurophysiology 99, 1008–1019.
- Dartnall, T.J., Rogasch, N.C., Nordstrom, M.A., and Semmler, J.G. (2009). Eccentric muscle damage has variable effects on motor unit recruitment thresholds and discharge patterns in elbow flexor muscles. Journal of Neurophysiology 102, 413–423.
- Dartnall, T.J., Nordstrom, M.A., and Semmler, J.G. (2011). Adaptations in biceps brachii motor unit activity after repeated bouts of eccentric exercise in elbow flexor muscles. Journal of Neurophysiology 105, 1225–1235.
- Deyhle, M.R., Gier, A.M., Evans, K.C., Eggett, D.L., Nelson, W.B., Parcell, A.C., and Hyldahl, R.D. (2015). Skeletal muscle inflammation following repeated bouts of lengthening contractions in humans. Frontiers in Physiology 6, 424.
- Duchateau, J. (2009). Stimulation conditions can improve the validity of the interpolated twitch technique. Journal of Applied Physiology (Bethesda, Md.: 1985) 107, 361; discussion 367–368.
- Enoka, R.M., and Duchateau, J. (2008). Muscle fatigue: what, why and how it influences muscle function: Muscle fatigue. The Journal of Physiology 586, 11–23.
- Fridén, J. (2002). Delayed onset muscle soreness. Scandinavian Journal of Medicine & Science in Sports 12, 327–328.
- Fridén, J., Sjöström, M., and Ekblom, B. (1983). Myofibrillar damage following intense eccentric exercise in man. Int J Sports Med 4, 170–176.
- Gandevia, S.C. (2001). Spinal and supraspinal factors in human muscle fatigue. Physiol. Rev. 81, 1725–1789.
- Goodall, S., Thomas, K., Barwood, M., Keane, K., Gonzalez, J.T., St Clair Gibson, A., and Howatson, G. (2017). Neuromuscular changes and the rapid adaptation following a bout of damaging eccentric exercise. Acta Physiologica 220, 486–500.
- Gordon, A.M., Huxley, A.F., and Julian, F.J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. The Journal of Physiology 184, 170–192.

- Gregory, J.E., Morgan, D.L., Allen, T.J., and Proske, U. (2007). The shift in muscle's length-tension relation after exercise attributed to increased series compliance. *European Journal of Applied Physiology* *99*, 431–441.
- Hedayatpour, N., Falla, D., Arendt-Nielsen, L., Vila-Chă, C., and Farina, D. (2009). Motor unit conduction velocity during sustained contraction after eccentric exercise. *Medicine and Science in Sports and Exercise* *41*, 1927–1933.
- Hill, A.V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond. B* *126*, 136–195.
- Hortobágyi, T., Barrier, J., Beard, D., Braspennincx, J., Koens, P., Devita, P., Dempsey, L., and Lambert, J. (1996). Greater initial adaptations to submaximal muscle lengthening than maximal shortening. *Journal of Applied Physiology* *81*, 1677–1682.
- Hortobagyi, T., Hill, J.P., Houmard, J.A., Fraser, D.D., Lambert, N.J., and Israel, R.G. (1996). Adaptive responses to muscle lengthening and shortening in humans. *Journal of Applied Physiology* *80*, 765–772.
- Hortobágyi, T., Lambert, N.J., and Hill, J.P. (1997). Greater cross education following training with muscle lengthening than shortening. *Medicine and Science in Sports and Exercise* *29*, 107–112.
- Hough, T. (1900). Ergographic studies in muscular fatigue and soreness. *Journal of the Boston Society of Medical Sciences* *5*, 81–92.
- Howatson, G., and Someren, K.A. van (2007). Evidence of a contralateral repeated bout effect after maximal eccentric contractions. *European Journal of Applied Physiology* *101*, 207–214.
- Huxley, A.F. (1957). Muscle structure and theories of contraction. *Progress in Biophysics and Biophysical Chemistry* *7*, 255–318.
- Huxley, A.F. (1998). Biological motors: energy storage in myosin molecules. *Current Biology: CB* *8*, R485–488.
- Hyldahl, R.D., Nelson, B., Xin, L., Welling, T., Groscost, L., Hubal, M.J., Chipkin, S., Clarkson, P.M., and Parcell, A.C. (2015). Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. *FASEB Journal* *29*, 2894–2904.
- Hyldahl, R.D., Chen, T.C., and Nosaka, K. (2017). Mechanisms and Mediators of the Skeletal Muscle Repeated Bout Effect: Exercise and Sport Sciences Reviews *45*, 24–33.
- Jones, D.A. (1996). High-and low-frequency fatigue revisited. *Acta Physiologica Scandinavica* *156*, 265–270.

- Jones, C., Allen, T., Talbot, J., Morgan, D.L., and Proske, U. (1997). Changes in the mechanical properties of human and amphibian muscle after eccentric exercise. *European Journal of Applied Physiology and Occupational Physiology* 76, 21–31.
- Jones, D., de Haan, A., and Round, J. (2004). *Skeletal Muscle from Molecules to Movement* (Elsevier).
- Katz, B. (1939). The relation between force and speed in muscular contraction. *J Physiol* 96, 45–64.
- Koch, A.J., Pereira, R., and Machado, M. (2014). The creatine kinase response to resistance exercise. *Journal of Musculoskeletal & Neuronal Interactions* 14, 68–77.
- Korthuis, R.J. (2011). *Anatomy of Skeletal Muscle and Its Vascular Supply* (Morgan & Claypool Life Sciences).
- Kufel, T.J., Pineda, L.A., and Mador, M.J. (2002). Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue. *Muscle & Nerve* 25, 438–444.
- Kyriakides, C., Austen, W., Wang, Y., Favuzza, J., Kobzik, L., Moore, F.D., and Hechtman, H.B. (1999). Skeletal muscle reperfusion injury is mediated by neutrophils and the complement membrane attack complex. *Am. J. Physiol.* 277, C1263–1268.
- Lanning, A.C., Power, G.A., Christie, A.D., and Dalton, B.H. (2017). Influence of sex on performance fatigability of the plantar flexors following repeated maximal dynamic shortening contractions. *Applied Physiology, Nutrition, and Metabolism* 42, 1118–1121.
- Lapier, T.K., Burton, H.W., Almon, R., and Cerny, F. (1995). Alterations in intramuscular connective tissue after limb casting affect contraction-induced muscle injury. *J. Appl. Physiol.* 78, 1065–1069.
- Linari, M., Lucii, L., Reconditi, M., Casoni, M.E.V., Amenitsch, H., Bernstorff, S., Piazzesi, G., and Lombardi, V. (2000). A combined mechanical and X-ray diffraction study of stretch potentiation in single frog muscle fibres. *The Journal of Physiology* 526, 589–596.
- Linari, M., Bottinelli, R., Pellegrino, M.A., Reconditi, M., Reggiani, C., and Lombardi, V. (2004). The mechanism of the force response to stretch in human skinned muscle fibres with different myosin isoforms. *The Journal of Physiology* 554, 335–352.
- Linari, M., Brunello, E., Reconditi, M., Fusi, L., Caremani, M., Narayanan, T., Piazzesi, G., Lombardi, V., and Irving, M. (2015). Force generation by skeletal muscle is controlled by mechanosensing in myosin filaments. *Nature* 528, 276–279.
- Mackey, A.L., Brandstetter, S., Schjerling, P., Bojsen-Moller, J., Qvortrup, K., Pedersen, M.M., Doessing, S., Kjaer, M., Magnusson, S.P., and Langberg, H. (2011). Sequenced response of

extracellular matrix deadhesion and fibrotic regulators after muscle damage is involved in protection against future injury in human skeletal muscle. *FASEB J.* 25, 1943–1959.

Mair, J., Mayr, M., Müller, E., Koller, A., Haid, C., Artner-Dworzak, E., Calzolari, C., Larue, C., and Puschendorf, B. (1995). Rapid adaptation to eccentric exercise-induced muscle damage. *International Journal of Sports Medicine* 16, 352–356.

Martin, V., Millet, G.Y., Martin, A., Deley, G., and Lattier, G. (2004). Assessment of low-frequency fatigue with two methods of electrical stimulation. *Journal of Applied Physiology* 97, 1923–1929.

McHugh, M.P., Connolly, D.A.J., Eston, R.G., and Gleim, G.W. (1999). Exercise-Induced Muscle Damage and Potential Mechanisms for the Repeated Bout Effect: *Sports Medicine* 27, 157–170.

Merton, P.A. (1954). Voluntary strength and fatigue. *The Journal of Physiology* 123, 553–564.

Millet, G.Y., Martin, V., Lattier, G., and Ballay, Y. (2003). Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *Journal of Applied Physiology* 94, 193–198.

Millet, G.Y., Martin, V., Martin, A., and Vergès, S. (2011). Electrical stimulation for testing neuromuscular function: from sport to pathology. *European Journal of Applied Physiology* 111, 2489–2500.

Morgan, D.L. (1990). New insights into the behavior of muscle during active lengthening. *Biophys J* 57, 209–221.

Nosaka, K., and Aoki, M.S. (2011). Repeated bout effect: research update and future perspective. 5, 12.

Nosaka, K., and Clarkson, P.M. (1995). Muscle damage following repeated bouts of high force eccentric exercise. *Med Sci Sports Exerc* 27, 1263–1269.

Nosaka, K., Clarkson, P.M., McGuiggin, M.E., and Byrne, J.M. (1991). Time course of muscle adaptation after high force eccentric exercise. *Eur J Appl Physiol Occup Physiol* 63, 70–76.

Nosaka, K., Sakamoto, K., Newton, M., and Sacco, P. (2001). The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *European Journal of Applied Physiology* 85, 34–40.

Nosaka, K., Newton, M., Sacco, P., Chapman, D., and Lavender, A. (2005). Partial protection against muscle damage by eccentric actions at short muscle lengths. *Med Sci Sports Exerc* 37, 746–753.

Oskouei, M. a. E., van Mazijk, B.C.F., Schuiling, M.H.C., and Herzog, W. (2003). Variability in the interpolated twitch torque for maximal and submaximal voluntary contractions. *Journal of Applied Physiology* *95*, 1648–1655.

Paleckis, V., Mickevičius, M., Snieckus, A., Streckis, V., Pääsuke, M., Rutkauskas, S., Steponavičiūtė, R., Skurvydas, A., and Kamandulis, S. (2015). Changes in indirect markers of muscle damage and tendons after daily drop jumping exercise with rapid load increase. *Journal of Sports Science & Medicine* *14*, 825–833.

Pasquet, B., Carpentier, A., Duchateau, J., and Hainaut, K. (2000). Muscle fatigue during concentric and eccentric contractions. *Muscle & Nerve* *23*, 1727–1735.

Paulin, D., and Li, Z. (2004). Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. *Exp. Cell Res.* *301*, 1–7.

Peake, J., Nosaka, K., and Suzuki, K. (2005). Characterization of inflammatory responses to eccentric exercise in humans. *Exerc Immunol Rev* *11*, 64–85.

Peake, J.M., Neubauer, O., Della Gatta, P.A., and Nosaka, K. (2016). Muscle damage and inflammation during recovery from exercise. *J. Appl. Physiol.* *122*, 559–570.

Peñailillo, L., Blazevich, A., Numazawa, H., and Nosaka, K. (2015). Rate of force development as a measure of muscle damage: Eccentric exercise and rate of force development. *Scandinavian Journal of Medicine & Science in Sports* *25*, 417–427.

Pizza, F.X., Koh, T.J., McGregor, S.J., and Brooks, S.V. (2002). Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. *Journal of Applied Physiology* (Bethesda, Md.: 1985) *92*, 1873–1878.

Pollack, G.H. (1983). The cross-bridge theory. *Physiological Reviews* *63*, 1049–1113.

Power, G.A., Dalton, B.H., Rice, C.L., and Vandervoort, A.A. (2010). Delayed recovery of velocity-dependent power loss following eccentric actions of the ankle dorsiflexors. *J. Appl. Physiol.* *109*, 669–676.

Power, G.A., Dalton, B.H., Rice, C.L., and Vandervoort, A.A. (2013). Peak power is reduced following lengthening contractions despite a maintenance of shortening velocity. *Applied Physiology, Nutrition, and Metabolism* *38*, 1196–1205.

Prasartwuth, O., Taylor, J., and Gandevia, S. (2005). Maximal force, voluntary activation and muscle soreness after eccentric damage to human elbow flexor muscles. *The Journal of Physiology* *567*, 337–348.

Proske, U., and Morgan, D.L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J. Physiol. (Lond.)* *537*, 333–345.

- Rebalka, I.A., and Hawke, T.J. (2014). Potential biomarkers of skeletal muscle damage. *Biomarkers in Medicine* 8, 375.
- Roth, S.M., Martel, G.F., and Rogers, M.A. (2000). Muscle biopsy and muscle fiber hypercontraction: a brief review. *European Journal of Applied Physiology* 83, 239–245.
- Rutherford, O.M., Jones, D.A., and Newham, D.J. (1986). Clinical and experimental application of the percutaneous twitch superimposition technique for the study of human muscle activation. *Journal of Neurology, Neurosurgery, and Psychiatry* 49, 1288–1291.
- Sargeant, A.J. (2007). Structural and functional determinants of human muscle power. *Experimental Physiology* 92, 323–331.
- Takagi, R., Ogasawara, R., Tsutaki, A., Nakazato, K., and Ishii, N. (2016). Regional adaptation of collagen in skeletal muscle to repeated bouts of strenuous eccentric exercise. *Pflugers Archiv: European Journal of Physiology* 468, 1565–1572.
- Takagi, R., Ogasawara, R., Takegaki, J., Tamura, Y., Tsutaki, A., Nakazato, K., and Ishii, N. (2018). Past injurious exercise attenuates activation of primary calcium-dependent injury pathways in skeletal muscle during subsequent exercise. *Physiological Reports* 6.
- Tidball, J.G. (2011). Mechanisms of muscle injury, repair, and regeneration. *Compr Physiol* 1, 2029–2062.
- Totsuka, M., Nakaji, S., Suzuki, K., Sugawara, K., and Sato, K. (2002). Break point of serum creatine kinase release after endurance exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985)* 93, 1280–1286.
- Tsuchiya, Y., Nakazato, K., and Ochi, E. (2018). Contralateral repeated bout effect after eccentric exercise on muscular activation. *European Journal of Applied Physiology*.
- Wallace, J.W., Power, G.A., Rice, C.L., and Dalton, B.H. (2016). Time-dependent neuromuscular parameters in the plantar flexors support greater fatigability of old compared with younger males. *Experimental Gerontology* 74, 13–20.
- Walsh, B., Tonkonogi, M., Söderlund, K., Hultman, E., Saks, V., and Sahlin, K. (2001). The role of phosphorylcreatine and creatine in the regulation of mitochondrial respiration in human skeletal muscle. *J. Physiol. (Lond.)* 537, 971–978.
- Warren, G.L., Lowe, D.A., and Armstrong, R.B. (1999a). Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 27, 43–59.
- Warren, G.L., Ingalls, C.P., Shah, S.J., and Armstrong, R.B. (1999b). Uncoupling of in vivo torque production from EMG in mouse muscles injured by eccentric contractions. *The Journal of Physiology* 515, 609–619.

Warren, G.L., Hermann, K.M., Ingalls, C.P., Masselli, M.R., and Armstrong, R.B. (2000). Decreased EMG median frequency during a second bout of eccentric contractions. Medicine and Science in Sports and Exercise 32, 820–829.

Weir, J.P., Housh, D.J., Housh, T.J., and Weir, L.L. (1995). The effect of unilateral eccentric weight training and detraining on joint angle specificity, cross-training, and the bilateral deficit. The Journal of Orthopaedic and Sports Physical Therapy 22, 207–215.

Westerblad, H., Duty, S., and Allen, D.G. (1993). Intracellular calcium concentration during low-frequency fatigue in isolated single fibers of mouse skeletal muscle. Journal of Applied Physiology (Bethesda, Md.: 1985) 75, 382–388.

Westerblad, H., Dahlstedt, A.J., and Lännergren, J. (1998). Mechanisms underlying reduced maximum shortening velocity during fatigue of intact, single fibres of mouse muscle. The Journal of Physiology 510 (Pt 1), 269–277.

Xin, L., Hyldahl, R.D., Chipkin, S.R., and Clarkson, P.M. (2014). A contralateral repeated bout effect attenuates induction of NF-κB DNA binding following eccentric exercise. Journal of Applied Physiology (Bethesda, Md.: 1985) 116, 1473–1480.

Zourdos, M.C., Henning, P.C., Jo, E., Khamoui, A.V., Lee, S.-R., Park, Y.-M., Naimo, M., Panton, L.B., Nosaka, K., and Kim, J.-S. (2015). Repeated bout effect in muscle-specific exercise variations. Journal of Strength and Conditioning Research 29, 2270–2276.

7 Appendices

7.1 Research Ethics Certification



RESEARCH ETHICS BOARDS

*Certification of Ethical Acceptability of Research
Involving Human Participants*

APPROVAL PERIOD:	December 8, 2016
EXPIRY DATE:	December 8, 2017
REB:	NPES
REB NUMBER:	16OC044
TYPE OF REVIEW:	Full Board
PRINCIPAL INVESTIGATOR:	Power, Geoffrey (gapower@uoguelph.ca)
DEPARTMENT:	Human Health & Nutritional Sciences
SPONSOR(S):	N/A
TITLE OF PROJECT:	Effect of the Repeated Bout Effect on Fatigue and Power Following Repetitive Eccentric Contractions

The members of the University of Guelph Research Ethics Board have examined the protocol which describes the participation of the human participants in the above-named research project and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement, 2nd Edition.

The REB requires that researchers:

- Adhere to the protocol as last reviewed and **approved** by the REB.
- Receive approval from the REB for any **modifications** before they can be implemented.
- Report any **change in the source of funding**.
- Report **unexpected events or incidental findings** to the REB as soon as possible with an indication of how these events affect, in the view of the Principal Investigator, the safety of the participants, and the continuation of the protocol.
- Are responsible for **ascertaining and complying with all applicable legal and regulatory requirements** with respect to consent and the protection of privacy of participants in the jurisdiction of the research project.

The Principal Investigator must:

- Ensure that the ethical guidelines and approvals of facilities or institutions involved in the research are obtained and filed with the REB prior to the initiation of any research protocols.
- Submit a **Status Report** to the REB upon completion of the project. If the research is a multi-year project, a status report must be submitted annually prior to the expiry date. Failure to submit an annual status report will lead to your study being suspended and potentially terminated.

The approval for this protocol terminates on the **EXPIRY DATE**, or the term of your appointment or employment at the University of Guelph whichever comes first.

Signature:

Date: December 8, 2016

L. Vallis
Chair, Research Ethics Board-NPES

APPROVAL PERIOD: December 8, 2016
EXPIRY DATE: December 7, 2018
REB: NPES
REB NUMBER: 16-12-743
TYPE OF REVIEW: Full Board
PRINCIPAL INVESTIGATOR: Power, Geoffrey (gapower@uoguelph.ca)
DEPARTMENT: Human Health & Nutritional Sciences
SPONSOR(S):
TITLE OF PROJECT: Effect of the Repeated Bout Effect on Fatigue and Power Following Repetitive Eccentric Contractions

CHANGES:

Type	Date
Annual Renewal	2017-11-06

The members of the University of Guelph Research Ethics Board have examined the protocol which describes the participation of the human participants in the above-named research project and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement, 2nd Edition.

The REB requires that researchers:

- Adhere to the protocol as last reviewed and **approved** by the REB.
- Receive approval from the REB for any **modifications** before they can be implemented.
- Report any **change in the source of funding**.
- Report **unexpected events or incidental findings** to the REB as soon as possible with an indication of how these events affect, in the view of the Principal Investigator, the safety of the participants, and the continuation of the protocol.
- Are responsible for **ascertaining and complying with all applicable legal and regulatory requirements** with respect to consent and the protection of privacy of participants in the jurisdiction of the research project.

The Principal Investigator must:

- Ensure that the ethical guidelines and approvals of facilities or institutions involved in the research are obtained and filed with the REB prior to the initiation of any research protocols.
- Submit an **Annual Renewal** to the REB upon completion of the project. If the research is a multi-year project, a status report must be submitted annually prior to the expiry date. Failure to submit an annual status report will lead to your study being suspended and potentially terminated.

The approval for this protocol terminates on the **EXPIRY DATE**, or the term of your appointment or employment at the University of Guelph whichever comes first.

Signature:

Date: November 6, 2017



L. Vallis
Chair, Research Ethics Board-NPES

7.2 Liquid Creatine Kinase Datasheet and Protocol



5449 Research Drive • Canton MI 48188 • USA

Creatine Kinase (Two Part Liquid) Reagent Set

Intended Use

For the quantitative determination of creatine kinase activity in serum.

Summary and Principle

Serum creatine kinase (CK) levels have proven valuable in the assessment of cardiac and skeletal muscle diseases, including myocardial infarction and muscular dystrophy.¹ Determination of creatine kinase and lactate dehydrogenase isoenzymes provides a definitive diagnosis of acute myocardial infarction.²

The kinetic procedure presented is a modification of Szasz³ of the Rosalki⁴ technique, which optimizes the reaction by reactivation of CK activity with N-acetyl-L-cysteine (NAC).

CK specifically catalyzes the transphosphorylation of ADP to ATP. Through a series of coupled enzymatic reactions, NADPH is produced at a rate directly proportional to the CK activity. The method determines the NADPH absorbance increase per min at 340 nm.

Reagents

CK R1 (buffer) contains: Imidazole buffer (pH 6.7) 100.0 mmol/L; NADP 2.0 mmol/L; HK (Baker's yeast) 2.5 KU/L; Glucose 20.0 mmol/L; Magnesium Acetate 10.0 mmol/L; EDTA 2.0 mmol/L and N-acetylcysteine (NAC) 20.0 mmol/L.

CK R2 (enzyme reagent) contains: Imidazole buffer (pH 6.7) 100.0 mmol/L; ADP 2.0 mmol/L; AMP 5.0 mmol/L; Diadenosine pentaphosphate 10.0 mmol/L; Creatine phosphate 30.0 mmol/L; G6PDH (Baker's yeast) 1.5 KU/L and EDTA 2.0 mmol/L.

Reagent Preparation

Reagents are supplied as ready to use liquids. To prepare working reagent, mix 4 parts of R1 (buffer) with 1 part R2 (enzyme).

Reagent Storage

1. Reagents should appear clear and colorless. Discard if either appears cloudy or contains particulate matter.
2. Store R1 and R2 at 2-8°C, protected from light. If stored as directed the reagents are stable until the expiration date.
3. Working reagent is stable for 3 weeks at 2-8°C or 2 days at room temperature (15-30°C).

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Normal precautions in handling laboratory reagents should be followed.
3. The reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

Specimen Collection and Handling

1. Clear unhemolyzed serum is the specimen of choice. No special additives or preservatives are required.
2. Whenever possible, specimens should be separated and analyzed on the day of collections and stored in capped tubes.

3. CK activity in serum is reportedly stable for three days at 2-8°C. Addition of sulphydryl agents preserves CK activity during prolonged storage.^{5,6} Some control sera, however, show a considerable decrease in CK activity only a few hours after reconstitution.

Interferences

1. Intramuscular injections and strenuous physical exercise may elevate serum CK.
2. Chloride and sulfate inhibit CK activity.
3. Bilirubin levels up to 40 mg/dl and triglyceride levels up to 2000 mg/dl show no interference in this test.⁹
4. Young, et al. have reviewed drug effects on serum CK levels.⁷

Materials Provided

CK R1 and R2 Reagent.

Materials Required but not Provided

1. Test tubes/rack
2. Pipetting devices
3. Spectrophotometer with the ability to read at 340nm and 1 cm light path.
4. Timer
5. Heating Block (37°C)

Procedure (Automated)

Refer to specific instrument application instructions.

Procedure (Manual)

1. Prepare working reagent according to instructions.
2. Pipette 1.0ml of working reagent into appropriate tubes and pre-warm at 37°C for 4 minutes.
3. Zero spectrophotometer with distilled water at 340nm.
4. Add 0.05 ml (50 ul) sample to its respective test tube and mix gently.
5. After two minutes, read and record the absorbance. Return tube to 37°C. Repeat readings every minute for the next two minutes. The rate should be constant.
6. Calculate the average absorbance difference per minute $\Delta A/\text{min}$.
7. Multiply the $\Delta A/\text{min}$ by the factor 3376 for results in U/L.

NOTE: If cuvette is not temperature controlled, incubate samples at 37°C between readings.

Calibration

CK activity is based on the "micromolar extinction coefficient" of NADP at 340 nm (see "Calculations" section). The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer. Assaying the CK contents of a control serum with known CK values can be used to assure instrument calibration has been performed correctly.

Calculations

Values are derived based on the "absorptivity micromolar extinction coefficient" of NADP at 340 nm (0.00622). A unit per liter (U/L) of CK activity is that amount of enzyme which oxidizes one $\mu\text{mol}/\text{L}$ of NADP per minute.

$$\text{U/L} = \frac{\Delta A/\text{Min}}{0.00622} \times \frac{1.05}{0.05}$$

Creatine Kinase (Two Part Liquid) Reagent Set

$$U/L = \frac{\Delta A/\text{Min}}{0.00622} \times \frac{\text{Total Volume}}{\text{Sample Volume}}$$

$$U/L = \Delta A/\text{Min} \times 3376$$

Limitations

If the $\Delta A/\text{Abs.}/\text{min}$ is greater than 0.345, dilute 1 part sample with 9 parts saline and re-assay. Multiply results by 10. CK values for neonatal patients have not been established with this procedure.

Quality Control

The validity of the reaction should be monitored by use of control sera with known normal and abnormal creatine kinase values. These conditions should be run at least with every working shift in which creatine kinase assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Expected Values⁸

Normal range: Males: 38-174 U/L (37°C)
Females: 26-140 U/L (37°C)

The range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories and local populations.

Performance Characteristics⁹

Comparison: A group of 77 sera ranging in CK activity from 3 - 700 U/L was assayed by the described CK method and by a similar commercially available CK reagent. Comparison of the results yielded a correlation coefficient of 0.998 and the regression equation was $y = 1.027x - 0.65$. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T).

Precision: Within-run precision was established by 20 assays on three different levels of commercial controls. Total Precision values were obtained by assaying 3 commercial controls for 5 consecutive days.

Within-Run	Serum 1	Serum 2	Serum 3
Mean CK (U/L)	159	220	508
Std. Deviation (U/L)	3.2	1.5	3.7
C.V. (%)	2.0	0.7	0.7
Total Precision	Serum 1	Serum 2	Serum 3
Mean CK (U/L)	50	157	228
Std. Deviation (U/L)	1.1	1.6	2.3
C.V. (%)	2.1	1.0	1.0

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.

Linearity: Linear from 1 to 1200 U/L at 37°C.⁸ Performed according to NCCLS Guideline EP6-P.

Sensitivity: Based on an instrument resolution of $A = 0.001$, the method presented shows a sensitivity of 1.0 U/L.

References

- Kachmar JF., Moss DW., In Fundamentals of Clinical Chemistry, 2nd ed. NW Tietz, Editor. WB Saunders, Philadelphia, 1976, p 682.
- Row CR et al., J Lab Clin. Med., 80:557, 1972.
- Szasz G., Proceedings of the Second International Symposium on Clinical Enzymology, Chicago, October 1975.
- Rosalki S.B., J Lab Clin. Chem., 23:646, 1977.
- Morin LG, Clin. Chem., 23:646, 1977.
- Nealon DA, Henderson AR., Clin. Chem., 23:646, 1977.
- Young DS et al., Clin. Chem., 21: 286D, 1975 (Special Issue).
- Tietz, Norbert W., Clinical Guide To Laboratory Tests, W.B. Saunders Company, Philadelphia, PA., (1995), p180.
- Manufacturer's Laboratory Data

Manufactured for Pointe Scientific, Inc.
5449 Research Drive, Canton, MI 48188



European Authorized Representative:
Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: (32)2.732.59.54 Fax:(32)2.732.60.03 email: mail@obelis.net

Rev. 10/11 P803-C7522-01