THE EFFECT OF ACUTE MILK-BASED CARBOHYDRATE/PROTEIN SUPPLEMENTATION ON THE ATTENUATION OF EXERCISE-INDUCED MUSCLE DAMAGE

EMMA COCKBURN

PhD
2010

THE EFFECT OF ACUTE MILK-BASED CARBOHYDRATE/PROTEIN SUPPLEMENTATION ON THE ATTENUATION OF EXERCISE-INDUCED MUSCLE DAMAGE

EMMA COCKBURN

A thesis submitted in partial fulfilment of the requirements of the University of Northumbria for the degree of Doctor of Philosophy

Research undertaken in the School of Psychology and Sports Sciences, Northumbria University

July 2010

Abstract

Eccentric exercise can be beneficial for both clinical and healthy populations. However, a common phenomenon associated with eccentric exercise is muscle damage, which leads to the disruption of the structures within the muscle. Exercise induced muscle damage (EIMD) is associated with reduced functional capacity and the delayed-onset of muscle soreness (DOMS). Therefore, research has investigated interventions to alleviate these symptoms. One such intervention that has received attention in the academic literature is the use of acute protein-carbohydrate supplementation. However, the results are equivocal, and the majority of studies reporting benefits have based their conclusions on measures of intramuscular proteins in serum. Furthermore, the variety of protein-carbohdrate supplements investigated makes it difficult to compare results and these supplements may not be accessible to athletes. Milk, which is convenient and accessible, contains carbohydrate in similar concentrations to many sports drinks, and protein. Therefore, the purpose of this thesis is to provide novel data to expand the existing body of knowledge and investigate the effect of acute milk ingestion on the attenuation of EIMD. This research focuses on the impact of milk in limiting decrements in muscle function.

Study 1: The aim of the first study was to investigate the effect of milk and milk-based drinks on the attenuation of EIMD. Twenty-four healthy male participants were divided into 4 equally matched independent groups, based on concentric knee flexion peak torque. Each group was provided with 1000 mL of their allocated supplement immediately following muscle damaging exercise: (i) milk, (ii) milk-based protein-carbohydrate, (iii) carbohydrate sports drink, or (iv) water. Passive muscle soreness, creatine kinase (CK), myoglobin (Mb), and peak torque and set total work of the hamstrings during concentric knee flexions were assessed immediately prior to muscle damaging exercise, and 24 and 48 h post. The primary finding of this study was that milk and milk-based protein-carbohydrate limited decrements in peak torque and total work of the set, and increases in CK and Mb 48 h after muscle damaging exercise.

Study 2: The aim of this study was to examine the optimal timing of milk-based protein-carbohydrate supplementation. Thirty-two healthy male participants were

divided into 4 equally matched independent groups: (i) milk-based protein-carbohydrate consumed pre-muscle damaging exercise, water consumed at all other time points, (ii) milk-based protein-carbohydrate consumed immediately post muscle damaging exercise, water consumed at all other time points, (iii) milk-based protein-carbohydrate consumed 24 h post muscle damaging exercise, water consumed at all other time points, or (iv) water consumed at all time points. Passive and active muscle soreness, CK, peak torque of the hamstrings during concentric knee flexions and reactive strength index (RSI) were assessed immediately prior to muscle damaging exercise, and 24, 48 and 72 h after. The primary finding of this study was that the consumption of milk-based protein-carbohydrate following muscle damaging exercise was beneficial for blunting increases in active muscle soreness and decreases in muscle function over 48 h in comparison to pre-exercise supplementation.

Study 3: The previous studies provided participants with 1000 mL of their allocated supplement. The aim of this study was to determine if the consumption of milk in smaller doses lead to the alleviation of EIMD. Twenty-four healthy male participants were divided into 3 equally matched independent groups: (i) 500 mL milk, (ii) 1000 mL milk, or (iii) water. Passive and active muscle soreness, CK, Mb, markers of inflammation (interleukin-6 and C-reactive protein), peak torque of the hamstrings and reactive strength index were assessed immediately prior to muscle damaging exercise, and 24, 48 and 72 h after. The primary finding of this study was that decrements in isokinetic muscle function could be reduced with the intake of less milk.

Study 4: The final study aimed to investigate the effect of milk supplementation following muscle damaging exercise on performance tests specific to field-based team sports. Fourteen healthy male footballers were divided equally into 2 independent groups: (i) milk, or (ii) water. Participants were provided with 500 mL of their allocated supplement immediately following muscle damaging exercise. Prior to muscle damaging exercise, and 24, 48 and 72 h after participants were assessed for speed (15 m), change of direction speed, countermovement jump (CMJ) height and reactive strength index. In addition, participants were required to complete the Loughborough Intermittent Shuttle Test (LIST) prior and 48 h following muscle damaging exercise. The findings of this study demonstrated that milk consumed immediately following muscle damaging exercise limited decrements in performance tests specific to field-based team sports.

These studies provide additional novel data regarding the impact of acute protein-carbohydrate ingestion on EIMD. Specifically, 500 mL of milk consumed immediately following muscle damaging exercise alleviates decrements in many facets of muscle function, such as sprinting and isokinetic muscle function. There is a possible benefit of milk for reducing increases in muscle soreness experienced during activity and intramuscular proteins measured in the serum. Future research is warranted to elucidate the underlying mechanisms responsible for these findings.

Peer reviewed publications arising from thesis

Cockburn, E. (2010) 'Acute protein-carbohydrate supplementation: effects on exercise-induced muscle damage', *Current Topics in Nutraceutical Research,* 8 (1), pp. 7-18.

Cockburn, E., Stevenson, E., Hayes, P.R., Robson-Ansley, P. & Howatson, G. (2010) 'Effect of milk-based carbohydrate-protein supplement timing on the attenuation of exercise-induced muscle damage', *Applied Physiology, Nutrition and Metabolism*, 35 (3), pp. 270-277.

Cockburn, E., Hayes, P.R., French, D.N., Stevenson, E. & St Clair Gibson, A. (2008) 'Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage', *Applied Physiology, Nutrition and Metabolism,* 33 (4), pp. 775-783.

Robson-Ansley, P., Cockburn, E., Walshe, I., Stevenson, E. & Nimmo, M. (2010) 'The effect of exercise on plasma IL-6 receptor concentration: a dichotomous Response', *Exercise Immunology Reviews,* 16, pp. 56-76.

Conference communications and published abstracts arising from thesis

Cockburn, E., Stevenson, E., Hayes, P.R. & Robson-Ansley, P. (2009) 'The effect of dose of milk consumed on exercise-induced muscle damage', *British Journal of Sports Medicine,* 43 (e2), pp. 12.

Robson-Ansley P., Cockburn, E., Hayes, P.R. & Stevenson, E. (2009) 'A reduction in plasma soluble IL-6 concentration during recovery from eccentric exercise', *British Journal of Sports Medicine,* 43 (e2), pp. 12-13.

Cockburn, E., Hayes, P.R., Stevenson, E. & St. Clair Gibson, A. (2008) 'Effect of milk-based protein/CHO supplement timing on the attenuation of exercise-induced muscle damage', Book of Abstracts of 13th Annual Congress of the European College of Sport Sciences (ECSS) in Estoril, Portugal from 9 – 12th July.

Cockburn, E., Hayes, P.R., French, D., Stevenson, E. & St. Clair Gibson, A. (2007) 'Milk-based protein supplementation aids recovery following exercise-induced muscle damage', *Journal of Sports Sciences*, 25 (S2), pp. S15-S16.

Cockburn, E., Hayes, P.R. & French, D. (2007) 'Effect of different nutritional supplements on recovery following exercise-induced muscle damage', *Journal of Sports Sciences*, 25 (3), pp. 256-257.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

TABLE OF ABBREVIATIONS

3-MH	3-methylhistadine
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
CHO	Carbohydrate
CI	Confidence Intervals
CK	Creatine Kinase
COX2	Cyclooxygenase-2
CRP	C-reactive Protein
DNA	Deoxyribonucleic Acid
DOMS	Delayed-Onset of Muscle Soreness
E-C	Excitation-Contraction
EIMD	Exercise Induced Muscle Damage
EMG	Electromyogram
GLUT	Glucose Transporter Type
IFCC	International Federation of Clinical Chemistry
IL	Interleukin
LDH	Lactate Dehydrogenase
LIST	Loughborough Intermittent Shuttle Test
Mb	Myoglobin
mRNA	Messenger Ribonucleic Acid
MSFT	Multi-Stage Fitness Test
mTOR	Mammalian Target of Rapamycin
MVC	Maximum Voluntary Contraction
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
$P70^{S6\kappa}$	P70 S6kinase
PCr	Phosphocreatine

PG	Prostaglandin
PI-3K	Phosphatidylinositol 3-kinase
PLA	Phospholipase
RNA	Ribonucleic Acid
RPE	Rating of Perceived Exertion
SD	Standard Deviation
SEM	Standard Error of the Mean
TBARS	Thiobarbituric Acid
Tn-Tm	Troponin-Tropomyosin
TNF	Tumor Necrosis Factor
tRNA	Transfer Ribonucleic Acid
Ub-P	Ubiquitin-Proteosome
VAS	Visual Analogue Scale
VO_2	Volume of Oxygen

Acknowledgements

It has taken 5 ½ years to get to this stage and there have been many ups and downs. Throughout this process I have received the guidance and support of many people whom I would like to thank.

Firstly, I would like to thank my supervision team, Dr Emma Stevenson, Phil Hayes and Dr Paula Robson-Ansley. A special thanks must be extended to Dr Emma Stevenson as we have been on the same 'wavelength' throughout the process, and even when you were on maternity leave and dealing with a new addition to the family, you were there to help me.

Secondly I must thank my fiancé, Phill Bell. You have put up with my stress and moaning, and without you I'm not sure that I would have completed the last study or found any participants.

I would also like to thank Kevin Thomas for answering my endless questions when confusion took over and helping with data collection.

Thanks also go to Dr Glyn Howatson, Dr Duncan French, Dr Lesley Fishwick and Professor Alan St Clair Gibson. You have provided me with guidance and advice during the long years.

Lastly, I must thank all my participants for completing the studies even when walking was an issue.

Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work.

Name:

Signature:

Date:

1. INTRODUCTION

1.1   Introduction

Eccentric muscle actions are defined as a lengthening of the muscle while tension is generated (Nosaka, Newton & Sacco, 2002). These types of muscle action occur frequently in daily activities, exercise and sport performance (Enoka, 1996). For example, walking down stairs requires the use of eccentric actions of the quadriceps, and during running and jumping lower limb muscles lengthen to provide deceleration of the body segments (Child, Saxton & Donnelly, 1998). In 1882, Fick observed that eccentric muscle actions could generate greater forces compared to concentric actions (Lindstedt, LaStayo & Reich, 2001). Many years later it was observed that eccentric muscle actions had another unique feature; a lower energy cost for the same amount of work completed (Abbott, Bigland & Ritchie, 1952). Bigland-Ritchie & Woods (1976) later reported that the oxygen requirement of submaximal eccentric cycling was 1/6 - 1/7 of that for concentric cycling at the same workload. Therefore, eccentric exercise can produce high forces with reduced strain on the cardiovascular system. This makes it a suitable form of exercise for both clinical and healthy populations. Eccentric exercise could be used for elderly and clinical populations with impaired lung function (Rooyackers, Berkeljen & Folgering, 2003) and mitochondrial disease (Taivassalo *et al.*, 1999) to increase their ability to function and improve their standard of living (Trenell *et al.*, 2006). Within healthy populations, eccentric exercise is commonly used to improve muscle strength and size, and alterations in the spring properties of the muscle (Lindstedt, LaStayo & Reich, 2001) used during stretch-shortening cycle exercise. Eccentric muscle actions lead to greater improvements in eccentric and isometric strength than concentric actions (Hortobagyi *et al.*, 1996a; Hortobagyi *et al.*, 1996b). This, therefore, makes eccentric exercise a desirable muscle action to use during training for sports performance and rehabilitation.

Eccentric exercise clearly has an important role in health, exercise and sport for a variety of populations. However, a common phenomenon associated with eccentric exercise is muscle damage. Exercise induced muscle damage (EIMD) occurs when the nature or magnitude of eccentric force production significantly changes (Lindstedt, LaStayo & Reich, 2001), such as a novel exercise stress. EIMD is ultimately the disruption of muscle structures and is associated with a number of symptoms. The delayed-onset of muscle soreness (DOMS) is commonly reported (Semark *et al.*, 1999; MacIntyre *et al.*, 2001) and functionally there is a decreased ability to produce force (Byrne & Eston, 2002a; b; Harrison & Gaffney, 2004; Twist & Eston, 2005). These

changes are also associated with an increase in intramuscular proteins measured in the plasma (Sorichter *et al.*, 2001; Seifert *et al.*, 2005). DOMS and reduced functional capacity can limit a person's ability to conduct everyday tasks, exercise and perform. Therefore, interventions to alleviate these symptoms have received a great deal of attention. Interventions are primarily focused around pharmaceutical, therapeutic and nutritional methods, which have been extensively reviewed elsewhere (Howatson & Van Someren, 2008).

The ingestion of a combination of carbohydrate (CHO) and protein alters protein metabolism (Borsheim, Aarsland & Wolfe, 2004; Tang *et al.*, 2007), which may limit the breakdown or increase repair of muscle protein structures. Therefore, this area of research has begun to receive a great deal of attention in the last few years (Wojcik *et al.*, 2001; Saunders, Kane & Todd, 2004; Seifert *et al.*, 2005; Baty *et al.*, 2007; Saunders, Luden & Herrick, 2007; Green *et al.*, 2008; Valentine *et al.*, 2008; White *et al.*, 2008; Betts *et al.*, 2009). The results are equivocal with some studies reporting no benefit of acute protein-CHO consumption (Green, 1997; Wojcik *et al.*, 2001; White *et al.*, 2008; Betts *et al.*, 2009), whilst others have demonstrated significant reductions in markers of muscle damage (Saunders, Kane & Todd, 2004; Seifert *et al.*, 2005; Baty *et al.*, 2007; Saunders, Luden & Herrick, 2007; Valentine *et al.*, 2008). Many researchers reporting benefits of acute protein-CHO supplementation have primarily based their conclusions on measures of intramuscular proteins. However, changes in DOMS and muscle function are of greater importance to clinical and healthy populations. Therefore, investigation into acute protein-CHO ingestion and its effect on DOMS and muscle function is required.

Previous studies have used a variety of protein-CHO supplements which makes it difficult to compare results and provide definitive advice for the population regarding choice of supplement. Milk has recently gained increasing interest as an exercise supplement (Roy, 2008). It is well known that milk has many health benefits, which have been reviewed elsewhere (Haug, Hostmark & Harstad, 2007). Milk is potentially a good choice to consume as a sports drink as it contains carbohydrates in amounts similar to many commercially available sports drinks, contains casein and whey proteins in a ratio of 3:1, and has a naturally high concentration of electrolytes (Roy, 2008). Milk has been shown to be a beneficial drink for hydration (Shirreffs, Watson & Maughan, 2007), recovery from glycogen depleting exercise (Thomas, Morris & Stevenson, 2009) and for enhancing protein

metabolism following resistance exercise (Elliot *et al.*, 2006). Milk also has the added benefit of providing additional nutrients and vitamins that are not present in commercial sport drinks (Roy, 2008), such as calcium, and vitamins A and E. Milk may be a viable supplement to ingest for the alleviation of EIMD, and from a practical perspective it is a supplement that is convenient, accessible and inexpensive. Finally, the research conducted on the use of protein-CHO supplements for attenuating EIMD have been extremely varied in the volume and timing of intake. Similar to the type of supplement this makes it increasingly difficult for athletes to apply these findings to their own practice. Therefore, from an applied perspective, milk is provided in volumes that are representative of standard servings, and that could be realistically consumed by athletes post exercise.

In light of all this information, the purpose of this thesis is to expand the current body of knowledge by investigating the effect of acute milk supplementation on alleviating symptoms of EIMD, with a specific focus on muscle function. This is conducted over four sequential investigations.

The first study investigates the effect of acute milk supplementation in attenuating the symptoms of EIMD. This study compares the ingestion of milk, a commercially available milk-based protein-CHO sports drink, a CHO sports drink or a control following resistance-based eccentric exercise on subsequent muscle function, DOMS and intramuscular proteins measured in the plasma over 48 h.

The first study provides participants with their allocated supplement immediately following muscle damaging exercise. Therefore, the second study investigates the optimal timing of acute milk ingestion in reducing the symptoms of EIMD. The study compares supplementation immediately prior, immediately following and 24 h following muscle damaging exercise. Changes in muscle function, DOMS and intramuscular proteins are assessed over 72 h.

The initial two studies provide participants with 1000 mL of milk. The third study investigates the optimal volume of milk ingestion to determine if the consumption of milk in smaller doses leads to the alleviation of EIMD. This study compares 500 mL and 1000 mL of milk, and EIMD is assessed by measuring muscle function, DOMS and intramuscular proteins over 72 h.

The final study applies the findings of the first three studies to a sporting situation by investigating the effect of acute milk supplementation on performance tests specific to field-based team sports. Participants either consume milk or a control following muscle damaging exercise, and performance tests of field-based team sports, DOMS and intramuscular proteins are assessed over 72 h.

2. LITERATURE REVIEW

2.1 Literature Review

This review of literature discusses muscle structure, action and maintenance, followed by the process of exercise induced muscle damage (EIMD) specifically highlighting mechanisms and markers. The review then focuses on the changes in protein metabolism following muscle damaging exercise with discussions based around acute protein-carbohydrate (CHO) supplementation for stimulating protein metabolism.

2.2 Anatomy, Architecture and Regulation of Skeletal Muscle

Skeletal muscle represents approximately 40 - 45% of total body mass (Snow, 2003), and it is skeletal muscle that produces joint movement and exerts force. In order to understand how muscles function and how that function can be disrupted, it is important to examine the structure of human muscle. Skeletal muscle is made up of a highly organised structure of proteins. It is this which permits the controlled generation of force. Figure 2.1 illustrates the structure of skeletal muscle from the gross to molecular level, as it is currently accepted.
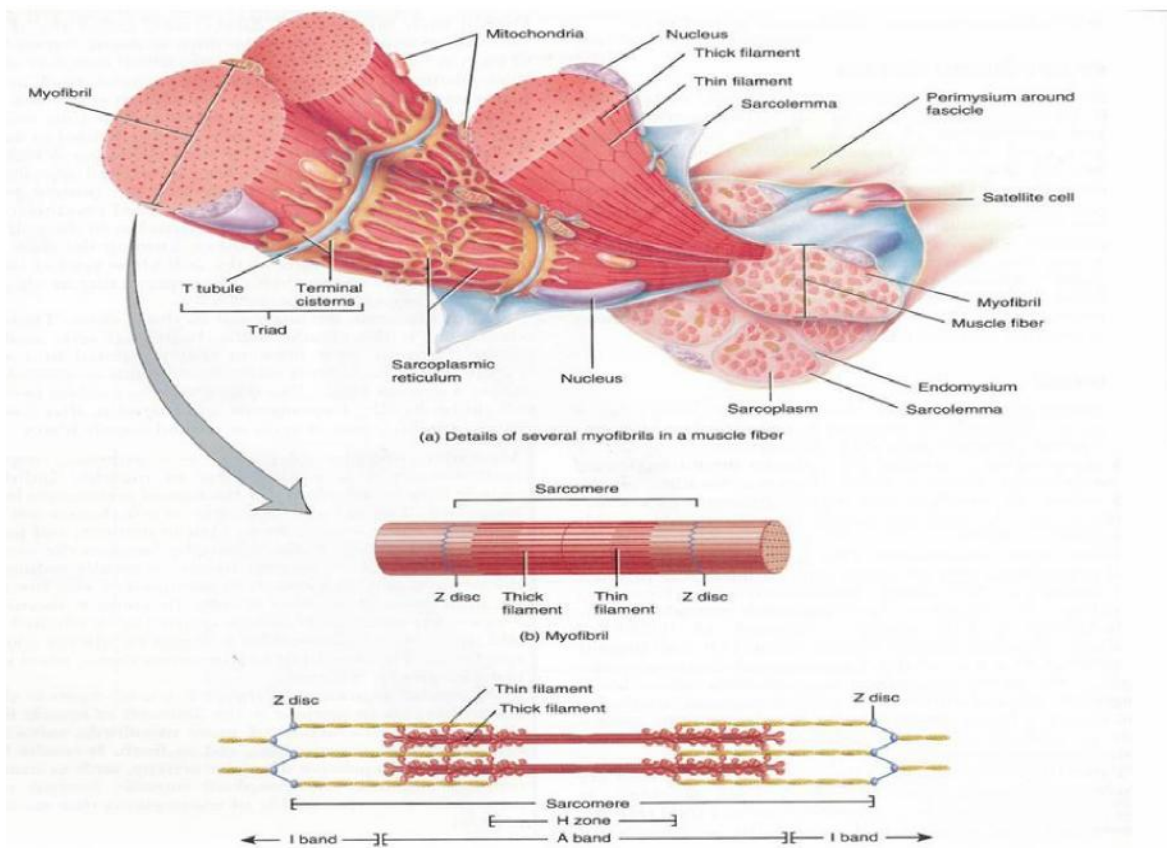
Figure 2.1 Organisation of skeletal muscle (Tortora & Grabowski, 2004)

2.2.1 Whole Muscle to Myofibril

Skeletal muscle is a discrete organ made up of hundreds of muscle fibres. They are grouped together in bundles termed fascicles (Jones, Round & de Haan, 2004). The fascicles are enveloped by connective tissue, which directly attaches to each muscle fibre and to all other fascicles within the muscle. All internal and external muscle connective tissue is continuous with tendons that are continuous with the external membrane of the skeleton, allowing force generated in the fibres to be transferred to the bones. Muscle fibres are surrounded by the sarcoplasm, which is the water based internal environment of the muscle cell containing ions, enzymes, fuels and molecular gases (Woledge, Curtin & Homsher, 1985). Each muscle fibre is separately enclosed by a plasma membrane (Widmaier, Raff & Strang, 2004) termed the sarcolemma, which is approximately 8 nm (Wilkie, 1985). The sarcolemma consists of a double layer of lipid molecules, the majority being phospholipids, and has proteins, referred to as membrane proteins, embedded within it. The sarcolemma provides a selectively permeable barrier between the fibre and extracellular fluid. It also plays a vital role in the transmission of the action potential to generate force. Each muscle fibre contains multiple nuclei, which store and transmit genetic information used to synthesise proteins determining the structure and function of the fibre. Thousands of myofibrils make up each muscle. These are 1 - 2 μm in diameter, and cause the contractile behaviour of the muscle (Woledge, Curtin & Homsher, 1985).

Surrounding each myofibril is the internal membrane system of the sarcoplasmic reticulum. The sarcoplasmic reticulum forms a sleeve like segment around each myofibril (Widmaier, Raff & Strang, 2004), with lateral sacs containing Ca^{2+}. The concentration of Ca^{2+} is around 10 000 times greater than the rest of the fibre (McComas, 1996). The main function of the sarcoplasmic reticulum is to release (via the ryanodine receptor) and re-sequester (via the Ca^{2+}-ATPase pump) Ca^{2+} during muscle actions. The transverse-tubules (t-tubules) pass between adjacent lateral sacs and their role is to conduct electrical changes (Huxley, 1971) by propagating action potentials into the interior of the muscle fibre to signal the release of Ca^{2+}.

2.2.1.1 Myofibril

Myofibrils contain a regular arrangement of hundreds of filaments in repeating units (Widmaier, Raff & Strang, 2004). The filaments are classed as thick (or anisotropic) or thin (or isotropic), which partially overlap, and one unit of these is

termed a sarcomere (Huxley, 1957). The arrangement of the thick and thin filaments within a sarcomere is shown in Figure 2.1. The I band (thin) is made up of actin, tropomyosin and troponin (Woledge, Curtin & Homsher, 1985). Tropomyosin is arranged along the surface of the actin filament, and troponin is a complex of three polypeptides (TnC, TnI and TnT) (Jones, Round & de Haan, 2004). Individual thin filaments are situated at either end of the sarcomere and are fixed in the centre of the I band. They are joined by α-actinin at each end of the sarcomere to the Z-line with the opposite end extending towards the centre of the sarcomere terminating just before the H zone. Thick filaments are primarily composed of myosin and are situated in the middle of each sarcomere. Thick filaments are joined at the M line, which is a transverse connective tissue filament in the centre of the sarcomere, and by titin filaments to the Z-line (McComas, 1996). Actin and myosin are the two principle protein molecules constituting approximately 80 % of total muscle protein mass (Jones, Round & de Haan, 2004). Actin and myosin interact to provide sliding of the filaments with troponin and tropomyosin having a control function (Woledge, Curtin & Homsher, 1985). TnT attaches to tropomyosin, and this troponin-tropomysosin (Tn-Tm) complex inhibits the binding sites on actin, therefore, preventing crossbridge attachment for muscle action (Jones, Round & de Haan, 2004). Ca^{2+} release from lateral sacs is required to remove this inhibitory effect and allow muscle action, which is discussed in section 2.2.1.2.

As well as contractile proteins, there are a number of structural proteins that play distinct physiological roles. There are two large proteins, titin and nebulin, that act to stabilise the highly ordered structure of the sarcomere, and are involved in the production of active and passive tension in the muscle (Horowits *et al*., 1986). Titin connects the Z-line to the myosin filaments, plays a role in locating thick filaments in the centre of the sarcomere (Morgan & Allen, 1999) and is responsible for the majority of the resting tension in stretched fibres (Magid & Law, 1985). Nebulin is associated with actin near the Z-line and may act to strengthen the thin filaments (Jones, Round & de Haan, 2004). Cytoskeleton proteins form a bi-dimensional lattice of the cytoplasmic side of the sarcolemma (attach sarcolemma and contractile proteins) and they include spectin, vinculin and talin (Small, Furst & Thornell, 1992). Transverse connections of this lattice exist between the myofibrils and the sarcolemma (Pardo, Siliciano & Craig, 1983), termed the costameres, which is partly made up of dystrophin. Finally, intermediate filaments provide a link between Z-lines and the sarcolemma. They act as an extrasarcomeric mechanical stabiliser of myofibrillar regularity and integrity

(Lazarides, 1980) that possibly may be important in limiting extreme sarcomere lengths (Wang & Ramirez-Mitchell, 1983). Intermediate filaments are composed mainly of desmin, along with vimentin and synemin. There are a number of other proteins present in skeletal muscle; however, to describe them all is beyond the scope of this thesis.

The thick and thin filaments and specific contractile proteins form the cytoskeleton which reinforces the muscle membrane, preventing it from tearing during muscle action. Therefore, damage or absence of structural proteins can signify structural damage to the muscle or membrane instability and, therefore, lead to the degradation of the muscle as is seen during EIMD.

2.2.1.2 Sliding Filament Theory

In the early 1950's the sliding filament theory was developed by Huxley A.F. and Huxley H.E. Using light (Huxley & Niedergerke, 1954; Huxley & Hanson, 1954) and electron (Page & Huxley, 1963) microscopy it was established that filament length remained constant during lengthening and shortening muscle actions. From this it was postulated that changes in muscle length to produce force occurred by relative lengthening of thick and thin filaments sliding past each other. The sliding of the filaments is widely accepted to occur by the attachment between the myosin head and actin, referred to as a crossbridge (Woledge, Curtin & Homsher, 1985). The details of crossbridge kinetics are beyond the scope of this thesis but in summary the interaction of actin, myosin and adenosine triphosphate (ATP) lead to crossbridge movement, stretch of the compliant portion of the crossbridge and subsequent force production. This was derived from proposals by Huxley (1957) and Huxley & Simmons (1971), and involves the requirement of ATP for detachment of the crossbridge. However, during lengthening actions very low heat values are measured (Fenn, 1923), there are low rates of ATP hydrolysis (Curtin & Davies, 1973), and there is a lower energy cost compared to shortening actions for the same amount of work completed (Abbott, Bigland & Ritchie, 1952). Therefore, during lengthening actions force production does not require as much ATP and thus does not fit with the original proposal. During lengthening actions, the detachment of the crossbridge would occur mechanically with forcible detachment, with ATP required for only some crossbridges.

Ca^{2+} is required to remove the inhibitory effect of the Tn-Tm complex and allow the

attachment of actin and myosin to create a crossbridge. Ca^{2+} release is stimulated via a sequence of events termed excitation-contraction (E-C) coupling. The details of this process are not within the scope of this thesis but briefly it involves a signal from the central nervous system to the muscle. At the muscle an action potential is propagated along the sarcolemma and through the t-tubules to release Ca^{2+} into the sarcoplasm, increasing cytosolic Ca^{2+} concentration (Widmaier, Raff & Strang, 2004). Troponin sensitises the thin filament to Ca^{2+} (Ebashi & Endo, 1968), removing the inhibitory effect, ultimately leading to sliding of the filaments and thus force production.

2.2.1.3 Length-Tension Relationship

The sliding filament theory suggests that the development of tension is dependent on the overlap of thick and thin filaments. Gordon, Huxley & Julian (1966) varied the stretch of a marked area of a fibre (assumed to represent one sarcomere) and measured the tension developed under tetanus. Figure 2.2 demonstrates the observed relationship between length and tension. At point C the greatest tension is developed, which relates to the greatest level of actin and myosin overlap allowing the maximum number of formed crossbridges. There is a plateau between points B and C as no new crossbridge sites are added. At lengths beyond point B (lengthening actions), tension declines linearly. It is now thought that at this point there is an increase in passive tension due to the elongation of titin filaments rather than active crossbridge cycling (Widmaier, Raff & Strang, 2004). Shortening of the fibre also reduces tension as the actin filaments interfere with each other. At point D the thick filaments collide with the Z-lines and tension drops to zero as no further sarcomere shortening is possible (Gordon, Huxley & Julian, 1966).

The length-tension relationship is an important concept within this thesis as it is the underlying theory of EIMD. It is acknowledged that tension produced can be influenced by a variety of other factors; intracellular Ca^{2+} concentration, crossbridge phosphorlylation, muscle cross-sectional area, number and size of active motor units, velocity of muscle action and fibre type (Snow, 2003). However, these mechanisms are not central to this thesis.
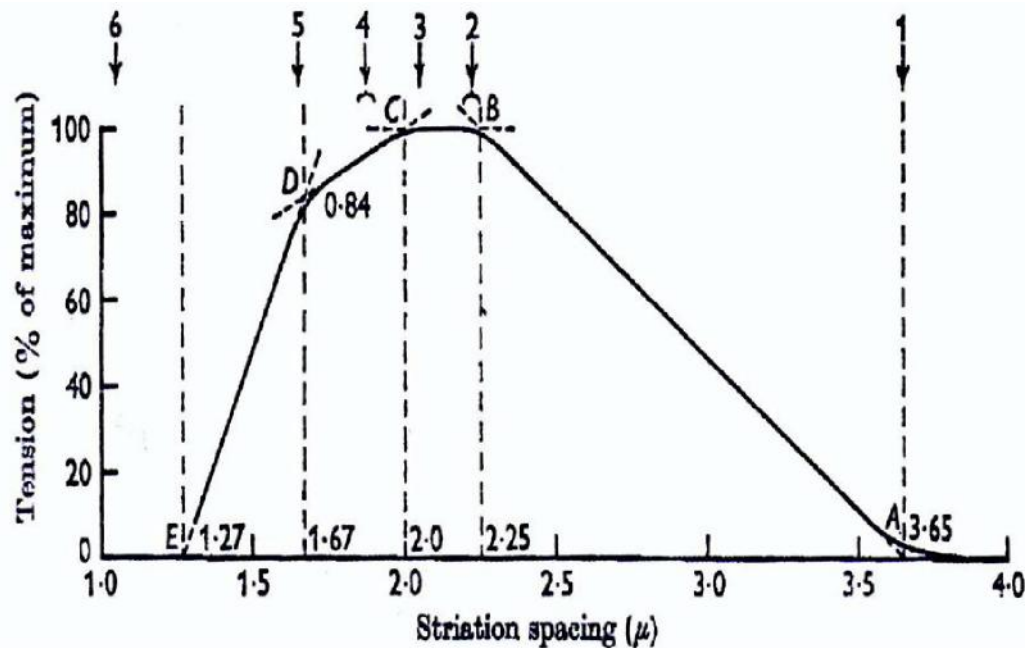
Figure 2.2 Gordon's length tension relationship (Gordon, Huxley & Julian 1966)

Force generation occurs by interaction of the thick and thin filaments and ATP, with a number of other muscle proteins providing functional and structural roles. Although during lengthening actions, ATP is not hydrolysed to as great an extent as shortening actions, it is important to understand how ATP is generated.

2.2.1.4 ATP Generation

Muscle actions require ATP for crossbridge cycling (attach and detach). ATP is the only energy source that can be used directly for contractile activities, however, intracellular concentrations of ATP are relatively low (2 - 8 mmol.L^{-1}) (Jones, Round & de Haan, 2004). Therefore, there is a need to generate ATP to allow for extended supply and thus continuous muscle activity. There are three main ATP generating pathways: ATP re-synthesis can occur locally on myofibrils and biomembranes due to activity of creatine kinase (ATP-PC) and glycolytic enzymes (glycolysis) (Korge, Byrd & Campbell, 1993); ATP can also be formed in the mitochondria (aerobic) and then diffuse to myofibrils. These pathways differ in two primary features; the maximum rate of ATP re-synthesis (power) and the maximum amount of ATP that can be produced by that system (capacity). The ATP-PC system is characterised by its high power but very low capacity of phosphocreatine (PCr) hydrolysis. The aerobic system is characterised by its low power but very high oxidative capacity of CHO and triacylclycerols. An important concept in this thesis is the re-synthesis of ATP via

glycolysis as EIMD has been shown to detrimentally affect this pathway. This process provides an intermediate pathway for ATP generation in terms of power and capacity.

In order for ATP re-synthesis to take place via glycolysis, glucose and/or glycogen are required. Glucose is the primary substrate and it can be formed via a number of processes, which are not within the scope of this thesis. However, exogenous CHO does increase blood glucose concentrations. Glycolytic ATP production requires the uptake of glucose into the muscle cell. There are currently five established glucose transporter isoforms (GLUT-1 to 4 and GLUTX1) (Watson & Pessin, 2001). GLUT4 is the major insulin responsive transporter that is predominantly restricted to striated muscle and adipose tissue (Watson & Pessin, 2001). The majority of GLUT4 resides within the cell interior, and the activation of insulin receptors, phosphatidylinositol 3-kinase (PI3K), triggers a large increase in the rate of GLUT4 vesicle exocytosis (Watson & Pessin, 2001). This leads to the translocation of GLUT4 storage compartments to the plasma membrane increasing levels on the cell surface, thereby, increasing the rate of glucose uptake (Watson & Pessin, 2001). Within the muscle cell, CHO (glucose/glycogen) is broken down via anaerobic processes to lactate and associated protons, providing ATP for muscle activity.

2.2.1.5 Fibre Type

An important factor in determining the muscles tension-producing ability is fibre type. Human skeletal muscle is composed of three main fibre types: type I (slow oxidative fibres), type IIA and type IIB/X (fast glycolytic fibres). These fibres differ substantially in their functional and metabolic characteristics (Table 2.1). Different fibre types are an important factor in this thesis as there is evidence of preferential damage to certain fibre types, which will be discussed in section 2.3.1.3.

Table 2.1. Functional and structural characteristics of skeletal muscle fibres (Cooke, 2005)

Characteristic	Slow Oxidative (Type I)	Fast Oxidative (Type IIA)	Fast Glycolytic (Type IIB/X)
Myosin ATPase activity	Low	High	High
Speed of Activity	Slow	Fast	Fast
Force of Activity	Low	Moderate	High
Fatigue Resistance	High	Moderate	Low
Oxidative Capacity	High	High	Low
Mitochondrial Density	High	High	Low
Myoglobin Content	High	High	Low
Anaerobic Enzyme Content	Low	Moderate	High
Capillary Density	High	High	Low
Glycogen	Low	Moderate	High
Z-line	Intermediate	Wide	Narrow
Fibre Diameter	Small	Moderate	Large

This section has discussed the structure of skeletal muscle and how muscle force is produced. These concepts are pertinent to this thesis due to the focus of the investigation, which is primarily the impact on muscle function. Force generated by the muscle can be affected by many variables, which have been previously stated. However, central to this thesis is the concept that the proteins within the muscle can be altered by muscle damaging exercise due to changes in protein metabolism, and that nutritional supplements can alter this impact.

2.2.2 Regulation of Skeletal Muscle

Protein metabolism is a continuous process regulating the protein structures within skeletal muscle. The object of protein turnover is to eliminate excess proteins and those that have been structurally or functionally altered (Andreu & Schwartz, 1995). Protein turnover involves continual synthesis and degradation, which are intrinsically linked. A relationship between fractional synthetic rate and fractional breakdown rate has been demonstrated in the fasted state (Phillips *et al.*, 1997). The primary link between synthesis and degradation is likely to be amino acid (AA) availability as it is the substrate for one process and the product of the other (Waterlow,

2006). Within the muscle there are intracellular pools of amino acids. Elevating muscle protein synthesis will reduce the amino acids within this pool, promoting inward amino acid transport and/or protein degradation with inhibition of amino acid oxidation (Wolfe & Miller, 1999). To maintain protein balance, synthesis and degradation must be equal; however, if one process is greater than the other then balance will become either negative or positive. This section outlines the processes of synthesis and degradation. Future sections discuss the impact of EIMD and protein-CHO supplements on these processes and thus protein balance.

2.2.2.1 Protein Synthesis

Muscle fibres contain multiple nuclei with each nucleus containing deoxyribonucleic acid (DNA). The information coded in the molecules of DNA is used to synthesise proteins from amino acids. This is a complex process and it would appear that protein synthesis and its regulation involves an array of proteins that undergo turnover, which is presumably regulated by other proteins, and the process continues (Waterlow, 2006). Therefore, protein synthesis is a continual process. It involves a number of processes; transcription, translation and initiation.

Transcription occurs primarily and involves the transfer of genetic information from DNA to ribonucleic acid (RNA) (Widmaier, Raff & Strang, 2004). Following this, RNA is modified to messenger RNA (mRNA), which holds the base sequence specifying the sequence of amino acids in the protein (Houston, 1995). Translation is the process of forming proteins using mRNA as a template (Waterlow, 2006), which involves three stages; initiation, elongation and termination. Initiation and elongation factor activity can be influenced by signals from hormone and nutrient receptors (Waterlow, 2006). Specifically, mRNA translation, thus protein synthesis, is regulated by p70 S6kinase (p70^{S6K}) which results in the capacity of the cell to synthesise protein (Ivy *et al.*, 2008). P70^{S6K} is an important factor of the hormone/nutrient sensing and signalling pathway that influences translation (Rennie & Tipton, 2000). Finally, initiation involves transfer RNA (tRNA) which attaches to specific amino acids and transfers them to the complex of mRNA and the ribosome on which the protein is formed (Houston, 1995). This is a process that continues until the ribosome reaches the termination sequence in mRNA, signalling the formation of the protein (Widmaier, Raff & Strang, 2004). This continual process of protein synthesis involving a multitude of different proteins aids in the regulation of structures within skeletal muscle. Protein degradation must also occur to regulate the protein structures

within the muscle.

2.2.2.2 Protein Breakdown

There are three main pathways of protein degradation; Ca^{2+} activated proteinases (calpain), lysosomes and the ubiquitin-proteasome (Ub-P) pathway. Protein degradation occurs via all three pathways but the contribution of each will be dependent on the type of tissue and stage of processing (Waterlow, 2006). All three pathways have been implicated in EIMD.

Increases in intracellular Ca^{2+} concentration will activate calcium activated proteinases. One of these is calpain, which is activated in the cytosol when bound to a cell membrane or to an activator associated with the cytoskeleton (Doherty & Mayer, 1992). In skeletal muscle, calpain will only play a minor part in protein degradation, but may still be important in the degradation of minor myofibrillar protein components such as nebulin, titin, tropinin and tropomysosin (Goll *et al.*, 1992; Attaix & Taillandier, 1998). Calpain will also degrade intermediate filament cytoskeleton proteins such as vimentin and desmin (Doherty & Mayer, 1992). Calpain has been implicated in the first step of myofibrillar degradation (Reddy *et al.*, 1975), as it is not a digestive process. Rather it proceeds in a limited manner and results in alteration rather than simple destruction of the substrate proteins (Saido, Sorimachi & Suzuki, 1994). This limited proteolysis is likely to cause destabilisation of structural rigidity, making it more sensitive to attacks by cellular proteases facilitating further degradation of the substrate protein (Saido, Sorimachi & Suzuki, 1994).

Protein degradation via the lysosomal pathway involves autophagy (Doherty & Mayer, 1992), which is the degradation of the cells own components. Protein substrates are sequestered into the vacuolar system, followed by lysosomal hydrolysis (Bechet *et al.*, 2005). The lysosomal pathway is involved in the degradation of non-myofibrillar proteins such as membrane associated proteins (Biolo *et al.*, 2000), and in the breakdown of cytoplasmic soluble constituents and cellular organelles (Bechet *et al.*, 2005) including the mitochondria (Doherty & Mayer, 1992). Skeletal muscle contains few lysosomes, only degrading sarcoplasmic proteins (Waterlow, 2006), and they do not significantly contribute to overall protein breakdown in muscles incubated under optimal conditions (tension, presence of insulin and amino acids) (Attaix & Taillandier, 1998). In the catabolic state, lysosomal

proteolysis is not quantitatively important in increased protein degradation (Mitch & Goldberg, 1996).

The Ub-P pathway involves the activation of ubiquitin by cytokines (Tumor Necrosis Factor (TNF) and interleukins (IL)) from activated macrophages (Mitch & Goldberg, 1996). Proteins targeted for degradation are covalently attached to multiple ubiquitin molecules (Attaix & Taillandier, 1998). The proteolysis of proteins involves 20S protesome (Attaix & Taillandier, 1998), and following proteolysis the ubiquitins are released and re-used (Waterlow, 2006). This pathway is responsible for the breakdown of a wide range of proteins in a variety of tissues (Herschko & Ciechanover, 1992). In the cytoplasm, this system degrades the majority of myofibrillar proteins (Biolo *et al.*, 2000). Actin and myosin light chains can be ubiquitylated (Attaix & Taillandier, 1998). However, these proteins must be destabilised to be ubiquitylated as this system does not degrade intact proteins. Therefore, calpain activation may render substrate proteins vulnerable to complete degradation via the Ub-P system. Both of these systems play a role during EIMD.

The processes of muscle protein synthesis and degradation are complex and are controlled by many factors including exercise, hormones and nutrition. Central to this thesis is the role of eccentric exercise, insulin from CHO intake and increased amino acid availability from protein intake in altering these processes. These factors will be discussed in section 2.5 and 2.6. The following section outlines the physiology of protein intake to increase amino acid availability.

2.2.2.3 Protein Digestion, Absorption and Uptake at the Muscle

Protein digestion breaks down proteins into tripeptides, dipeptides and simple amino acids within the stomach and small intestine, and these are then released into the blood stream. The digestion of protein begins in the stomach (Erickson & Kim, 1990). In the stomach, pepsin, which is activated by hydrochloric acid, breaks down proteins into large polypeptides, smaller oligopeptides and some free amino acids (Erickson & Kim, 1990). The pancreas synthesises and releases proteolytic enzymes that are important in converting ingested protein into a mixture of small oligopeptides and free amino acids (Erickson & Kim, 1990). The final stage in the digestion of peptides is associated with the small intestine (Erickson & Kim, 1990). Within the small intestine, proteins and polypeptides are broken down by trypsin and chymotrypsin

(Widmaier, Raff & Strang, 2004). Cells of the small intestine contain a brush border membrane that has a number of peptidases (Erickson & Kim, 1990). The result of gastric, pancreatic and brush border membrane proteases is to reduce dietary protein to a mixture of free amino acids, dipeptides and tripeptides (Erickson & Kim, 1990), which are then available for transport around the body. Amino acids are transferred from the enterocyte to the blood by the basolateral membrane and this occurs primarily via facilitated and simple diffusion (Erickson & Kim, 1990). Amino acids are then actively transported around the body by carrier mediated transports systems coupled to sodium transport (Erickson & Kim, 1990). Amino acids are delivered to the liver were they can be either converted to glucose or fats, or can be directly released into the bloodstream as plasma protein albumin or as free amino acids.

Direct release of free amino acids into the bloodstream allows amino acid uptake at the muscle. The transport of amino acids into the muscle cell takes place via diffusion across the capillary wall into the interstitial fluid by secondary active transport and then amino acids gain entry into the muscle cell. This process of amino acid transport is governed by a variety of amino acid transporters (Miller *et al.*, 2004), and operates at rates directly proportional to amino acid concentrations inside and outside of the sarcoplasmic reticulum (Rennie & Tipton, 2000). Protein synthesis rates are directly affected by intracellular amino acid availability, therefore, the transporters have an important physiological role (Miller *et al.*, 2004). Transport through the interstitial fluid is an important process governing the rate of amino acid uptake by the muscle (Miller *et al.*, 2004). Amino acids can be transported out of the muscle cell also via secondary active transport. The size and composition of the muscle free amino acid pool will depend on the bodies nutritional state, plasma amino acid availability and hormonal milieu (Rennie & Tipton, 2000).

Muscle structure and regulation has been discussed. These processes are central to this thesis as following muscle damaging exercise the structure of the muscle is disrupted and changes in protein metabolism occur. The following section discusses the mechanisms underlying the disruption of the muscle with future sections discussing the impact on protein metabolism.

2.3   Mechanisms of Exercise Induced Muscle Damage

A vast amount of research has been conducted investigating the mechanisms underlying EIMD. Despite this, the findings remain inconclusive, and the exact sequence of events remains to be elucidated (MacKey *et al.*, 2008). Many researchers have reviewed the mechanisms of EIMD (Armstrong, 1990; Armstrong, Warren & Warren, 1991; Clarkson & Sayers, 1999; Lieber & Friden, 1999; Proske & Morgan, 2001). Armstrong (1990) proposed an integrated model of EIMD which included four stages: initial; autogenic; phagocytic; and regenerative. For the purposes of this review these stages are simplified into primary and secondary phases. The primary phase is characterised by direct damage to the muscle during the exercise bout with the secondary phase exacerbating damage via increased protein degradation or change in myofibrillar protein metabolism rate (Trappe *et al.*, 2002a). Figure 2.3 outlines the postulated processes and demonstrates their interaction as well as the effects they may have on the muscle. These processes are discussed in the following sections.
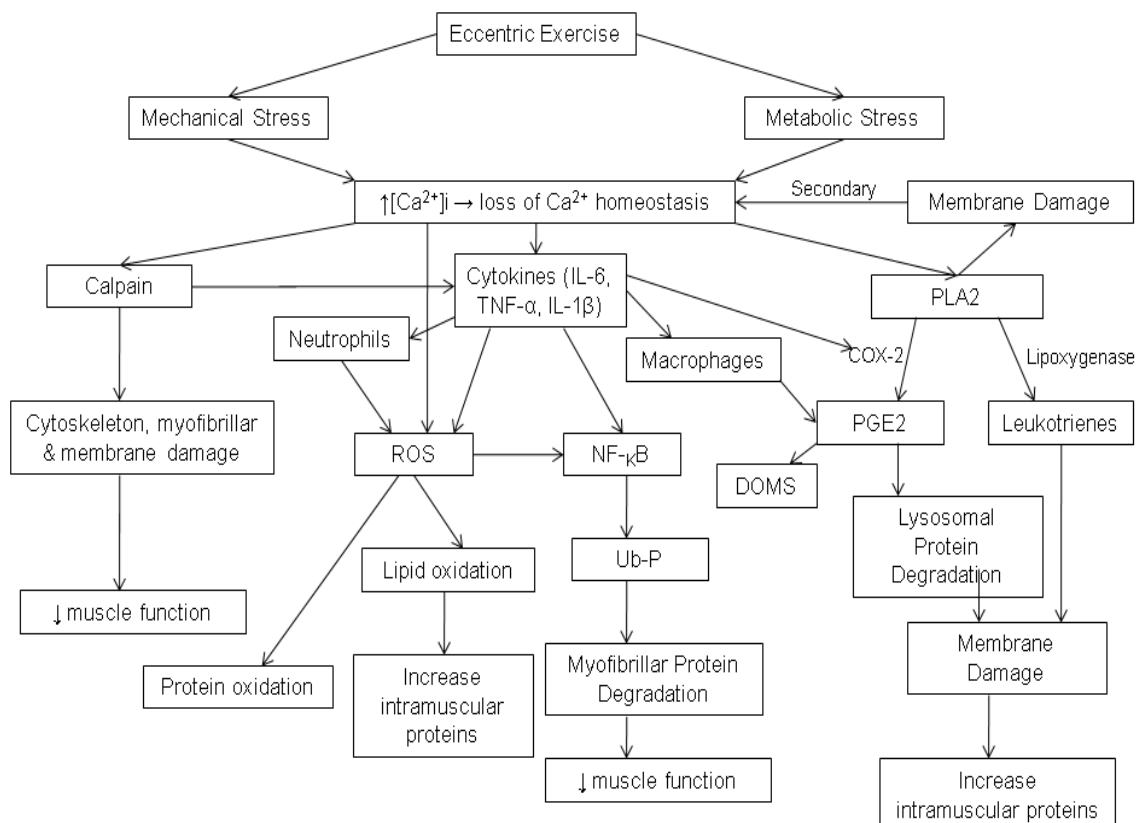
Figure 2.3 An overview of the postulated processes occurring during exercise-induced muscle damage.
COX-2 = cyclooxygenase-2; **DOMS** = delayed-onset of muscle soreness; **IL-6** = interleukin-6; **IL-1β** = interleukin-1beta; **NF-κB** = nuclear factor kappa-light-chain-enhancer of activated B cells; **PGE2** = prostaglandin E2; **PLA2** = phospholipase A2; **ROS** = reactive oxygen species; **TNF-α** = tumor necrosis factor-alpha; **Ub-P** = ubiquitin proteasome;

2.3.1 Primary Event

EIMD is common following muscle activity with a high degree of eccentric actions such as resistance exercise, plyometrics, downhill running and prolonged intermittent shuttle running (Eston *et al.*, 1996; Thompson, Nicholas & Williams, 1999; Byrne & Eston, 2002b; a; Twist & Eston, 2005; Nosaka *et al.*, 2006; Twist & Eston, 2009). Other forms of exercise without a high degree of eccentric muscle actions, such as endurance cycling, can also cause EIMD (Saunders, Kane & Todd, 2004; Saunders, Luden & Herrick, 2007; Valentine *et al.*, 2008). There are two main competing hypotheses for the primary event, which are summarised as mechanical stress or metabolic stress. This section will review literature for both proposals.

2.3.1.1 Mechanical Damage

Mechanical stress is the most widely accepted proposal (Tee, Bosch & Lambert, 2007), and relates to the damage caused by direct mechanical loading on the muscle fibre. This model focuses on the distinguishing aspects of eccentric muscle actions compared to concentric and isometric muscle actions (Armstrong, Warren & Warren, 1991).

During eccentric muscle actions excessive strain may be placed on the sarcomere due to the existence of sarcomere length inhomogeneties (Julian & Morgan, 1979). On the descending limb of the length-tension curve (Figure 2.2) some sarcomeres are unstable, and during an eccentric action some will undergo rapid lengthening becoming weaker until the point of no myofilament overlap (Armstrong, Warren & Warren, 1991; Proske & Allen, 2005). Beyond the point of no myofilament overlap, the passive structures compensate for falling active tension (Morgan & Proske, 2004) and undergo 'popping' (Morgan, 1990). This process may be progressive with more sarcomeres becoming overstretched during a number of eccentric muscle actions. Upon relaxation, overstretched sarcomeres may not be able to reinterdigitate and become disrupted (Talbot & Morgan, 1996). Disruption of the sarcomere may involve the non-contractile and intermediate filaments, and sufficient disruption may lead to sarcolemma or sarcoplasmic reticulum damage through tearing (Morgan, 1990). Damage to the sarcolemma or sarcoplasmic reticulum may lead to increases in intracellular Ca^{2+} concentration and as a consequence initiate a number of degradative pathways, which will be discussed in section 2.3.2. There is ultrastructural evidence of disrupted sarcomeres with other sarcomeres

remaining intact (Friden, Sjostrom & Ekblom, 1981; Newham *et al.*, 1983; Nurenberg *et al.*, 1992) distributed throughout the muscle (Talbot & Morgan, 1996). Newham *et al* (1983) compared muscle biopsy samples of the quadriceps following concentric and eccentric actions during bench stepping. They observed no abnormalities in samples from the concentrically exercised leg but observed many disrupted sarcomeres (disorganised myofilaments, Z-line streaming) immediately after eccentric exercise.

Eccentric muscle actions do not have to be maximal to elicit damage. Lieber & Friden (1993) found that contractile and morphological measures were altered with differences in magnitude of strain but not with large changes in force. Similarly, Talbot & Morgan (1998) demonstrated that the shift in optimum length (marker of EIMD) of toad muscle was not related to the magnitude of tension developed or velocity of lengthening. Other research is in agreement that the velocity of lengthening is not a major determinant of muscle damage (McCully & Faulkner, 1986; Talbot & Morgan, 1998). Talbot & Morgan (1998) attributed the shift in optimum length to the range of sarcomere lengths involved in muscle lengthening. Furthermore, other research provides evidence that a greater degree of damage may be induced by eccentric actions at long rather than short muscle lengths (Newham *et al.*, 1988; Child, Saxton & Donnelly, 1998). Based on the evidence, it would appear that the nature of eccentric actions induces sarcomere disruption due to excessive strain during lengthening on the descending limb of the length-tension curve leading to myofibrillar disruption.

Alternatively, rather than damage to the sarcomere occurring through mechanical stress, it has been suggested that the primary event is due to E-C coupling failure (Warren *et al.*, 2001). Evidence for this hypothesis comes from measures of intracellular Ca^{2+} (Warren *et al.*, 1993; Balnave & Allen, 1995; Ingalls *et al.*, 1998). Evidence of abnormal t-tubular arrangement after eccentric exercise may also provide the basis for this hypothesis (Takekura *et al.*, 2001). However, the evidence presented does not provide support for E-C coupling as the primary event. Firstly, it is difficult to envisage as it is hard to account for t-tubule disruption as the primary event, and why damage only occurs beyond optimum length (Proske & Morgan, 2001). Damage to the t-tubules and increases in intracellular Ca^{2+} could be secondary to sarcomere damage. Secondly, Proske and Allen (2005) stated that this theory cannot explain the shift in the length-tension relationship of the muscle

that is observed following eccentric muscle actions. The more likely explanation is that membrane damage occurs due to sarcomere disruption, which then leads to damage of the E-C coupling system (Proske & Morgan, 2001).

2.3.1.2 Metabolic Damage

The model of metabolic damage proposes that the primary event in the process of EIMD is caused by metabolic deficiencies in the working muscle (Tee, Bosch & Lambert, 2007). There are a number of theories within this model which have been extensively reviewed (Armstrong, Warren & Warren, 1991): insufficient mitochondria respiration, the production of free radicals and high temperatures. These processes are out of the scope of this thesis.

There is little experimental and scientific evidence in support of metabolic damage. This is mainly because eccentric muscle actions have a lower metabolic cost than both concentric (Asmussen, 1952; Bigland-Ritchie & Woods, 1976) and isometric muscle actions when working at the same absolute loads, yet concentric actions do not induce EIMD (Newham *et al.*, 1983). Offering further support against this theory was research by Armstrong, Ogilvie & Schwane (1983). They demonstrated an increased metabolic cost of uphill running compared to downhill running in rats; however, downhill running was associated with a higher incidence of damage. More, recently, Beltman *et al* (2004) demonstrated a lower metabolic energy cost per force time integral for electrically stimulated lengthening actions in rats, compared to electrically stimulated concentric and isometric actions. However research utilising endurance cycling, which is predominantly concentric in nature, has demonstrated increases in intramuscular proteins measured in the plasma (Saunders, Kane & Todd, 2004; Saunders, Luden & Herrick, 2007; Rowlands *et al.*, 2008; Valentine *et al.*, 2008). This is indicative of damage to the sarcolemma likely initiated by metabolic stress due to the low eccentric involvement. Therefore, it is unlikely that metabolic factors play a role in EIMD occurring from eccentric biased exercise, but it may underlie damage occurring from high intensity, long duration exercise of a concentric nature.

2.3.1.3 Preferential Damage of Fast Twitch Fibres

There is a primary event that may be mechanical or metabolic in nature and this initiates a process whereby the muscle fibre becomes damaged. There has been evidence that the damage to the muscle preferentially occurs in the fast twitch fibres,

specifically type IIB/X. A number of studies have demonstrated ultrastructural changes indicative of the fast contracting fibres being selectively damaged (Friden, Seger & Ekblom, 1981; Friden, Sjostrom & Ekblom, 1983; Lieber, Woodburn & Friden, 1991; Vijayan *et al.*, 2001). More recent observations demonstrate that the largest diameter fibres had the greatest intracellular $[Ca^{2+}]$, consistent with preferential damage to fast twitch fibres (Sanobe *et al.*, 2008).

Theories exist as to why fast twitch fibres may be more vulnerable to damage. Friden & Lieber (Friden & Lieber, 1992; 1998; Lieber & Friden, 1999) have mainly implicated the low oxidative capacity of fast twitch fibres that renders them vulnerable to damage. They hypothesise that there is a significant metabolic demand on fast twitch fibres early in an exercise bout so that they become stiff (Friden & Lieber, 1992). On subsequent eccentric actions these fibres become mechanically disrupted (Friden & Lieber, 1992). There are a number of other factors that may render fast twitch fibres more vulnerable: higher tensions generated (Appell, Soares & Duarte, 1992); and narrower Z-lines, which means there will be less thick and thin filament attachments and, therefore, weaker sarcomere connections (Friden, Sjostrom & Ekblom, 1983; Friden & Lieber, 1992). It is likely, however that selective damage of fast twtich fibres will only occur when those fibres are lengthened and stressed by the exercise bout.

In summary, there are a number of theories relating to the primary event and the exact mechanisms remain inconclusive, although there is stronger support for mechanical stress. However, it is unlikely that either type of stress to the muscle occurs in isolation since all forms of exercise will incorporate a degree of both components (Tee, Bosch & Lambert, 2007). The contribution of each will be dependent upon the exercise bout used to induce EIMD (Tee, Bosch & Lambert, 2007).

2.3.2 Secondary Event

Following the primary event there appears to be mechanisms occurring in the muscle that exacerbate the evidence of damage over time. The most commonly accepted event is the disturbance of Ca^{2+} homeostasis, which consequently initiates a number of proteolytic and lipolytic pathways. These pathways include the non-lysosomal protease calpain, phospholipases stimulating prostaglandins and leukotrienes, free radicals and the Ub-P pathway. This section will review these

processes.   A number of cytokines are also involved in signalling some of these events.

2.3.2.1 Disturbance of Ca^{2+} Homeostasis

The disturbance of Ca^{2+} homeostasis is viewed as the likely intermediate event between early mechanical processes and inflammation (Balnave & Allen, 1995). During muscle actions the intracellular Ca^{2+} concentration is high enough to stimulate damage (Armstrong, Warren & Warren, 1991).   However, as Ca^{2+} is rapidly bound to tropinin and other calcium binding proteins, or is removed by Ca^{2+} stimulated ATPase pumps of the sarcoplasmic reticulum during muscle relaxation (Byrd, 1992) this transient change may not be sufficient to cause damage (Gissel & Clausen, 2001).   Following eccentric exercise there may be prolonged increases in intracellular Ca^{2+} concentration at the site of the lesion (Armstrong, Warren & Warren, 1991) due to membrane (sarcoplasmic reticulum and/or sarcolemma) damage, failure of the sarcoplasmic reticulum to resequester Ca^{2+} and/or Ca^{2+} uptake via stretch activated channels.   An increase in intracellular Ca^{2+} concentration, from intra- or extra-cellular sources, may activate a number of degradative pathways. It is likely that the duration and magnitude of Ca^{2+} movement across key membranes is more important than the concentration of Ca^{2+} (Duncan, 1987).

Evidence implicating increased Ca^{2+} concentration in the damage process comes from a wide range of studies (Publicover, Duncan & Smith, 1978; Jackson, Jones & Edwards, 1984; Jones *et al.*, 1984; Duncan & Jackson, 1987; Duan *et al.*, 1990; Warren *et al.*, 1995; Lynch, Fary & Williams, 1997; Zhang *et al.*, 2008).   However, most are conducted using animals and this hypothesis has rarely been examined in humans. Overgaard and colleagues (Overgaard *et al.*, 2002; Overgaard *et al.*, 2004) have demonstrated an increase in cellular Ca^{2+} accumulation following long distance running. Beaton, Tarnapolsky & Phillips (2002a) reported reduced disruption of desmin and Z-band streaming with the use of a calcium channel blocker, implicating a role of calcium in the damage process. However, other studies have reported no change in total Ca^{2+} content (Fredsted, Clausen & Overgaard, 2008; Vissing *et al.*, 2008; Raastad *et al.*, 2010) following maximal voluntary eccentric muscle actions or eccentric bench stepping. However, it was stated that there may have been a change in cytostolic Ca^{2+}, which could not be measured (Vissing *et al.*, 2008; Raastad *et al.*, 2010). The difference in findings may be due to the duration of exercise (Fredsted, Clausen & Overgaard, 2008). During running a significant increase in Ca^{2+} was not

observed until 20 km (Overgaard *et al.*, 2004). It is unlikely that voluntary eccentric muscle actions or eccentric bench stepping would be of such duration.

The proposed theories regarding an increase in intracellular Ca^{2+} concentration include damage to the sarcoplasmic reticulum, failure of the sarcoplasmic reticulum to resequester Ca^{2+}, Ca^{2+} uptake via stretch activated channels and/or diffusion of extracellular Ca^{2+} through the damaged sarcolemma (Evans & Cannon, 1991; Clarkson & Sayers, 1999; Yeung & Allen, 2004; Allen, Whitehead & Yeung, 2005), as previously discussed. Damage to the sarcolemma would increase its permeability thus allowing the influx of extracellular Ca^{2+} (Armstrong, Warren & Warren, 1991; Allen, Whitehead & Yeung, 2005). Studies have provided evidence of membrane permeability soon after exercise in mice (Straub *et al.*, 1997; Hamer *et al.*, 2002), however, permeability was not changed via mechanical tears, and this may not be applicable to humans. Damage to the sarcoplasmic reticulum, specifically the membranes, may lead to Ca^{2+} leakage from the sarcoplasmic reticulum lumen (Yasuda *et al.*, 1997). Both types of damage may increase Ca^{2+} concentration in the compartments of degradative enzymes, which would allow Ca^{2+} to contact the binding sites (Armstrong, Warren & Warren, 1991). Another mechanism related to sarcoplasmic reticulum damage, for which there is evidence, may be that the sarcoplasmic reticulum fails to resequester Ca^{2+} (Duncan & Smith, 1978; Duncan, Greenaway & Smith, 1980), exposing fibres to intracellular Ca^{2+} for prolonged periods of time (Byrd, 1992). Duncan & Smith (1980) provided evidence demonstrating that the Ca^{2+} initiating muscle breakdown, originates in the sarcoplasmic reticulum. Mammalian muscle was exposed to caffeine, which stimulates Ca^{2+} release from the sarcoplasmic reticulum (Sorensen, Coelho & Reuben, 1986), and this was involved in the breakdown of the muscle. Finally, it is postulated that an increase in strain on the fibres would trigger stretch activated channels in the membrane allowing increased Ca^{2+} influx into the muscle cell (Lieber, Thornell & Friden, 1996). Studies that have used blockers of stretch activated channels in *mdx* mice in vitro (Yeung, Head & Allen, 2003; Yeung *et al.*, 2005) or made measures of membrane potential in rat muscles in vivo (McBride *et al.*, 2000) provide evidence for a role of stretch activated channels following eccentric exercise. More recently, research under in vivo conditions in rats, demonstrated reduced intracellular Ca^{2+} accumulation with stretch activated channel blockers, suggesting stretch activated channels are largely responsible for eccentric action induced intracellular Ca^{2+} concentration elevations (Sanobe *et al.*, 2008). However, McBride *et al* (2000) demonstrated that stretch

activated channels did not contribute to the force deficit observed.

This process is viewed as the intermediate event. However, a viscous cycle has been proposed (Gissel & Clausen, 2001; Allen, Whitehead & Yeung, 2005; Gissel, 2005). It is hypothesised that the first increase in Ca^{2+} concentration results from stretch activated channels, which then activates a number of degradative pathways including phospholipases and proteases (calpain), resulting in membrane and/or sarcoplasmic reticulum damage allowing further influx of Ca^{2+} (Gissel, 2000; Yeung & Allen, 2004; Gissel, 2005). Further influx of Ca^{2+} may lead to the accumulation of Ca^{2+} in the mitochondria (Gissel & Clausen, 2001), of which there has been some evidence (Duncan, 1987; Duan *et al*., 1990). Duan *et al* (1990) found a 3-fold increase in mitochondrial Ca^{2+} in rats exposed to 2 h of downhill walking, which further increased 2 days post. Accumulation of mitochondrial Ca^{2+} could contribute to further muscle damage via the activation of reactive oxygen species.

Following increases in intracellular and mitochondrial Ca^{2+}, a number of degradative pathways are activated (Figure 2.3). The activation of these pathways would lead to the degeneration of the muscle fibre possibly through an increase in protein turnover rate (Rodemann & Goldberg, 1982; Byrd, 1992). The following sections will outline these processes leading to damage of the muscle fibre and the evidence for their occurrence.

2.3.2.2 Calpain

Calpain is the next sequential pathway in the process of EIMD, stimulated by increased intracellular Ca^{2+}. Calpain is located at the I and Z regions of the skeletal muscle (Belcastro, Shewchuk & Raj, 1998), and there are three types in the muscle: the ubiquitous calpains 1 (μ-calpain) and 2 (m-calpain), and muscle specific calpain 3 (p94) (Gissel, 2005). Calpain activation would lead to the initial proteolytic dismantling of the sarcomere before major degradation by the Ub-P pathway (Goll *et al*., 2003). Therefore, the activation of calpain is likely necessary for the release of disrupted filaments so that complete degradation by the Ub-P system can occur (Raastad *et al*., 2010). The Ub-P system will be discussed in section 2.3.2.6.

Damage to the Z-disks is the most commonly observed ultrastructural damage in

human muscle (Friden, Sjostrom & Ekblom, 1981; Newham *et al.*, 1983; Nurenberg *et al.*, 1992; Fielding *et al.*, 1993; Crenshaw, Thornell & Friden, 1994; Gibala *et al.*, 1995; Gibala *et al.*, 2000). Calpain has been implicated in this damage (Belcastro, 1993; Verburg *et al.*, 2005; Zhang *et al.*, 2008; Salazar, Michele & Brooks, 2010); however, this evidence comes from animal muscle. Specifically, Salazar, Michele & Brooks (2010) demonstrated maintenance of force production and sarcomere ultrastructure when calpain was inhibited (via over-expression of calpastatin) in mice exposed to hindlimb suspension. Recently studies have measured calpain activity after eccentric exercise in humans. Raastad *et al* (2010) demonstrated a significant increase in total calpain activity in the exercised leg, relative to the control leg, 30 min after isokinetic eccentric exercise. Total calpain activity in the exercised leg remained increased for 7 days (Raastad *et al.*, 2010). Other studies have observed an increase in activated calpain-3 24 h following isokinetic eccentric exercise (Murphy *et al.*, 2007), and an increase in mRNA levels of calpain 2 following eccentric stepping exercise (Vissing *et al.*, 2008). The difference in the time responses observed may be due to the measurement of total calpain rather than specific calpains, and the measurement technique (western blotting and colorimetric assay) used. Although the studies exhibit different time responses, they do suggest an involvement of calpain in EIMD. However, the lack of correlations between it and the degree of fibre disruption make it difficult to draw definitive conclusions on the importance of this protease for the observed ultrastructural damage in humans (Raastad *et al.*, 2010).

If calpain is involved in sarcomere damage, specifically the Z-disk, then the most common proposed mechanism for this involves the cleaving of desmin. However, again this evidence comes from animal studies. Loss of desmin staining has been observed in animal muscle (Lieber *et al.*, 1994; Lieber, Thornell & Friden, 1996; Friden & Lieber, 1998; 2001; Zhang *et al.*, 2008). Desmin attaches adjacent myofibrils at Z-disks (Patel & Lieber, 1997) and so loss of desmin would render the Z-disks vulnerable to damage. Studies of human muscle have shown that there is no loss of desmin staining although the desmin cytoskeleton was altered (Yu, Malm & Thornell, 2002; Yu & Thornell, 2002). This was proposed to be related to an increased synthesis of desmin and remodelling of myofibrils rather than degeneration of the desmin cytoskeleton (Yu & Thornell, 2001; 2002; Yu, Furst & Thornell, 2003). It was proposed by Yu and colleagues that the sequence of events involved in muscle damage in animal models is not representative of occurrence's in humans, and this may possibly contribute to the differences

observed. However, the protocol used in these studies did not include maximal or near maximal eccentric actions over a large range of movement (Lauritzen *et al.*, 2009), which may have impacted on the observations that damage, per se, did not occur. Therefore, the vast amount of literature conducted on animals should still be accounted for in attempting to understand the human response to novel eccentric exercise (Lauritzen *et al.*, 2009).

The loss of desmin may not be implicated in ultrastructural damage observed following eccentric exercise in humans. Titin is a known substrate of calpain (Goll *et al.*, 2008) and cleavage of it would lead to sarcomere disruption. Yu, Furst & Thornell (2003) observed a lack of staining for α-actinin, titin and nebulin in muscle biopsies taken 2 - 3 and 7 - 8 days post eccentric exercise in humans. Similarly, Trappe *et al* (2002a) found decreased titin and nebulin contents of 30% and 15%, respectively, 24h following eccentric exercise. Although these studies provide evidence of ultrastructural damage it is unknown whether this loss is due to direct damage, degradation or changes in myofibrillar protein rate (Trappe *et al.*, 2002a).

Calpain may not only be implicated in damage to the sarcomere directly but it may also be involved in inducing further degradation of the muscle. Calpain may act as a chemotactic signal that attracts neutrophils, via cytokines, to the site of damage immediately following exercise (Belcastro, Shewchuk & Raj, 1998; Raj, Booker & Belcastro, 1998). Therefore, calpain may initially be activated, subsequently leading to further muscle protein degradation via activation of different degradative pathways, initiated by cytokines.

2.3.2.3 Cytokines

Cytokines may be activated by calpain, as previously discussed, or by increases in Ca^{2+} concentration, the perturbation of the muscle fibre or sensing of damage by resident macrophages (Butterfield, Best & Merrick, 2006). The main cytokines thought to be involved in EIMD are tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), with the early release of TNF-α and IL-1β stimulating the inflammatory response and the production of IL-6 (Miles *et al.*, 2008). TNF-α and IL-1β are thought to represent the most pro-inflammatory combination of all cytokines (Pyne, 1994). It is thought that IL-6 is the main systemic mediate of the acute phase response following exercise (Pyne, 1994).

All three of these cytokines have been shown to significantly increase 48 h following downhill running in rats (Davis *et al.*, 2007). Similarly, Liao *et al* (2010) observed significant increases in TNF-α mRNA, protein content and serum concentration 6 and 24 h following 2 h of downhill running in rats. In humans, in vitro TNF-α and IL-1β have been shown to significantly increase 24 h following downhill running, with no significant increase in IL-6 although it was increased above baseline (Cannon *et al.*, 1991). Buford *et al* (2009) found no significant up-regulation of TNF-α and IL-1β following downhill running. Differences in these studies may be due to the measurement of mRNA content in Buford *et al* (2009) study compared to protein content; the intensity of downhill running with participants in Cannon *et al* (1991) study running at a greater intensity; and the use of in vitro versus plasma analysis. In humans, IL-6 has been shown to significantly increase in the hours (Rohde *et al.*, 1997; Dousset *et al.*, 2007; Miles *et al.*, 2008; Buford *et al.*, 2009), 24 h (Buford *et al.*, 2009) and 48 h (Philippou *et al.*, 2009) post. Hirose *et al* (2004) utilised eccentric actions of the elbow flexors and found a significant decrease in TNF-α but no change in IL-1 β. Different findings may be attributed to the mode of exercise.

The up-regulation of both TNF-α and IL-1 β may be implicated in the breakdown of the muscle proteins. Muscle protein wasting was reduced when animals were treated with a TNF-α antibody (Costelli *et al.*, 1993), implicating TNF-α as a mediator of proteolytic pathways. TNF-α has also been shown to inhibit basal protein synthesis and the ability of insulin-like growth factor-I to stimulate protein synthesis in vitro (Frost, Lang & Gelato, 1997). Frost, Lang & Gelato (1997) also found that TNF-α acts rapidly with impaired protein synthesis for 48 h after transient exposure. It may also increase protein degradation in vivo and in vitro (Andreu & Schwartz, 1995). Similarly, IL-1β may increase protein turnover by inhibiting the secretion of insulin-like growth factor-I.

IL-6 can act as both a pro and anti-inflammatory cytokine. Its up-regulation may result in the loss of myofibrillar protein by suppressing the effects of IGF (Caiozzo *et al.*, 1996) and the production of C-reactive protein (CRP) (Peterson & Pederson, 2005). CRP has been shown to significantly increase 48 h post eccentric exercise (Dousset *et al.*, 2007) and this has been associated with increases in IL-6 (Miles *et al.*, 2008). IL-6 has also been associated with TNF-α production in humans (Starkie *et al.*, 2003), which potentially creates a viscous cycle (Liao *et al.*, 2010). However, branched chain amino acid supplementation has been shown to limit muscle proteolysis, which was independent of the cytokine response demonstrating that IL-6 does not play a role in

protein degradation during EIMD in humans (Rohde *et al.*, 1997).

Cytokines regulate the phagocytic phase which is the process of acute inflammation, characterised by the infiltration of neutrophils and macrophages. Following muscle damaging exercise increases in circulating neutrophils have been found between 2h and 12h (Pizza *et al.*, 1995; Malm, Lenkei & Sjodin, 1999; MacIntyre *et al.*, 2001), 24 h (McLoughlin *et al.*, 2003; Tsivitse *et al.*, 2003; Milias *et al.*, 2005), 48 h (Milias *et al.*, 2005) and at 3 days (Pizza *et al.*, 2002) post exercise. The majority of studies showing an early response (2 - 12 h) are studies using humans whereas those with a delayed response are those conducted with rats or mice. Results from animals may not apply to voluntary exercise in humans (Malm, 2001). This may impact on results as firstly voluntary muscle actions can be highly variable in inexperienced participants (Brown *et al.*, 1997). Secondly, exercise in animals is not voluntary exertion. Studies using electrical stimulation can result in higher force production causing changes in muscle that would not happen with voluntary exercise (Malm, 2001). However, Rohde *et al* (1997) found no change in circulating neutrophils following prolonged eccentric actions of the quadriceps. Similarly, Lapointe, Frenette & Cote (2002) found no evidence of neutrophils in the muscle. It appears that the role of neutrophils in exercise and eccentric muscle actions is not clear (St. Pierre Schneider & Tiidus, 2007).

Following neutrophil invasion it is thought that the invasion of macrophages is the next phase in the inflammatory response. Specifically, ED1$^+$ macrophages may be responsible for further damage in the muscle. These are the first macrophages to accumulate in skeletal muscle following damage induced by hindlimb suspension reloading (Tidball, Berchenko & Frenette, 1999; Frenette *et al.*, 2002). These macrophages appear as early as 1 day after neutrophil invasion (McLennan, 1996), with their highest activity observed between 24 and 72 h (Marsolais, Cote & Frenette, 2001; Pizza *et al.*, 2002; McLoughlin *et al.*, 2003; Tsivitse *et al.*, 2003). However, St. Pierre Schneider, Find & Tiidus (2005) found no evidence of macrophage infiltration 1 day following hindlimb reloading in mice. These studies have been conducted using animals and induced damage in different ways including electrical stimulation and hindlimb unloading. Therefore, it is unknown whether these same changes occur in humans following voluntary eccentric exercise.

In summary, the cytokines, specifically TNF-α may initiate a number of degradative pathways. Its proteolytic function may be attributed to reactive oxygen species generation (Li *et al.*, 1999), which can activate the NF-κB pathway (Liao *et al.*, 2010). TNF-α may also play a role in initiating the cyclooxygenase-2 (COX-2) pathway. Cytokines may also regulate the infiltration of neutrophils and macrophages. Neutrophils can induce a respiratory or oxidative burst, and secrete proteolytic enzymes (Rossi, 1986; Smith *et al.*, 2008). Macrophages may activate prostaglandin E_2 (PGE_2) (Scott *et al.*, 2004). The activation of reactive oxygen species, NF-κB, COX-2 and PGE_2 can lead to further damage in the muscle. These processes will be discussed in the following sections.

2.3.2.4 Reactive Oxygen Species

Reactive oxygen species produced during the respiratory burst may be activated by increases in intracellular Ca^{2+} concentration, TNF-α and/or neutrophils. The respiratory burst involves an increase in non-mitochondrial oxidative metabolism which results in the production of superoxide anion (O_2^-) and associated reactive oxygen species (Halliwell & Gutteridge, 1986). This respiratory burst has been shown to occur 3 days post eccentric muscle actions directly using mouse models (Zerba, Komorowski & Faulkner, 1990; McArdle *et al.*, 1999). Specifically, superoxide anion has been shown to be generated during 2 h of downhill running in rats (Liao *et al.*, 2010). Superoxide is converted to hydrogen peroxide, which can peroxidise lipids and damage cell membranes (Hampton, Kettle & Winterbourn, 1998). Liao *et al* (2010) also demonstrated an increase in hydrogen peroxide 24 h post downhill running compared to rest. Hydrogen peroxide can also form hypochloride acid, which can damage cell membranes (Tidball, 2005). It is, therefore, feasible that reactive oxygen species may cause lipid oxidation and exacerbate damage in the days following eccentric exercise (Close *et al.*, 2005b). Serum thiobarbitoric acid (TBARS) concentration, which is a marker of lipid oxidation, was increased immediately, 6 and 24 h post exercise in rats following 2 h of downhill running (Liao *et al.*, 2010), which may have been linked to the activation of reactive oxygen species. In humans, there is evidence of lipid oxidation. Following muscle damaging exercise, serum malondialdehyde (MDA) is increased immediately (Sacheck *et al.*, 2003), 48 h (Goldfarb, Bloomer & McKenzie, 2005), 72 h (Close *et al.*, 2004; Close *et al.*, 2005a; Close *et al.*, 2006) and 96 h (Close *et al.*, 2005a; Close *et al.*, 2006) post. Furthermore, $F_{2\alpha}$-isoprostanes are increased up to 72 h following muscle damaging exercise (Sacheck *et al.*, 2003), and serum TBARS are significantly

increased from 48 to 96 h (Nikolaidis *et al.*, 2007).

Proteins can also be oxidised, which is indicated by the presence of carbonyl groups. Following isokinetic lengthening muscle actions, plasma protein carbonyls are increased from 48 to 96 h post in females (Nikolaidis *et al.*, 2007), and at 24 and 48 h in males (Goldfarb, Bloomer & McKenzie, 2005). Differences in time lines may be attributed to gender or the muscle group used during the damaging protocol. It is difficult to identify which proteins are being oxidised as different proteins may differ greatly in their susceptibility to oxidative damage (Nikolaidis *et al.*, 2008).

In summary, the activation of reactive oxygen species may lead to lipid and protein oxidation, causing further damage to the muscle. They may also activate the signalling pathway, NF-κB, which may up-regulate the Ub-P pathway.

2.3.2.5 Phospholipases

The activation of phospholipases (PLA), specifically, PLA2 can be stimulated by increased intracellular Ca^{2+} concentration (Chang, Musser & McGregor, 1987; Duncan, 1988), as it contains a Ca^{2+} binding site (Armstrong, 1990). PLA2 can use membrane phospholipids as substrates for the production of arachidonic acid (Trappe *et al.*, 2001) and subsequent to this production of prostaglandins and leukotrienes (Duncan, 1988; Armstrong, Warren & Warren, 1991). The activation of PLA2 may lead to the degradation of membrane phospholipids (Jackson, Jones & Edwards, 1984), which may lead to increased permeability of the sarcolemma (Gissel & Clausen, 2001). This may allow the efflux of intracellular proteins and allow further Ca^{2+} to enter the cell (Armstrong, 1990) giving rise to more degradation, leading to a secondary increase in Ca^{2+} that may accumulate in the mitochondria. Duncan & Jackson (1987) demonstrated, in vitro, that PLA2 inhibiting agents protected against intracellular enzyme efflux. Ultrastructural damage was still present indicating different degradative pathways play a role in membrane and myofibrillar damage. The production of prostaglandins and leukotrienes in the context of EIMD will be discussed.

Prostaglandins

Prostaglandins can be generated from PLA2, as previously stated. Prostaglandins play a central role in inflammation. COX-2 catalyses the reaction of arachidonic acid to

PGH_2, which is subsequently converted to a variety of prostaglandins (Dubois *et al.*, 1998). COX-2 can be induced by inflammatory factors and cytokines. Specifically, TNF-α has been shown to induce COX-2 expression (Dubois *et al.*, 1998). Following eccentric exercise, COX-2 mRNA expression was significantly increased 4 and 24 h post, with no change in COX-2 protein (Weinheimer *et al.*, 2007). Although there was no change in protein content of COX-2, it was concluded that it was still available for the production of prostaglandins. Similarly, Buford *et al* (2009) found a significant up-regulation of COX-2 mRNA 3 h following downhill running. The formation of prostaglandins via COX-2 activity may be both detrimental (Buford *et al.*, 2009) and vital (Bondesen *et al.*, 2004) for muscle structures.

The two prostaglandins researched in the context of EIMD have been $PGF_{2\alpha}$ and PGE_2, which are involved in protein synthesis and degradation, respectively (Rodemann & Goldberg, 1982). $PGF_{2\alpha}$ has been shown to significantly increase 24 h following eccentric resistance exercise (Trappe *et al.*, 2001). The role of PGE_2 has attracted more attention due to its potential involvement in the breakdown of the muscle. In humans, PGE_2 has been shown to significantly increase 2 h post sledge drop jumping exercise (Dousset *et al.*, 2007), and 24 and 48 h following downhill running (Cannon *et al.*, 1991). The fact that these studies did not utilise a concentric control group may limit findings. Peake *et al* (2005) found that downhill running significantly increased PGE_2 immediately post but that this was no different to a high-intensity level running group. However, measures were not taken past 1 h in this study and differences may have been apparent at later time points. Increases in PGE_2 may increase protein turnover (Evans & Cannon, 1991). Rodemann & Goldberg (1982) observed increased PGE_2 synthesis and in turn increased protein degradation rates when rat muscles were incubated in vitro with arachidonic acid.

It is thought that increased protein degradation may be via lysosomal proteases, however, Furano & Goldberg (1986) demonstrated that the effect of Ca^{2+} on muscle protein does not involve a lysosomal enzyme. Other studies have found no significant changes in PGE_2 up to 96 h post eccentric exercise (Croisier *et al.*, 1996; Trappe *et al.*, 2001). Differences in these studies may be attributed to the mode of exercise with these studies utilising local eccentric exercise. Therefore, PGE_2 increase may be in response to whole body exercise. However, Trappe *et al* (2001) did induce a 60 % increase in PGE_2, and Croisier *et al* (1996) measured plasma levels of PGE_2 in blood samples from a forearm vein which may not have reflected local

production at the active muscle.

In summary, there is a potential role of PGE_2 in the breakdown of the muscle during EIMD, but its role may be dependent on the mode of exercise. Its increased activation may lead to lysosomal degradation, which would lead to non-myofibrillar protein breakdown and damage to the sarcolemma.

Leukotrienes

Leukotrienes are also derived from PLA_2, and the key enzyme regulating this process is lipoxygenase (Armstrong, 1990). The activation of leukotrienes may also lead to damage of the sarcolemma, increasing its permeability. There has been little research conducted on leukotriene activation following muscle damaging exercise. Peake *et al* (2005) demonstrated that following downhill running there was no change in leukotriene B_4 concentration. However, measures were only made immediately and 1 h post exercise. Changes may have been observed if it was measured at future time points. Leukotrienes can also act as chemoattractants to amplify phagocyte numbers at cells (Pyne, 1994), indicating a role in the stimulation of the inflammatory response (Peake *et al.*, 2005).

2.3.2.6 Ubiquitin-proteosome Pathway

The signal for initiating the ubiquitin-proteosome (Ub-P) system following eccentric exercise is unknown but cytokines have attracted a lot of attention, specifically TNF-α (Murton, Constantin & Greenhaff, 2008). TNF-α has been shown to activate NF-κB pathway in a variety of cells, including muscle cells (Ghosh & May, 1998), which in turn activates the Ub-P system (Jackman & Kandarian, 2004). NF-κB can also be stimulated by increased intracellular Ca^{2+} concentration (Hughes, Antonsson & Grundstrom, 1998) and reactive oxygen species accumulation (Muller, Rupec & Baeuerle, 1997). NF-κB is a major regulator of gene transcription and metabolism in response to oxidative, energetic and mechanical stress in skeletal muscle (Kramer & Goodyear, 2007), and it up-regulates expression of genes for ubiquitin (Reid, 2005). Research has demonstrated an increase in NF-κB activation (Garcia-Lopez *et al.*, 2007; Liao *et al.*, 2010). However, Buford *et al* (2009) found no statistically significant changes in the transcription of NF-κB 24 h following downhill running. However, it was not measured at 48 and 72 h post exercise, and it could have potentially been significantly increased at these time points as there was a marked increase at 24 h

(Buford *et al.*, 2009). There is evidence for increased NF-κB activation during EIMD. If unrestrained then this increased signalling may perpetuate insulin resistance and protein catabolism (Wellen & Hotamisligil, 2005; Shoelson, Lee & Goldfine, 2006).

Further support for TNF-α and reactive oxygen species stimulating the Ub-P pathway comes from research demonstrating that both hydrogen peroxide and TNF-α induced the expression of MAFbx/atrogin-1 and MuRF1 (Li *et al.*, 2003; Li *et al.*, 2005). These ubiquitin-ligases are required for ubiquitin proteolysis, and this research demonstrates that they are sensitive to inflammatory mediators and reactive oxygen species.

The pathway for stimulating the Ub-P system is activated during EIMD. There is also evidence of Ub-P activation. A number of studies in humans have demonstrated an increase in mRNA and protein expression of free ubiquitin, components of the 20S protesome and ubiquitin conjugated protein levels in skeletal muscle between 24 and 48 h following eccentric exercise (Thompson & Scordilis, 1994; Stupka *et al.*, 2001; Willoughby, Rosene & Myers, 2003; Willoughby, Taylor & Taylor, 2003), which may be indicative of Ub-P activation. The activation of the Ub-P system will degrade myofibrillar proteins in skeletal muscle (Jagoe & Goldberg, 2001). In Willoughby's studies (Willoughby, Rosene & Myers, 2003; Willoughby, Taylor & Taylor, 2003) when increases in mRNA and protein levels of ubiquitin and 20S proteasome were observed at 24 – 48 h, myofibrillar protein content was significant less than a control group, which may have been due to ubiquitin-mediated proteolysis. This system is unable to degrade intact myofibrils (Solomon & Goldberg, 1996), suggesting that an alternative system is responsible for initial myofibril disruption (Murton, Constantin & Greenhaff, 2008). The suggested process is the activation of calpain, which will degrade and release proteins from myofibrillar structures, rendering them more vulnerable to damage. Degradation is then completed by the Ub-P system.

Summarising the process of EIMD, there is strong evidence that increases in intracellular Ca^{2+} concentration provide an intermediate event that stimulates a number of other degradative pathways, either directly or indirectly (Figure 2.3). Calpain may lead to early disruption of the muscle fibre, which is then further

degraded via other processes. Cytokines, especially TNF-α appear to play a significant role in stimulating reactive oxygen species, which may then initiate the NF-κB pathway that up-regulates the Ub-P system leading to myofibrillar degradation. TNF-α may also stimulate COX-2, which is the key regulating enzyme of prostaglandin production. Prostaglandin and leukotriene generation may also be due to the activation of PLA$_2$ directly stimulated by increased intracellular Ca^{2+} concentration. These pathways may lead to sarcolemma damage, which may further initiate influx of extracellular Ca^{2+}. Although muscle damage can be divided into separate processes, it is clear that they overlap and the exact mechanisms responsible and processes involved are not fully understood (Kendall & Eston, 2002). It is difficult to fully understand the processes as studies in the area of EIMD differ greatly in their methodological approaches. For example, the mode of exercise, muscle(s) used, timing of measurement, age, training status of participants, and the use of animals or humans.

2.4   Markers of EIMD

The process of EIMD involves a number of pathways with research suggesting it is bi-phasic. The primary event may cause direct damage with the secondary phase exacerbating it via increased protein degradation or change in myofibrillar protein metabolism rate (Trappe *et al.*, 2002a). The extent to which each is involved in the process remains to be determined. What is agreed upon is the structural damage observed in the muscle. Direct measures of muscle damage can be expensive and difficult to measure, therefore, many researchers use indirect markers of EIMD.

2.4.1 Direct Markers

To measure damage to the muscle structures, electron and/or light microscopy and histological techniques provide the only direct measures of changes. Histochemical abnormality has been shown to occur early post exercise (12 h) with peak damage at 48 h (Yasuda *et al.*, 1997); evidence of processes exacerbating the damage. Through these methods, two main signs of EIMD have been observed; presence of disrupted sarcomeres and damage to the E-C coupling system (Proske & Morgan, 2001). Recently, Lauritzen *et al* (2009) demonstrated in humans, that following eccentric exercise of the elbow flexors 85 %, 65 % and 38 % of fibres contained focal, moderate and extreme disruptions, respectively.

Sarcomere disruption is observed in humans following bench stepping exercise immediately post eccentric exercise, with the damage focal and only involving one or two adjacent sarcomeres and myofibrils (Newham *et al.*, 1983). This study demonstrated development of sarcomere disruption to involve more sarcomeres and widespread areas in the 24 - 48 h following EIMD (Newham *et al.*, 1983). One of the main signs of sarcomere disruption involves damage to the Z-disk. A study utilising voluntary eccentric actions of the elbow flexors demonstrated that the exercised arm contained significantly more Z-disks that could be classified as disrupted or destroyed (Lauritzen *et al.*, 2009). Similarly, Hansen *et al* (2009) observed significant increases in disrupted Z-lines 5 and 24 h following maximal isokinetic eccentric actions, and small but significant increases in destroyed Z-lines at 24 h. Damage to the Z-disk may initially involve Z-disk streaming (Friden & Lieber, 2001) but progresses to the extension of the Z-band into the A-band and the loss of Z-disk material (Friden, Sjostrom & Ekblom, 1983; Newham *et al.*, 1983; Nurenberg *et al.*, 1992; Friden & Lieber, 1996; 1998; Beaton, Tarnapolsky & Phillips, 2002b; Hansen *et al.*, 2009). The most common change at the level of the Z-disk is the loss of desmin. Desmin loss has been shown to occur rapidly (within 5 - 15 min) following eccentric exercise (Lieber, Thornell & Friden, 1996), which is the earliest documented change. The loss of desmin is also observed 1 - 3 days post EIMD (Friden & Lieber, 1998), indicative of damage occurring and/or progressing over a number of days following the primary event. Sarcomere disruption is also evident as disorganisation of the thick and thin filaments, loss of myofibrillar band registry (A-band distorted/sliding) and disturbances of the regular titin lattice (Friden, Sjostrom & Ekblom, 1983; Newham *et al.*, 1983; Friden & Lieber, 1996; 1998). Titin and nebulin have been shown to be significantly reduced 24 h following eccentric exercise (Trappe *et al.*, 2002a), indicative of myofibrillar damage. In this study it was suggested that the decrease of these proteins may have been via direct damage (primary event), degradation following the exercise (secondary event) or decrease in synthesis relative to other muscle proteins (Trappe *et al.*, 2002a). These different studies have utilised animals, electrical stimulation and voluntary muscle actions in humans, providing evidence that muscle damage occurs following a range of modes.

Damage to the E-C coupling system is evident as structural changes involving the t-tubules, abnormal membrane systems involved in the process (Takekura *et al.*, 2001), and rounded sarcoplasmic reticulum and t-tubules (Friden & Lieber, 1996). Similarly to sarcomere disruption, the amount of damage to the t-tubules

increased in the 2 - 3 days following eccentric muscle actions (Takekura *et al.*, 2001). These studies have both involved the use of animals and this damage may not be evident in humans. However, similar sarcomere damage is observed in human and animal studies and, therefore, this damage may be evident in humans following muscle damaging exercise.

It is clear that there are early signs of damage to the muscle structures, which are likely due to the primary and some of the secondary phase. A common observation is that the damage to the muscle structures progresses over a number of days. This is indicative of a secondary phase during EIMD that further degrades the muscle following the primary event.

2.4.2 Indirect Markers

Indirect markers of EIMD provide researchers with the ability to draw conclusions about damage to the muscle. The main markers used are measures of intramuscular proteins in serum, muscle soreness and muscle function. This section will review literature on each of these three measures.

2.4.2.1 Intramuscular Proteins in Serum

There is a number of blood serum markers used to identify muscle damage. The most common muscle proteins used as an indication of EIMD are the cytosolic enzymes; creatine kinase (CK), myoglobin (Mb) and lactate dehydrogenase (LDH).

CK is found in skeletal muscle but also in the brain, mitochondria and cardiac muscle. It is the most commonly measured intramuscular protein in serum to examine damage. CK does not typically leak out of undamaged cells (Lee *et al.*, 2002), therefore an increase is primarily interpreted as an increased permeability or breakdown of the muscle cell membrane (Friden & Lieber, 2001). Further to this, it is often assumed to indicate the magnitude of damage (Friden & Lieber, 2001). However, concentrations of CK also reflect clearance by the reticuloendothelial system (Clarkson, Nosaka & Braun, 1992), therefore, its use as a measure of magnitude should be used cautiously.

Increases in CK following exercise with a high component of eccentric muscle

actions are common but the response is variable, which may partly depend on the mode of exercise and muscle group damaged. Following downhill running (Sorichter *et al.*, 2001), squats (Garcia-Lopez *et al.*, 2006; Davies, Rowlands & Eston, 2009), and eccentric actions of the elbow extensors (Shahbazpour *et al.*, 2004) and knee extensors (Eston *et al.*, 1996) CK levels are low (<1000 IU). Following vertical jumps (drop and countermovement) CK levels are varied between 332 IU and 1400 IU (Harrison & Gaffney, 2004; Skurvydas *et al.*, 2006a; Marcora & Bosio, 2007). Higher CK levels are observed following eccentric actions of elbow flexors and have been shown to be anything from 1600 IU (Lee *et al.*, 2002) to 4000 IU (Sayers, Clarkson & Lee, 2000b). Protocols using electrical stimulation induce higher responses with Brown *et al* (1996) observing peaks of 12540 IU. These peaks are shown to occur between 24 and 72 h. CK also has high intersubject variability in comparison to other enzymes and Mb (Nosaka & Clarkson, 1996). Explanations for this high variability include the concept of individuals being high or low responders, which may be related to training level, muscle size, fibre type and/or genetics but this requires further investigation (Brancaccio, Maffuli & Limongelli, 2007). Activity level following muscle damaging exercise has also been shown to play a role in creatine kinase variability (Sayers, Clarkson & Lee, 2000a). This study compared CK levels in a group which had their eccentrically exercised arm immobilised, a control group and a light exercise group (50 bicep curls daily) for 4 days post. The immobilisation group had significantly lower CK levels than the other two groups, with no significant differences observed between the control and light exercise group. Therefore, the use of CK to compare between studies and individuals should be used with caution as a number of factors appear to affect its variability.

Mb increases following eccentric exercise are common. Studies have demonstrated peaks of Mb at 48 h (Lowe *et al.*, 1995; Childs *et al.*, 2001), 72 h (Childs *et al.*, 2001) and 96 h (Childs *et al.*, 2001; Lee & Clarkson, 2003). Assays used to determine Mb concentrations cannot, however, determine between Mb release from the heart or skeletal muscle (Sorichter, Puschendorf & Mair, 1999), and therefore researchers must use it with caution.

LDH is another intramuscular protein measured in the serum, however, it is not as commonly used in muscle damage studies as creatine kinase and myoglobin. LDH in serum increases in the 6 – 12 h following muscle damaging exercise with a return to baseline within 8 – 14 days due to its slow catabolization (Sorichter, Puschendorf &

Mair, 1999). LDH is suggested to be a sign that the muscle cell is no longer temporally damaged but irreversibly (Gissel & Clausen, 2001).

The increases in intramuscular proteins observed may be due to a number of processes that disrupt the sarcolemma and increase its permeability: TNF-α, reactive oxygen species, COX-2, PGE_2 and leukotrienes have all been implicated as discussed in section 2.3. Reactive oxygen species can increase lipid oxidation (Close *et al.*, 2005b). Sacheck *et al* (2003) demonstrated correlations between a marker of lipid oxidation (iPF_{2a}) at 72 h and CK values at both 24 and 72 h (R^2 = 0.44 and 0.51). This may provide evidence that changes in lipid oxidation leads to increases in CK measured in the plasma, although there is a large amount of unexplained variance. Reactive oxygen species production may be due to cytokines, especially TNF-α (Li *et al.*, 1999). Cytokines may also lead to the production of COX-2 (Dubois *et al.*, 1998), which is the key enzyme regulating prostaglandin production. However, cytokines may not be an underlying mechanism in the increase in intramuscular proteins measured in the plasma. Both Miles *et al* (2008) and Buford *et al* (2009) observed no association between cytokines (IL-6, IL-1β, TNF-α) and CK. Buford *et al* (2009) also found no relationship between COX-2 and CK, which may imply that changes in CK cannot be attributed to cytokines and increased prostaglandin production. Early studies did demonstrate that inhibiting PLA_2 protected against intracellular enzyme efflux (Duncan & Jackson, 1987). More recently, Milias *et al* (2005) demonstrated a positive correlation between platelet activating factor at 96 h, and CK and LDH at 48 and 72 h. Platelet activating factor can be synthesised from PLA_2 activation (Milias *et al.*, 2005). This may provide further evidence for a role of PLA_2 in increases in intramuscular proteins in the plasma, however, PLA_2 was not measured in this study. Therefore, reactive oxygen species and/or PLA_2, from increases in intracellular Ca^{2+} concentration, may be the underlying mechanism(s) contributing to the release of intramuscular proteins.

Although these proteins can be measured as an indirect marker of EIMD, many researchers only analyse one. Measures of intramuscular proteins in the serum must be analysed in parallel with other indirect markers to provide a complete overview of EIMD as there is little relationship between CK and maximal isometric force (Nosaka & Clarkson, 1996). There is also a lack of correlation between CK and direct markers of EIMD (Fielding *et al.*, 1993; Malm *et al.*, 2000; Beaton, Tarnapolsky & Phillips, 2002b). However, it should be noted that a lack of correlation may be due to other

factors: CK data is obtained from venous blood were as muscle histology such as Z-band streaming is assessed by muscle biopsies and there is high interindividual variability of CK, both of which may reduce correlations (Saunders, 2005).

2.4.2.2 Delayed-Onset of Muscle Soreness

Following exercise involving eccentric muscle actions, muscle soreness is evident. Muscle soreness is evident as a dull aching pain (Armstrong, 1984) combined with tenderness and stiffness (Gulick & Kimura, 1996; Lieber & Friden, 2002). Following muscle damaging exercise, significant increases in soreness from 24h to 96 h, with peaks between 24 and 48 h (Semark *et al.*, 1999; Lee *et al.*, 2002; Harrison & Gaffney, 2004; Marginson *et al.*, 2005; Twist & Eston, 2005; Garcia-Lopez *et al.*, 2006; Twist, Gleeson & Eston, 2008; Davies, Rowlands & Eston, 2009), and a return to baseline within 5 - 7 days (Ebbeling & Clarkson, 1989) have been observed. It is possible that muscle soreness is initially concentrated in the distal portions of the muscle and diffuses to the centre over time (MacIntyre, Reid & McKenzie, 1995; Cheung, Hume & Maxwell, 2003). However, this has not been investigated in all muscle groups and should, therefore, not be generalised (MacIntyre, Reid & McKenzie, 1995).

It is generally accepted that the delayed-onset of muscle soreness (DOMS) is a consequence of eccentric muscle actions, however, it should be used with caution to reflect the magnitude of muscle damage (Nosaka, Newton & Sacco, 2002). Nurenberg *et al* (1992) demonstrated weak correlations between DOMS and the degree of ultrastructural damage. Although DOMS may be viewed as a negative consequence of EIMD, it could function as a protective mechanism to allow adequate time for muscle recovery and regeneration as it may decrease the likelihood of a person undertaking physical exercise (Malm, 2001). However, poor non-significant correlations (r = 0.07 to 0.47) have been observed between DOMS and functional performance following downhill running when all time points are combined (isometric strength and peak power) (Nottle & Nosaka, 2007). In part agreement, Cleak & Eston (1992) found no relationship between soreness and strength 24 h following eccentric actions of the biceps. However, there was a significant relationship (r = -0.60) at 72 h when soreness was at its peak. Similarly, peak palpatation and extension soreness were significantly correlated (r = -0.26 to -0.56) with maximum voluntary isometric action immediately, 24 and 96 h following eccentric actions of the elbow flexors (Nosaka *et al.*, 2006). These studies differ in terms of significant and non-significant findings which may be

41

attributed to different participant numbers. However, all correlations would be classified as weak to moderate with R^2 values below 36 %, demonstrating high amounts of unexplained variance. Therefore, DOMS is unlikely to provide an indication of exercise readiness. A possible reason for the low shared variance is the subjective nature of DOMS, which makes it difficult to compare independent participants. Although DOMS may not indicate exercise readiness, athletes' perception of muscle soreness is important as it can impact on their decision to exercise. Therefore, the measurement of DOMS and the investigation of interventions to alleviate it is important.

2.4.2.3 Theories of Muscle Soreness

There are a number of theories to explain muscle soreness, which have previously been reviewed (Cheung, Hume & Maxwell, 2003). MacIntyre, Reid & McKenzie (1995) reviewed two of the main mechanisms underlying DOMS: tissue oedema and inflammation. Tissue oedema may lead to muscle stiffness that increases the mechanical sensitivity of the muscle receptors. Soreness is then experienced when activated by pressure or stretching (Jones, Newham & Clarkson, 1987). Weerakkody *et al* (2001) have shown that muscle mechanoreceptors, including muscle spindles, contribute to muscle soreness. However, Nosaka, Newton & Sacco (2002) stated that oedema could not be one of the main mechanisms underlying DOMS as when upper arm circumference peaks, DOMS is subsiding.

The inflammatory response is a common mechanism to explain DOMS (MacIntyre, Reid & McKenzie, 1995). Increased COX at the site of damage is thought to be hyperalgesic (Dubois *et al.*, 1998), and subsequent PGE_2 production may sensitise type III and IV afferent receptors to mechanical, chemical or thermal stimuli (Armstrong, 1984; Smith, 1991) experienced during movement, thereby inducing soreness. Providing participants with a COX inhibiting agent (Flurbiprofen) did not influence muscle soreness following eccentric cycling (Kuipers *et al.*, 1985). Furthermore, there was no evidence of an inflammatory response using histological investigation. More recently, following isokinetic eccentric actions, muscle soreness peaked at 48 h, however, PGE_2 remained unchanged (Croisier *et al.*, 1996). This may provide evidence that PGE_2 is not involved in pain. Similarly, Buford *et al* (2009) found no significant correlations between muscle soreness and COX-2, TNF-α, IL-1β and IL-6 following downhill running. In support of this, other studies have found no evidence of a relationship between markers of inflammation and DOMS

(Malm *et al.*, 2000; Miles *et al.*, 2008; Paulsen *et al.*, 2010). Milias *et al* (2005) did observe significant correlations between neutrophils at 2 and 24 h and muscle soreness at 48 h, however, the strength of the relationship was not stated. There appears to be a lack of evidence in favour of inflammation as the underlying cause of DOMS; it may be that DOMS is related to inflammation of the connective tissue around the muscle (Jones & Round, 1990).

Although these are the two most common mechanisms reviewed in the literature, it still remains controversial whether oedema formation as well as inflammatory cell infiltration is the mechanism responsible for DOMS.

2.4.2.4 Muscle Function

The measurement of muscle performance is commonly used as an indirect marker of EIMD. Maximum voluntary contractions (MVC) are one of the most widely used measures. MVCs can take the form of isometric, concentric and/or eccentric actions. Isometric muscle actions appear to be routinely used and following eccentric muscle actions they have been shown to significantly decrease immediately, 24, 48 and 72 h (Cleak & Eston, 1992; Behm *et al.*, 2001; Harrison & Gaffney, 2004; Shahbazpour *et al.*, 2004; Marginson *et al.*, 2005; Skurvydas *et al.*, 2006a) and remain decreased for as long 11 days (Cleak & Eston, 1992), with peak decrements occurring between 24 and 72 h. Concentric and eccentric MVCs are less used, which is surprising as these muscle actions are commonly used during exercise. The studies conducted demonstrate that EIMD leads to a decrease in these measures that is similar to the loss occurring during isometric actions (Byrne & Eston, 2002a). Peak torque has been shown to be significantly reduced from 30 min to 48 h following eccentric muscle actions, with largest decrements observed between 24 and 48 h (Eston *et al.*, 1996; Behm *et al.*, 2001; Twist, Gleeson & Eston, 2008). MVCs can be measured via isokinetic dynamometry which has limited ecological validity when extrapolating to a sporting context, as exercise very rarely involves these isolated muscle actions (Komi, 2000). Those experiencing EIMD need to be able to optimally function in the days post muscle damaging exercise to continue training and/or competing. With this in mind researchers have examined the effect of eccentric muscle actions on dynamic measures of muscle function. The stretch-shortening cycle provides a good model to investigate normal and damaged muscle (Komi, 2000) as it is involved in many sporting movements, including running and jumping.

Vertical Jumps are commonly measured with researchers examining squat, countermovement and drop jumps. The use of these different vertical jumps allows researchers to examine the effect of EIMD on stretch-shortening cycle exercise. Byrne & Eston (2002a) have observed decrements in all forms of vertical jumps from 1 h to 72h following muscle damaging exercise. This is in agreement with other studies (Horita *et al.*, 1999; Harrison & Gaffney, 2004; Marginson *et al.*, 2005; Garcia-Lopez *et al.*, 2006; Skurvydas *et al.*, 2006a; Skurvydas *et al.*, 2006b). Studies examining all three vertical jumps have found that squat jumps are affected to a greater extent than either countermovement and/or drop jumps (Byrne & Eston, 2002a; Harrison & Gaffney, 2004). It has been proposed that the stretch shortening cycle used during countermovement and drop jumps may provide potentiating mechanisms that attenuate the detrimental effects of EIMD (Byrne & Eston, 2002a), therefore, decrements in performance are attenuated.

Other facets of high intensity muscle function have also been shown to be detrimentally affected following muscle damaging exercise. Peak power measured during a Wingate test or repeated cycle sprints decreases from 30 min through to 72 h with largest decrements observed between 24 and 48 h following squats and countermovement jumps (Byrne & Eston, 2002b; Twist & Eston, 2005). However, Nottle & Nosaka (2007) did not observe any prolonged reductions in peak power measured during a 30 sec Wingate test following downhill running. Sprint times have also been evaluated with significant reductions in 10 m sprint time from 30 min to 48 h being found (Twist & Eston, 2005). However, Semark *et al* (1999) did not find a reduction in 30 m sprint times. Although muscle soreness increased in this study, there was no change in CK possibly indicating a lack of structural muscle damage. Fatigue during repeated cycle sprints has also been evaluated (Byrne & Eston, 2002b; Twist & Eston, 2005). Both of these studies demonstrate a significant reduction in fatigue index from 24 – 48 h following muscle-damaging exercise, which may be due to an inability to generate high force and power at the start of exercise (Byrne & Eston, 2002b). Therefore, the power produced at the start of the exercise bout would be lower than that prior to muscle damage. During the exercise bout following muscle-damaging exercise the change in power output from the start to the end would be smaller, which would subsequently translate into a reduced fatigue index.

Most of the research in the area of EIMD and muscle performance has focused on high intensity exercise (MVCs, peak power, vertical jumps). Central to this thesis is the

effect of EIMD on high intensity exercise and the role of milk in altering this response. However, the effect of EIMD on endurance exercise is also of primary importance. Many sports, especially team sports, require the athlete to be able to perform both high intensity and endurance exercise for optimal performance. Therefore, it is important to understand the effect of EIMD on endurance exercise. Overall, EIMD has a negative impact on endurance performance 48 h following muscle damaging exercise (Marcora & Bosio, 2007; Davies, Rowlands & Eston, 2009; Twist & Eston, 2009). In terms of performance, distance covered during a time trial (Marcora & Bosio, 2007; Twist & Eston, 2009) and time to exhaustion is reduced (Davies, Rowlands & Eston, 2009). During these performance tests (Davies, Rowlands & Eston, 2009) and moderate intensity exercise (Twist & Eston, 2009) ratings of perceived exertion (RPE) increase. The change in RPE may contribute to reduced performance as participants perceive a higher level of effort for lower metabolic cost and work (Twist & Eston, 2009). In line with this, the ventilatory response has been shown to increase during moderate intensity exercise (Davies, Rowlands & Eston, 2009; Twist & Eston, 2009).

It is clear that EIMD reduces many aspects of muscle performance. The underlying mechanisms remain unclear, however, the following section will discuss some factors contributing to reduced performance.

2.4.2.5 Theories of Reduced Muscle Function

There are a number of theories regarding mechanisms underlying reduced performance. These include central, peripheral, impaired metabolism and selective fibre damage. Reduced performance is a result of a complex interaction of a number of mechanisms (Warren *et al.*, 2002). Peripheral damage is the most common theory, widely researched and reviewed in the literature. It must be noted that most of the evidence is taken from animal models, therefore, caution must be taken when generalising to humans (Warren *et al.*, 2002).

Peripheral damage includes a failure in the E-C coupling system or structural damage, which have been previously reviewed in this chapter. Warren *et al* (1993) suggested that a failure to activate intact contractile proteins may contribute to immediate decrements in power output. From their study it appeared that the loss was not due to depolarization of the sarcolemma or to sarcoplasmic reticulum dysfunction

and more likely lay in the E-C coupling process. E-C coupling may contribute to decreased muscle performance via reduced Ca^{2+} release per action potential (Warren *et al.*, 1993; Balnave & Allen, 1995). These findings were taken from mouse muscle, where as studies in amphibian muscle have found that reduced force could not be accounted for by changes in Ca^{2+} release (Morgan, Claflin & Julian, 1996). Reduced Ca^{2+} release may occur due to damage to the triads (connections between the sarcoplasmic reticulum and t-tubules), sarcoplasmic reticulum (Clarkson, Nosaka & Braun, 1992; Allen, 2001) and/or t-tubule voltage sensor. Indirect support for reduced Ca^{2+} may come from the finding that using the stretch shortening cycle blunts decreases in vertical jump performance (Byrne & Eston, 2002a; Harrison & Gaffney, 2004). Pre-activation during the stretch shortening cycle may counter the reduced availability of Ca^{2+} to the myofibrils by increasing the intensity or duration of the active state (Byrne, Twist & Eston, 2004).

Damage to the sarcomere (contractile, regulatory and structural proteins) via the secondary phase would limit the muscles ability to produce and transmit force. There is a progressive loss of contractile protein, with actin and myosin found to be reduced by 20% 5 days after muscle damaging exercise (Ingalls, Warren & Armstrong, 1998), which may limit the muscles ability to function optimally. A number of studies have observed relationships between muscle performance and sarcomere disruption. Raastad *et al* (2010) demonstrated significantly strong correlations (r = 0.89) between myofibrillar disruption and decrements in peak torque throughout all time points following eccentric actions of the knee extensors. Similarly, Lauritzen *et al* (2009) demonstrated that the reduction in force generating capacity at 2 and 48 h post eccentric actions of the elbow flexors correlated with the percentage of fibres with moderate (r = 0.93) and extreme (r = 0.94) myofibrillar disruption. These studies indicate a role of structural damage in the ability of the muscle to produce force. Disrupted fibres may include the loss of or damage to the force transmitting and generating structures via the Ub-P pathway, which is responsible for myofibrillar degradation as discussed in section 2.3. The Ub-P pathway may be stimulated by increases in intracellular Ca^{2+} concentration, reactive oxygen species and/or TNF-α, as discussed in section 2.3. Paulsen *et al* (2010) observed strong significant correlations between leukocytes and muscle weakness, which may indicate a role of the inflammatory response. However, Miles *et al* (2008) found that changes in IL-6 and CRP were independent of the magnitude of strength loss. Differences may be attributed to the different measures of inflammation used and how

they were measured (biopsy v blood samples). Neither of these studies assessed TNF-α, which appears to be the key cytokine for exacerbating damage. They also did not measure any factors linked to myofibrillar protein degradation.

Warren *et al* (2002) concluded that the majority of strength loss (~75 %) in the first 3 days following muscle damaging exercise could be ascribed to E-C coupling failure. Furthermore, for at least the first 2 days decrements unaccounted for by this could be attributed to damage of the force generating and transmitting structures. By the third day, the loss unaccounted for by E-C coupling would be due to decreased contractile protein content most likely due to the removal of damaged force generating structures (Warren *et al.*, 2002), and prolonged recovery could be explained by a significant loss of contractile protein (Ingalls, Warren & Armstrong, 1998). More recent evidence supports this. Reduced 20:50 Hz force ratio has been found immediately after exercise (Raastad *et al.*, 2010), demonstrating that disrupted E-C coupling function contributed to reduced force generating capacity. However, by 2 days this was no longer significantly reduced from baseline, whilst decrements in force generating capacity reached a peak, indicating that the role of E-C coupling in decrements in muscle performance was reduced in the days following muscle damaging exercise.

Changes in the central nervous system may be one of the underlying mechanisms of reduced muscle function. Evidence for this comes from electromyogram (EMG) measures. Changes may occur in neural activation patterns that would 'bypass' the severely damaged fibres (Clarkson, Nosaka & Braun, 1992). Research has demonstrated a change in EMG pattern up to 24 h (Dartnell *et al.*, 2009) and 48 h after muscle damaging exercise (Komi & Viitasalo, 1977). Behm *et al* (2001) concluded that neuromuscular inactivation was not a significant contributor to EIMD. It is also suggested that centrally mediated pain or soreness (Twist & Eston, 2009) experienced during EIMD reduces the ability to voluntary produce maximum force. However, it has been concluded that the data available do not support the notion of soreness causing decreased force production (Cleak & Eston, 1992). The time course of muscle soreness and decreased force production are different (Clarkson, Nosaka & Braun, 1992) and force produced during electrically stimulated muscle actions leads to decreased force (Newham, Jones & Clarkson, 1987).

Another postulated theory of reduced muscle performance relates to impaired

metabolism, as previously stated.   Decreased glycogen re-synthesis following muscle damaging exercise has been well documented (O'Reilly *et al.*, 1987; Costill *et al.*, 1990; Asp, Daugaard & Richter, 1995; Asp, Rohde & Richter, 1997; Asp *et al.*, 1998; Zehender *et al.*, 2004).   Performance that relies on muscle glycogen, such as high intensity, endurance and/or intermittent sports, will be affected by impaired re-synthesis of glycogen (Byrne, Twist & Eston, 2004).   Reduced glycogen re-synthesis has been observed in males 48 h post maximal voluntary eccentric actions of the quadriceps (Doyle, Sherman & Strauss, 1993; Asp *et al.*, 1998), with glycogen content of type II fibres being particularly affected, which may be associated with reduced exercise capacity (Asp *et al.*, 1998).   When measurement points are extended beyond 48 h, glycogen re-synthesis remains reduced for up to 10 days (O'Reilly *et al.*, 1987).   By increasing CHO intake, Costill *et al* (1990) found that glycogen storage increased indicating that EIMD does not completely inhibit glycogen re-synthesis.   However, 24 h post eccentric exercise, a 35 % reduction from pre-exercise levels in glycogen content was observed, despite a high CHO intake (Zehender *et al.*, 2004).

Reduced glycogen re-synthesis is assumed to be related to decreased glucose uptake into the cell (Tee, Bosch & Lambert, 2007).   Inflammatory cells are known to increase glucose utilisation via oxidation (Bergstrom & Hultman, 1966; Jansson, Hjemdahl & Kaijser, 1986), therefore, reduced glycogen re-synthesis may be a result of the competition between the inflammatory and glycogen depleted muscle cells for available glucose (Costill *et al.*, 1990).   Disruption of the muscle cell membrane may also reduce insulin-stimulated glucose transport (Doyle, Sherman & Strauss, 1993).   Decreased insulin sensitivity may be due to TNF-α (Kirwan & Del Aguila, 2003).   However, it is possible that glucose transport into the muscle cell is the rate-limiting step (O'Reilly *et al.*, 1987).   GLUT4 is one of the major glucose transporter proteins (Tee, Bosch & Lambert, 2007) and the muscle content of GLUT4 has shown to be decreased 48 h following eccentric exercise in both rats (Asp, Kristiansen & Richter, 1995; Kristiansen, Asp & Richter, 1996; Kristiansen *et al.*, 1997) and humans (Asp, Daugaard & Richter, 1995).   However, Asp *et al* (1998) found no difference in glucose uptake between eccentric exercise and control groups.   Differences in findings may be due to methodological reasons (Byrne, Twist & Eston, 2004), with decreases in GLUT4 content being reduced to a greater extent in rat muscle after electrically induced eccentric in situ muscle actions (Asp, Kristiansen & Richter, 1995) than in vivo human muscle actions (Asp, Daugaard & Richter, 1995).   If GLUT4 is

reduced then this may be linked to the inflammatory response with TNF-α (Stephens & Pekala, 1991) being shown to cause transcriptional repression of the GLUT4 gene (Stephens & Pekala, 1991).

Lastly, if type II fibres are selectively damaged then their contribution during maximal intensity activities will be reduced (Byrne, Twist & Eston, 2004), either via reduced glycogen concentration or an inability to generate and transmit force. The findings of muscles being less fatigable (Byrne & Eston, 2002b; Twist & Eston, 2005) may link to selective type II fibre damage. Type II fibres are associated with high activation and rapid fatigue, therefore, if they are selectively damaged then this response may not be apparent and it will appear as if muscles are less fatigable (Byrne, Twist & Eston, 2004).

In conclusion, EIMD directly affects the structure of the muscle and has a number of indirect consequences. Indirect markers of EIMD are commonly measured and muscle function is of primary importance as losses of it will affect a person's ability to not only exercise, but may impact on their general daily activities. The processes underlying each of these measures are unknown but it appears that different mechanisms contribute to the different indirect markers. Therefore, interventions to alleviate decrements in muscle function are likely to be different from those that attenuate increases in DOMS, for example.

2.5   Effect of Eccentric Exercise on Protein Metabolism

It is evident from the observed ultrastructural changes that there is a breakdown of protein structures in the muscle, and there is evidence of processes occurring in the muscle that can affect protein metabolism. Furthermore, the structures that produce and transmit force are proteins; therefore, it is reasonable to predict that changes in protein metabolism may be related to decrements in muscle function. However, it is not known if there is an association between the processes that lead to myofibrillar damage and the breakdown of muscle proteins (Phillips *et al.*, 1997). Following maximal eccentric exercise of the elbow flexors, increases in 3 protein bands of 40 - 80 % have been observed at 48 h (Reichsman *et al.*, 1991). It was suggested that these proteins could be myofibrillar related to protein degradation, such as heat shock, stress proteins or ubiquitin (Reichsman *et al.*, 1991). The increase in protein may have indicated an increase in intracellular concentrations suggesting a change in synthesis and

degradation. This section will discuss the changes in protein metabolism following muscle damaging exercise, as these changes may have an impact on direct and indirect markers of EIMD.

2.5.1 Protein Synthesis

Research examining the influence of eccentric exercise on protein synthesis has on the whole demonstrated an increase. Trappe *et al* (2002b) reported a significant increase in mixed muscle protein fractional synthetic rate measured via a stable isotope infusion of phenylalanine 24 h following eccentric exercise. Similarly, Phillips *et al* (1997) demonstrated a significant increase in mixed muscle fractional synthetic rate, measured via phenylalanine infusion, 3, 24 and 48 h following eccentric resistance exercise. Lowe *et al* (1995) found a decrease in mixed muscle protein synthesis, using phenylalanine incorporation, immediately post in vivo electrically stimulated eccentric muscle actions in mouse EDL muscles. However, this increased over time crossing baseline levels between 6 and 24 h post and was still rising 5 days later. Although muscle protein synthesis increased in this study, muscle protein content was significantly reduced 14 days later (Lowe *et al*., 1995). Myofibrillar protein synthesis has been shown to increase at 4.5 and 8.5 h following maximal eccentric actions of the knee extensors in a fed state (Moore *et al*., 2005). However, one study has demonstrated that muscle protein synthesis in fast twitch fibres is depressed 24 and 48 h following electrically stimulated eccentric actions in rats (Fluckey *et al*., 2001). The difference between this study and other studies is likely to be due to the use of electrical stimulation versus voluntary high intensity eccentric actions, and possibly the time points used to measure changes (Fluckey *et al*., 2001).

Research has compared eccentric and concentric actions effects on muscle protein synthesis. It was found that in recreational body builders mixed muscle protein synthesis, measured via leucine infusion, 24 h following eccentric exercise was no different to that after concentric exercise even though significant myofibrillar disruption was only observed following eccentric exercise (Gibala *et al.*, 2000). However, this study did not measure baseline muscle protein synthesis and it differs too many of the other studies in this area by its use of trained participants. Phillips *et al* (1997) demonstrated no difference between concentric and eccentric actions in muscle protein synthesis rate in untrained participants. However, the bout of eccentric exercise used in this study may not have been severe enough to induce damage as there was no change in CK or myofibrillar damage (Phillips *et al*., 1997).

These studies would appear to suggest that the response of protein synthesis is similar between muscle action modes. However, at 4.5 h following eccentric actions, myofibrillar protein synthesis was significantly greater than concentric actions when both bouts of exercise were matched for total work (Moore *et al.*, 2005). In this study moderate Z-band streaming following eccentric actions was significantly greater than rest and concentric actions. The difference in results between this study and others (Phillips *et al.*, 1997; Gibala *et al.*, 2000) may be due to the measurement of myofibrillar versus mixed muscle protein synthesis, matching muscle action modes for total work, the recruitment of greater amounts of active muscle (Moore *et al.*, 2005), time points of measurement, and fasted versus fed states.

Muscle protein synthesis increases following eccentric muscle actions even when there is evidence of myofibrillar disruption. Therefore, myofibrillar disruption may be due to enhanced rates of protein breakdown.

2.5.2 Protein Breakdown

Protein degradation rates have been shown to increase following muscle damaging exercise. Lowe *et al* (1995) measured protein degradation in mice via tyrosine release and found increases at 24 h, which reached a plateau of a 60 % increase by 48 h and remained at this level for a further 3 days. In humans, Phillips *et al* (1997) demonstrated a significant increase in mixed muscle fractional breakdown rate 3 and 24 h following eccentric exercise, and by 48 h it had returned to resting levels. Lastly, Fielding *et al* (1991) demonstrated an increase in leucine release from protein breakdown immediately post exercise and remaining increased for 10 days following eccentric cycling. These studies have used isotopes for measuring muscle protein breakdown. Changes in 3-methylhistadine (3-MH) have also been used as a measure of myofibrillar protein breakdown, with 24 h urine collections the most commonly used method.

Measurements of 3-MH are based on the assumption that its rate of production is proportional to the rate at which actin and myosin are broken down and excreted from the muscle (Hansen *et al.*, 2009). Results from studies utilising 3-MH are equivocal. In rats, 3-MH excretion was increased following downhill running with peak excretion occurring 2 days after (Kasperek & Snider, 1985). This change was greater and longer lasting than rats completing level running (Kasperek & Snider, 1985). However, in a

later study 3-MH release following downhill running in rats was no different to rats that had completed level running (Kasperek *et al.*, 1992). Differences were likely due to the protocols used; the recent investigation had control rats which were pair fed with the older study using exercised rats that served as their own control (Kasperek *et al.*, 1992). However, this study did observe an increase in the rate of non-myofibrillar protein degradation during exercise as measured by tyrosine release (Kasperek *et al.*, 1992). In young untrained men, urinary 3-MH has been found to significantly increase 10 days post eccentric cycling, demonstrating a prolonged change in protein degradation. The findings of Fielding *et al* (1991) demonstrate longer alterations with the differences possibly attributed to the use of human participants versus mice and the method to measure protein degradation. When using labelled isotopes, Fielding *et al* (1991) found immediate increases in protein degradation. In opposition to these findings, Plante & Houston (1984) found no change in 3-MH excretion following eccentric cycling in young untrained males. Furthermore, in untrained males, 3-MH in the interstitial space of skeletal muscle measured via microdialysis did not change over 96 h following voluntary maximal eccentric actions even though there were significant increases in disrupted Z-lines 5 and 24 h after (Hansen *et al.*, 2009). These results are surprising considering that protein degradation measured via isotopes has been shown to increase following resistance exercise (Biolo *et al.*, 1995; Phillips *et al.*, 1997). Specifically, in Hansen *et al* (2009) study there were significant increases in disrupted Z-lines 5 and 24 h after, which may indicate increased protein breakdown. However, there were no gross morphology changes as indicated by no differences in desmin, dystrophin and vimentin, which may be indicative of severe myofibril damage not being caused. Therefore, the method of 3-MH quantification may not have been sensitive enough to detect small local increases (Hansen *et al.*, 2009). The study by Phillips *et al* (1997) also provides evidence against the use of 3-MH. They demonstrated significant increases in protein breakdown using isotopes but no significant increases in 3-MH and cited that 24 h urine collections for 3-MH determination may be an insensitive marker of myofibrillar degradation. It has been stated that without measures of tracer dilution, muscle blood flow and muscle microvascular blood flow this method is unreliable (Kumar *et al.*, 2009). It has been stated that 3-MH is a poor indicator of muscle protein breakdown since small rapidly turned over pools of 3-MH substantially contribute to its excretion (Rennie & Millward, 1983). However, this method may still provide a useful non-invasive indication of protein degradation (Ballard & Tomas, 1983). The use of 3-MH for quantification of myofibrillar protein breakdown may be limited and the controversy surrounding its use may help explain the equivocal results.

Studies measuring whole body protein breakdown following eccentric exercise have found no change when measured via the rate of phenylalanine appearance 24 h post (Phillips *et al.*, 1997; Trappe *et al.*, 2002b). However, muscle protein metabolism reflects only a third of whole body metabolism (Nair, 1995), therefore, using whole body measures may not identify local muscle changes. Furthermore, in the study by Phillips *et al* (1997) muscle protein breakdown was significantly increased.

The majority of evidence seems to support an increase in muscle protein breakdown occurring after eccentric actions. It may be that myofibrillar damage is due to a greater increase in protein breakdown during eccentric compared to concentric actions, leading to a net negative muscle protein balance. Very few studies have researched this. In untrained participants, muscle protein breakdown was not different between eccentric and concentric actions (Phillips *et al.*, 1997). However, the exercise bout utilised in this study may not have been severe enough to induce muscle damage as evidenced by no increase in creatine kinase and no significant differences in myofibrillar damage (Phillips *et al.*, 1997). More research is required in this area.

2.5.3 Theories of Change in Protein Metabolism

Changes in protein metabolism may be due to a number of processes that have been previously discussed in section 2.3. Changes in protein synthesis may be regulated by $PGF_{2\alpha}$ as it has been demonstrated that by providing participants with COX inhibiting drugs, fractional synthetic rate and $PGF_{2\alpha}$ were inhibited (Trappe *et al.*, 2002b). Therefore, interventions to alleviate the negative aspects of EIMD should possibly avoid those that may inhibit the COX pathway. Protein synthesis may be increased by the phosphorylation of $p70^{S6K}$. One of the only studies to investigate the effect of maximal eccentric muscle actions on the phosphorlyation of this pathway in humans without nutritional supply demonstrated a fourfold increase in $p70^{S6K}$ phosphorylation for 2 h (Eliasson *et al.*, 2006). Both maximal concentric and submaximal eccentric actions did not increase the phosphorylation of $p70^{S6K}$ (Eliasson *et al.*, 2006). An increase in $p70^{S6K}$ would stimulate translation initiation. Akt phosphorylation was not affected by concentric or eccentric muscle actions, therefore $p70^{S6K}$ is phosphorylated via another signalling pathway (Eliasson *et al.*, 2006). The increase from eccentric actions may have been due to greater force production and stretching of the muscle (Eliasson *et al.*, 2006). It is postulated that the increase in synthesis may be due to an increase in the intracellular availability of amino acids (Phillips *et al.*, 1997) due to increases in protein degradation. A strong significant correlation between fractional

synthesis and breakdown rate (r = 0.88) has been found, indicating the two processes are tightly related and one may drive the other (Phillips *et al.*, 1997).

PGE_2 has been found to be significantly correlated (r = 0.742) with 3-MH 12 days following downhill running (Cannon *et al.*, 1991), which may implicate PGE_2 in myofibrillar breakdown rather than non-myofibrillar. This may imply that the COX pathway does need to be inhibited to prevent damage. However, this finding must be viewed with caution as although 3-MH peaked at 12 days, there was no significant change in this variable at any time point. Kasperek & Snider (1985) suggested that lysosomal enzymes were involved in increases in muscle protein degradation, which may have been activated by macrophage infiltration. Cytokine production may impact on protein degradation, with specific roles of IL-1β and TNF-α. Significant longitudinal and cross-sectional relationships at 12 days between 3-MH and IL-1β have been observed following downhill running, however, there was no significant correlation between 3-MH and TNF-α (Cannon *et al.*, 1991). However, the significant correlations in the study by Cannon *et al* (1991) study were low to moderate (0.257 and 0.479) indicative of a relatively high percentage of unexplained variance. Cytokines are mediators of the inflammatory response, and Lowe *et al* (1995) provided further evidence of inflammation playing a role in changes in muscle protein degradation. They observed a significant correlation (r = 0.75) between muscle protein degradation and myeloperoxidase activity, which is a marker of phagocytic infiltration.

Most of these studies implicate processes occurring during the secondary phase of EIMD, specifically those activated by cytokines, which can activate the Ub-P pathway and elevate myofibrillar protein synthesis. The interesting finding of Lowe *et al* (1995) study was that protein degradation was not changed immediately (0 – 6 h) following exercise suggesting that the intrinsic proteases such as calpain have no role in changes in protein metabolism following eccentric exercise. However, even though changes in protein degradation were not detected total protein content was still 12% lower at this time point (Lowe *et al.*, 1995). Phillips *et al* (1997) did find an early increase in protein degradation following eccentric exercise suggesting a rapid activation of a mechanism, which may have involved calpain.

Changes in protein degradation may be a cause of the microscopic injury observed as these have been shown to follow similar time courses (Lowe *et al.*, 1995). However,

Lowe *et al* (1995) stated that altered protein metabolism does not exacerbate damage. Furthermore, it has been suggested that the losses of titin and nebulin may be due to degradation following eccentric exercise (Trappe *et al.*, 2002a). If the changes in protein degradation are a causative factor of ultrastructural damage then it is likely that decrements in muscle function and increases in intramuscular proteins in serum are a consequence of it. However, Lowe *et al* (1995) found no significant correlation between mean muscle protein degradation and power output over time (r = -0.73).

There is little research regarding what state of balance the muscle is in following eccentric exercise. However, following resistance exercise, protein balance remains negative in the absence of nutrient ingestion (Biolo *et al.*, 1995), and Phillips *et al* (1997) demonstrated a negative balance following concentric or eccentric exercise. It is, therefore, assumed that following eccentric exercise the muscle is in a catabolic state. Therefore, nutritional interventions that increase muscle protein balance to an anabolic state may be beneficial for attenuating EIMD.

2.6   Acute Protein-CHO Supplementation

Quantity, quality and timing of dietary intake around exercise will influence nutrient and hormone availability at specific receptors on target tissues (Chandler *et al.*, 1994; Tipton *et al.*, 2001). This in turn may influence muscle protein metabolism. This section will focus on the impact of acute dietary supplementation (amino acids, protein, CHO and a combination) on protein metabolism following resistance exercise, with reference to the impact of timing and dosage, and muscle damaging exercise. Studies utilising resistance exercise will only be referred to as this thesis utilised eccentric resistance exercise to induce muscle damage. Lastly, although many studies have infused amino acids or insulin these studies will not be discussed as the interest is nutrient ingestion.

2.6.1 Effect of Amino Acid/Protein on Protein Metabolism

The ingestion of essential amino acids or mixed amino acids following leg resistance exercise has been shown to lead to a positive net muscle protein balance, primarily through increases in muscle protein synthesis (Tipton *et al.*, 1 999a; Borsheim *et al.*, 2002; Tipton *et al.*, 2003). There does not appear to be any effect of amino acid ingestion on muscle protein breakdown (Borsheim *et al.*, 2002). Muscle protein

synthesis is greater during the 2 - 3 h following resistance exercise (Borsheim *et al.*, 2002; Tipton *et al.*, 2003) and this is reflective of changes occurring over 24 h (Tipton *et al.*, 2003). This stimulation of muscle protein synthesis does not require nonessential amino acids (Tipton *et al.*, 1 999a; Tipton *et al.*, 2003).

Ingesting amino acids or protein may stimulate muscle protein synthesis by increasing the availability of intracellular amino acids (Tipton *et al.*, 1999a). The pathway by which muscle protein synthesis may be stimulated is via the increased phosphorylation of p70^{S6K}. The ingestion of branched chain amino acids following a leg resistance exercise bout lead to a significantly greater phosphorylation of p70^{S6K} 1 and 2 h post compared to a placebo group (Karlsson *et al.*, 2004). However, this study did not measure muscle protein synthesis, making it difficult to conclude if this change lead to increased protein synthesis. However, p70^{S6K} is an important factor of the hormone/nutrient sensing and signalling pathway that influences translation (Rennie & Tipton, 2000).

2.6.2 Effect of Carbohydrate (insulin) on Protein Metabolism

CHO ingestion can influence muscle protein metabolism. Providing CHO in the hours following leg resistance exercise has been shown to improve muscle net protein balance, likely due to a progressive reduction of muscle protein breakdown (Miller *et al.*, 2003; Borsheim *et al.*, 2004). However, although net muscle protein balance was significantly greater than a placebo in the 3rd hour following exercise, it did not reach positive values (Borsheim *et al.*, 2004). Although, Miller *et al* (2003) did indicate moderate anabolism in the 3 h post-exercise period. Similarly, Roy *et al* (1997) demonstrated significantly lower myofibrillar protein degradation following ingestion of a glucose supplement immediately and 1 h following resistance leg exercise. Significantly lower urea nitrogen excretion was also observed with glucose ingestion, indicative of enhanced muscle protein balance (Roy *et al.*, 1997). In these studies increase in net muscle protein balance was likely due to changes in protein breakdown as CHO did not influence muscle protein synthesis (Roy *et al.*, 1997; Miller *et al.*, 2003; Borsheim *et al.*, 2004). However, Thyfault *et al* (2004) observed no influence of CHO ingestion 10 minutes before and after a whole body resistance exercise session on protein degradation measured by urinary nitrogen excretion even though insulin concentrations were increased. Differences between this study and the others may have due to the measure of protein degradation, which may have been insensitive (Thyfault *et al.*, 2004) or the timing of supplementation.

It is apparent that CHO ingestion following resistance exercise lowers muscle protein degradation with no effect on muscle protein synthesis. This leads to an improvement in muscle protein balance that does not reach positive values. Protein degradation may be decreased via increased plasma insulin concentration, which may possibly decrease the proteolytic activity of lysosomes (Mortimore, Ward & Schworer, 1978), with the Ub-P system insensitive to insulin (Kettlehut, Wing & Goldberg, 1988). Changes in protein breakdown may be attenuated as insulin may decrease plasma amino acid concentration by increasing amino acid uptake from the plasma (Borsheim, Aarsland & Wolfe, 2004). This is why sufficient amino acids are required for increased insulin to be reflected in elevated synthesis (Biolo *et al.*, 1999; Borsheim, Aarsland & Wolfe, 2004; Volek, 2004). If there were sufficient amino acids, it is likely that insulin would exert its effect on translation through the mammalian target of rapamycin (mTOR) via the activation of PI3-kinase and Akt (Kimball, Farrell & Jefferson, 2002). Lastly, studies (Miller *et al.*, 2003; Borsheim *et al.*, 2004) have demonstrated a delayed effect of CHO ingestion, therefore, insulin possibly causes a delayed effect on protein metabolism.

2.6.3 Effect of Amino Acid/Protein-CHO on Protein Metabolism

Independent ingestion of either amino acids or CHO influences protein metabolism, therefore, a combination may amplify the response following resistance exercise. Miller *et al* (2003) demonstrated that a combination of CHO and amino acid ingestedfollowing a bout of leg resistance exercise stimulated muscle protein synthesis that was approximately equivalent to the sum of the independent effect of either ingested alone. Bird, Tarpenning & Marino (2006) demonstrated significant reductions in myofibrillar protein degradation 48 h following a bout of whole body resistance exercise in comparison to a placebo. Although both CHO and essential amino acids attenuated the increase in myofibrillar protein degradation compared to a placebo, the combination lead to significant reductions (Bird, Tarpenning & Marino, 2006). These findings demonstrate a greater influence of CHO-amino acid compared to either nutrient alone on protein metabolism. In comparison to a placebo, Rasmussen *et al* (2000) demonstrated muscle anabolism resulting from increased muscle protein synthesis in the hours following resistance exercise when CHO-amino acids were ingested 1 and 3 h post. This study did not observe any changes in muscle protein degradation. Differences in findings may be attributed to the use of mixed muscle versus myofibrillar protein measurements, methodology (isotopes versus 3-MH excretion) and/or muscle mass utilised during the exercise bout.

It is questionable whether results from studies using amino acids are translatable to intact proteins. This is because differences in amino acid uptake have been reported with either pre or post exercise ingestion of amino acids (Tipton *et al.*, 2001) versus whey protein (Tipton *et al.*, 2007). This is important as studies using intact proteins are likely to be more relevant to the exercising individual (Tang *et al.*, 2007), as athletes regularly consume protein supplements (Froiland *et al.*, 2004; Kristiansen *et al.*, 2005). Casein and whey protein are regularly investigated. Whey protein results in a rapid and transient increase in amino acids in the plasma which is associated with increased whole body protein synthesis (Boirie *et al.*, 1997). Casein results in slower and prolonged increases in plasma amino acids leading to a marked inhibition of whole body protein breakdown, possibly due to a slower gastric emptying rate (Boirie *et al.*, 1997). Therefore, the consumption of these proteins may have different impacts on the postprandial metabolic response. This study was conducted at rest and, therefore, the results may not be transferrable to changes following resistance exercise.

The consumption of both casein and whey protein following resistance exercise has been shown to stimulate net muscle protein synthesis (Tipton *et al.*, 2004; Tang *et al.*, 2009). The study by Tang *et al* (2009) demonstrated that whey hydrolysate protein stimulated muscle protein synthesis to a greater extent than casein following resistance exercise, which may be related to the leucine content of the protein and how quickly it is digested (Tang *et al.*, 2009). The ingestion of whey protein following resistance exercise results in a pronounced elevation of circulating amino acids (Tang *et al.*, 2009) and significantly greater leucine concentration (Tipton *et al.*, 2004; Tang *et al.*, 2009) compared to casein. However, Tipton *et al* (2004) observed no significant difference in net muscle protein synthesis between the two proteins. There may have been a difference but due to insufficient sample size and type II error this was not significantly different (Tipton *et al.*, 2004). Whey protein ingestion following a bout of leg resistance exercise has been shown to significantly increase myofibrillar protein synthesis throughout a 5 h recovery period (Moore *et al.*, 2009b). Sarcoplasmic protein synthesis was also increased at 3 h but this was not different to feeding without exercise (Moore *et al.*, 2009b).

Many studies have investigated the influence of CHO and whey protein following resistance exercise (Borsheim, Aarsland & Wolfe, 2004; Koopman *et al.*, 2005; Tang *et al.*, 2007). The ingestion of whey protein and CHO following a unilateral leg

resistance exercise bout has been shown to lead to greater increases in muscle protein synthesis in comparison to an isoenergetic CHO supplement (Tang *et al.*, 2007). Similarly, consuming a CHO-whey protein-amino acid supplement following leg resistance exercise leads to a positive net muscle protein balance, compared to a CHO supplement (Borsheim, Aarsland & Wolfe, 2004). This was likely attributed to the significantly greater increase in muscle protein synthesis as although muscle protein breakdown was lower following ingestion of either supplement there was no difference between them (Borsheim, Aarsland & Wolfe, 2004). Koopman *et al* (2005) did demonstrate significantly lower whole body protein breakdown with the consumption of CHO-whey protein compared to a CHO supplement following leg resistance exercise. The contrasting results may be attributed to the use of whole body protein measures versus muscle protein measures. This study also demonstrated higher whole body protein synthesis, which lead to a positive whole body protein balance with CHO-whey protein supplementation (Koopman *et al.*, 2005).

It is clear that CHO and whey protein can lead to an anabolic state in the muscle. However, due to the different metabolic properties of whey and casein proteins, a combination of these proteins with CHO may be optimal. Bovine milk presents an ideal supplement to investigate as it contains these nutrients. Elliot *et al* (2006) demonstrated that whole or fat-free milk consumed following leg resistance exercise results in a positive net muscle protein balance that may have been due to increased muscle protein synthesis and/or decreased breakdown. However, the exact mechanism could not be discerned as no measures of muscle protein synthesis and breakdown were made. Roy *et al* (2000) demonstrated no difference in 3-MH excretion with a CHO-protein-fat supplement compared to a CHO or placebo supplement. There was a trend for lower 3-MH with CHO-protein-fat and CHO ingestion, which may have been clinically relevant (Roy *et al.*, 2000). There was also no difference in whole body protein synthesis between CHO-protein-fat and CHO supplements, which may have been indicative of insulin being more important than circulating amino acids for increases in whole body protein synthesis (Roy *et al.*, 2000). Myofibrillar protein synthesis following resistance exercise was responsive to ingestion of a CHO-protein-fat supplement with significantly higher increases compared to a fasted state (Holm *et al.*, 2010). Collagen protein synthesis was not further increased following exercise with the ingestion of CHO-protein-fat (Holm *et al.*, 2010).

The mechanism underlying greater muscle anabolism with CHO-protein/amino aicd supplements may be related to increases in insulin coinciding with increases in amino acid availability that would allow insulin to exert its effect on muscle protein synthesis. Muscle protein breakdown may then be attenuated due to the action of insulin. Therefore, the effect of protein-CHO on intracellular signalling may be attributed to greater amino acid availability and/or increased plasma insulin concentration (Ivy *et al.*, 2008).

The findings of these studies provide evidence that amino acids or protein should be an essential component in a recovery supplement to promote muscle anabolism after exercise. Including CHO may be important to improve palatability and stimulate small increases in net protein balance via insulin (Borsheim, Aarsland & Wolfe, 2004). Furthermore, CHO in a recovery supplement is required for glycogen resynthesis (Van Hall, Shirreffs & Calbet, 2000). Protein-CHO supplements will provide the substrate for these metabolic process and possibly increase activity of key enzymes controlling these metabolic processes (Ivy *et al.*, 2008).

2.6.4 Influence of Timing of Supplementation

As there is an interactive effect of exercise and nutrient supplementation, it follows that the nature of that interaction will be dependent, to some extent, on the timing of ingestion in relation to the bout of exercise (Rasmussen *et al.*, 2000).

Levenhagen *et al* (2001) have demonstrated that leg and whole body protein synthesis was greater with consumption of a protein-CHO supplement consumed immediately rather than 3 h following 60 min of moderate intensity cycling. This was despite similar substrate (amino acid and glucose concentration) and hormonal (insulin) milieu following consumption of either supplement. It was thought that exercise increased insulin sensitivity, and this was a central component involved in the timing of supplementation. Protein degradation was not different between timing of supplementation. However, protein metabolism is not different when an essential amino acid/CHO supplement is consumed 1 or 3 h following resistance exercise (Rasmussen *et al.*, 2000). This is a similar finding to Borsheim *et al* (2002) who found a similar response to essential amino acids consumed 1 and 2 h post resistance exercise. Fractional synthetic rate remains elevated for more than 48 h after resistance exercise (Phillips *et al.*, 1997) so an effect of timing of supplement ingestion

after resistance exercise is less likely (Borsheim *et al.*, 2002). However, there may be a different response to ingestion before exercise.

Tipton *et al* (2001) has shown that ingestion of an essential amino acid/CHO supplement either immediately before or after resistance exercise leads to a positive net muscle protein balance, primarily due to increased protein synthesis. However, the total response is greater with consumption prior to exercise. The greater response may be due to a greater delivery of amino acids to the muscle due to a greater availability of amino acids and their provision when blood flow is elevated (Tipton *et al.*, 2001). However, a later study (Tipton *et al.*, 2007) found no difference in the anabolic response to 20 g of whey protein when ingested immediately before or 1 h after resistance exercise. Therefore, although net muscle protein balance was positive the timing of ingestion was not as important as it was for essential amino acid-CHO ingestion. It is possible that the delivery of amino acids explains the difference between the two studies, which may be linked to the digestion of protein. Digestion of protein may limit the availability of amino acids during exercise when blood flow is increased. The other difference may be due to a lack of CHO, which may have altered the response of insulin. However, the insulin response was similar in both studies. Finally, there was a large variability in the response of participants that may have contributed to a lack of statistical significance (Tipton *et al.*, 2007). Therefore, the effect of protein-CHO timing on protein metabolism requires further investigation.

2.6.5 Influence of Dose

Research suggests that muscle protein synthesis appears to respond in a dose-dependent manner to essential amino acid consumption (Tipton *et al.*, 1999b; Borsheim *et al.*, 2002; Miller *et al.*, 2003). Muscle anabolism has been shown to be promoted with as little as 6 g amino acids and 35 g of sucrose (Rasmussen *et al.*, 2000; Tipton *et al.*, 2001). This demonstrates that very little protein-CHO is required to bring about muscle anabolism. There may in fact be an upper limit where by consuming more protein does not elicit greater increases in protein synthesis. It has been demonstrated that muscle protein synthesis reaches maximal stimulation after the consumption of 20g high-quality intact protein (~8.6g essential amino acids), suggesting an upper limit for the incorporation of amino acids into proteins (Moore *et al.*, 2009a). Any extra protein consumed is likely to be oxidised (Moore *et al.*, 2009a).

2.6.6 Effect of Acute Protein-CHO on EIMD

The results of the studies investigating acute protein-CHO supplementation and EIMD (Table 2.2) are equivocal. Some studies report no benefit (Wojcik *et al.*, 2001; Green *et al.*, 2008; White *et al.*, 2008; Betts *et al.*, 2009) and others demonstrate reductions in markers of EIMD (Saunders, Kane & Todd, 2004; Seifert *et al.*, 2005; Baty *et al.*, 2007; Saunders, Luden & Herrick, 2007; Rowlands *et al.*, 2008) following acute protein-CHO supplementation. The studies that have been conducted have utilised different methodologies (model of EIMD; markers of EIMD and when measured; study design; type of CHO and protein in supplement; amount of supplement; timing of consumption; matching of supplements), and is likely why results are equivocal.

Table 2.2 Studies reporting effects of acute protein-CHO supplementation on markers of EIMD

Study	Supplement	Exercise Protocol	Effect Post Exercise
Wojcik et al., (2001)	CHO-P (0.875 g/kg CHO, 0.375 g/kg PRO) immediately and 2 h post exercise Protein: skim milk	10 x 10 eccentric actions of quadriceps at 120% 1RM	CK ↔[*a] IL-6 ↔[*a] 3-MH ↔[*a] DOMS ↔[*a] Isometric Peak Torque ↔[*a]
Saunders et al., (2004)	CHO-P (26g CHO, 6.5g PRO per 355 mL) consumed during (1.8 mL/kg every 15 min) and within 30 min (10 mL/kg) post exercise Protein: whey	Cycle to fatigue at 75 % VO_{2peak}	CK ↓ at 15 h[a]
Seifert et al., (2005)	CHO-P consumed before, during and after downhill skiing (total 98 g CHO, 24 g PRO) Protein: whey	Downhill skiing (8 – 12 runs)	CK ↓ at 2 h[*] Mb ↓ at 2 h[*]
Baty et al., (2007)	CHO-P (6.2 % CHO, 1.5 % PRO) 30 min before (355 mL) immediately before (177 mL), during (177 mL) and post (355 mL) exercise Protein: whey	2 x 8 reps at 8RM resistance exercises, 3rd set as many reps as possible	Mb ↓ at 6 h[*] CK ↓ at 24 h[*] DOMS ↓ at 24 h[*]
Saunders et al., (2007)	CHO-P gel (0.146 g/kg BM CHO, 0.0365 g/kg BM PRO) during (2 mL/kg BM every 15 min) and post (5 mL/kg BM) exercise Protein: whey	Cycle to fatigue at 75 % VO_{2peak}	CK ↓ at 15 h[a]
Green et al., (2008)	CHO-P immediately, 30 min (1.2 g/kg BM CHO, 0.3 g/kg BM PRO) and 60 min (0.6 g/kg BM CHO, 0.15 g/kg BM PRO) post exercise Protein: whey	30 min downhill running (-12 %) at 8mph	CK ↔[*a] DOMS ↔[*a] Isometric Peak Torque ↔[*a]
Rowlands et al., (2008)	Protein enriched recovery meal (1.6 g/kg FFM/h CHO, 0.8 g/kg FFM/h PRO) consumed post exercise Protein: milk based	2.5 h interval cycling at relative intensities of W_{max}	CK possible ↓ over 60 h[b] IL-6 ↔[b] TNF-α ↔[b] CRP ↔[b]
Valentine et al., (2008)	CHO-P (77.5 g/h CHO, 19.4 g/h PRO) consumed during exercise Protein: whey	Cycle to fatigue at 75 % VO_{2peak}	CK ↓ at 24 h[*a] Mb ↓ at 9 h[*a] Concentric leg extensions to fatigue at 70 % 1RM ↑ at 24 h[*a] DOMS ↔[*a]
White et al., (2008)	CHO-P (75 g CHO, 23 g PRO) consumed immediately before or post exercise Protein: whey	50 eccentric actions of quadriceps at 1.05 rad/s	CK ↔[*] DOMS ↔[*] Isometric MVC ↔[*]
Betts et al., (2009)	CHO-P (1.2 g/kg BM/h CHO, 0.4 g/kg BM/h PRO) before, during and for 4 h post exercise Protein: whey protein isolate	90 min LIST	CK ↔[a] Mb ↔[a] LDH ↔[a] IL-6 ↔[a] IL-10 ↔[a] IL-1 ↔[a] DOMS ↔[a] Isometric peak torque hip and knee extensors and flexors ↔[a]

3-MH = 3 methylhistadine; **BM** = body mass; **CHO** = carbohydrate; **CK** = creatine kinase; **CRP** = C reactive protein; **DOMS** = delayed onset muscle soreness; **FFM** = fat free mass; **IL** = interleukin; **LDH** = lactate dehydrogenase; **LIST** = loughborough intermittent shuttle test; **MVC** = maximum voluntary contraction; **PRO** = protein; **RM** = repetition maximum; **TNF-α** = tumor necrosis factor-α; ↑ = indicates higher values; ↓ = indicates lower values; ↔ = indicates no difference between groups; [*] = compared to control/placebo; [a] = compared to CHO; [b] = compared to low protein

The majority of studies demonstrating a benefit of acute protein-CHO supplementation have centred on indirect markers of EIMD following endurance cycling (Saunders, Kane & Todd, 2004; Saunders, Luden & Herrick, 2007; Rowlands *et al.*, 2008; Valentine *et al.*, 2008). These studies have demonstrated attenuations in increased CK between 15 - 24 h (Saunders, Kane & Todd, 2004; Saunders, Luden & Herrick, 2007; Valentine *et al.*, 2008) and Mb at 9 h (Valentine *et al.*, 2008) following consumption of protein-CHO before, during and/or after endurance cycling in comparison to CHO or a control supplement. However, the applicability of these studies to all forms of EIMD is questionable. Endurance cycling is largely concentric (Tee, Bosch & Lambert, 2007) and it is likely that the damage predominantly occurs from metabolic stress. Therefore, these findings may not be generalised to other models of EIMD, specifically those involving a high degree of mechanical stress. However, studies using different models of EIMD have demonstrated similar results. Increases in CK and Mb at 24 h and 6 h, respectively, have been reduced following consumption of protein-CHO before, during and after a bout of resistance exercise compared to a control (Baty *et al.*, 2007). Similarly, the intake of protein-CHO before, during and after alpine skiing reduced CK and Mb 2 h post exercise (Seifert *et al.*, 2005).

Conclusions of these studies have, however, primarily been based on measures of intramuscular proteins in the serum, with CK frequently used. Basing conclusions on CK is problematic for a number of reasons. Firstly, CK is a highly variable marker of EIMD, as previously reviewed. Secondly, CK is poorly correlated to direct markers of EIMD (Fielding *et al.*, 1993; Malm *et al.*, 2000). Lastly, from an applied perspective, the exercising individual needs to know if acute protein-CHO supplementation will alleviate soreness, stiffness and the reduced capacity to exercise. It is difficult to know if an attenuated CK response will be functionally relevant as there is a little relationship between CK and maximal isometric force (Nosaka & Clarkson, 1996).

Few studies have assessed muscle soreness (Wojcik *et al.*, 2001; Baty *et al.*, 2007; Valentine *et al.*, 2008). Baty *et al* (2007) reported reduced muscle soreness at 24 h following muscle damaging exercise. However, Valentine *et al* (2008) and Wojcik *et al* (2001) found muscle soreness was not reduced. The measurement of muscle soreness is subjective (Rodenburg, Bar & De Boer, 1993), which may explain the equivocal results.

More recently, studies have begun to investigate changes in muscle function. Valentine *et al* (2008) investigated muscle function by measuring leg extensions to fatigue at 70 % of participants 1 repetition maximum (1RM) and found more repetitions were complete 24 h following muscle damaging exercise with protein-CHO compared to CHO consumption. However, it is difficult to state with certainty that these results were due to a reduction in EIMD. The improved muscle function with protein-CHO ingestion may have been due to an improvement in glycogen re-synthesis due to the addition of protein. Measuring muscle function in conjunction with a variety of other indirect markers (CK, IL-6, 3-MH, DOMS, isometric peak torque), no beneficial effects of protein-CHO consumed post eccentric exercise were observed compared to CHO and a control group (Wojcik *et al.*, 2001). However, eccentric actions of the quadriceps to induce EIMD were completed following a bout of glycogen depleting exercise. Temporary damage of the muscles in a glycogen depleted state may have confounded results. More importantly from an applied perspective, it could be argued that exercising individuals do not train in a glycogen depleted state and therefore, this does not reflect practices that could be generalised to applied settings. 3-MH was assessed and found to increase 24 h post when compared to 1 day prior to eccentric exercise with no effect of nutrient intake. This is in contrast to the observed reduction in 3-MH excretion 48 h post resistance exercise with consumption of a CHO/essential amino acid supplement (Bird, Tarpenning & Marino, 2006). 3-MH values on day 1 may not have been reflective of baseline samples as participants had completed the bout of muscle glycogen depleting exercise. Similarly, other investigations have observed no alleviation of EIMD using functional, biochemical and subjective markers (Green *et al.*, 2008; White *et al.*, 2008; Betts *et al.*, 2009).

Methodological differences can confound results for a number of reasons and this is a likely reason for why there is inconsistent information regarding the benefits of acute protein-CHO supplementation for alleviating EIMD. Firstly, the accurate interpretation of the effectiveness of protein-CHO intake is difficult because timing of ingestion differs between studies, occurring before, during and/or after exercise. If supplements are to be investigated to improve recovery then ingestion at different time points may confound findings as it is difficult to determine if the positive effects are due to a reduction in the initial damage, enhanced recovery or a combination of both (Green *et al.*, 2008). Furthermore, if benefits are a result of altered protein metabolism, then protein-CHO intake at different time points may alter the response (Tipton *et al.*, 2001).

Secondly, a number of studies have assessed indirect markers of EIMD at one time point. It is accepted that the histological evidence of damage is exacerbated over a number of days (Newham *et al.*, 1983). Therefore, to assess the attenuation of EIMD, markers of EIMD should be assessed over days to provide an accurate picture of the change occurring. Furthermore, by assessing only one time point, potential benefits of supplementation may be missed. Thirdly, using different models of EIMD may affect results due to the different type (metabolic v mechanical) of muscle stress. Prolonged endurance exercise is likely to primarily induce metabolic stress, which may influence the level of activation and types of degradative pathways. The degree of damage may also affect results. The majority of studies providing evidence in support of the use of protein-CHO show low to moderate levels of muscle damage (Baty *et al.*, 2007; Saunders, Luden & Herrick, 2007; Rowlands *et al.*, 2008; Valentine *et al.*, 2008), as assessed by CK levels. Investigations demonstrating no positive effect primarily produce high degrees of damage (Wojcik *et al.*, 2001; Green *et al.*, 2008; White *et al.*, 2008; Betts *et al.*, 2009). This may imply that acute protein-CHO supplementation alleviates EIMD, when damage is not severe. However, Saunders, Kane & Todd (2004) demonstrated benefits of protein-CHO intake with severe damage. Using CK to determine the magnitude of damage is problematic as it does not provide this information (Friden & Lieber, 2001). Furthermore, using CK to assess differences between studies should be done with caution as CK has high interindividual variance and it may not have been measured at standardised temperatures (Betts *et al.*, 2009).

There is some evidence that acute protein-CHO supplementation has the potential for attenuating indirect markers of EIMD. This may be via an improved protein balance possibly through the ingestion of protein-CHO increasing the extracellular amino acid concentration and driving protein synthesis whilst inhibiting increases in protein degradation. However, there is clearly a requirement for more research in this area.

2.7   Milk as a Recovery Supplement from EIMD

Milk is a nutrient dense food containing numerous essential nutrients: protein, CHO, fat, vitamins, minerals and antioxidants (Haug, Hostmark & Harstad, 2007). Milk protein has a high biological value making it a good source of essential amino acids and branched chain amino acids (Haug, Hostmark & Harstad, 2007). Two of the main proteins in milk are casein and whey, making up 80 % and 20 % of milk proteins, respectively. Casein is considered a 'slow' protein (Tipton *et al.*, 2004) as it slowly

empties from the stomach (Bos, Gaudichon & Tome, 2000) leading to a slow and prolonged appearance of amino acids in the blood (Tipton *et al.*, 2004). Whey is considered a 'fast' protein due to its rapid digestion (Haug, Hostmark & Harstad, 2007) that provides higher concentrations of amino acids in the blood, however, this response is short-lived (Tipton *et al.*, 2004). Both proteins ingested after resistance exercise lead to net muscle protein synthesis (Tipton *et al.*, 2004). Milk contains CHO in the form of lactose and has been shown to be a potent insulin secratogue (Gannon *et al.*, 1986).

Due to milk providing both amino acids and CHO in similar concentrations to many commercially available sports drinks, it could provide the ideal supplement to be used within sport and exercise. Milk ingestion has been shown to have potential benefits for hydration (Shirreffs, Watson & Maughan, 2007) and endurance capacity following glycogen depleting exercise (Thomas, Morris & Stevenson, 2009). In terms of recovery from muscle damaging exercise there have been few studies that have utilised milk as a recovery supplement, with many studies utilising whey protein supplementation. Protein from food is equally effective as that in supplements for stimulating muscle hypertrophy, and there is no apparent reason why muscle growth cannot be optimised by eating foods of high quality proteins such as milk (Tipton, 2009). Therefore, milk may be as beneficial as whey protein supplementation for recovery from EIMD. In fact, milk ingestion 1 h following resistance exercise has been shown to result in an increase in a positive net muscle protein balance, possibly through increased protein synthesis and a decrease in breakdown (Elliot *et al.*, 2006). This result suggests milk may be suitable for consumption during recovery (Elliot *et al.*, 2006), and potentially beneficial for recovery from EIMD.

2.8 Summary

The process of EIMD likely involves two phases: primary and secondary, that lead to damage of the membrane and protein structures within the muscle. As a consequence, increases in intramuscular proteins in the serum and muscle soreness, and decreases in muscle function are observed. One postulated reason for these changes, that would be detrimental to the exercising individual, is a net negative protein balance. Protein-CHO supplements have been shown to result in muscle anabolism following resistance exercise. There is very little evidence on the effect of acute protein-CHO supplementation on protein metabolism following muscle damaging

exercise.   However, many researchers have investigated the impact these supplements may have on recovery from EIMD using indirect markers. The findings are equivocal and there is clear need for further research focusing on the effect on muscle function.   Further to this, many of the studies in this area have focused on the use of whey protein.   Milk is a convenient, accessible and relatively cheap product that may provide an effective source of nutrients to alleviate EIMD.   This area also requires investigation.

3. GENERAL METHODS

3.1 General Methods

This thesis consists of four progressive studies designed to examine the effects of acute milk supplementation on the attenuation of exercise-induced muscle damage (EIMD). The methods described in this chapter are those that are generic to the majority of studies. Each investigation was conducted in the Sports Sciences Laboratories of Northumbria University, following institutional ethical approval. The target population were males who regularly competed in a variety of sports (team and individual), except for the final study where non-league footballers were used. Participants were recruited by emails sent to the undergraduate students, specifically the sports science students, and via poster advertisements within the University. After volunteering for a study, participants were briefed verbally and in writing regarding what would be required of them for that particular study. They were also informed of the associated risks and benefits, before providing informed consent (Appendix 1) and completing a health questionnaire (Appendix 2). Participants were fully familiarised with all testing procedures prior to commencing the study. For each study, participants were asked to maintain their habitual diet throughout, and record it in the provided food diary (except study 1). Participants were asked to arrive at the laboratory in a rested state having avoided strenuous physical activity, caffeine and alcohol for at least 48 h and not taken any nutritional supplements or anti-inflammatory drugs. Participants were tested within 30 minutes of the same time each day to minimise diurnal variation.

3.2   Experimental Design

Each study followed an independent design. This design was selected due to the phenomenon of the repeated bout effect associated with eccentric exercise (McHugh, 2003). Studies 1, 2 and 3 equally matched participants into groups based on concentric knee flexion peak torque recorded from 6 leg extension-flexions during preliminary testing. Study 4 randomly allocated participants into 1 of 2 independent groups.

For each study, participants attended the laboratory on either 3 or 4 consecutive days, depending on the study. During the first visit, participants completed baseline tests followed by a bout of muscle damaging exercise. The exception was study 4,

whereby participants completed baseline tests one week prior to completing the muscle damage protocol. Prior to baseline tests, participants' stature (Seca, Bonn, Germany) and body mass (Seca, Bonn, Germany) were recorded. On the days following muscle damaging exercise, participants returned to the laboratory on consecutive days to repeat baseline tests, with the exception of the muscle damaging protocol. Baseline tests consisted of participants firstly having a venous blood sample collected for analysis. Following this, participants rated their perceived level of muscle soreness in their hamstrings when standing using a visual analogue scale (VAS) (Semark *et al.*, 1999; Close *et al.*, 2004; Twist & Eston, 2005) (Appendix 6). Participants then completed a standardised warm-up, the relevant muscle performance measures and any other appropriate measures specific to that study.

3.3   Nutritional Supplement

The nutritional content of the supplements used in the studies is shown in Table 3.1. The milk-based protein-carbohydrate (CHO) supplement was a commercially available low-fat chocolate milkshake marketed to facilitate an athlete's recovery following exercise (For Goodness Shakes, My Goodness Ltd., London, UK). This product provided protein in the form of semi-skimmed milk and CHO in the forms of lactose, sucrose, fructose, maltodextrin and cellulose. It also contains NutrixMix^{TM} which is a unique combination of vitamins and minerals. The milk supplement was semi-skimmed (Rock Farm Dairy, Durham, UK) and provided CHO in the form of lactose. The CHO supplement was a commercially available sports drink (Lucozade Sport, GlaxoSmithKline, UK) providing CHO in the form of glucose and maltodextrin. The control supplement used throughout the studies was water.

Table 3.1 Nutritional Content of Supplements

	CHO-P	M	CHO
Energy (kcal)	707	480	280
Protein (g)	33	34	Trace
Casein (g)	26.4	27.2	
Whey (g)	6.6	6.8	
CHO (g)	118.2	49	64
Fat (g)	16.4	17	Trace
Vitamin A (µg)	498	Trace	Nil
Vitamin C (mg)	7	20	Nil
Vitamin E (mg)	12	0.4	Nil
Selemium (µg)	62	10	Nil

Amounts are per 1000 mL. CHO-P = milk-based protein-CHO supplement; M = semi-skimmed milk; CHO = carbohydrate sports drink

3.4   Dietary Control

To ensure there were no differences between groups in macronutrient and energy content of participants habitual diets, they were provided with a food diary (Appendix 3) and weighing scales (Microtonic Kitchen Scale, Salter, Kent, England) with which to record their dietary intake for 48 h prior to baseline tests and throughout testing.   Each dietary record was assessed using a nutritional software package (Microdiet V2, Downlee Systems Ltd., UK). Results from each study demonstrated that there were no differences between groups in total energy intake or macronutrient content of the diets (Appendix 4).

3.5   Muscle Damage Protocol

The same muscle damage protocol was used in all studies in this thesis. The protocol to induce muscle damage was adapted from previous research (Harrison & Gaffney, 2004) to illicit damage in the hamstrings rather than the quadriceps. The hamstrings were damaged as eccentric exercise of this muscle group was novel in the

participant group, were as eccentric actions of the quadriceps was a frequent activity and, therefore, did not illicit substantial muscle damage. Pilot testing demonstrated that this protocol resulted in muscle damage of the hamstrings (i.e. increased creatine kinase (CK) and reduced isokinetic muscle performance). Participants completed 6 sets of 10 repetitions, with 90 s rest between sets, of unilateral eccentric-concentric actions of the knee flexors at a test speed of 1.05 rad.s^{-1} using a Cybex Isokinetic Dynamometer (Cybex Norm, Cybex International, New York, N.Y.). This was conducted on one side of the body and then repeated on the contralateral leg. This process took approximately 30 minutes to complete. Participants were instructed to provide a maximal effort throughout their full range of movement during the eccentric phase of each leg flexion. During the concentric phase, participants were instructed to return their leg to the starting position with minimum effort. Previous studies have used the non-dominant limb (Sayers, Clarkson & Lee, 2000a; Sayers & Clarkson, 2001) or have not specified the use of the dominant or non-dominant limb (Cleak & Eston, 1992; Howell, Chleboun & Conatser, 1993; Rodenburg, Bar & De Boer, 1993; Bowers, Morgan & Proske, 2004; Harrison & Gaffney, 2004), assuming that both limbs respond similarly. This assumption has not been investigated; therefore both legs were tested throughout all studies.

3.6   Muscle Soreness Measurement

The degree of muscle soreness experienced was measured on a VAS (Appendix 6). In all studies, participants were required to rate the level of soreness, combined for both legs, that they perceived to have in their hamstrings when standing, from 0 (no pain-soreness) to 10 (pain-soreness as bad as it could be). This is referred to as passive soreness. Using similar scales, previous investigators have shown significant increases in the delayed-onset of muscle soreness (DOMS) following EIMD (Semark *et al.*, 1999; Close *et al.*, 2004; Twist & Eston, 2005). During studies 2, 3 and 4, participants were also asked to rate the level of soreness, using the same scale, they perceived to have in their hamstrings when conducting some of the muscle function measures. This is referred to as active soreness.

3.7   Muscle Function

Throughout studies 1, 2 and 3, muscle function was examined using isokinetic muscle dynamometry. Studies 2, 3 and 4 investigated measures of dynamic

muscle function using reactive strength index.   The final study examined other facets of muscle performance but these are discussed in the relevant chapter.

3.7.1 Isokinetic Muscle Function

Participants completed 6 concentric maximal-effort knee flexion repetitions at a test speed of 1.05 rad.s^{-1} on a Cybex Isokinetic Dynamometer (Cybex Norm) in a sitting position.   Participants were required to maximally extend and flex their leg over their maximum range of movement for 6 repetitions.   This was measured sequentially on both legs.   From this, peak torque of the best repetition and total work of the set were calculated for study 1.   Due to a change in software, studies 2 and 3 analysed peak torque only.   Coefficient of variations for this protocol, calculated from reliability trials in Northumbria University Laboratories, are reported at 4.5 – 4.9 %.

3.7.2 Reactive Strength Index

Each participant conducted three separate drop jumps from a height of 43 cm on to a force plate (Kistler Instrumente AG, Winterthur, Switzerland).   Participants were instructed to drop from the box and upon landing jump for maximum height with minimum contact time (Young, 1995).   Reactive strength index was calculated from jump height (cm) / contact time (s) (Young, 1995) and the mean was used for analysis.   Reactive strength index is a measure of an athlete's ability to utilise the stretch shortening cycle (Young, 1995) and provides a measure of dynamic muscle actions that can be related to sports involving running and jumping.   Although this does not provide a measure in which the hamstrings are isolated, the studies aimed to investigate a global picture of performance that could be applied to a variety of athletes. The coefficient of variation for this protocol, calculated from reliability trials in Northumbria University Laboratories, is reported at 7.3 %.

3.8   Blood Sampling and Analysis

During each study blood samples were collected via venipuncture from a forearm vein into a serum gel monovette (9 mL). Participants from study 3 also had blood collected into an EDTA gel monovette (9 mL). The samples were centrifuged at 3000 r.min^{-1} for 10 min (Allegra X-22 Centrifuge, Beckman Coulter, Bucks, UK).   Samples were

then aliquoted and stored at -80 °C for later analysis.

3.8.1 Creatine Kinase Analysis

Following completion of studies 1 and 2, thirty µL of serum was used for the analysis of total creatine kinase (CK), measured at 37 °C using the Reflotron (Reflotron Plus System, Bio-Stat Diagnostic Systems, Stockport, UK). Roche Diagnostics Ltd report intra-assay and inter-assay coefficients of variation for this system at 3.5 % and 3.7 %, respectively.

Study 3 analysed total CK activity using high sensitivity procedures (Advia 2400, Seimens Healthcare Diagnositics, UK). This method is adapted from the International Federation of Clinical Chemistry (IFCC) reference method. Seimens Healthcare Diagnostics report intra-assay and inter-assay coefficients of variation for this system at 0.5 – 0.8 % and 0.9 – 1.6 %, respectively.

Study 4 analysed total CK activity using kinetic UV tests (Olympus analyser, Olympus Diagnostica GmbH, Hamburg). This method is based on the IFCC reference method. Olympus Diagnostica report intra-assay and inter-assay coefficients of variation for this system at 0.64 – 2.37 % and 3.2 – 4.55 %, respectively.

To allow the comparisons of methods, during study 3 baseline CK samples were analysed using both methods (reflotron and high sensitivity). The intraclass correlation coefficient (ICC) was 0.56, demonstrating a low level of agreement between methods.

3.8.2 Myoglobin Analysis

Myoglobin (Mb) was analysed using an assay kit (Myoglobin Enzyme Immunoassay Test Kit, Oxford Biosystems Ltd., Wheatley, Oxon, UK). Absorbance was read using an Anthos 2010 Microplate reader (Anthos, Labtec Instruments, Salzberg, Germany). Biomerica report intra-assay and inter-assay coefficients of variation at 3.9 % - 6.6 %

and 5.2 % - 11.8 %, respectively.

3.9   Statistical Analysis

Study 1 presented results as means and standard error of the mean (SEM).   All studies utilised inferential statistics. Mauchley's test was used to check the sphericity of the data. Differences in isokinetic muscle performance measures were determined using a factorial analysis of variance (ANOVA) with repeated measures on 2 factors (time and leg).   Differences in other performance measures (reactive strength index, countermovement jump height, 15 m sprint, change of direction), DOMS, intramuscular proteins and inflammatory markers were determined using a factorial ANOVA with repeated measures on one factor (time). Significant within effects were analysed using Bonferonni step-wise calculation (Field, 2005).   Significant between effects were analysed using a Games Howell post-hoc test (Field, 2005).   Significant interaction effects were analysed using Tukey's honestly significant difference (HSD) test. Statistical significance was set at $p < 0.05$.

Studies 2, 3 and 4 reported inferential statistics, however, conclusions were based on statistical analysis that reports uncertainty of outcomes as 90% confidence intervals (CI), making probabilistic magnitude based inferences about true values of outcomes using methods described by Batterham & Hopkins (2006). The move towards this method of analysis is in keeping with recent trends in methods of inferential statistics (Sterne & Smith, 2001; Batterham & Hopkins, 2006).   This method allows the emphasis of effect magnitudes and estimate precision rather than the traditional null-hypothesis testing based on an arbitrary *p* value which focuses on absolute effect versus non-effect interpretation (Rowlands *et al.*, 2008) and does not deal with the real world significance of an outcome (Batterham & Hopkins, 2006). This method defines the smallest biological or practical effect, allowing the researcher to qualify the probability of a worthwhile effect with inferential descriptors to aid interpretation (Rowlands *et al.*, 2008).   Magnitude based inferences recognise sample variability (Rowlands *et al.*, 2008) and provide scientists, support staff and athletes with an indication of the practical meaningfulness of the results.   Traditional inferential statistics do not allow for this and can be misleading depending on the magnitude of statistic, error of measurement and sample size (Batterham & Hopkins, 2006). The selection of this inferential method suits the applied nature of this thesis.

Each dependent variable was analysed using a published spreadsheet (Hopkins, 2006) to determine the effect of the independent variable as the difference in the change between each group. The analysis of dependent variables were conducted on log-transformed values to overcome heteroscedastic error (Nevill & Lane, 2007), except muscle soreness data and rating of perceived exertion. These variables were not log-transformed as it is inappropriate due to interval scaling (Nevill & Lane, 2007). Participant descriptive data and muscle soreness data are presented as absolute means ± standard deviations (SD). Means derived from the analysis of log-transformed variables were back transformed to provide mean percentage change and percentage SD, except intramuscular protein values which were reported as factors due to the large percentage changes (Hopkins, 2003).

For calculation of the chances of benefit and harm, the smallest worthwhile or important effect for each dependent variable was the smallest standardised (Cohen) change in the mean: 0.2 times the between-subject SD for baseline values of all participants (Batterham & Hopkins, 2006), which has been used elsewhere in a similar investigation (Rowlands *et al.*, 2008). Practical inferences were drawn using the approach identified by Batterham & Hopkins (2006). Quantitative chances of benefit and harm were assessed qualitatively: <1% almost certainly not; 1-5% very unlikely; 5-25% unlikely; 25-75% possibly; 75-95% likely; 95-99% very likely; >99% almost certainly (Hopkins, 2002). This method provides a way to qualify clear outcomes with a descriptor that represents the likelihood that the true value will have the observed magnitude (Batterham & Hopkins, 2006). They are also free of the burden of type I and II errors as they are probabilistic rather than definitive statements (Batterham & Hopkins, 2006). Due to there being a large number of comparisons that could be reported, only changes from baseline to 48 and 72 h have been reported. These time points were chosen as the first study demonstrated that milk supplementation was not beneficial until 48 h following muscle damaging exercise. Throughout the results, *p* values for the main interaction effects (time x group), have also been stated.

4. EFFECT OF ACUTE MILK-BASED PROTEIN-CHO SUPPLEMENTATION ON THE ATTENUATION OF EIMD

4.1   Introduction

Exercise induced muscle damage (EIMD) has been shown to be caused by activities involving eccentric muscle actions (Semark *et al.*, 1999; Byrne & Eston, 2002a; b; Harrison & Gaffney, 2004; Twist & Eston, 2005), as previously discussed. EIMD has a number of consequential effects including the delayed-onset of muscle soreness (DOMS) (Semark *et al.*, 1999; MacIntyre *et al.*, 2001; Nosaka, Newton & Sacco, 2002), increased release of intramuscular proteins into the serum (Clarkson *et al.*, 1986; Sorichter *et al.*, 2001; Seifert *et al.*, 2005) and most importantly, in terms of sport performance, decrements in muscle function (Byrne & Eston, 2002a; b; Harrison & Gaffney, 2004; Twist & Eston, 2005). These measures change over a number of days, peaking between 24 and 72 h, possibly due to proteolytic and lipolytic pathways initiated by disturbances in Ca^{2+} homeostasis.

Decrements in muscle function are of primary concern to the athlete. These decrements, occurring in the days following muscle damaging exercise, may be due to the disruption of force generating and/or force transmitting structures (Warren *et al.*, 2001). Changes in protein metabolism (Fielding *et al.*, 1991; Lowe *et al.*, 1995) and losses in myofibrillar protein (Willoughby, Rosene & Myers, 2003; Willoughby, Taylor & Taylor, 2003), possibly due to the secondary phase (Cannon *et al.*, 1991; Lowe *et al.*, 1995) rather than the primary mechanical event, may be a causal factor of the ultrastructural damage observed following muscle damaging exercise, and therefore decrements in muscle function. In support of this, Raastad *et al* (2010) observed significantly, strong correlations between myofibril disruption and decrements in peak torque.

Nutritional supplements may minimise changes in protein metabolism. A combination of protein or amino acids and carbohydrate (CHO) has been found to be optimal for eliciting a positive effect on protein balance following exercise (Rasmussen *et al.*, 2000; Miller *et al.*, 2003; Bird, Tarpenning & Marino, 2006; Elliot *et al.*, 2006; Tang *et al.*, 2007; Beelen *et al.*, 2008). Therefore, the consumption of a protein-CHO supplement may limit decrements in muscle function through changes in myofibrillar protein synthesis and/or protein degradation rates. There may also be beneficial effects in muscle soreness and increases in intramuscular proteins in the plasma.

The use of acute protein-CHO consumption, in the context of EIMD, has been extensively studied (Wojcik *et al.*, 2001; Saunders, Kane & Todd, 2004; Baty *et al.*, 2007; Luden, Saunders & Todd, 2007; Saunders, Luden & Herrick, 2007; Green *et al.*, 2008; Valentine *et al.*, 2008). The results are equivocal with some studies reporting no benefit of protein-CHO consumption (Wojcik *et al.*, 2001; Green *et al.*, 2008) whilst others have demonstrated significant reductions in markers of muscle damage (Saunders, Kane & Todd, 2004; Romano-Ely *et al.*, 2006; Baty *et al.*, 2007; Luden, Saunders & Todd, 2007; Saunders, Luden & Herrick, 2007; Etheridge, Philp & Watt, 2008; Valentine *et al.*, 2008). Many researchers reporting benefits of protein-CHO consumption have derived their conclusions from measures of intramuscular proteins in the serum (creatine kinase (CK) and myoglobin (Mb)). Changes in biochemical markers are likely to be functionally irrelevant, as there is little relationship observed between CK and maximal isometric force (Nosaka & Clarkson, 1996). Furthermore, the impact of acute protein-CHO supplementation on alleviating muscle soreness and the reduced capacity to exercise is important to athletes. Only one study to date has measured muscle performance and observed a beneficial effect of acute protein-CHO supplementation following muscle-damaging exercise (Valentine *et al.*, 2008). Studies evaluating muscle function along with a variety of other indicators of EIMD found no beneficial effect of acute supplementation with a protein-CHO drink (Wojcik *et al.*, 2001; Green *et al.*, 2008; White *et al.*, 2008; Betts *et al.*, 2009). Methodological differences may have confounded results, such as the measurement of EIMD markers at only one time point, timing of supplementation, and the mode of exercise used to initiate EIMD. Many studies observing a benefit of protein-CHO supplementation have used endurance cycling to initiate damage (Saunders, Kane & Todd, 2004; Saunders, Luden & Herrick, 2007; Rowlands *et al.*, 2008; Valentine *et al.*, 2008). This mode of exercise is predominantly concentric in nature (Tee, Bosch & Lambert, 2007) and, therefore, damage may be primarily due to metabolic processes rather than mechanical damage.

The evidence for acute protein-CHO supplementation reducing EIMD has been contradictory. Many studies have only measured one indirect marker, which is not sufficient to provide overall conclusions with regards to the effect on EIMD as different degradative pathways are likely responsible for each indirect marker (refer to Figure 2.3). Furthermore, it is apparent that more research using muscle function as an indirect marker of EIMD is required, which from an applied perspective is important to investigate. Many of the studies conducted in this area have used whey

or milk-based protein. Milk and milk-based drinks are readily available and are a high-quality, inexpensive (Wojcik *et al.*, 2001) source of amino acids, with a similar CHO concentration to many sports drinks, that result in a positive protein balance after resistance exercise (Elliot *et al.*, 2006). The benefits of consuming milk-based drinks in other areas of sport nutrition have been investigated (Wojcik *et al.*, 2001; Karp *et al.*, 2006; Shirreffs, Watson & Maughan, 2007). Previously, studies investigating the effect of protein-CHO and CHO drinks on recovery from muscle damaging exercise have matched for energy intake (Wojcik *et al.*, 2001) or CHO content (Luden, Saunders & Todd, 2007) resulting in different volumes of drink being consumed. This study compared commercially available drinks in volumes that athletes could realistically consume post exercise. The aim of this study was to investigate the use of commercially available milk and milk-based protein-CHO supplements in attenuating EIMD following resistance- based eccentric exercise.

4.2   Methods

4.2.1 Participants

Twenty-four healthy male participants (age 21 ± 3 years; stature 180.8 ± 5.7 cm; mass 80.2 ± 9.1 kg) who regularly competed in team sports (football, rugby, hockey and cricket) volunteered to take part in the study. Participants were tested within 30 minutes of the same time of day to minimise diurnal variation.

4.2.2 Experimental Design

Participants were assigned to 1 of 4 independent groups: (i) milk-based protein-CHO (CHO-P), (ii) milk (M), (iii) CHO, or (iv) control (CON). A one-way ANOVA revealed no group differences in baseline participant characteristics ($p > 0.05$). There were no significant differences between groups in peak torque values used for group allocation ($p > 0.05$).

Participants attended the laboratory on 3 consecutive days. The procedure for this is outlined in section 3.2. Following completion of the muscle damaging exercise bout on the initial day, participants immediately consumed 500 mL of their allocated nutritional supplement and again within 2 h post muscle damaging exercise.

4.2.3 Nutritional Supplement

The supplements compared are described in section 3.3. Nutrient content of the drinks was not standardised, as part of the purpose of this study was to use commercially available drinks. Each supplement is commercially available in 500 mL bottles, therefore participants were provided with 2 standard servings equating to 1000 mL.

4.2.4 Muscle Damaging Exercise

Muscle damage of the hamstrings was induced via unilateral eccentric-concentric knee flexions on the Cybex Isokinetic Dynamometer (Cybex Norm). Please refer to section 3.5 for more detail.

4.2.5 Muscle Soreness Measurement

This study assessed passive DOMS. Please refer to section 3.6 for more information.

4.2.6 Isokinetic Muscle Function

Participants' peak torque and total work of the set for 6 maximal concentric knee flexions was measured. Please refer to section 3.7.1 for more detail.

4.2.7 Blood Sampling and Analysis

Venous blood was collected as outlined in section 3.8. This was used for the analysis of CK (Section 3.8.1) and Mb (Section 3.8.2).

4.2.8 Statistical Analysis

All dependent variables were analysed using factorial ANOVA's and appropriate post-hoc tests. Please refer to section 3.9 for details.

4.3   Results

4.3.1 Evidence of Muscle Damage

For all participants the protocol was deemed to have caused EIMD in both legs. This was evident from reductions in isokinetic muscle performance and increases CK, Mb, and DOMS over the 2 subsequent days of testing (Table 4.1).

Table 4.1 Muscle soreness, blood markers and isokinetic muscle function following EIMD in all groups combined

Variable	baseline	24 h	48 h
DOMS (cm)	0.7 ± 0.2	$5.1 \pm 0.4^*$	$7.1 \pm 0.4^{**}$
Creatine Kinase (U/l)	96.6 ± 18.7	$331.8 \pm 65.7^*$	$539.1 \pm 104.4^{**}$
Myoglobin (ng/ml)	55.1 ± 14.8	$240.6 \pm 68.7^*$	$331.8 \pm 82.7^*$
Peak Torque Dominant (Nm)	123 ± 3	115 ± 5	$100 \pm 7^{**}$
Peak Torque Non-Dominant (Nm)	118 ± 3	$101 \pm 4^*$	$90 \pm 6^{**}$
Total Work of Set Dominant (J)	981 ± 28	$798 \pm 41^*$	$681 \pm 61^{**}$
Total Work of Set Non-Dominant (J)	920 ± 31	$718 \pm 35^*$	$608 \pm 53^{**}$

Values presented as mean ± standard error of the mean
*Significantly different from 0 h ($p < 0.05$)
**Significantly different from 0 h and 24 h ($p < 0.05$)

4.3.2   Effects of Nutritional Supplement

4.3.2.1 Muscle Soreness

No significant differences between groups ($F_{3, 20} = 2.617$, $p = 0.079$) or significant interaction effects between time and group ($F_{6, 40} = 1.323$, $p = 0.270$) were observed for DOMS (Figure 4.1).

Figure 4.1 Muscle soreness response to exercise-induced muscle damage in the M (n = 6), CHO-P (n = 6), CHO (n = 6) and CON (n = 6) groups. No significant differences were observed ($p > 0.05$). Values presented as mean ± standard error of the mean. M = milk; CHO-P = milk-based CHO-protein; CHO = carbohydrate sports drink; CON = control

4.3.2.2 Isokinetic Muscle Function

A significant main effect for leg ($F_{1, 18}$ = 22.115, $p < 0.001$) and a significant leg x group interaction ($F_{3, 18}$ = 5.397, $p = 0.008$) were found for peak torque. There were significant decreases in peak torque for the dominant leg between baseline and 48 h in the control group only. Post hoc tests demonstrated that for the dominant leg, peak torque was significantly higher after 48 h in the milk-based protein-CHO group compared with the control and CHO groups, and in the milk group compared with the CHO group (Figure 4.2).
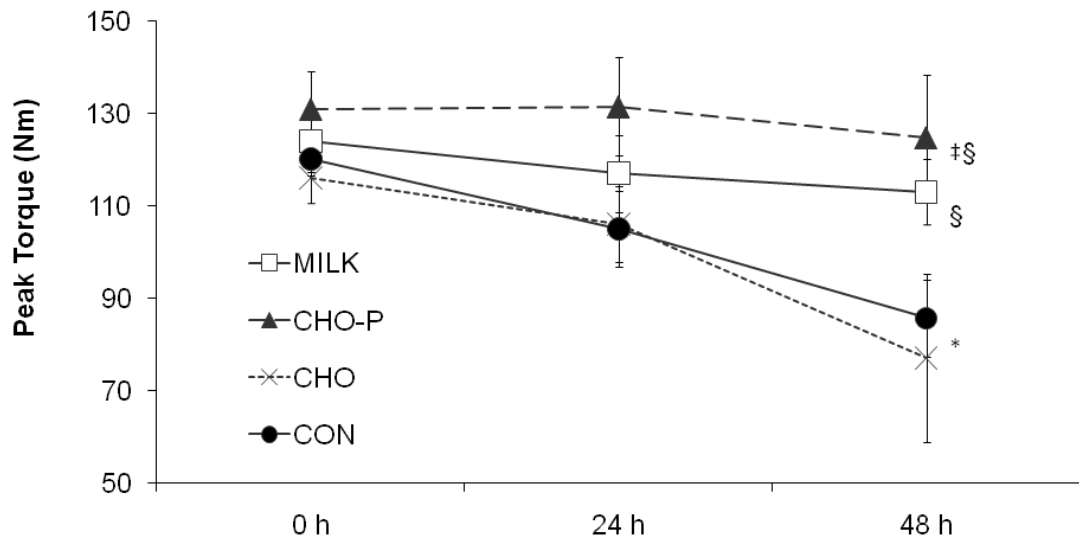
Figure 4.2 Peak torque of the dominant leg in response to exercise–induced muscle damage in the M (n = 5), CHO-P (n = 6), CHO (n = 6), and CON (n = 5) groups. *, significantly different from 0 h ($p < 0.05$); ‡, significantly different from CON ($p < 0.05$); §, significantly different from CHO ($p < 0.05$). Values presented as mean ± standard error of the mean. M = milk; CHO-P = milk-based protein-CHO; CHO = carbohydrate sports drink; CON = control

There were significant decreases of peak torque for the non-dominant leg between baseline and 48 h in the control group only. Post hoc tests demonstrated that for the non-dominant leg, peak torque was significantly higher at 48 h in the milk-based protein-CHO and milk groups than in the control group.

A significant main effect for leg ($F_{1, 18}$ = 30.645, $p < 0.001$) and significant leg x group interaction ($F_{3, 18}$ = 7.010, $p = 0.003$) were found for total work of the set. There were significant decreases in total work of the set over time for the dominant leg in the control (baseline - 24 h and baseline - 48 h), CHO (24 - 48 h and baseline - 48 h), milk (baseline - 48 h), and milk-based protein-CHO (baseline - 48 h) groups. Post hoc tests revealed that for the dominant leg, total work of the set at 48 h was significantly higher in the milk-based protein-CHO and milk groups than in the CHO and control groups (Figure 4.3).
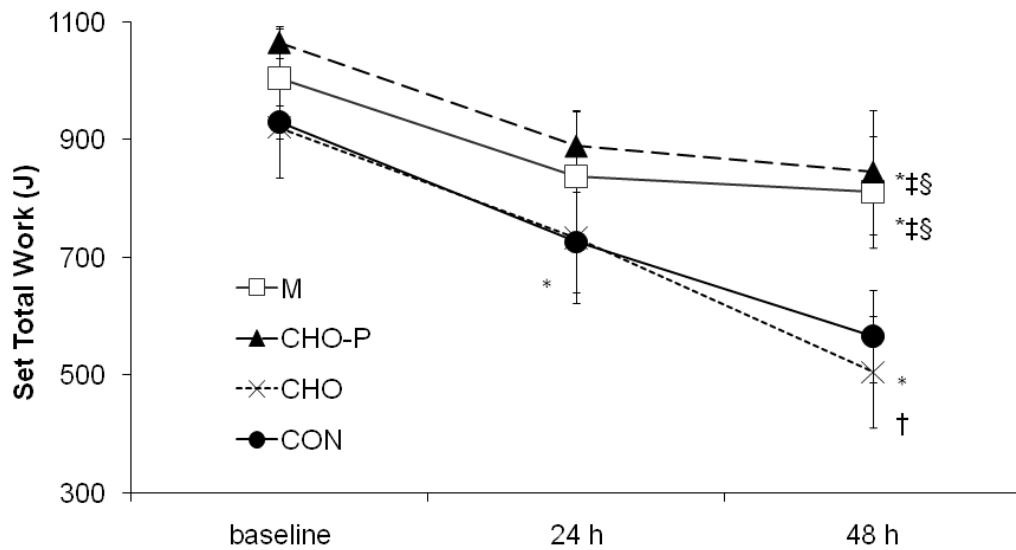
Figure 4.3 Total work of the set of the dominant leg in response to exercise-induced muscle damage in the M (n = 5), CHO-P (n = 6), CHO (n = 6), and CON (n = 5) groups. *, significantly different from 0 h ($p < 0.05$); †, significantly different from 0 and 24 h ($p < 0.05$); ‡, significantly different from CON ($p < 0.05$); §, significantly different from CHO ($p < 0.05$). Values presented as mean ± standard error of the mean. M = milk; CHO-P = milk-based protein-CHO; CHO = carbohydrate sports drink; CON = control

There were significant decreases in total work of the set over time for the non-dominant leg in the control (baseline - 24 h and baseline - 48 h), CHO (baseline - 48 h and 24 - 48 h), milk (baseline - 24 h and baseline - 48 h), and milk-based protein-CHO (baseline - 24 h and baseline - 48 h) groups. Post hoc tests revealed that for the non-dominant leg, total work of the set at 48 h was significantly higher in the milk-based protein-CHO group than in the control group.

4.3.2.3 Intramuscular Proteins in the Serum

CK analysis identified no significant main effect for groups ($F_{3, 18} = 2.093$, $p = 0.137$). A significant time x group interaction effect over the 3 days of testing ($F_{6, 36} = 3.124$, $p = 0.014$) was observed for CK. Post hoc tests revealed significantly lower CK values at 48 h in the milk and milk-based protein-CHO groups than in the CHO group (Figure 4.4). CK concentrations significantly increased from baseline to 48 h in the control and CHO groups only. Significant increases in CK were observed between 24 and 48 h for the CHO group only.
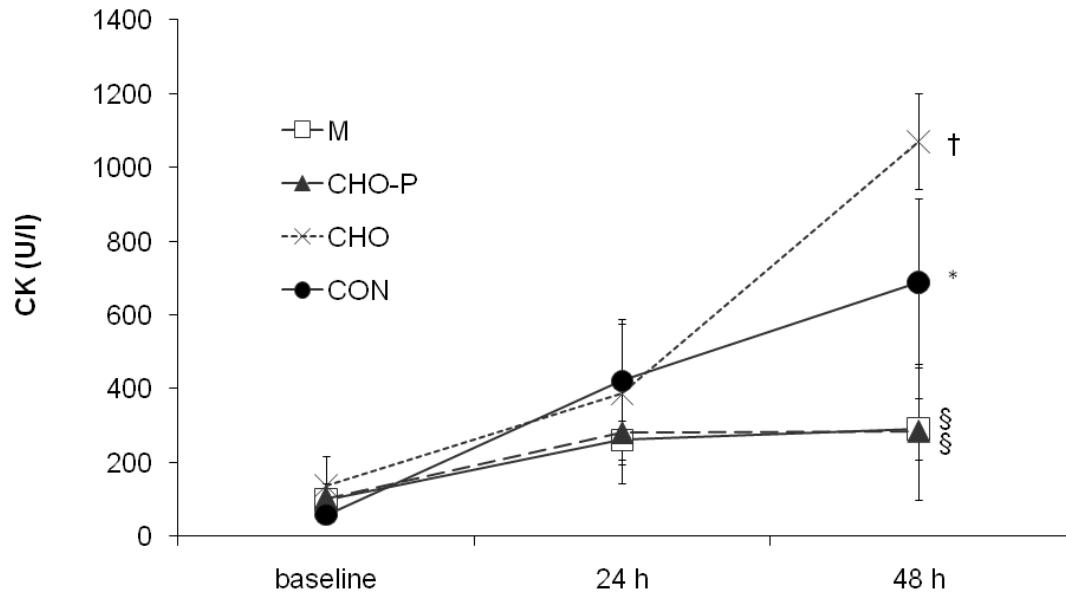
Figure 4.4 Creatine Kinase (CK) response to exercise-induced muscle damage in the M (n = 6), CHO-P (n = 6), CHO (n = 4), and CON (n = 6) groups. *, significantly different from 0 h ($p < 0.05$); †, significantly different from 0 and 24 h ($p < 0.05$); §, significantly different from CHO ($p < 0.05$). Values presented as mean ± standard error of the mean. M = milk; CHO-P = milk-based protein-CHO; CHO = carbohydrate sports drink; CON = control

A significant main effect for group ($F_{3, 18}$ = 3.399, p = 0.040) was found for Mb. Post hoc tests revealed that Mb concentrations in the milk and milk-based protein-CHO groups were significantly lower than in the CHO group (Figure 4.5). Running a one-way ANOVA for changes in baseline after 48 h revealed a significant effect for group ($F_{3, 18}$ = 3.628, p = 0.033). Post hoc tests revealed that Mb concentration in the milk-based protein-CHO group was significantly lower than in the CHO group ($p < 0.05$).
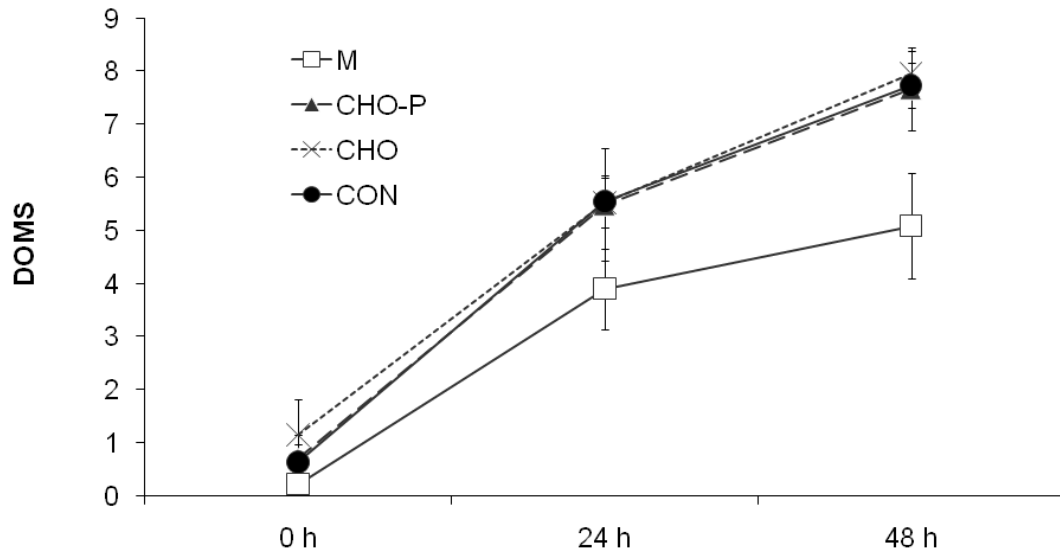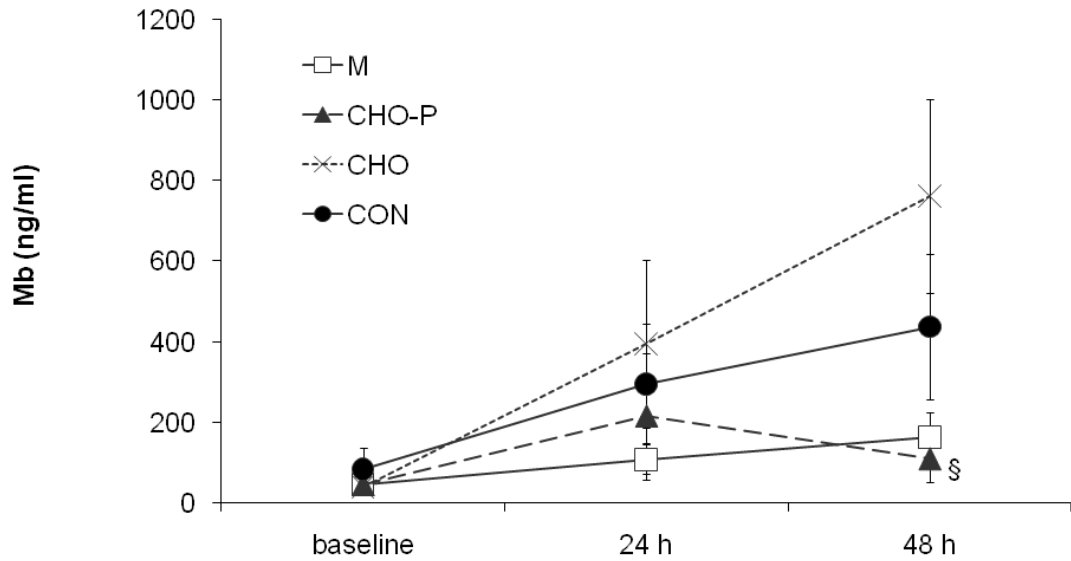
Figure 4.5 Myoglobin (Mb) response to exercise-induced muscle damage in the M (n = 6), CHO-P (n = 6), CHO (n = 4), and CON (n = 6) groups. §, significantly different from CHO ($p < 0.05$). Values presented as mean ± standard error of the mean. M = milk; CHO-P = milk-based protein-CHO; CHO = carbohydrate sports drink; CON = control

4.4   Discussion

The primary finding of this study demonstrated that milk and milk-based protein-CHO supplementation attenuated changes in peak torque, total work of the set, CK and Mb 48 h after muscle damaging exercise in comparison to CHO and a control. There were no beneficial effects of consuming milk or milk-based protein-CHO on perceptions of passive muscle soreness.

There are several potential mechanisms underlying these findings. The main theory is that the intake of protein and CHO lead to a positive net muscle protein balance. This has been previously observed with the intake of milk following resistance exercise (Elliot *et al.*, 2006). Protein intake will increase amino acid availability (Tipton *et al.*, 2007) and CHO will provide the optimal hormonal environment by increasing insulin concentrations (Miller *et al.*, 2003; Borsheim *et al.*, 2004). Increased exogenous amino acid availability could coincide with increases in insulin concentrations that would allow insulin to exert its effect on protein synthesis without the need for elevated protein degradation rates. Thus

muscle protein synthesis will be increased (Rasmussen *et al.*, 2000; Miller *et al.*, 2003; Tang *et al.*, 2007) with no concomitant increases in muscle protein breakdown (Bird, Tarpenning & Marino, 2006).

Following eccentric exercise the muscle is in a state of catabolism, therefore, ingesting milk and milk-based protein-CHO may have lead to a state of anabolism or a reduced state of catabolism. A positive protein balance could limit ultrastructural damage or promote repair. As a consequence, myofibrillar disruption and cell membrane integrity would be maintained. Myofibrillar disruption occurs via the ubiquitin-proteosome (Ub-P) pathway and the increase in myofibrillar disruption following resistance exercise is reduced following the consumption of a CHO-amino acid supplement (Bird, Tarpenning & Marino, 2006). Limiting myofibrillar disruption would impact on muscle function as these two variables have been shown to be significantly related (Lauritzen *et al.*, 2009; Raastad *et al.*, 2010). Furthermore, a relationship between muscle function and the percentage of desmin-negative fibres has been observed (Lieber *et al.*, 1994). With regards to limiting increases in Ck and Mb, changes in these variables are primarily due to the degradation of membrane phospholipids via cytokines and PLA_2 activation. PLA_2, which is significantly correlated to CK (Milias *et al.*, 2005), leads to PGE_2 production resulting in lysosomal protein degradation. The lysosomal pathway is responsible for non-myofibrillar protein degradation, and is responsive to changes in amino acid concentration (Fulks, Li & Goldberg, 1975) and insulin (Mortimore, Ward & Schworer, 1978) that would result from protein-CHO ingestion. Therefore, milk ingestion would reduce lysosomal protein degradation and/or increase non-myofibrillar protein synthesis resulting in reduced membrane degradation or enhanced repair. The maintenance of myofibrillar protein and membrane integrity is in agreement with other studies who have found beneficial effects of protein-CHO ingestion (Saunders, Kane & Todd, 2004).

There were no significant differences in isokinetic muscle performance, CK or Mb between milk and milk-based protein-CHO. Both of these nutritional supplements contained similar amounts of protein. Thus any effect on attenuating EIMD based on protein ingestion would be similar for both groups. The milk and milk-based protein-CHO supplements did differ in terms of CHO content with milk-based protein-CHO containing approximately 2.5 times more CHO. CHO increases insulin concentrations (Miller *et al.*, 2003; Borsheim *et al.*, 2004), which can increase the

muscles' capacity for protein synthesis (Jefferson, Li & Rannels, 1977) possibly through increased amino acid uptake (Borsheim, Aarsland & Wolfe, 2004), and may attenuate increases in degradation (Denne *et al.*, 1991). Previous research has indicated that as little as 35 g of sucrose in combination with 6 g of amino acid will promote muscle anabolism (Rasmussen *et al.*, 2000; Tipton *et al.*, 2001). Both these supplements had a greater amount of CHO than this. There may be a ceiling effect to CHO content; therefore, any effect of CHO on protein metabolism would be similar for both supplements. The CHO content of the milk and milk-based supplements may not have influenced protein metabolism, however, the supplements contained different types of CHO with different glycemic effects that could have affected postprandial insulin response, thereby impacting on protein metabolism. However, postprandial insulin response is not significantly different following the consumption of different CHO types (sucrose, maltodextrin and honey powder containing fructose, glucose and maltose) co-ingested with whey protein after resistance exercise (Kreider *et al.*, 2007). Therefore, the different CHO types would not affect protein metabolism and thus the attenuation of EIMD differently. Furthermore, Bird, Tarpenning & Marino (2006) have indicated that insulin does not play a role in the regulation of myofibrillar protein degradation, possibly as the Ub-P pathway is insensitive to insulin (Kettlehut, Wing & Goldberg, 1988). This may explain why there were no differences between the two groups in some of the measured variables.

The blunting of EIMD following milk and milk-based protein-CHO supplementation was not apparent until 48 h after muscle-damaging exercise. Ultrastructural damage becomes progressively worse in the days following eccentric exercise, with more damage being observed during 24 - 48 h after exercise (Newham *et al.*, 1983) or 3 days later (Friden, Sjostrom & Ekblom, 1983). This may partly be because protein degradation rates do not increase until 24 h later (Lowe *et al.*, 1995; Wojcik *et al.*, 2001), peaking at 48 h (Lowe *et al.*, 1995). Overall, protein balance would remain negative. This may imply that changes in the measured variables before 48 h are not due to ultrastructural damage via protein degradation, initiated during the secondary phase, but to other processes that may be mechanical in nature. With regards to muscle function, excitation-contraction (E-C) coupling disruption is primarily responsible for the initial decrements observed, with damage to the force generating and transmitting structures implicated from 48 h (Warren *et al.*, 2002; Raastad *et al.*, 2010). Altering protein metabolism would not significantly impact on E-C coupling; therefore, there

would be no influence of protein-CHO ingestion during this phase of EIMD. In support of this, increases in urinary 3-methylhistadine (3-MH) excretion are not significantly limited until 48 h after resistance exercise with the consumption of a CHO-essential amino acid supplement (Bird, Tarpenning & Marino, 2006). This may be why, in the present study, the attenuation of EIMD was not observed until 48 h after muscle damaging exercise.

Attenuations of CK and Mb are in agreement with previous work (Saunders, Kane & Todd, 2004; Seifert *et al.*, 2005; Baty *et al.*, 2007; Saunders, Luden & Herrick, 2007; Valentine *et al.*, 2008). However, this is only the second investigation to show a reduction in the impact of EIMD on isokinetic muscle performance, CK, and Mb following acute milk and milk-based protein-CHO consumption. Similar studies previously conducted found no beneficial effects of protein-CHO ingestion on a number of measures of EIMD including isokinetic muscle performance (Wojcik *et al.*, 2001; Green *et al.*, 2008; White *et al.*, 2008; Betts *et al.*, 2009). These studies differed in their methodological approaches (mode of muscle damaging exercise; type of supplement; timing of ingestion; measurement points), which may explain the contradictory findings. For example, the mode of muscle damaging exercise may affect results due to the different types (metabolic or mechanical) of muscle stress experienced which may influence the level of activation and type of degradative pathways. Furthermore, a number of studies have measured variables at only one time point. It is accepted that damage is exacerbated over a number of days, therefore, these studies may have missed the time point at when protein-CHO supplements could have been beneficial.

Milk and milk-based protein-CHO had no significant effect on the DOMS. It has been suggested that functional and biochemical measures are preferred when comparing group differences in EIMD (Rodenburg, Bar & De Boer, 1993). The reason for this finding may be two-fold. Firstly DOMS is subjective (Rodenburg, Bar & De Boer, 1993), making it difficult to compare between independent groups. This may be the reason for no observed differences between groups. EIMD was only induced in the hamstrings. The hamstrings are only a small muscle group in relation to the whole body, which may have affected individual perceptions of DOMS. Secondly, the pathway responsible for DOMS is different to those that are implicated in the changes in intramuscular proteins and muscle performance (refer to Figure 2.3). It is

unlikely that alterations in protein metabolism would impact on perceptions of muscle soreness.

The results demonstrate that the intake of CHO alone had no beneficial effect on attenuating EIMD. This is partly in agreement with Dalton *et al* (1999) who found CHO intake had no beneficial effect on EIMD as indicated by measurements of CK. This is an interesting finding, as athletes traditionally consume CHO when recovering from exercise (Millard-Stafford *et al.*, 2005). CHO is a necessary component for a recovery drink as it is required for glycogen re-synthesis (Van Hall, Shirreffs & Calbet, 2000). However, consuming CHO and protein will be more beneficial for recovery with regards to glycogen re-synthesis and protein metabolism. This indicates that athletes may benefit more from consuming milk or a milk-based protein-CHO supplement after eccentric exercise. The Ub-P pathway, which is speculated to lead to decrements in muscle function, is insensitive to insulin (Kettlehut, Wing & Goldberg, 1988). This explains why there was no beneficial effect of CHO intake on muscle performance. The proteolytic activity of lysosomes can be affected by insulin, however, it is interesting to note that based on CK and Mb data, CHO consumption appeared to exacerbate the damage. It has previously been stated that differences in CK levels do not provide information on the differences in magnitude of EIMD (Friden & Lieber, 2001). This is an important finding considering the 'normal' dietary practices of athletes during recovery, which is likely to include a CHO sports drink (Millard-Stafford *et al.*, 2005).

Participants were required to maintain their normal diet to replicate free living, however, this was not recorded. The limitation of this is that diet was not strictly controlled and caloric and macronutrient content of each group's diet could not be assessed. However, it would be expected that participants with high protein intake would be equally distributed across groups. Furthermore, both groups consuming protein and CHO demonstrated similar patterns of results. This would be unlikely if participants with high habitual protein intakes were allocated to one group. The sample size for peak torque, CK and Mb was reduced. However, significant differences were still observed which implies that the sample size provided sufficient power.

In conclusion, milk and milk-based protein-CHO drinks consumed immediately after

resistance-based eccentric muscle damaging exercise leads to the reduction of EIMD 48 h later. This may be due to altered protein metabolism limiting the disruption of myofibrillar protein and membrane structures and thus reducing the impact of EIMD on physical performance and biochemical markers.

94

5. EFFECT OF ACUTE MILK-BASED PROTEIN-CHO SUPPLEMENT TIMING ON THE ATTENUATION OF EIMD

5.1   Introduction

The first study of this thesis demonstrated that consuming milk or milk-based protein-carbohydrate (CHO) immediately following muscle damaging exercise limited decrements in muscle function and increases in creatine kinase (CK) and myoglobin (Mb) over 48 h, with no effect on passive muscle soreness. Exercise induced muscle damage (EIMD) may increase degradation of the protein and membrane structures leading to myofibrillar disruption, and the loss of cell membrane integrity (Armstrong, Warren & Warren, 1991; Evans & Cannon, 1991; Gissel, 2005; Zhang *et al.*, 2008). The benefit derived from milk-based protein-CHO may be from altered protein balance by providing additional protein to increase amino acid availability (Tipton *et al.*, 2007) and CHO to increase insulin concentrations (Miller *et al.*, 2003). Changes in protein balance may limit the degradation of protein and membrane structures or enhance their repair, and as a consequence limit changes in muscle performance and intramuscular protein release.

Research investigating the effect of protein-CHO on recovery from EIMD have provided the supplement at different times around muscle damaging exercise (before, during and/or after), and hence there is very little consistency in ascertaining the optimal timing of protein-CHO ingestion. The effects of supplement timing before and following resistance exercise on muscle protein balance have been researched (Tipton *et al.*, 2001; Tipton *et al.*, 2007); consuming essential amino acids and CHO prior to exercise results in greater amino acid uptake by the muscle in comparison to ingestion following exercise (Tipton *et al.*, 2001). However, the response to timing of whey protein ingestion was not different between pre and post exercise consumption (Tipton *et al.*, 2007). The reason for the different findings may be twofold. Firstly, CHO intake increases insulin concentrations (Miller *et al.*, 2003), which has been shown to increase protein synthesis via improved amino acid availability (Biolo, Fleming & Wolfe, 1995) and limit myofibrillar breakdown (Roy *et al.*, 1997), but CHO was not consumed with whey protein (Tipton *et al.*, 2007). Secondly, whey protein needs to be digested before amino acids are available for protein metabolism; therefore, the time of amino acid availability may be responsible for the difference. It is therefore, plausible that timing of protein-CHO ingestion may impact on protein metabolism following muscle damaging exercise and, therefore, may affect changes in intramuscular protein release into the plasma and muscle function.

To date there has been only one study investigating the timing of protein-CHO supplementation on indirect markers of muscle damage (White *et al.*, 2008). White *et al* (2008) found no effect on the reduction of muscle damage indices when comparing a whey protein-CHO supplement against a control in the 96 h following damaging exercise. As a consequence no effect of timing on response was found, thereby limiting any conclusions that can be drawn. The first study provided evidence that milk-based protein-CHO has a beneficial impact on EIMD. However, there is a lack of data concerning the optimal timing of milk-based protein-CHO supplementation for limiting changes in muscle damage indices. Therefore, the aim of this investigation was to compare pre and post milk-based protein-CHO supplementation on the alleviation of EIMD.

5.2   Methods

5.2.1 Participants

Thirty-two healthy male participants (age 20 ± 2 years; stature 180.3 ± 4.8 cm; mass 78.5 ± 9.0 kg) who regularly competed in a variety of sports (team and individual) volunteered to take part in the study. Participants were tested in the morning to minimise diurnal variation.

5.2.2 Experimental Design

Participants were assigned to 1 of 4 independent groups: (i) milk-based protein-CHO consumed pre-muscle damaging exercise, water consumed at all other time points (PRE), (ii) milk-based protein-CHO consumed immediately post-muscle damaging exercise, water consumed at all other time points (POST), (iii) milk-based protein-CHO consumed 24 h post-muscle damaging exercise, water consumed at all other time points (TWENTY-FOUR), or (iv) water consumed at all time points (CON). A one-way ANOVA revealed no group differences in baseline participant characteristics ($p > 0.05$). There were no significant differences between groups in peak torque values used for group allocation ($p > 0.05$).

All participants were required to attend the laboratory on 4 consecutive days. The procedure for this is outlined in section 3.2. Following baseline testing, participants consumed 1000 mL of their allocated supplement within 30 min, and then immediately completed a bout of exercise designed to induce acute muscle damage. Upon completing the exercise bout they immediately consumed 1000 mL of their allocated supplement within 30 min. At 24 h post muscle damaging exercise, prior to any testing, participants consumed 1000 mL of their allocated supplement.

5.2.3 Nutritional Supplement

This study provided participants with the milk-based protein-CHO supplement used in study 1 (Chapter 4). Participants were provided with 1000 mL of this supplement as the first study demonstrated that this supplement and volume resulted in the significant attenuation of decreases in isokinetic muscle function and increases in creatine kinase and myoglobin. Please refer to section 3.3 for nutritional content of this supplement.

5.2.4 Muscle Damaging Exercise

Muscle damage of the hamstrings was induced via unilateral eccentric-concentric knee flexions on the Cybex Isokinetic Dynamometer (Cybex Norm). Please refer to section 3.5 for more detail.

5.2.5 Muscle Soreness Measurement

Participants were required to rate both passive and active muscle soreness. Participants were asked to rate perceived muscle soreness in their hamstrings during concentric knee flexions. Please refer to section 3.6 for further information.

5.2.6 Muscle Function

5.2.6.1 Peak Torque

Participant's peak torque of 6 maximal concentric knee flexions was measured. Please refer to section 3.7.1 for more detail.

5.2.6.2 Reactive Strength Index

Reactive strength index was calculated from the mean of 3 drop jumps. Please refer to section 3.7.2 for more detail.

5.2.7 Blood Sampling and Analysis

Venous blood was collected as described in section 3.8. This was used for the analysis of CK (section 3.8.1).

5.2.8 Statistical Analysis

Dependent variables were analysed using magnitude based inferences. Please refer to section 3.9 for more detail.

5.3   Results

5.3.1 Muscle Soreness

All groups showed an increase in passive and active muscle soreness (DOMS) for both legs. Passive and active muscle soreness peaked at 48 h for all groups and at 72 h each group began to return to baseline levels. For changes in passive DOMS all comparisons between baseline and 48 h, and baseline and 72 h were unclear. The exception was that consuming milk-based protein-CHO before muscle damaging exercise had a likely benefit for reducing soreness in comparison to consuming milk-based protein-CHO 24 h following muscle damaging exercise between baseline and 72 h. The p value for the main interaction effect was 0.972. For changes in active DOMS (dominant) from baseline to 48 h consuming milk-based protein-CHO immediately following muscle damaging exercise had a likely and possible benefit for reducing increases in soreness compared to the control group and consuming milk-based protein-CHO before muscle damaging exercise, respectively. Consuming milk-based protein-CHO before muscle damaging exercise also had a possible beneficial effect for reducing increases in soreness in comparison to the control group. Between baseline and 72 h, consuming milk-based protein-CHO before muscle damaging exercise had a likely beneficial effect for recovery of active DOMS in comparison to the control group and consuming milk-based protein-CHO 24 h following muscle damaging exercise. All

other comparisons were unclear (Figure 5.1). A summary of the statistical analysis for active DOMS is shown in Table 5.1. The *p* value for the main interaction effect was 0.827.

Figure 5.1 Active muscle soreness of the dominant leg in response to exercise-induced muscle damage in the CON (n = 8), PRE (n = 8), POST (n = 8), and TWENTY-FOUR (n = 8) group. Values presented as mean ± standard deviation. CON = control; PRE = milk-based protein-CHO consumed before muscle damaging exercise; POST = milk-based protein-CHO consumed immediately following muscle damaging exercise; TWENTY-FOUR; milk-based protein-CHO consumed 24 h following muscle damaging exercise

Table 5.1 Effect of supplement timing on increases in active muscle soreness of the dominant leg following muscle damaging exercise

Comparison	Mean effect [a]± 90% CI[b]	Qualitative Inference
baseline - 48 h		
PRE v CON	-0.6 ± 1.9	Decrease Possible
POST v CON	-1.4 ± 2.4	Decrease Likely
TWENTY-FOUR v CON	-0.6 ± 2.3	Unclear
POST v PRE	-0.8 ± 2.3	Decrease Possible
TWENTY-FOUR v PRE	0.0 ± 2.2	Unclear
TWENTY-FOUR v POST	0.8 ± 2.6	Unclear
baseline - 72 h		
PRE v CON	-1.2 ± 2.1	Decrease Likely
POST v CON	-0.8 ± 2.7	Unclear
TWENTY-FOUR v CON	-0.2 ± 2.4	Unclear
POST v PRE	0.4 ± 2.2	Unclear
TWENTY-FOUR v PRE	1.1 ± 1.9	Increase Likely
TWENTY-FOUR v POST	0.6 ± 2.5	Unclear

[a]Mean effect refers to the first named group minus second named; [b]±90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude. CON = control group; PRE = milk-based protein-CHO consumed before muscle damaging exercise; POST = milk-based protein-CHO consumed immediately following muscle damaging exercise; TWENTY-FOUR = milk-based protein-CHO consumed 24 h following muscle damaging exercise

5.3.2 Muscle Function

A summary of the statistical analysis is shown in Table 5.2.

Table 5.2 Effect of supplement timing on decreases in muscle function following muscle damaging exercise

Muscle Performance	Comparison	Mean effect[a] ± 90% CI[b]	Qualitative Inference
baseline - 48 h			
Peak Torque (DOM)	PRE v CON	2 ± 26	Unclear
	POST v CON	22 ± 30	Increase Likely
	TWENTY-FOUR v CON	16 ± 28	Increase Likely
	POST v PRE	20 ± 21	Increase Likely
	TWENTY-FOUR v PRE	14 ± 18	Increase Likely
	TWENTY-FOUR v POST	-5 ± 24	Unclear
RSI	PRE v CON	-8 ± 25	Unclear
	POST v CON	17 ± 24	Increase Likely
	TWENTY-FOUR v CON	22 ± 19	Increase Likely
	POST v PRE	26 ± 27	Increase Likely
	TWENTY-FOUR v PRE	32 ± 23	Increase Very Likely
	TWENTY-FOUR v POST	3 ± 21	Unclear
baseline - 72 h			
Peak Torque (DOM)	PRE v CON	24 ± 28	Increase Likely
	POST v CON	29 ± 29	Increase Likely
	TWENTY-FOUR v CON	16 ± 30	Increase Possible
	POST v PRE	4 ± 16	Unclear
	TWENTY-FOUR v PRE	-7 ± 18	Unclear
	TWENTY-FOUR v POST	-11 ± 19	Decrease Possible
RSI	PRE v CON	-13 ± 31	Decrease Possible
	POST v CON	8 ± 27	Unclear
	TWENTY-FOUR v CON	17 ± 19	Increase Likely
	POST v PRE	25 ± 35	Increase Likely
	TWENTY-FOUR v PRE	35 ± 29	Increase Likely
	TWENTY-FOUR v POST	8 ± 25	Unclear

[a]Mean effect refers to the first named group minus second named; [b]±90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude. CON = control group; PRE = milk-based protein-CHO consumed before muscle damaging exercise; POST = milk-based protein-CHO consumed immediately following muscle damaging exercise; TWENTY-FOUR = milk-based protein-CHO consumed 24 h following muscle damaging exercise.

5.3.2.1 Peak Torque

Baseline peak torque values for consuming milk-based protein-CHO before, immediately or 24 h following muscle damaging exercise, and the control group were 128 Nm, 127 Nm, 137 Nm and 131 Nm, respectively. At 48 h peak torque had reduced to 100 Nm, 121 Nm, 121 Nm and 103 Nm for the groups consuming milk-based protein-CHO before, immediately or 24 h following muscle damaging exercise, and the control group, respectively. At 72 h peak torque values for the groups consuming milk-based protein-CHO before, immediately or 24 h following muscle damaging exercise, and the control group were 119 Nm, 120 Nm, 115 Nm and 99 Nm, respectively.

There was a likely benefit of consuming milk-based protein-CHO immediately (-7 ± 30 %) and 24 h (-12 ± 25 %) following muscle damaging exercise for limiting decreases

in peak torque of the dominant leg between baseline and 48 h in comparison to consumption before muscle damaging exercise (-22 ± 14 %) and the control group (-24 ± 39 %).   There were no clear effects of the control group versus consuming milk-based protein-CHO before muscle damaging exercise or consuming milk-based protein-CHO immediately versus 24 h following muscle damaging exercise.   For changes between baseline and 72 h there was a likely benefit of consuming milk-based protein-CHO before (-10 ± 16 %) and immediately following (-6 ± 20 %) muscle damaging exercise, and a possible benefit of consuming milk-based protein-CHO 24 h following muscle damaging exercise (-16 ± 24 %) for limiting decreases in peak torque in comparison to the control group (-27 ± 42 %).   There was also a possible benefit of consuming milk-based protein-CHO immediately following muscle damaging exercise in comparison to consumption 24 h later (Figure 5.2).   The *p* value for the main interaction effect was 0.198.

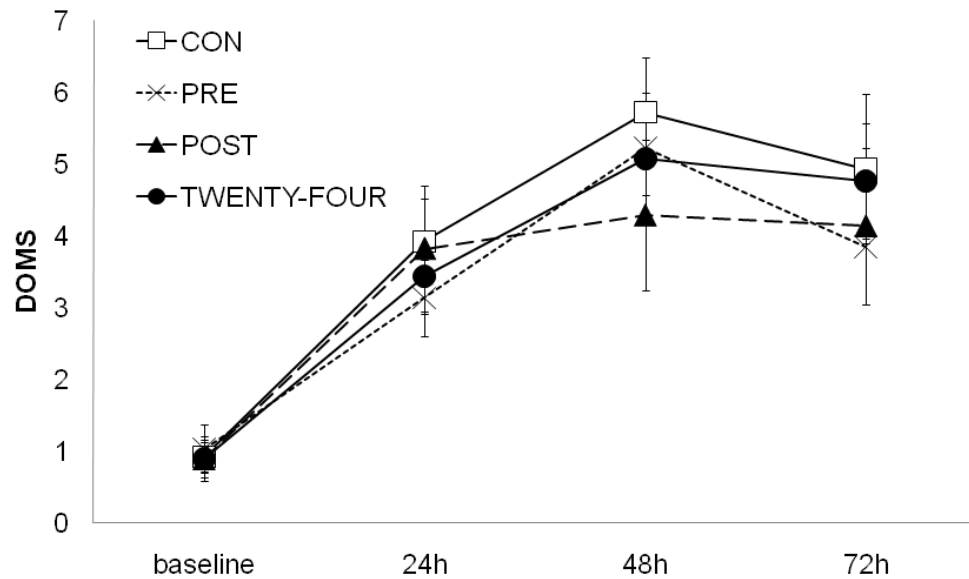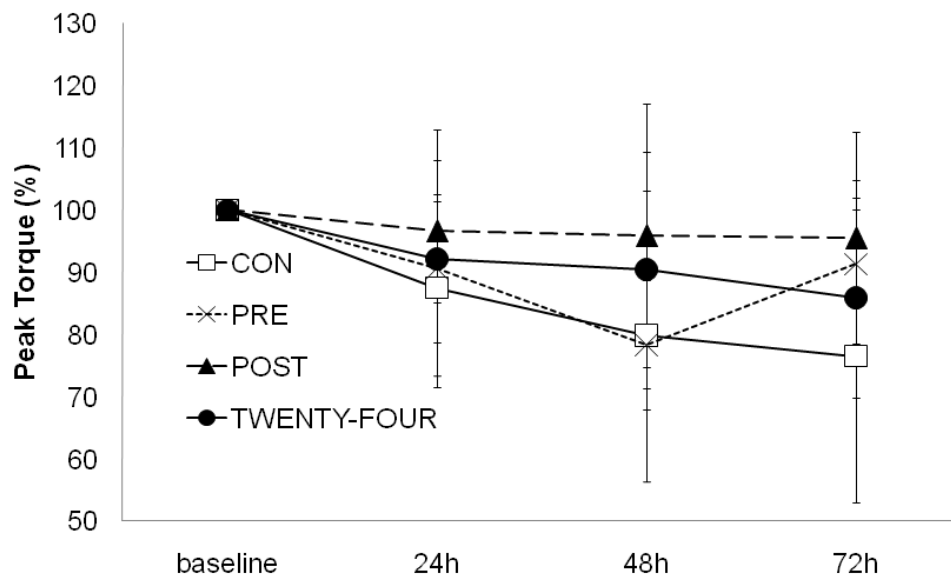Figure 5.2 Relative peak torque of the dominant leg in response to exercise-induced muscle damage in the CON (n = 8), PRE (n = 8), POST (n = 8), and TWENTY-FOUR (n = 8) group. Values are presented as mean ± standard deviation. CON = control; PRE = milk-based protein-CHO consumed before muscle damaging exercise; POST = milk-based protein-CHO consumed immediately following muscle damaging exercise; TWENTY-FOUR = milk-based protein-CHO consumed 24 h following muscle damaging exercise

5.3.2.2 Reactive Strength Index

Baseline reactive strength index values for consuming milk-based protein-CHO before, immediately or 24 h following muscle damaging exercise, and the control group were 118 cm.s^{-1}, 117 cm.s^{-1}, 95 cm.s^{-1} and 110 cm.s^{-1}, respectively. At 48 h reactive strength index had decreased to 85 cm.s^{-1}, 111 cm.s^{-1}, 87 cm.s^{-1} and 85 cm.s^{-1} for the groups consuming milk-based protein-CHO before, immediately or 24 h following muscle damaging exercise, and the control group, respectively. At 72 h reactive strength index for the groups consuming milk-based protein-CHO before, immediately or 24 h following muscle damaging exercise, and the control group were 89 cm.s$^{-1,}$ 112 cm.s^{-1}, 91 cm.s^{-1} and 91 cm.s^{-1}, respectively.

Over the first 48 h there was a likely benefit of consuming milk-based protein-CHO immediately following muscle damaging exercise (-11 ± 30 %) compared to the control group (-24 ± 24 %) and consumption before muscle damaging exercise (-30 ± 32 %) for attenuating decreases in reactive strength index. Consuming milk-based protein-CHO 24 h following muscle damaging exercise (-7 ± 18 %) also demonstrated a likely and very likely benefit for attenuating decreases in reactive strength index in comparison to the control group and consumption before muscle damaging exercise, respectively. Again there were no clear effects of consuming milk-based protein-CHO before muscle damaging exercises versus the control group and consumption of the supplement immediately following versus 24 h following muscle damaging exercise. Decrements in reactive strength index from baseline to 72 h were likely blunted by consuming milk-based protein-CHO 24 h following muscle damaging exercise (- 4 ± 16 %) compared to the control group (-18 ± 25 %) and consuming milk-based protein-CHO before muscle damaging exercise (-29 ± 43 %) and by consumption of the supplement immediately following (-11 ± 36 %) in comparison to consumption before muscle damaging exercise. There was also a possible harmful effect of consuming milk-based protein-CHO before muscle damaging exercise in comparison to the control group (Figure 5.3). The *p* value for the main interaction effect was 0.214.
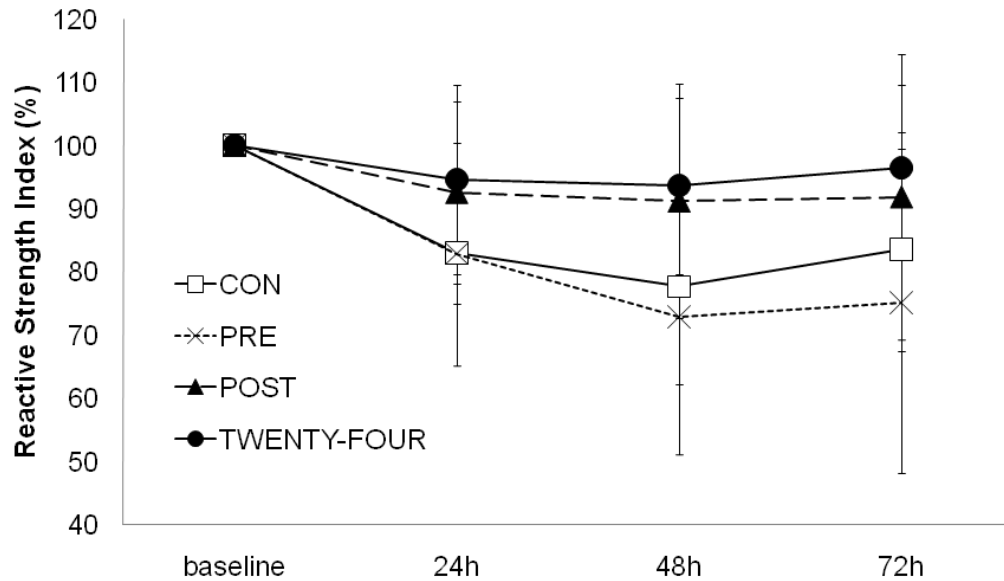
Figure 5.3 Relative reactive strength index in response to exercise-induced muscle damage in the CON (n = 8), PRE (n = 8), POST (n = 8), and TWENTY-FOUR (n = 7) groups. Values are presented as mean ± standard deviation. CON = control; PRE = milk-based protein-CHO consumed before muscle damaging exercise; POST = milk-based protein-CHO consumed immediately following muscle damaging exercise; TWENTY-FOUR = milk-based protein-CHO consumed 24 h following muscle damaging exercise

5.3.3 Creatine Kinase

Mean baseline CK values for each group were 154 $U.L^{-1}$, 307 $U.L^{-1}$, 218 $U.L^{-1}$ and 220 $U.L^{-1}$ for the control group and the groups consuming milk-based protein-CHO before, immediately following or 24 h following muscle damaging exercise, respectively. There was a likely benefit of consuming milk-based protein-CHO both immediately (6.31 ×/÷ 3.06) and 24 h (4.99 ×/÷ 7.62) following muscle-damaging exercise in comparison to the control group (16.48 ×/÷ 7.43) for blunting increases in CK between baseline and 48 h. There was also a possible benefit of consuming milk-based protein-CHO before muscle damaging exercise (7.54 ×/÷ 5.16) for blunting increases in CK in comparison to the control group. All other comparisons were unclear. Between baseline and 72 h there was a possible benefit of consuming milk-based protein-CHO immediately following (12.55 ×/÷ 3.59) and before (9.89 ×/÷ 7.05) muscle damaging exercise for recovery of CK in comparison to the control group (21.49 ×/÷ 7.51). There was no clear benefit of consuming the supplement 24 h following muscle damaging exercise (14.64 ×/÷

10.76) compared to the control group (Figure 5.4). A summary of the statistical analysis is shown in Table 5.3. The *p* value for the main interaction effect was 0.832.

Table 5.3 Effect of supplement timing on increases in Creatine Kinase (CK) following muscle damaging exercise

CK	Comparison	Mean effect[a] ×/÷ 90% CI[b]	Qualitative Inference
baseline - 48 h			
	PRE v CON	-1.54 ×/÷ 6.53	Possible Decrease
	POST v CON	-1.62 ×/÷ 5.57	Likely Decrease
	TWENTY-FOUR v CON	-1.70 ×/÷ 7.59	Likely Decrease
	POST v PRE	-1.16 ×/÷ 3.79	Unclear
	TWENTY-FOUR v PRE	-1.34 ×/÷ 5.87	Unclear
	TWENTY-FOUR v POST	-1.21 ×/÷ 4.87	Unclear
baseline - 72 h			
	PRE v CON	-1.54 ×/÷ 7.39	Possible Decrease
	POST v CON	-1.42 ×/÷ 5.87	Possible Decrease
	TWENTY-FOUR v CON	-1.32 ×/÷ 9.05	Unclear
	POST v PRE	1.30 ×/÷ 4.80	Unclear
	TWENTY-FOUR v PRE	1.48 ×/÷ 8.04	Unclear
	TWENTY-FOUR v POST	1.17 ×/÷ 6.45	Unclear

[a]Mean effect refers to the first named group minus second named; [b]×/÷90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude. CON = control group; PRE = milk-based protein-CHO consumed before muscle damaging exercise; POST = milk-based protein-CHO consumed immediately following muscle damaging exercise; TWENTY-FOUR = milk-based protein-CHO consumed 24 h following muscle damaging exercise.
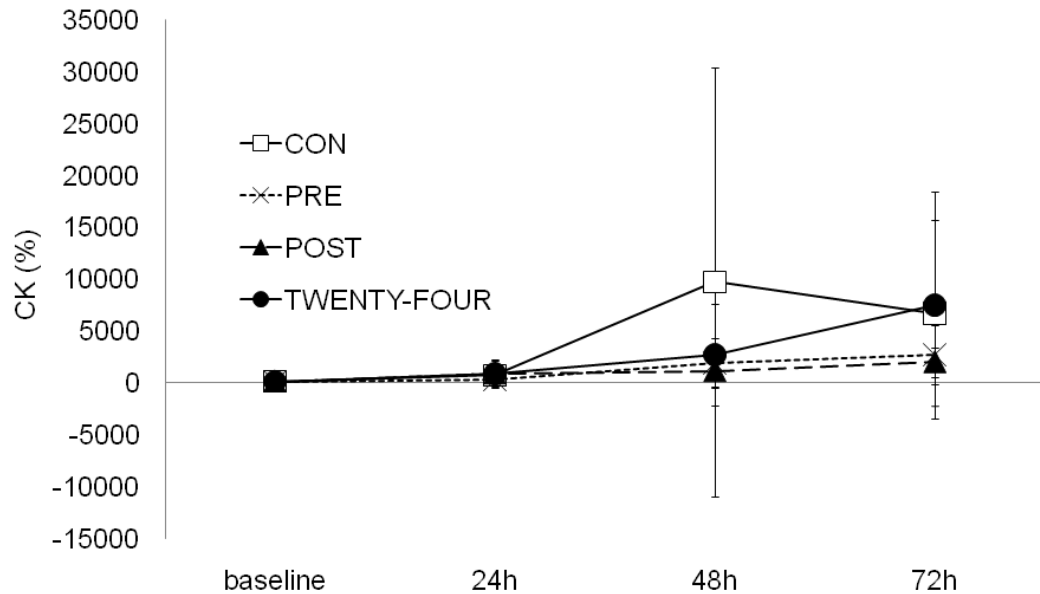
Figure 5.4 Relative Creatine Kinase (CK) in response to exercise-induced muscle damage in the CON (n = 6), PRE (n = 7), POST (n = 8), and TWENTY-FOUR (n = 7) groups. Values are presented as mean ± standard deviation. CON = control; PRE = milk-based protein-CHO consumed before muscle damaging exercise; POST = milk-based protein-CHO consumed immediately following muscle damaging exercise; TWENTY-FOUR = milk-based protein-CHO consumed 24 h following muscle damaging exercise

Only the results for the dominant leg have been presented as following analysis similar responses in both legs were evident.

5.4   Discussion

This study compared the consumption of milk-based protein-CHO before muscle damaging exercise to consumption immediately and/or 24 h following on the alleviation of indices of EIMD. The primary finding of the study was that consumption of a milk-based protein-CHO supplement immediately or 24 h following muscle damaging exercise was beneficial for blunting increases in active DOMS and decreases in muscle function over 48 h compared to pre-exercise supplementation. Over 72 h following muscle damaging exercise, pre-exercise supplementation was more beneficial for limiting changes in active DOMS and peak torque compared to a control. Finally, consuming milk-based protein-CHO at any time point was beneficial for attenuating increases in CK activity compared to the control group. In agreement with the first study there was no apparent benefit of milk-based protein-CHO ingestion at any time point on passive DOMS.

Previous studies (Saunders, Kane & Todd, 2004; Romano-Ely *et al.*, 2006; Baty *et al.*, 2007; Saunders, Luden & Herrick, 2007; Etheridge, Philp & Watt, 2008; Valentine *et al.*, 2008) have shown that the consumption of a protein-CHO supplement before, during and/or after exercise, leading to changes in indices of muscle damage, attenuates those markers. The current study provides further support for the use of protein-CHO supplements for the attenuation of EIMD. The reason for this blunting is speculated to be due to the provision of CHO and protein, which improves muscle protein balance by increasing synthesis and/or limiting increases in degradation. This may lead to blunting or enhanced repair of myofibrillar and membrane proteins reducing changes in indices of muscle damage.

The current study demonstrated a benefit of consuming milk-based protein-CHO post muscle damaging exercise on peak torque, reactive strength index and active DOMS. Consuming milk-based protein-CHO post exercise reduced the impact of muscle damage, possibly due to the replacement of amino acids lost to the increases in protein degradation that have been previously reported (Lowe *et al.*, 1995). In turn this could have limited ultrastructural damage and resulted in the observed effect on the measured variables. Previous research has demonstrated that milk ingestion post resistance exercise results in a positive net muscle protein balance (Elliot *et al.*, 2006). It is possible that consuming milk-based protein-CHO at 24h post had a positive effect as protein degradation rates start to increase at 24h (Lowe *et al.*, 1995; Wojcik *et al.*, 2001) peaking at 48h (Lowe *et al.*, 1995) following damaging exercise and protein synthesis rates remain below baseline (Lowe *et al.*, 1995). Therefore, consuming milk at a time point when the secondary phase is responsible for changes in EIMD indices rather than the primary mechanical event would allow the actions of CHO and protein to coincide with changes in protein balance. However, this hypothesis has yet to be investigated.

Consuming protein-CHO pre-muscle damaging exercise was not beneficial for reducing increases in active DOMS and decreases in muscle performance up to 48 h, with the response being similar to the control group. The reason for this may be linked to the availability of nutrients when they are required. To promote changes in protein metabolism within the muscle, a change in the intracellular pool of amino acids must occur. The process of whole protein intake to increased availability of amino acids involves gastric emptying which could indirectly effect the rate of amino

acid intestinal absorption (Gary, 1991) and thus the availability of amino acids. The gastric emptying halftime for a milk protein solution has been shown to be 26 minutes (Calbet & MacLean, 1997). The muscle damaging exercise trial lasts approximately 30 minutes which means that pre-exercise ingestion provides amino acids at a time when primary mechanical factors influence changes in EIMD indices. By providing milk-based protein-CHO following exercise, it is possible that amino acids are available at a time when the proteolytic and lipolytic pathways are activated. It is acknowledged that the exercise bout may have delayed gastric emptying. Previous research has demonstrated an increase in gastric emptying half-time of a CHO solution during intermittent high-intensity exercise compared to rest (Lieper, Broad & Maughan, 2001). Over 72 h, pre-exercise ingestion of milk-based protein-CHO was possibly beneficial for limiting changes in EIMD. However, values at this time point remained below both groups that consumed milk-based protein-CHO following muscle damaging exercise. It is not known why consumption pre-exercise may have had this effect and this area needs to be researched further. Lastly, over 48 h milk-based protein ingestion pre-exercise had a possible benefit for blunting increases in CK. The proteolytic pathways responsible for membrane damage (and hence increased CK in plasma), and myofibrillar protein loss (decreased muscle performance) are not the same, which may explain the different response. The intake of protein-CHO prior to muscle damaging exercise may allow the availability of amino acids and increased insulin concentration at a time that influences protein degradation via lysosomal processes.

It has been demonstrated that consuming milk-based protein-CHO immediately or 24 h post muscle damaging exercise can limit decreases in isokinetic peak torque and dynamic muscle performance (reactive strength index), which may be of practical benefit to the exercising individual. Furthermore, increases in active DOMS were reduced with the consumption of the supplement post muscle damaging exercise. However, in agreement with the first study there was no effect on passive DOMS. The reason for this may be two-fold. Firstly, during measurement of active DOMS the hamstrings were activated and isolated which may have made participants more consciously aware of the soreness present in them, in comparison to passive DOMS. Secondly, research has suggested that the process underlying the two aspects of muscle soreness are different (Nosaka, Newton & Sacco, 2002), and that passive DOMS may be related to inflammatory processes where as DOMS during activation may be reflex mediated pain (Lieber & Friden, 2002). The attenuation of

active DOMS is of practical interest, as for the exercising individual soreness present during muscle actions is more important as it could potentially limit performance. However, muscle soreness may also provide a protective mechanism to allow sufficient time for recovery (Malm, 2001).

It is proposed that athletes with EIMD can, therefore, limit performance decrements in the days following muscle damaging exercise through the consumption of milk-based protein-CHO after exercise. Although pre-exercise ingestion may be potentially beneficial at 72 h this is of limited benefit as the ability to conduct everyday activities and perform will be reduced for longer. It is, therefore, suggested that those exercising consume milk-based protein-CHO immediately following training or competition to limit the negative impact of EIMD on subsequent functional capacity. This is in agreement with the results of the first study, but the novel outcome of this study is the benefit to stretch shortening cycle exercise, and that benefits are observed over 72 h.

In conclusion milk-based protein-CHO consumed immediately or 24 h post muscle damaging exercise hastens recovery by 72 h. The exact mechanisms are presently unclear but may be due to limiting increases in protein degradation and/or stimulating protein synthesis. However, further research is required to examine this theory.

6. EFFECT OF VOLUME OF MILK CONSUMED ON THE ATTENUATION OF EIMD

6.1   Introduction

The first two studies of this thesis have demonstrated that acute supplementation with milk or milk-based protein-carbohydrate (CHO) attenuates exercise-induced muscle damage (EIMD), and that the optimal timing of this supplementation should be immediately or 24 h following the exercise bout. The benefits may be derived from altered protein metabolism during the secondary phase, limiting myofibrillar disruption and the loss of cell membrane integrity. As a consequence, increases in intramuscular protein release and decrements in muscle function are limited. There is also a benefit of supplementation on active muscle soreness but it is unlikely that this is derived from altered protein metabolism.

The previous studies have provided participants with 1000 mL of the supplement, equating to 33 g protein and 118 g CHO for the milk-based protein-CHO supplement, and 34 g protein and 49 g CHO in milk. This volume is quite large, especially in terms of caloric content. Furthermore, following exercise this large volume may lead to stomach fullness and discomfort. Other investigations demonstrating a positive impact of protein-CHO supplementation have provided participants with varying amounts of CHO and protein (Wojcik *et al.*, 2001; Baty *et al.*, 2007; Valentine *et al.*, 2008). If the benefit of protein-CHO supplementation is derived from changes in protein metabolism then it can be hypothesised that less CHO and protein can be consumed to elicit similar effects, which may also be more beneficial for the athlete in terms of reduced caloric intake, and less stomach fullness and discomfort. Rasmussen *et al* (2000) and Tipton *et al* (2001) have both demonstrated that as little as 6 g amino acids and 35 g of sucrose will promote muscle anabolism. Furthermore, Moore *et al* (2009a) demonstrated that muscle protein synthesis reaches maximal stimulation after the consumption of 20 g high-quality intact protein, suggesting an upper limit for the incorporation of amino acids into proteins. Consuming protein in greater amounts than this leads to no further increase in protein synthesis, with additional amino acids oxidised (Moore *et al.*, 2009a). By reducing the amount of protein and CHO through decreasing the volume of supplement ingested it may be hypothesised that benefits to changes in muscle function and intramuscular proteins will still be observed. There have not been any other studies investigating the dose response of protein-CHO supplementation on recovery from EIMD.

It has been speculated, throughout the first two studies, that the benefits derived from acute milk-based protein-CHO supplementation are due to an impact on myofibrillar and membrane protein metabolism during the secondary phase of EIMD. Changes in protein metabolism following EIMD may be related to phospholipase A_2 (PLA_2) and the ubiquitin-protesome (Ub-P) pathway, which have been associated with the activation of cytokines, specifically tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). To date only three studies in this research area have assessed cytokines to investigate underlying mechanisms (Wojcik *et al.*, 2001; Rowlands *et al.*, 2008; Betts *et al.*, 2009). Both Wojcik *et al* (2001) and Betts *et al* (2009) found no benefit of protein-CHO supplementation on any indirect markers of EIMD, including cytokines (IL-6, IL-10, IL-1), thereby limiting any conclusions that can be drawn. Rowlands *et al* (2008) observed a possible decrease in creatine kinase (CK) with no changes in cytokines and markers of inflammation (IL-6, TNF-α, C-reactive protein (CRP)). However, basing outcomes of protein-CHO supplementation on measures of CK limits conclusions as it is a highly variable marker of EIMD.

IL-6 is thought to be the main systemic mediate of the acute inflammatory response following exercise (Pyne, 1994) with increases observed following eccentric exercise (Dousset *et al.*, 2007; Miles *et al.*, 2008; Buford *et al.*, 2009; Philippou *et al.*, 2009). The up-regulation of IL-6 may result in the loss of myofibrillar protein (Caiozzo *et al.*, 1996), possibly through the activation of the Ub-P system, as it has attracted attention as a factor initiating this pathway (Murton, Constantin & Greenhaff, 2008). Another reason may be via the production of CRP (Peterson & Pederson, 2005), which also increases following eccentric exercise (Dousset *et al.*, 2007).

Therefore, the aims of this study were two-fold. Firstly, to determine if a reduced volume of milk ingested immediately following muscle damaging exercise reduces indices of EIMD; secondly, to investigate the effect of acute milk ingestion on IL-6 and CRP.

6.2   Method

6.2.1 Participants

Twenty-four healthy male participants (age 21 ± 3 years; stature 181.4 ± 6.5 cm; mass 79.7 ± 9.3 kg) who regularly competed in a variety of sports (team and individual) volunteered to take part in the study.   Participants were tested in the morning, following an overnight fast, to minimise diurnal variation.

6.2.2 Experimental Design

Participants were assigned to 1 of 3 independent groups: (i) 500 mL milk (FIVE), (ii) 1000 mL milk (LITRE), or (iii) 1000 mL of water (CON).   A one-way ANOVA revealed no group differences in baseline participant characteristics, or peak torque values used for group allocation ($p < 0.05$).

All participants were required to attend the laboratory on 4 consecutive days, the procedure for which is outlined in section 3.2.   Following muscle damaging exercise, participants consumed their allocated supplement in the relevant volume.

6.2.3 Nutritional Supplement

Participants were provided with semi-skimmed milk, the details of which are described in section 3.3.   This was altered from study 2; however, study 1 demonstrated no differences between these two supplements, both being concluded as beneficial.

6.2.4 Muscle Damaging Exercise

Muscle damage of the hamstrings was induced via unilateral eccentric-concentric knee flexions on the Cybex Isokinetic Dynamometer (Cybex Norm). Please refer to section 3.5 for more detail.

6.2.5 Muscle Soreness Measurement

Participants were required to rate both passive and active muscle soreness. Active muscle soreness was assessed during concentric knee flexions. Please refer to section 3.6 for further information.

6.2.6 Muscle Function

6.2.6.1 Peak Torque

Participants' peak torque of 6 maximal concentric knee flexions was measured. Please refer to section 3.7.1 for more detail.

6.2.6.2 Reactive Strength Index

Reactive strength index was calculated from the mean of 3 drop jumps. Please refer to section 3.7.2 for more detail.

6.2.7 Blood Sampling and Analysis

Venous blood was collected as described in section 3.8. This was used for the analysis of CK (section 3.8.1), Mb (section 3.8.2), IL-6 and CRP.

Plasma IL-6 concentrations were analysed from K_3EDTA treated venous blood using an enzyme linked immunosorbent assay (R&D Systems, Minneapolis, USA). R&D Systems report intra- and inter-assay coefficient of variations for IL-6 at less than 5.8 % and 9.6 %, respectively. CRP activity was analysed using high sensitivity techniques (Advia 2400, Seimens Healthcare Diagnostica, Australia). R&D Systems report intra- and inter-assay coefficient of variations at less than 5.3 % and 6.8 %, respectively.

6.2.8 Statistical Analysis

Dependent variables were analysed using magnitude based inferences. Please refer

to section 3.9 for more detail.

6.3   Results

6.3.1 Muscle Soreness

All groups demonstrated an increase in passive and active muscle soreness that peaked at 48 h and started returning to baseline levels by 72 h. For changes in passive muscle soreness (DOMS), all comparisons were unclear except between 0 and 48 h, which showed a possible benefit for participants consuming 500 mL of milk in comparison to those consuming 1000 mL of milk. The *p* value for the main interaction effect was 0.601. For changes in active DOMS, all comparisons were unclear in both legs (Figure 6.1). The *p* value for the main interaction effect was 0.454.   A summary of the statistical analysis for DOMS is shown in table 6.1.

Figure 6.1 Active muscle soreness of the dominant leg in response to exercise-induced muscle damage in the CON (n = 8), FIVE (n = 8) and LITRE (n = 8) groups. Values presented as mean ± standard deviation. CON = control; FIVE = 500 mL of milk consumed immediately following muscle damaging exercise; LITRE = 1000 mL of milk consumed immediately following muscle damaging exercise

Table 6.1 Effect of supplement volume on increases in muscle soreness following muscle damaging exercise

	Comparison	Mean effect[a] ± 90% CI[b]	Qualitative Inference
baseline - 48 h			
Passive DOMS	FIVE v CON	-0.6 ± 1.9	Unclear
	LITRE v CON	0.7 ± 1.9	Unclear
	LITRE v FIVE	1.3 ± 1.3	Increase Possible
Active DOMS (DOM)	FIVE v CON	-0.9 ± 2.5	Unclear
	LITRE v CON	-0.5 ± 1.7	Unclear
	LITRE v FIVE	0.4 ± 2.3	Unclear
Active DOMS (NON)	FIVE v CON	-0.4 ± 2.2	Unclear
	LITRE v CON	-0.3 ± 2.0	Unclear
	LITRE v FIVE	0.1 ± 1.9	Unclear
baseline - 72 h			
Passive DOMS	FIVE v CON	-0.1 ± 2.2	Unclear
	LITRE v CON	1.1 ± 2.4	Unclear
	LITRE v FIVE	1.1 ± 1.9	Unclear
Active DOMS (DOM)	FIVE v CON	-1.5 ± 2.6	Unclear
	LITRE v CON	-0.9 ± 2.3	Unclear
	LITRE v FIVE	0.6 ± 2.0	Unclear
Active DOMS (NON)	FIVE v CON	-0.4 ± 2.7	Unclear
	LITRE v CON	0.0 ± 2.8	Unclear
	LITRE v FIVE	0.4 ± 2.4	Unclear

[a]Mean effect refers to the first named group minus second named; [b]±90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude. CON = control group; FIVE = 500 mL of milk consumed immediately following muscle damaging exercise; LITRE = 1000 mL of milk consumed immediately following muscle damaging exercise

6.3.2 Muscle Function

A summary of the statistical analysis is shown in Table 6.2.

Table 6.2 Effect of supplement volume on decreases in muscle function following muscle damaging exercise

Muscle Performance	Comparison	Mean effect[a] ± 90% CI[b]	Qualitative Inference
baseline - 48 h			
Peak Torque (DOM)	FIVE v CON	12 ± 23	Unclear
	LITRE v CON	12 ± 23	Unclear
	LITRE v FIVE	0 ± 9	Unclear
Peak Torque (NON)	FIVE v CON	-3 ± 18	Unclear
	LITRE v CON	1 ± 18	Unclear
	LITRE v FIVE	7 ± 18	Unclear
RSI	FIVE v CON	4 ± 14	Unclear
	LITRE v CON	-4 ± 32	Unclear
	LITRE v FIVE	-6 ± 32	Unclear
baseline - 72 h			
Peak Torque (DOM)	FIVE v CON	21 ± 20	Likely Decrease
	LITRE v CON	23 ± 21	Likely Decrease
	LITRE v FIVE	-2 ± 13	Unclear
Peak Torque (NON)	FIVE v CON	2 ± 24	Unclear
	LITRE v CON	6 ± 19	Unclear
	LITRE v FIVE	3 ± 18	Unclear
RSI	FIVE v CON	0 ± 16	Unclear
	LITRE v CON	-11 ± 22	Unclear
	LITRE v FIVE	-11 ± 23	Unclear

[a]Mean effect refers to the first named group minus second named; [b]±90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude. CON = control group; FIVE = 500 mL of milk consumed immediately following muscle damaging exercise; LITRE = 1000 mL of milk consumed immediately following muscle damaging exercise

6.3.2.1 Peak Torque

Baseline values for peak torque of the dominant leg for the control group, and the groups consuming 500 mL or 1000 mL of milk immediately following muscle damaging exercise were 146 Nm, 130 Nm and 136 Nm, respectively. At 48 h, peak torque had decreased to 120 Nm, 113 Nm and 123 Nm for the control group, and the groups consuming 500 mL or 1000 mL of milk immediately following muscle damaging exercise, respectively. At 72 h the control group demonstrated a decrease to 112 Nm, however, the group consuming 500 mL of milk and the group consuming 1000 mL of milk returned to baseline values of 130 Nm and 137 Nm, respectively.

Changes in peak torque of the dominant leg between baseline and 48 h for the control group, those consuming 500 mL of milk and those consuming 1000 mL of milk were -19 ± 33 %, -10 ± 9 % and -10 ± 10 %, respectively. All effects were unclear. Between baseline and 72 h there was a likely benefit of both groups consuming 500 mL (-3 ± 13 %) and 1000 mL (-3 ± 14 %) of milk in comparison to the control group (-20 ± 28 %) for changes in peak torque of the dominant leg. There were no clear effects of

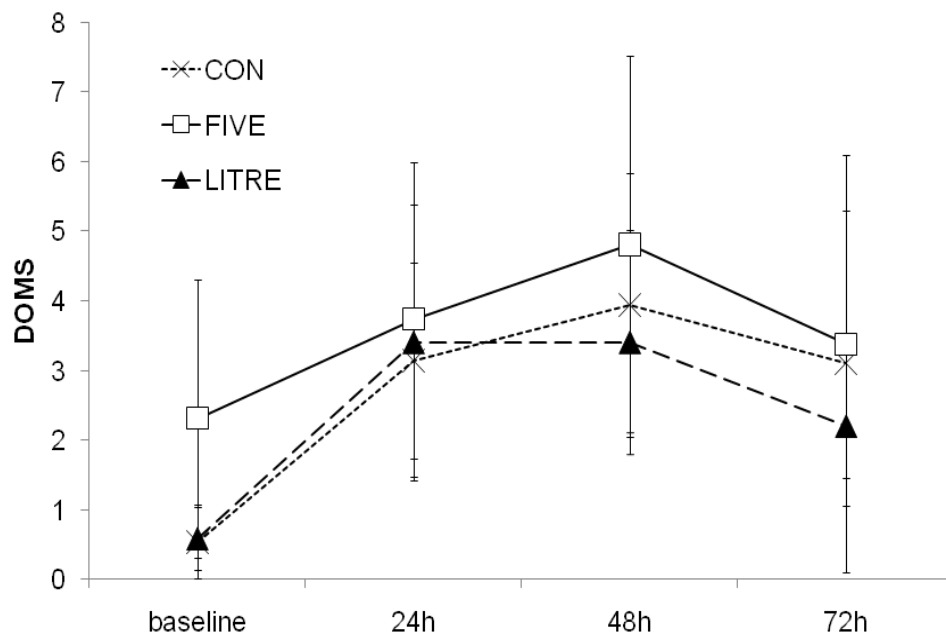participants consuming 500 mL of milk v 1000 mL of milk (Figure 6.2).

Figure 6.2 Relative peak torque of the dominant leg in response to exercise-induced muscle damage in the CON (n = 8), FIVE (n = 7), and LITRE (n = 7) group. Values presented as mean ± standard deviation. CON = control; FIVE = 500 mL of milk consumed immediately following muscle damaging exercise; LITRE = 1000 mL of milk consumed immediately following muscle damaging exercise

Baseline values of peak torque of the non-dominant leg were 128 Nm, 129 Nm and 136 Nm for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. Peak decrements occurred at 48 h for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk which were 110 Nm, 105 Nm and 117 Nm, respectively. At 72 h values were 113 Nm, 117 Nm and 123 Nm for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively.

Changes in peak torque of the non-dominant leg between baseline and 48 h for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk were -15 ± 20 %, -18 ± 22 % and -15 ± 18 %, respectively. Changes between baseline and 72 h were -14 ± 28 %, -11 ± 27 % and -9 ± 8 % for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. All comparisons between baseline and 48 h, and baseline and 72 h for

changes in peak torque of the non-dominant leg were unclear. The *p* value for the main interaction effect was 0.135.

6.3.2.2 Reactive Strength Index

Baseline values for reactive strength index for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk were 110 cm.s^{-1}, 118 cm.s^{-1} and 111 cm.s^{-1}, respectively. At 48 h these decreased to 101 cm.s^{-1}, 113 cm.s^{-1} and 104 cm.s^{-1} for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. At 72 h reactive strength index for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk were 108 cm.s^{-1}, 115 cm.s^{-1} and 99 cm.s^{-1}, respectively.

Between baseline and 48 h, changes for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk were -8 ± 16 %, -4 ± 14 % and -11 ± 47 %, respectively. Changes between baseline and 72 h were -2 ± 18 %, -2 ± 18 % and -12 ± 30 % for the control group, the group consuming 500 mL of milk and 1000 mL of milk, respectively. All comparisons were unclear and the *p* value for the main interaction effect was 0.786.

6.3.3 Intramuscular Proteins in the Serum

A summary of the statistical analysis is shown in Table 6.3.

Table 6.3 Effect of supplement volume on increases in intramuscular proteins following muscle damaging exercise

Intramuscular Proteins	Comparison	Mean effect[a] $\times/\div$ 90% CI[b]	Qualitative Inference
baseline - 48 h			
CK	FIVE v CON	0.1 $\times/\div$ 79.2	Unclear
	LITRE v CON	0.0 $\times/\div$ 24.8	Very likely decrease
	LITRE v FIVE	0.9 $\times/\div$ 7.9	Unclear
Mb	FIVE v CON	1.2 $\times/\div$ 9.8	Unclear
	LITRE v CON	0.3 $\times/\div$ 10.9	Unclear
	LITRE v FIVE	0.5 $\times/\div$ 8.4	Unclear
baseline - 72 h			
CK	FIVE v CON	0.0 $\times/\div$ 99.0	Possible decrease
	LITRE v CON	0.2 $\times/\div$ 10.8	Unclear
	LITRE v FIVE	3.0 $\times/\div$ 6.1	Unclear
Mb	FIVE v CON	0.1 $\times/\div$ 7.7	Very likely decrease
	LITRE v CON	1.1 $\times/\div$ 6.6	Unclear
	LITRE v FIVE	17.3 $\times/\div$ 4.2	Almost certain decrease

[a]Mean effect refers to the first named group minus second named; [b]±90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude. CON = control group; FIVE = 500 mL of milk consumed immediately following muscle damaging exercise; LITRE = 1000 mL of milk consumed immediately following muscle damaging exercise; CK = creatine kinase; Mb = myoglobin

6.3.3.1 Creatine Kinase

Mean baseline CK values were 540.5 $U.L^{-1}$, 113.4 $U.L^{-1}$ and 150.4 $U.L^{-1}$ for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. Baseline CK values for the control group are relatively high and this is due to one participant. However, as results are analysed as the difference between groups in change over time, this participant was not removed from the analysis.

Between baseline and 48 h, changes in CK were 6.7 $\times/\div$ 13.3, 0.5 $\times/\div$ 6.6 and 0.1 $\times/\div$ 9.3 for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. There was a very likely benefit of the group consuming 1000 mL of milk in comparison to the control group, and all other effects were unclear for changes between baseline and 48 h.

Changes for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk between baseline and 72 h were 12.9 ×/÷ 10.2, 0.2 ×/÷ 9.1 and 3.0 ×/÷ 5.2, respectively. There was a possible benefit of the group consuming 500 mL of milk in comparison to the control group, and all other effects were unclear for changes between baseline and 72 h (Figure 6.3). The *p* value for the main interaction effect was 0.956.
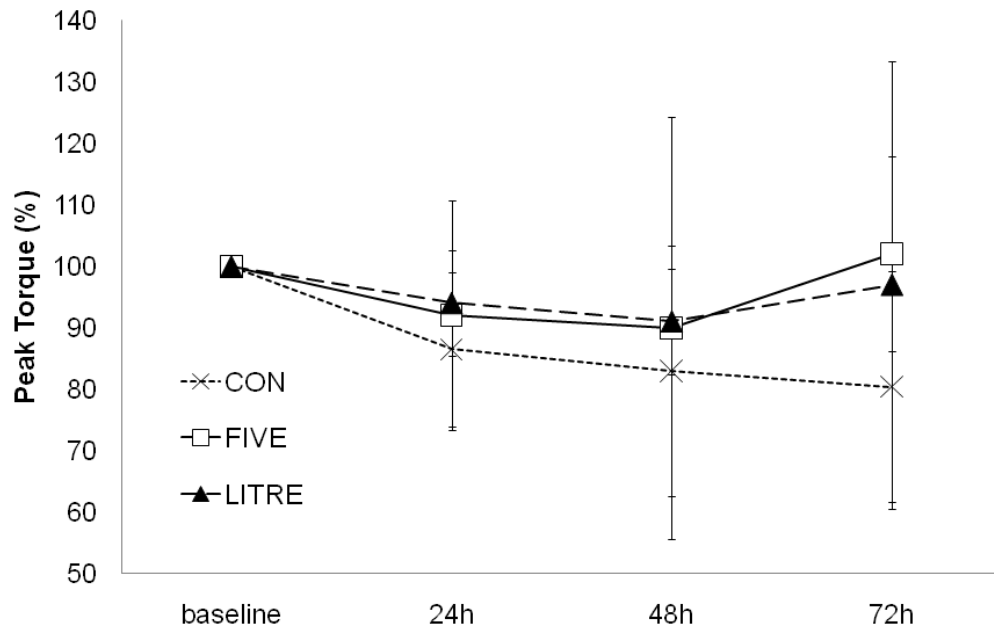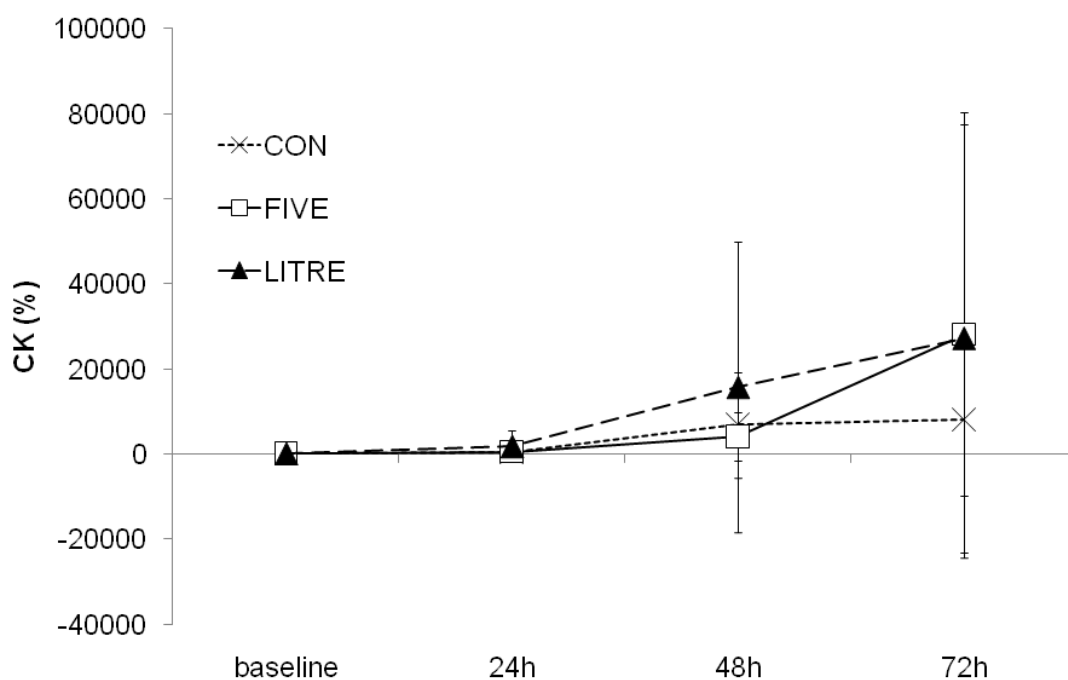

Figure 6.3 Relative Creatine Kinase (CK) in response to exercise-induced muscle damage in the CON (n = 6), FIVE (n = 7) and LITRE (n = 7) groups. Values presented as mean ± standard deviation. CON = control; FIVE = 500 mL of milk consumed immediately following muscle damaging exercise; LITRE = 1000 mL of milk consumed immediately following muscle damaging exercise

6.3.3.2 Myoglobin

Mean baseline Mb values were 29.1 ng.mL^{-1}, 21.8 ng.mL^{-1} and 30.2 ng.mL^{-1} for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. Changes for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk between baseline and 48 h were 7.0 ×/÷ 13.1, 8.1 ×/÷ 6.1 and 1.6 ×/÷ 7.8, respectively. All effects were unclear for changes between baseline and 48 h. Changes in Mb for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk were 15.5 ×/÷

8.2, 1.2 $\times\div$ 4.6 and 14.7 $\times\div$ 3.6 between baseline and 72 h, respectively. There was a very likely benefit for the group consuming 500 mL of milk in comparison to the control group, and the group consuming 500 mL of milk was almost certainly beneficial in comparison to the group consuming 1000 mL of milk for changes between baseline and 72 h. All other effects were unclear. The *p* value for the main interaction effect was 0.859.

6.3.4 Markers of Inflammation

6.3.4.1 Interleukin-6

IL-6 did not change significantly over time. Baseline values for IL-6 were 1.86 pg.mL^{-1}, 1.28 pg.mL^{-1} and 0.90 pg.mL^{-1} for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. Between baseline and 48 h, changes in IL-6 for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk were 27.9 ± 134.7 %, -32.4 ± 595.2 % and -44.5 ± 130.4 %, respectively. There was a likely benefit of the group consuming 1000 mL of milk in comparison to the control group, with all other effects unclear. Changes in IL-6 between baseline and 72 h were 11.1 ± 413.2 %, 23.5 ± 449.6 % and -49.0 ± 200.7 % for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. All comparisons were unclear. The *p* value for the main interaction effect was 0.634.

6.3.4.2 C-Reactive Protein

Baseline values for CRP were 2.7 mg.L^{-1}, 2.8 mg.L^{-1} and 1.7 mg.L^{-1} for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. Changes in CRP between baseline and 48 h were -19.4 ± 30.1 %, 10.3 ± 43.3 % and -4.3 ± 75.8 % for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk, respectively. Between baseline and 72 h changes in CRP for the control group, the group consuming 500 mL of milk and the group consuming 1000 mL of milk were 52.6 ± 323.4 %, 25.2 ± 45.4 % and 59.1 ± 58.8 %, respectively. The effects of all comparisons for both time points were unclear. The *p* value for the main interaction effect was 0.028.

6.4   Discussion

The findings of this study demonstrate that decrements in isokinetic muscle function, and increases in CK can be blunted with the intake of less milk than has been previously shown. There was no effect of milk ingestion on passive or active muscle soreness, reactive strength index or Mb. Changes in IL-6 can be altered with the consumption of one litre of milk immediately following exercise, however, there were no effects on CRP.

Reducing the volume of milk consumed and, therefore, the amount of protein and CHO had no negative effects for attenuating decrements in isokinetic muscle function and increases in CK. It has been proposed that the benefit derived from milk is due to increases in protein synthesis and/or limiting increases in protein degradation. It has been demonstrated that there is a dose-response relationship between muscle protein synthesis and amino acid intake after resistance exercise (Borsheim *et al.*, 2002; Miller *et al.*, 2003). Moore *et al* (2009a) demonstrated that muscle protein synthesis is not further stimulated with intakes of protein above 20 g, and that this may be the upper limit for incorporation of amino acids into protein pools. This study compared 17 g to 34 g protein and found no difference, suggesting that, if the benefits are due to increased protein synthesis, then consuming 34 g does not provide extra amino acids that can be incorporated into new proteins to preserve myofibrillar and membrane proteins. The excess protein consumed with 1000 mL of milk will not be utilised for the synthesis of new proteins, but is more likely to be oxidised (Moore et al, 2009). A change in CHO intake through reduced supplement volume also occurred. CHO increases insulin that can increase the capacity for muscle protein synthesis, however, sufficient amino acids are required for this to be reflected in elevated synthesis (Biolo *et al.*, 1999; Volek, 2004). Therefore, although CHO ingestion was reduced this is likely to have minimal effect on protein metabolism. Furthermore, there is likely to be a ceiling effect to CHO intake were by consuming more does not provide greater effects. Therefore, there is no additional benefit to attenuating EIMD with the consumption of larger volumes of milk.

Milk, in either quantity, had no effect on increases in passive muscle soreness. This is in agreement with the results of the previous two studies. In contrast to the second study, there was no benefit of milk on reactive strength index, active muscle soreness

or Mb. With regards to changes in reactive strength index, it has a coefficient of variation of 7.4 % which indicates a high degree of variability in measurement. Therefore, the different findings may be attributed to the low reliability of reactive strength index. Secondly, the decrements observed in the control group over 48 and 72 h in the second study were greater than those observed in the current study. Changes with consumption of one litre of milk consumed immediately following muscle damaging exercise were similar in both studies. This may have masked any effect of milk supplementation. A similar phenomenon was observed for active DOMS with participants perceiving less muscle soreness during the current study. It is unknown why the control group demonstrated less muscle damage, based on measures of muscle soreness and reactive strength index, and this requires further investigation. Regarding effects on Mb, there was a benefit of 500 mL of milk but not a litre over 72 h. Again it is difficult to know why this occurred as if less protein and CHO intake limits increases in Mb then consuming more of the supplement should produce similar benefits. The use of Mb, however, should be used with caution as assays to determine concentrations cannot determine between Mb released from the heart or skeletal muscle (Sorichter, Puschendorf & Mair, 1999).

To investigate possible underlying mechanisms, IL-6 and CRP were assessed. This is because cytokines have been implicated in many of the pathways responsible for myofibrillar and membrane damage and thus changes in indirect markers. There was a likely benefit of a litre of milk in comparison to the control group over 48 h on IL-6. However, altering the IL-6 response did not appear to have any additional benefits for changes in muscle function, as there were no differences in changes of peak torque between both milk groups. Research has shown that muscle proteolysis is limited with branched chain amino acids supplementation, but this response was independent of the IL-6 response (Rohde *et al.*, 1997). More recently, it was shown that CHO supplementation for 48 h following high force eccentric exercise did not attenuate the response of IL-6 (Miles *et al.*, 2007). However, there was no impact of CHO on other indirect markers of EIMD (Miles *et al.*, 2007). Therefore, the relevance of this finding requires further investigation. There were no effects of milk on CRP over 48 or 72 h. Taken collectively, the mechanisms underlying the benefits of acute milk supplementation require further investigation as the results presented here are inconclusive. Future studies should concentrate on measuring TNF-α and IL-1β as these are the most pro-

inflammatory combination of cytokines (Pyne, 1994), both with potential for limiting protein synthesis (Frost, Lang & Gelato, 1997) and increasing degradation (Andreu & Schwartz, 1995). Furthermore, the current study measured IL-6 and CRP systematically and not locally, therefore, future studies should possibly use microdialysis techniques.

In conclusion, decrements in muscle function following muscle damaging exercise can be limited with the immediate consumption of 500 mL of milk. The attenuation of EIMD, specifically isokinetic muscle function, with protein-CHO is not novel; however, this study provides athletes with important information regarding the volume to be consumed. Consuming a supplement with more than 20g of protein may not provide any extra benefit for blunting EIMD. An insight into the role of cytokines and markers of inflammation in the limitation of EIMD with milk has been provided. However, this is preliminary research and further investigation is warranted investigating the role of the inflammatory process and protein metabolism.

7. EFFECT OF ACUTE MILK INTAKE ON PHYSIOLOGICAL ASPECTS OF FIELD-BASED TEAM SPORTS FOLLOWING EIMD

7.1   Introduction

The studies in this thesis have provided information regarding type, timing and amount of milk supplementation required to attenuate exercise-induced muscle damage (EIMD).   Five hundred millilitres of milk consumed immediately following muscle damaging exercise has a beneficial effect, over 48 h and 72 h, on isokinetic muscle function and creatine kinase (CK), and in some studies, myoglobin (Mb) and active muscle soreness (DOMS) have been attenuated.   Limiting decrements in muscle function following muscle damaging exercise is important to the athlete.   The first three studies utilised isokinetic dynamometry to assess changes in muscle function. Isokinetic dynamometry has limited external validity when extrapolating to a sporting context (Baltzopoulos & Gleeson, 2001), as it does not produce velocities used in sport or utilise the stretch shortening cycle.   The second study demonstrated benefits to stretch shortening cycle exercise; however, this was not reproduced in the third study.   It is clear that to apply the findings of the first three studies to athletes then the effects of milk on many different facets of exercise performance requires investigation.

Field-based team sports such as soccer, rugby and hockey are popular throughout the world (Spencer *et al.*, 2005). The competitive demands of these sports may impose strains on various physiological systems to a point where recovery strategies post-exercise become influential in preparing for the next match (Reilly & Ekblom, 2005). Soccer matches have been shown to induce decrements in hamstring peak torque and increases in 20 m sprint time and CK concentrations up to 72 h post-match (Ascensao *et al.*, 2008).   Following a rugby match, CK is increased from 24 h through to 84 h (Suzuki *et al.*, 2004; Gill, Beaven & Cook, 2006), and lactate dehydrogenase (LDH) is increased at 48 h (Suzuki *et al.*, 2004). The Loughborough Intermittent Shuttle Test (LIST), which simulates the physiological demands of field-based team sports (Nicholas, Nuttall & Williams, 2000) has been shown to lead to increases in EIMD (CK, aspartate aminotransferase) and muscle soreness up to 72 h (Thompson, Nicholas & Williams, 1999).   Reported muscle soreness is highest in the hamstrings (Thompson, Nicholas & Williams, 1999).   It is therefore apparent that field-based team sport athletes will suffer from a degree of muscle damage that can potentially hinder subsequent training and performance, and that this damage is most pronounced in the hamstrings. During the competitive season the usual weekly cycle of training, taper, competition and recovery can be altered (Ascensao *et al.*, 2008).

The match day may not necessarily be the same from week to week (Ascensao *et al.*, 2008) and a number of matches could take place in one week, alongside training. Therefore, these athletes need to be able to limit the consequences of muscle damage so that they can perform optimally during subsequent training and matches. Therefore, this study aims to apply the findings of the first three studies to a sporting situation and investigate the effect of milk supplementation following muscle damaging exercise on performance tests specific to field-based team sports.

7.2   Methods

7.2.1 Participants

Fourteen healthy male participants (age 24 ± 4 years; stature 183.1 ± 7.1 cm; mass 79.9 ± 8.4 kg) who competed in non-league (Northern League) soccer volunteered to take part in the study. In addition to recording their diet throughout the study, participants were asked to record their diet one day prior to performing their baseline LIST and repeat this the day prior to completing the LIST following muscle damaging exercise. Participants were tested in the morning, following an overnight fast, to minimise diurnal variation.

7.2.2 Experimental Design

Participants were assigned to one of two independent groups: (i) 500 mL semi-skimmed milk (MILK), (ii) 500 mL water (CON). An independent t-test revealed no group differences in baseline participant characteristics (age and body mass), except height ($p < 0.05$). However, body mass index was not significantly different between groups ($p < 0.05$).

Prior to any testing, participants were asked to attend a familiarisation session, which involved completion of the multi-stage fitness test (MSFT). This was used to determine the intensity of the LIST. All participants were required to attend the laboratory on 5 days. On the first visit participants completed baseline performance tests (countermovement jump height, reactive strength index, 15 m sprint, change of direction speed) and the LIST. Participants then reported to the laboratory a week later for 4 consecutive days. The first day involved completing the muscle damaging protocol and

immediately following this, participants consumed their allocated supplement. At 24, 48 and 72 h following muscle damaging exercise, participants returned to the laboratory to complete baseline performance tests. At 48 h, participants completed the LIST. A schematic representation of the testing protocol is shown in Figure 7.1.
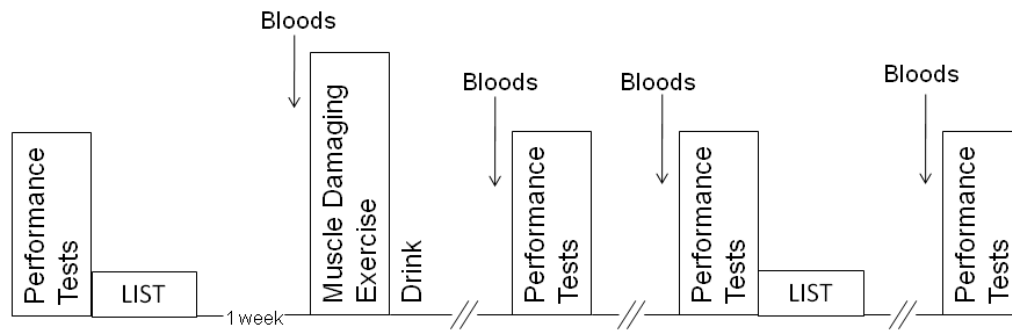
Figure 7.1 Schematic Representation of Protocol. LIST = Loughborough intermittent shuttle test; Performance Tests = 15 m sprint, T-test, countermovement jump and reactive strength index; Bloods = analysed for creatine kinase and myoglobin

7.2.3 Nutritional Supplement

Participants were provided with semi-skimmed milk, the details of which are described in section 3.3.

7.2.4 Muscle Damaging Exercise

Muscle damage of the hamstrings was induced via unilateral eccentric-concentric knee flexions on the Cybex Isokinetic Dynamometer (Cybex Norm). Please refer to section 3.5 for more detail.

7.2.5 Muscle Soreness Measurement

Participants were required to rate passive soreness and active muscle soreness of the hamstrings during all performance measures. Please refer to section 3.6 for further information.

7.2.6 Multi-Stage Fitness Test

The MSFT was completed to gain predictive measures of VO_2max in order to calculate the running speeds required to elicit 55 % and 95 % of VO_2max for performance of the LIST (Ramsbottom, Brewer & Williams, 1988). This involved participants running 20 m shuttles, signalled by audio bleeps, which increased in running velocity until volitional exhaustion or the participant failed to finish a shuttle in time with the bleep on two consecutive occasions.

7.2.7 Muscle Function

7.2.7.1 Countermovement Jump

Vertical jump height is commonly used when assessing performance in team-based field sports (Keogh, Weber & Dalton, 2003; Wisloff *et al.*, 2004; Gabbett, 2006), and can be used as a measure of explosiveness. In order to assess this, countermovement jump height was assessed. Using a force plate (Kistler Instrumente AG, Winterthur, Switzerland), participants were required to place their hands on their hips to minimise impact of arm swing, and in one flowing movement bend their knees to approximately 90° and then jump straight into the air for maximum height. Participants jump height was calculated from flight time and the mean of 2 jumps was used for analysis. The coefficient of variation for this protocol calculated from reliability trials conducted in Northumbria University Laboratories is 1.9 %.

7.2.7.2 Reactive Strength Index

Reactive strength index was calculated from the mean of 3 drop jumps. Please refer to section 3.7.2 for more detail.

7.2.7.3 15 m Sprint

Speed is a fitness prerequisite for field-based team sport athletes (Reilly, Bangsbo & Franks, 2000; Keogh, Weber & Dalton, 2003; Gabbett, 2006). A 15 m sprint was used to assess this physiological parameter. This distance was chosen as it is reported to be the average sprint distance covered during soccer match analysis (Stolen *et al.*, 2005). Timing gates (Brower Timing Systems, Utah, USA) were placed at 0, 5, 10

and 15 m in order to gain split times. Participants were instructed to sprint through all timing gates as fast as possible from a standing start positioned 30 cm behind the first timing gate. Participants completed this twice and the mean of two performances was used for analysis. Coefficient of variations for this test, calculated from reliability trials conducted at Northumbria University Laboratories are between 0.6 and 1.5 %.

7.2.7.4 Change of Direction Speed

During field-based team sports, players are continually involved in sudden directional changes in order to be effective during a match (Mujika *et al.*, 2009), and change of direction speed has been shown to discriminate between elite and sub- elite soccer players (Reilly *et al.*, 2000). The T-Test was used as it includes forwards, backwards and lateral movements, and acceleration and deceleration, which are all involved during field-based team sports. Timing gates were set up at 0 m, with a cone set out 9.14 m forward of this. At 90° to the left and right of this cone, cones were set at 4.57 m (Figure 7.2). Participants were required to sprint straight to the first cone, side-step left, change direction and side-step to the other cone, change direction side-step to the central cone and then run backwards through the timing gates. Participants were instructed to complete this in the quickest time possible adhering to the following rules: must face forward at all times; during side-stepping legs must not cross; each side cone must be touched using the hand; when returning to the central cone participants must not cut the corner. Non-adherence to these rules resulted in that measurement being removed from analysis. The mean of two T-tests was used for analysis, and the coefficient of variation for this protocol calculated from reliability trials conducted in Northumbria University Laboratories is 0.6 %.
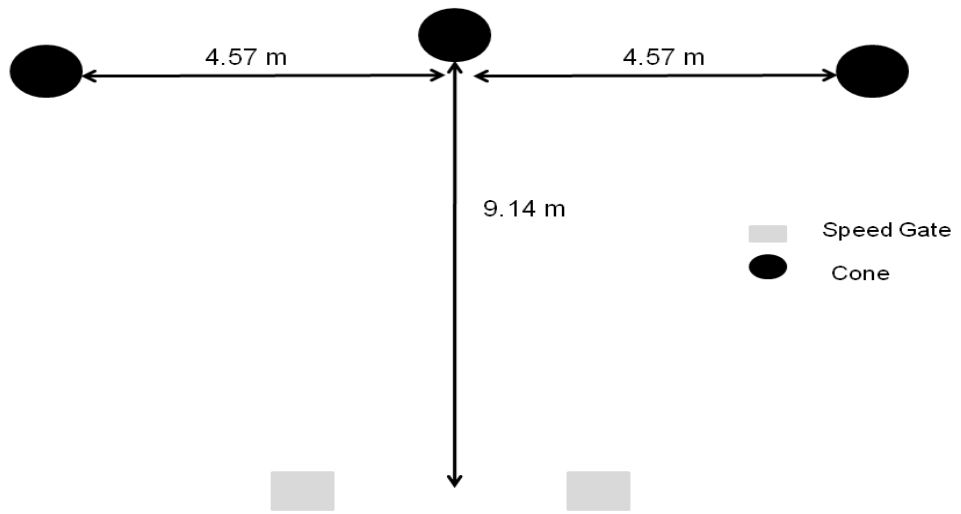
Figure 7.2 Schematic representation of the T-Test

7.2.7.5 Loughborough Intermittent Shuttle Test

The LIST is a 90 minute shuttle run test designed to elicit the physiological demands of field-based team sports (Nicholas, Nuttall & Williams, 2000). The test is divided into 6, 15 minute cycles that consist of; 3 x 20 m walk at 1.5 m.s^{-1}, 1 x 15 m sprint (recovery), 3 x 20 m run at 95 % of VO$_2$max, 3 x 20 m cruise at 55 % of VO$_2$max (Figure 7.3).

Each sprint throughout the LIST was timed using timing gates (Brower Timing Systems, Utah, USA) set up at 0 and 15 m, which were used to calculate mean sprint times for each 15 minute cycle. Participants were also required to wear a heart rate monitor and watch (Polar Electro, Oy, Finland) throughout the LIST to determine mean heart rate, which was averaged over 5 s for each cycle. Lastly, after each 15 min cycle participants were asked to rate their perceived levels of exertion on a rating of perceived exercise (RPE) scale (Borg, 1998). During the LIST, participants were allowed to drink water ad libitum, the volume of which was recorded. During subsequent testing participants were asked to consume the same volume.

The LIST has been shown to be reproducible (Nicholas, Nuttall & Williams, 2000) and, therefore, is a valuable tool to investigate the effects of muscle damage on field-based team sports.
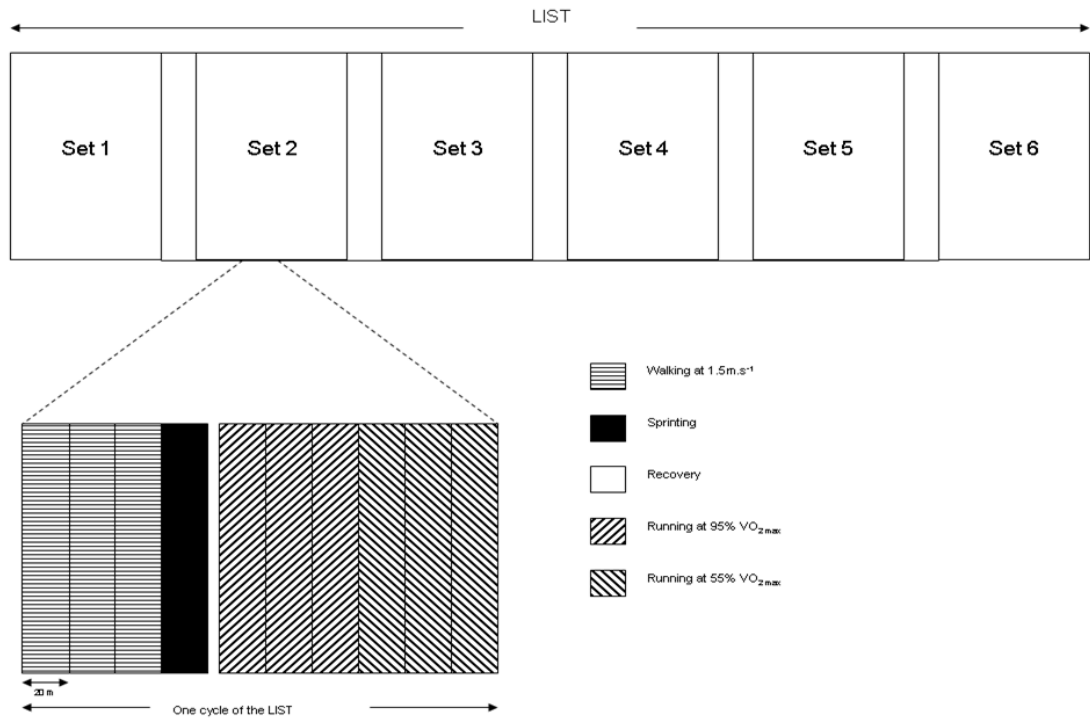
Figure 7.3 Schematic representation of the Loughborough Intermittent Shuttle Test (Nicholas, Nuttall & Williams, 2000)

7.2.8 Blood Sampling and Analysis

Venous blood was collected as described in section 3.8. This was used for the analysis of CK (section 3.8.1) and Mb (section 3.8.2).

7.2.9 Statistical Analysis

Dependent variables were analysed using magnitude based inferences. Please refer to section 3.9 for more detail.

7.3   Results

7.3.1 Muscle Soreness

Both groups showed an increase in passive and active DOMS (all performance measures) up to 48 h. At 72 h this started to return to baseline levels (Figure 7.4).
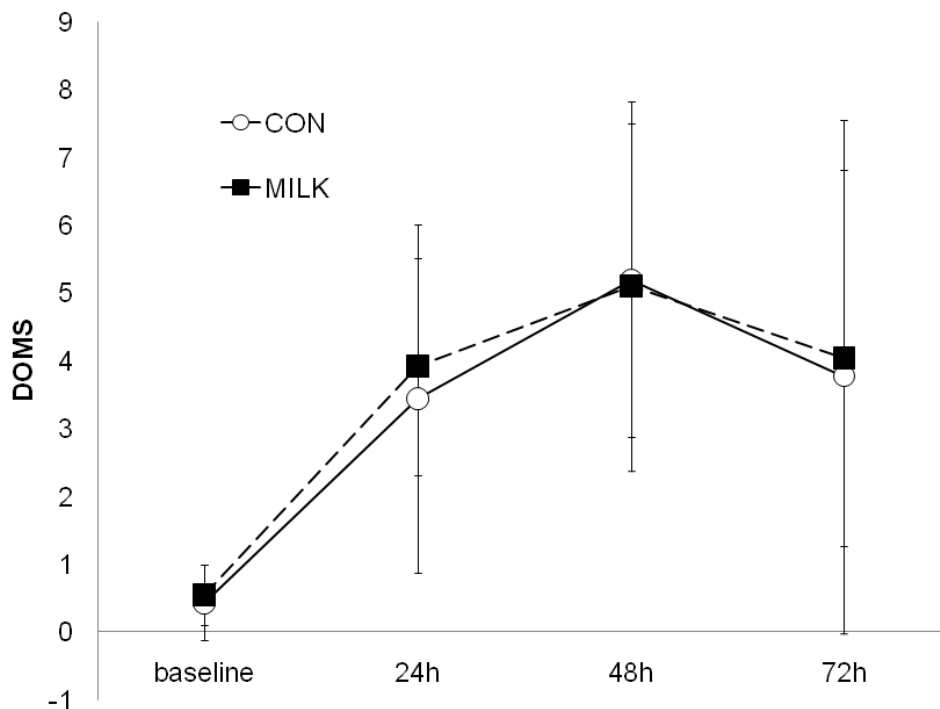
Figure 7.4 Perceived muscle soreness during a 15 m sprint in response to exercise-induced muscle damage in the control (n = 7) and milk (n = 7) groups. Values presented as mean ± standard deviation

All effects at both time points were unclear. The *p* values for all main interaction effects were 0.643, 0.860, 0.744, 0.973 and 0.944 for muscle soreness measured passively and during a countermovement jump, drop jump, 15 m sprint and the T-test,

respectively. A summary of the statistical analysis is shown in Table 7.1.

Table 7.1 Effect of milk on increases in muscle soreness following muscle damaging exercise

Variable	Mean effect[a] ± 90% CI[b]	Qualitative Inference
baseline - 48 h		
Passive DOMS	0.3 ± 1.9	Unclear
Active DOMS CMJ	0.5 ± 2.0	Unclear
Active DOMS DJ	0.9 ± 2.3	Unclear
Active DOMS 15 m	-0.2 ± 2.2	Unclear
Active DOMS T-test	0.3 ± 2.3	Unclear
baseline - 72 h		
Passive DOMS	-0.6 ± 2.9	Unclear
Active DOMS CMJ	0.2 ± 2.8	Unclear
Active DOMS DJ	0.5 ± 3.0	Unclear
Active DOMS 15 m	0.2 ± 3.1	Unclear
Active DOMS T-test	0.1 ± 2.9	Unclear

[a]Mean effect refers to the milk group minus the control group; [b]± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude. DOMS = delayed-onset muscle soreness; CMJ = countermovement jump; DJ = drop jump

7.3.2 Muscle Function

A summary of the statistical analysis is shown in Table 7.2.

Table 7.2 Effect of milk on changes in performance tests specific to field-based team sports following muscle damaging exercise

Muscle Performance	Mean effect[a] ± 90% CI[b]	Qualitative Inference
baseline - 48 h		
Countermovement Jump Height	1.6 ± 6.1	Unclear
Reactive Strength Index	-5.5 ± 20.9	Unclear
5 m	-3.0 ± 6.1	Unclear
10 m	-3.1 ± 3.8	Possible Decrease
15 m	-1.5 ± 3.4	Unclear
T-Test	-1.3 ± 3.6	Unclear
baseline - 72 h		
Countermovement Jump Height	2.2 ± 7.2	Unclear
Reactive Strength Index	-6.2 ± 13.4	Unclear
5 m	-1.3 ± 5.1	Unclear
10 m	-1.1 ± 2.2	Unclear
15 m	-3.1 ± 3.7	Possible Decrease
T-Test	-3.9 ± 3.6	Likely Decrease

[a]Mean effect refers to the milk group minus the control group; [b]±90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude.

7.3.2.1 Countermovement Jump

Baseline jump height for the control and milk groups were 32.8 cm and 33.2 cm, respectively. Jump height decreased for both groups over 72 h. Changes between baseline and 48 h for the control and milk groups were -6.9 ± 6.3 % and -5.5 ± 6.0 %, respectively. Between baseline and 72 h changes in jump height were -9.0 ± 9.2 % and -7.0 ± 3.4 % for the control and milk groups, respectively. All effects for both time points were unclear. The p value for the main interaction effect was 0.695.

7.3.2.2 Reactive Strength Index

Reactive strength index decreased for both groups over 48 h and at 72 h levelled off. Baseline values were 124 cm.s^{-1} and 126 cm.s^{-1} for the control and milk groups, respectively. Changes between baseline and 48 h for the control and milk groups were -18.2 ± 20.1 % and -22.8 ± 22.6 %, respectively. Between baseline and 72 h changes in jump height were -16.3 ± 12.5 % and -21.5 ± 14.8 % for the control and milk groups, respectively. All effects for both time points were unclear.

The *p* value for the main interaction effect was 0.223.

7.3.2.3 15 m Sprint

At baseline the time taken to cover 5 m was 1.14 s and 1.11 s for the control and milk groups, respectively. Between baseline and 48 h there were no clear effects of the control group (4.5 ± 7.4 %) in comparison to the milk group (1.4 ± 3.2 %) for attenuating increases in the time to cover 5 m. This was the same for changes between baseline and 72 h where the change in the control group was 4.8 ± 4.8 % and in the milk group was 3.4 ± 5.3 %. The *p* value for the main interaction effect was 0.748.

At baseline the time taken to cover 10 m was 1.89 s and 1.86 s for the control and milk groups, respectively. Between baseline and 48 h there was a possible benefit of the milk group (1.7 ± 1.8 %) in comparison to the control group (5.0 ± 4.8 %) for blunting increases in the time to cover 10 m. The effect was unclear for changes between baseline and 72 h where the change in the control group was 5.2 ± 2.1 % and in the milk group was 4.0 ± 1.9 %. The *p* value for the main interaction effect was 0.860.

At baseline the time taken to cover 15 m was 2.57 s and 2.53 s for the control and milk groups, respectively. Between baseline and 48 h there were no clear effects of the milk group (3.5 ± 2.5 %) in comparison to the control group (5.1 ± 4.0 %) for attenuating increases in the time to cover 15 m. There was a possible benefit of the milk group (2.6 ± 2.5 %) in comparison to the control group (5.9 ± 4.3 %) for blunting increases in the time to cover 15 m between baseline and 72 h (Figure 7.5). The *p* value for the main interaction effect was 0.720.

Figure 7.5 Relative 15 m time in response to exercise-induced muscle damage in the control (n = 7) and milk (n = 7) groups. Values presented as mean ± standard deviation.

7.3.2.4 Change of Direction Speed

Baseline values for change of direction speed were 10.61 s and 10.31 s for the control and milk groups, respectively. Between baseline and 48 h there were no clear effects of the milk group (2.0 ± 3.4 %) in comparison to the control group (3.4 ± 3.5 %) for blunting increases in change of direction speed. There was, however, a likely benefit of the milk group (0.7 ± 3.9 %) in comparison to the control group (4.8 ± 3.1 %) for attenuating increases in change of direction speed between baseline and 72 h (Figure 7.6). The *p* value for the main interaction effect was 0.086.
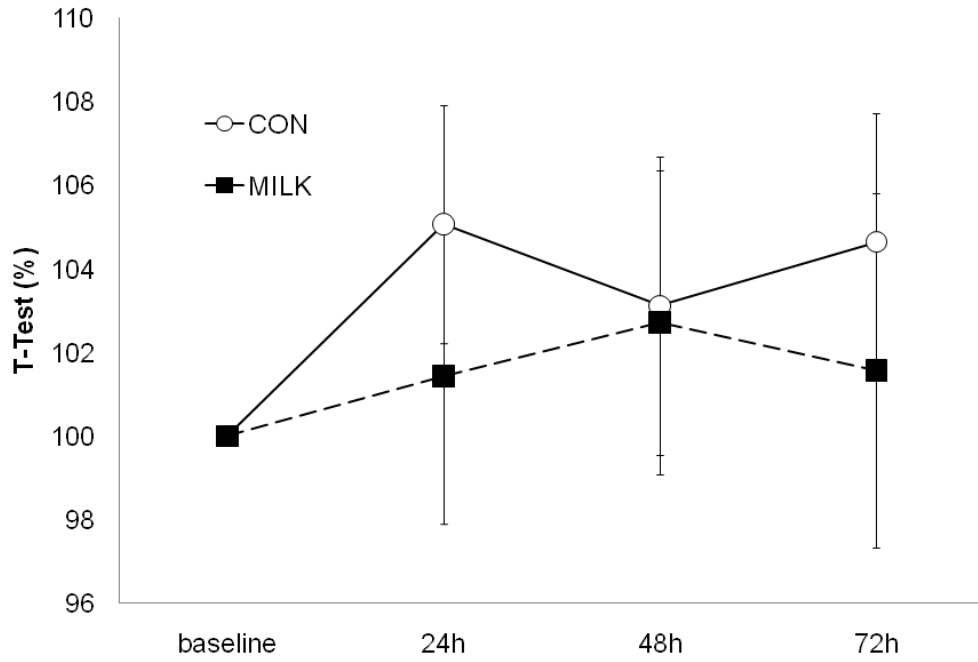
Figure 7.6 Relative T-test time in response to exercise-induced muscle damage in the control (n = 7) and milk (n = 7) groups. Values presented as mean ± standard deviation.

7.3.3 LIST Performance

A summary of the statistical analysis is shown in Table 7.3.

Table 7.3 Effect of milk on changes in variables measured during the Loughborough Intermittent Shuttle Test before and following muscle damaging exercise

LIST	Mean effect[a] ± 90% CI[b]	Qualitative Inference
Heart Rate	8 ± 13	Unclear
RPE	2 ± 2	Unclear
15 m	-2.3 ± 2.2	Likely Decrease

[a]Mean effect refers to the milk group minus the control group; [b]±90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude.

7.3.3.1 Heart Rate

Heart rate during the LIST before muscle damaging exercise was 158 bpm and 150 bpm for the control and milk groups, respectively. There was no clear effect of the milk group (4 ± 14 bpm) in comparison to the control group (-4 ± 9 bpm) for attenuating changes in heart rate. The *p* value for the main interaction effect was 0.533.

7.3.3.2 Rating of Perceived Exertion

RPE before muscle damaging exercise was 16 and 15 for the control and milk groups, respectively. There was no clear effect of the milk group (1 ± 1) in comparison to the control group (0 ± 2) for attenuating changes in RPE. The *p* value for the main interaction effect was 0.197.

7.3.3.3 15 m Sprint

The mean time to cover 15 m during 90 minutes of the LIST prior to muscle damaging exercise for the control and milk groups was 2.93 s and 2.86 s, respectively. There was a likely benefit of the milk group (0.0 ± 2.0 %) in comparison to the control group (2.4 ± 1.9 %) for blunting increases in the mean time to cover 15 m during 90 minutes of the LIST (Figure 7.7). The *p* value for the main interaction effect was 0.009.
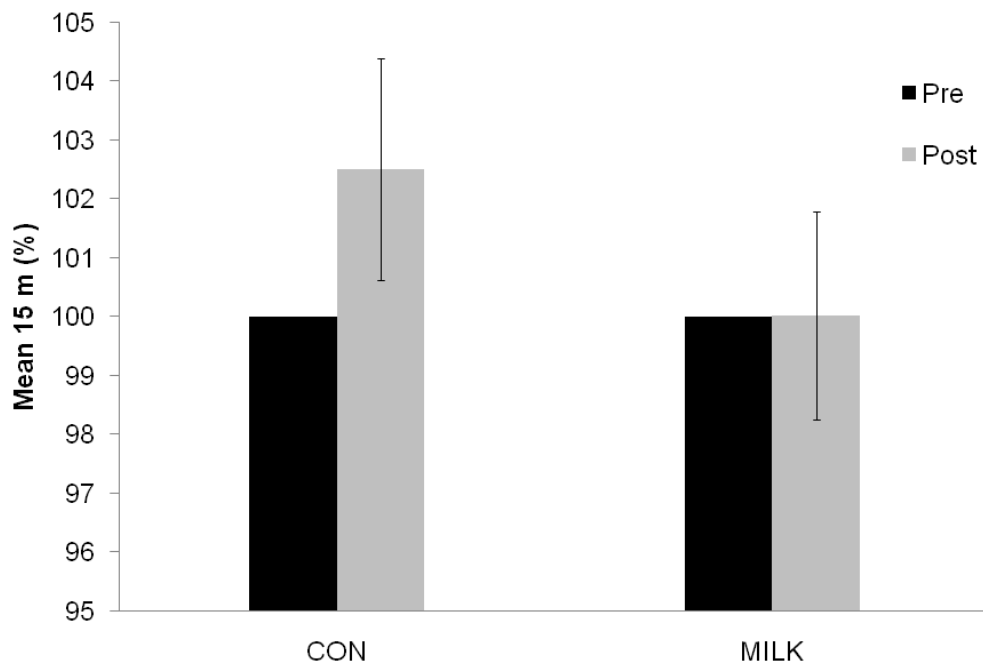
Figure 7.7 Relative mean time to cover 15 m during 90 min shuttle running in response to exercise-induced muscle damage in the control (n = 6) and milk (n = 6) groups. Values presented as mean ± standard deviation.

7.3.4 Intramuscular Proteins in the Serum

A summary of the statistical analysis is shown in Table 7.4.

Table 7.4 Effect of milk on increases in intramuscular proteins following muscle damaging exercise

Variable	Mean effect[a] ×/÷90% CI[b]	Qualitative Inference
baseline - 48 h		
CK	3.36 ×/÷ 11.50	Unclear
Mb	0.96 ×/÷ 3.07	Unclear
baseline - 72 h		
CK	2.34 ×/÷ 69.2	Unclear
Mb	0.63 ×/÷ 4.17	Unclear

[a]Mean effect refers to the milk group minus the control group; [b]×/÷90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference. Qualitative inference represents the likelihood that the true value will have the observed magnitude. CK = creatine kinase; Mb = myoglobin

7.3.4.1 Creatine Kinase

Mean baseline CK values for the the control and milk groups were 321 U.L^{-1} and 174 U.L^{-1}, respectively. Baseline CK values for the control group are relatively high but as results are analysed as the difference between groups in change over time this would not alter results. For both groups CK increased over 72 h. There were no clear effects of the milk group (5.0 $\times/\div$ 3.1) in comparison to the control group (1.5 $\times/\div$ 2.3) for limiting increases in CK between baseline and 48 h. Between baseline and 72 h there were no clear effects of the milk group (11.4 $\times/\div$ 7.0) in comparison to the control group (4.9 $\times/\div$ 5.2) for blunting increases in CK. The *p* value for the main interaction effect was 0.655.

7.3.4.2 Myoglobin

Mean baseline Mb values were 41.8 ng.mL^{-1} and 34.1 ng.mL^{-1} for the control and milk groups, respectively. Both groups showed an increase in Mb up to 72 h (Figure 7.8). Between baseline and 48 h changes in Mb were 1.4 $\times/\div$ 2.6 and 1.3 $\times/\div$ 3.3 for the control and milk groups, respectively. Changes between baseline and 72 h in the control and milk group were 3.1 $\times/\div$ 4.1 and 1.9 $\times/\div$ 3.4, respectively. There were no clear effects at any time point. The *p* value for the main interaction effect was 0.549.
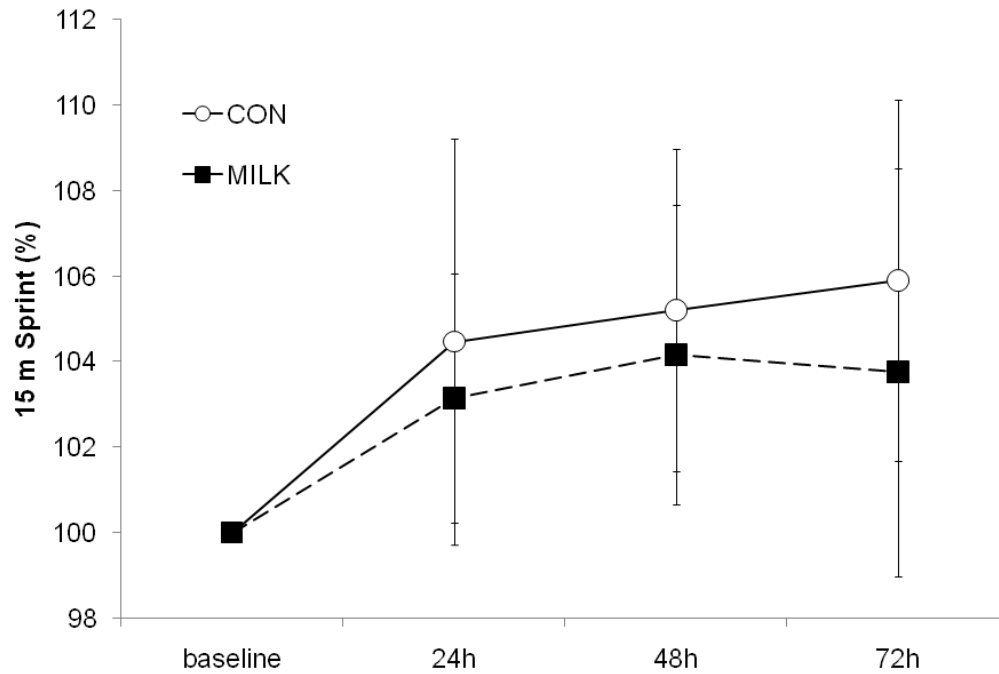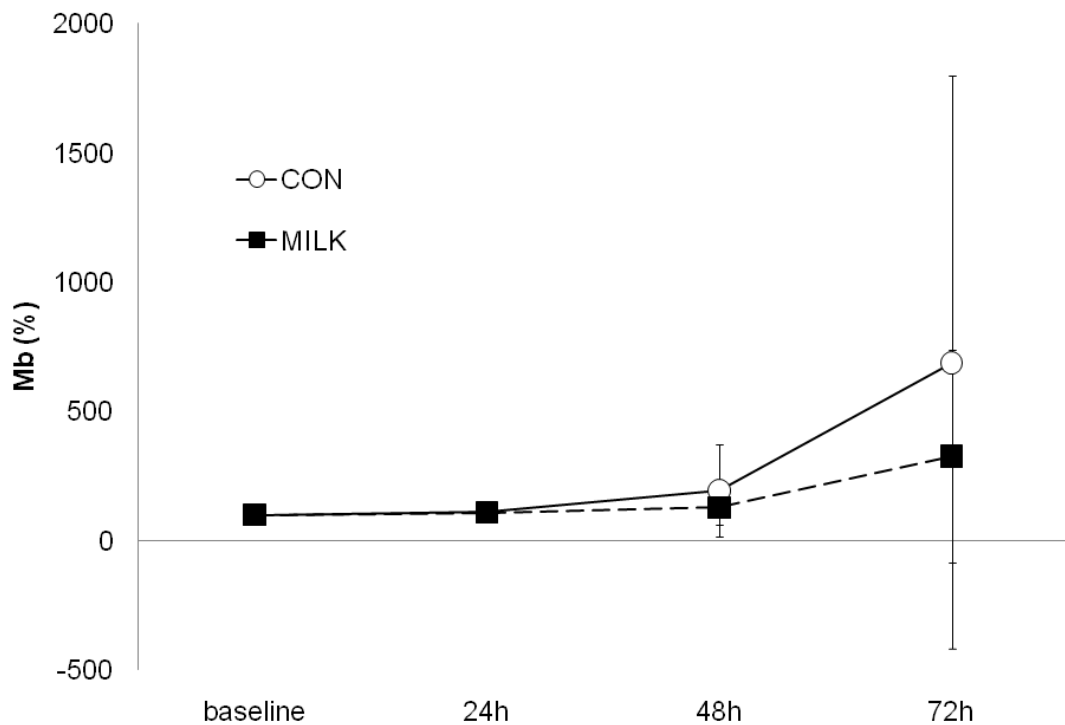
Figure 7.8 Relative Myoglobin (Mb) in response to exercise-induced muscle damage in the control (n = 7) and milk (n = 6) groups. Values presented as mean ± standard deviation.

7.4   Discussion

The primary finding of this study was that milk consumed immediately following muscle damaging exercise limited decrements in physiological aspects of field-based team sports. A benefit was observed for the time to cover 10 and 15 m, change of direction speed and mean 15 m sprint performance during the LIST. There was no benefit for active and passive muscle soreness, increases in intramuscular proteins in the serum, reactive strength index, countermovement jump height, and RPE and heart rate during the LIST.

The finding of attenuated muscle function is in agreement with the first three studies. However, the previous studies have demonstrated benefits to isokinetic concentric muscle actions, whereas the current study demonstrated a benefit for dynamic sporting movements. The ingestion of milk may have increased myofibrillar protein synthesis and/or limited increases in degradation that may have maintained the force transmitting and/or force generating protein structures, or enhanced repair. This would allow subsequent performance to take place at closer to

optimal levels.   These structural factors are only one hypothesised reason why decrements in muscle function occur following muscle damaging exercise, and is a likely mechanism underlying the attenuation of one-off performance.   A reduction in glycogen re-synthesis has also been observed following muscle damaging exercise (O'Reilly *et al.*, 1987; Asp *et al.*, 1998; Zehender *et al.*, 2004).   Muscle glycogen is important for performance in intermittent sports and, therefore, impairment of glycogen re-synthesis will limit performance (Byrne, Twist & Eston, 2004).   The LIST replicates the physiological demands of field-based team sports (Nicholas, Nuttall & Williams, 2000), and muscle glycogen is depleted during it (Nicholas *et al.*, 1999).   Therefore, if muscle glycogen is primarily utilised during the LIST and the re-synthesis of it is inhibited, the ability to perform repeated sprints will be reduced.   The intake of milk could have limited these changes.   Reduced glucose uptake into the muscle cell due to inflammatory processes (Jansson, Hjemdahl & Kaijser, 1986; Costill *et al.*, 1990; Kirwan & Del Aguila, 2003) and decreased insulin sensitivity due to disruption of the muscle cell membrane (Doyle, Sherman & Strauss, 1993), and reduced glucose transport via GLUT4 (Asp, Daugaard & Richter, 1995) have all been implicated in reduced glycogen re-synthesis.   The intake of milk may limit disruption to the muscle membrane, decreases in GLUT4 content or impact on the proteolytic pathways stimulated via inflammation, all of which may impact on the muscles ability to re-synthesise glycogen.   In turn this would allow participants to maintain their ability to perform repeated sprints to a greater extent, which is what has been observed in this study.

There was no impact of milk on the physiological responses (RPE and heart rate) to performance of the LIST.   Muscle damaging exercise has been shown to increase RPE during endurance exercise performed 48 h later (Davies, Rowlands & Eston, 2009; Twist & Eston, 2009).   A change in RPE is implicated in reduced performance (Twist & Eston, 2009).   RPE did not increase in either group; therefore, this will not have impacted on performance.   Heart rate averaged over each 15 min cycle also did not change between pre and post muscle damaging exercise in either group.   Therefore, participants' ability to exercise at a relative intensity was not altered due to EIMD.   This provides indirect evidence against a centrally regulated mechanism of reduced performance.

Similar to previous studies there was no benefit of milk on passive or active muscle soreness. During measurement of either aspect of muscle soreness the

hamstrings were not isolated. This may have, therefore, affected individual perceptions of DOMS. Secondly, milk supplementation may not impact on the processes leading to DOMS. The lack of impact of milk supplementation on intramuscular proteins measured in the serum is in contrast to previous studies. The degradative pathways impacting on membrane disruption are different from those that lead to myofibrillar disruption, and thus muscle function. Milk may only impact on myofibrillar protein metabolism and not lysosomal degradation, which is why only benefits were observed to most aspects of muscle performance. However, this does not explain why differences were observed in previous studies. CK is a highly variable indirect marker of EIMD and Mb should be used with caution as previously discussed; this may have impacted on the contrasts observed. From an applied perspective, changes in intramuscular proteins are likely to be functionally irrelevant; therefore, this finding will be of no concern to athletes involved in field-based team sports.

Similar to study 3 there was no benefit of milk intake on measures of reactive strength index, and in addition there was no benefit to countermovement height. The decrements observed in reactive strength index were similar to those for the control group in study 2. During jumping activities, the hamstrings do not play a significant role in performance; therefore, if milk ingestion does limit myofibrillar damage then an impact on performance will not be observed. This is because other major muscle groups impacting on jumping ability may mask the effects. During sprinting activities the hamstrings are used to a greater extent than the quadriceps (Mann, Moran & Dougherty, 1986), which may be why benefits to this aspect of performance were observed. However, there was no effect of milk supplementation on the time to cover 5 m. The electromyogram (EMG) activity of the hamstrings increases as speed increases (Mero & Komi, 1987), therefore, during the initial 5 m other lower limb muscles may have a more predominant role in performance.

This study has demonstrated that 500 mL of milk consumed immediately following muscle damaging exercise limits decrements in one-off sprinting performance and change of direction speed, and the ability to perform repeated sprints during the physiological replication of field-based team sports. These athletes would, therefore, be able to limit performance decrements following training or matches that could impact on subsequent performance. During the competitive season, this is

important as the match day does not always occur on the same day each week (Ascensao *et al.*, 2008) and on many occasions a number of matches take place in one week.   This information provides coaches and sports scientists with the knowledge of how long performance can be affected after a match or training, and use it to inform recovery strategies. The hypothesised reasons may be linked to alterations in protein metabolism and the maintenance of muscle glycogen resynthesis.

8. GENERAL SUMMARY AND DIRECTIONS FOR FUTURE INVESTIGATION

8.1   General Discussion

The overall purpose of this thesis was to investigate the effect of acute milk supplementation on the attenuation of Exercise Induced Muscle Damage (EIMD). The main finding was that 500 mL of milk consumed immediately following muscle damaging exercise reduces decrements in many aspects of muscle function between 48 and 72 h, including peak torque, sprints, change of direction ability and repeated sprints during physiological replication of field-based team sports. There was some evidence that this intervention also limited decreases in reactive strength index and increases in active muscle soreness and intramuscular proteins, but this was not conclusive. There was no benefit of milk on measures of passive muscle soreness or countermovement jump height.

A number of studies have previously demonstrated beneficial effects of protein-carbohydrate (CHO) on EIMD (Saunders, Kane & Todd, 2004; Seifert *et al.*, 2005; Baty *et al.*, 2007; Saunders, Luden & Herrick, 2007; Valentine *et al.*, 2008), and this thesis adds to the existing literature. However, the majority of these studies have based their conclusions on measures of intramuscular proteins in the serum, as previously discussed. Very few studies measuring muscle function following EIMD have demonstrated beneficial effects of acute protein-CHO supplementation (Valentine *et al.*, 2008). This thesis is, therefore, novel in its findings of attenuated decrements in muscle function and has extended the literature to findings that can be applied to field-based team sports. Furthermore, there is no existing literature investigating volume of supplementation and only one other study (White *et al.*, 2008) that has examined timing of supplementation. Therefore, the findings reported in this thesis can be practically applied to provide specific advice for athletes.

There are a number of proposed theories as to why supplementation with 500 mL of milk immediately following muscle damaging exercise reduces EIMD. These are highlighted in Figure 8.1. Throughout this thesis it has been proposed that the primary benefit is due to changes in protein metabolism. Following eccentric exercise there are increases in mixed muscle (Phillips *et al.*, 1997; Trappe *et al.*, 2002b) and myofibrillar (Moore *et al.*, 2005) protein synthesis, which is possibly linked to an increase in $p70^{S6K}$. Following eccentric exercise $p70^{S6K}$ phosphorylation increases for 2 h in the absence of nutritional supply (Eliasson *et al.*, 2006). Mixed muscle protein degradation is elevated following eccentric exercise

(Fielding *et al.*, 1991; Lowe *et al.*, 1995; Phillips *et al.*, 1997). Results surrounding the effect of eccentric exercise on myofibrillar protein degradation are equivocal likely due to the difficulty of using 3-methylhistadine (3-MH) as a measure. These changes in muscle protein metabolism will result in a negative protein balance in the absence of nutritional supply, as this has been observed following resistance exercise (Biolo *et al.*, 1995; Phillips *et al.*, 1997). The catabolic state of the muscle is likely responsible for the sarcomere damage that has been observed (Newham *et al.*, 1983; Lauritzen *et al.*, 2009), specifically damage to the Z-disks (Hansen *et al.*, 2009) resulting from the loss of desmin (Friden & Lieber, 1998), actin, myosin (Ingalls, Warren & Armstrong, 1998), titin and nebulin (Trappe *et al.*, 2002a). However, relationships between protein metabolism and damage have not been investigated. It is postulated that the co-ingestion of CHO and protein in the form of milk leads to a positive protein balance following eccentric exercise due to an increase in protein synthesis and/or limiting increases in protein breakdown.
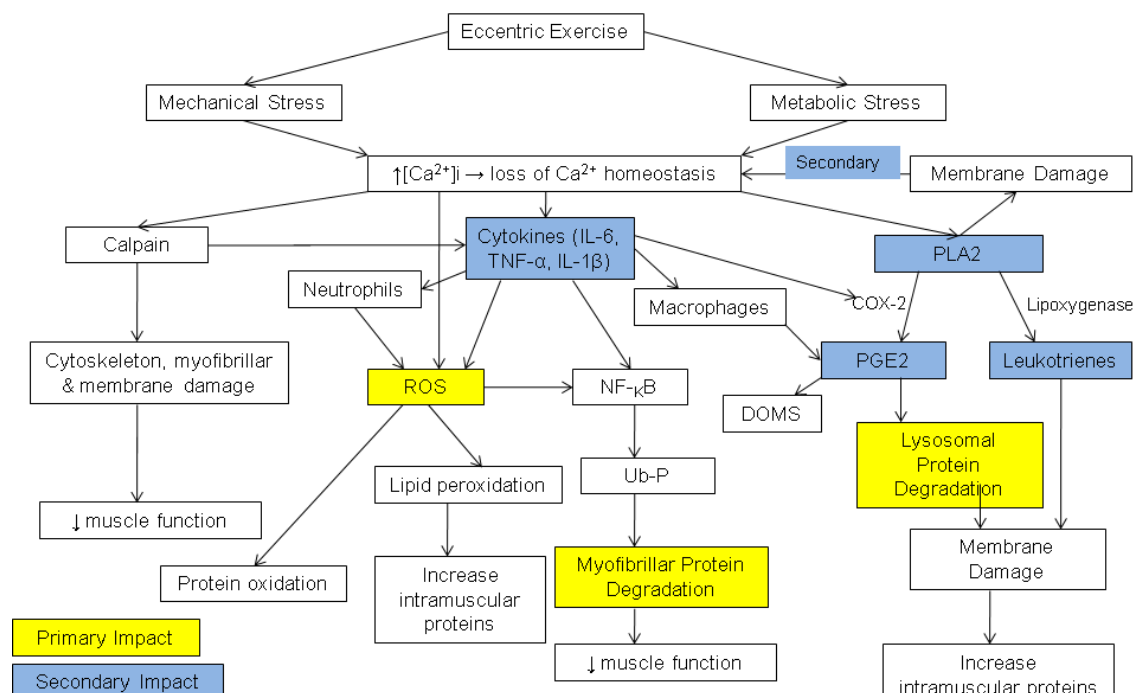
Figure 8.1 Proposed theories of benefits of milk on processes occurring during exercise-induced muscle damage. **COX-2** = cyclooxygenase-2; **DOMS** = delayed-onset of muscle soreness; **IL-6** = interleukin-6; **IL-1β** = interleukin-1beta; **NF-κB** = nuclear factor kappa-light-chain-enhancer of activated B cells; **PGE2** = prostaglandin E2; **PLA2** = phospholipase A2; **ROS** = reactive oxygen species; **TNF-α** = tumor necrosis factor-alpha; **Ub-P** = ubiquitin proteasome

Milk ingestion following resistance exercise has been shown to lead to a positive net muscle protein balance (Elliot *et al.*, 2006). CHO intake reduces the negative protein balance observed due to a progressive and delayed reduction of protein breakdown (Miller *et al.*, 2003; Borsheim *et al.*, 2004). CHO intake increases insulin

concentrations (Miller *et al.*, 2003; Borsheim *et al.*, 2004), which could decrease lysosomal activity (Mortimore, Ward & Schworer, 1978). Protein intake stimulates net muscle protein synthesis (Tipton *et al.*, 2004; Tang *et al.*, 2009) from a combination of myofibrillar and sarcoplasmic protein synthesis (Moore *et al.*, 2009b). The co-ingestion of these nutrients is, therefore, optimal for eliciting an anabolic state. Following eccentric exercise, the intake of milk leading to a positive protein balance could enhance repair (Blacker *et al.*, 2010) and/or limit damage of muscle proteins.

Following a combination of resistance exercise and amino acids or protein and CHO intake, a positive protein balance is mainly attributed to increases in protein synthesis (Borsheim, Aarsland & Wolfe, 2004). The ingestion of milk could stimulate protein synthesis by providing the substrate (amino acids) and the signal required for protein synthesis to occur. The intake of protein will increase the availability of intracellular amino acids, which would allow protein synthesis to take place. Consuming protein will increase amino acid plasma concentrations that will stimulate amino acid transporters to increase the uptake of amino acids into the muscle cell, increasing intracellular amino acid availability and thus protein synthesis (Miller *et al.*, 2004). This will be a delayed response due to the time required for milk proteins to be digested and go through the process of gastric emptying, which has a half-time of 26 minutes (Calbet & MacLean, 1997). This may allow amino acid availability to coincide with increased insulin concentration, therefore, allowing insulin to exert its effect on muscle protein synthesis. This is postulated to be the reason why milk should be consumed following exercise as the availability of nutrients will coincide with the secondary phase exacerbating the damage, rather than the primary mechanical event. Milk could also provide a signal for increasing muscle protein synthesis via the Akt-mTOR-p70^{S6K} pathway. Branched chain amino acid ingestion following resistance exercise leads to significant increases in p70^{S6K} phosphorylation (Karlsson *et al.*, 2004). This could increase messenger ribonucleic acid (mRNA) translation and thus muscle protein synthesis, however, this is not clear from the research. Increasing muscle protein synthesis, both myofibrillar and non-myofibrillar, would enhance repair of the damaged structures, and thus changes in muscle function and intramuscular proteins measured in the plasma would be reduced. The finding that only 500 mL of milk is required to elicit positive effects on EIMD provides indirect support for increases in protein synthesis being the underlying mechanism. Muscle protein synthesis is not further stimulated by consuming more than 20 g of protein (Moore *et al.*, 2009a), therefore, consuming 1000 mL of milk containing 34 g

protein would not provide any extra benefit to attenuating EIMD. The third study of this thesis demonstrated this was the case. However, increasing muscle protein synthesis is unlikely to be the sole underlying cause as increasing myofibrillar protein synthesis is unlikely to repair enough damaged muscle proteins within 48 h (Tipton, personal communication).

Providing protein and CHO may also limit muscle protein breakdown, thus limiting ultrastructural damage and, therefore, changes in indirect markers of EIMD. Providing a CHO/amino acid supplement following resistance exercise has been shown to significantly reduce myofibrillar protein breakdown (Bird, Tarpenning & Marino, 2006). CHO may attenuate muscle protein degradation through the action of insulin. Protein intake will provide exogenous amino acid that can be utilised for increased synthesis rather than elevating breakdown to provide the substrate, therefore, suppressing the signal for muscle protein breakdown (Biolo *et al.*, 1997; Greer *et al.*, 2007).

The intake of milk limits decrements in muscle function. Disruption to the sarcomere and excitation-contraction (E-C) coupling damage can both limit the ability of the muscle to produce force (Warren *et al.*, 2002). Initial decrements in force production are postulated to be due to E-C coupling damage (Warren *et al.*, 2002). In the days following muscle damaging exercise, myofibrillar damage is thought to play an increased role in reduced muscle function (Warren *et al.*, 2002; Raastad *et al.*, 2010). Research has found significantly, strong correlations between muscle function and myofibrillar disruption (Lauritzen *et al.*, 2009; Raastad *et al.*, 2010). Myofibrillar protein breakdown occurs via the activation of the ubiquitin-proteosome system (Ub-P). Calpain activation is the first step in myofibrillar degradation (Saido, Sorimachi & Suzuki, 1994) and is probably necessary for complete disruption via the Ub-P pathway. Therefore, the intake of milk may suppress protein degradation occurring via this pathway. Limiting myofibrillar protein breakdown would lead to greater preservation of the myofibrillar proteins responsible for force transmission and generation, therefore, limiting decrements in muscle function. Reduced decrements in muscle function were not observed until 48 - 72 h, which may provide indirect evidence that it is the blunting of myofibrillar protein degradation which is the underlying mechanism. At these time points loss of muscle proteins is likely to have a greater role in reduced muscle function than damage to the E-C coupling system. However, milk consumption may alter metabolism of the proteins

involved in E-C coupling; therefore, milk impacting on E-C coupling damage should not be ruled out. The muscle function measures utilised in this thesis would predominantly rely on fast twitch muscle fibres. Following muscle damaging exercise, damage may have predominantly occurred in these fibres; therefore, muscle function relying on these fibres for force production would be detrimentally affected.

There was evidence that acute milk supplementation limited increases in intramuscular proteins measured in the serum. The lysosomal pathway leads to the breakdown of non-myofibrillar proteins (Biolo *et al.*, 2000; Waterlow, 2006), and this may be stimulated during EIMD. Similar to the discussion of the Ub-P pathway, the intake of milk could limit protein degradation via this pathway leading to better maintenance of the membrane structures within the muscle, and thus reducing increases in intramuscular proteins released into the plasma. The lysosomal system is sensitive to changes in amino acids concentration (Fulks, Li & Goldberg, 1975), and insulin secreted by CHO can decrease the proteolytic activity of lysosomes (Mortimore, Ward & Schworer, 1978). Furthermore, mTOR, part of the Akt-mTOR-p70^{S6K} intracellular signalling pathway of protein synthesis, inhibits the autophagy process (Negro *et al.*, 2008). Therefore, the intake of milk that signals this pathway may limit lysosomal protein degradation.

Myofibrillar and lysosomal protein breakdown are stimulated by a number of processes during EIMD (Figure 2.3). Specifically, the ubiquitin-proteosome pathway is initiated by reactive oxygen species, cytokines and/or prostaglandin E_2 (PGE_2) via phospholipase A_2 (PLA_2) production. Lysosomal protein degradation can be stimulated by cytokines and PLA2. It is possible that milk consumption has a secondary impact on signalling in one of these pathways (Figure 8.1). The cytokines have begun to attract attention in this area of research. Tumor necrosis factor-α (TNF-α), which increases following muscle damaging exercise (Cannon *et al.*, 1991; Hamada *et al.*, 2005), has been implicated as a mediator of proteolytic pathways (Costelli *et al.*, 1993; Andreu & Schwartz, 1995; Frost, Lang & Gelato, 1997). Rowlands *et al* (2008) analysed TNF-α following interval cycling with a protein enriched meal and found no difference between a high protein and low protein meal even though there was a possible attenuation of increases in creatine kinase (CK). Furthermore, Buford *et al* (2009) found no relationship between TNF-α and CK, implying that this cytokine does not play a role in membrane damage. Other studies (Wojcik *et al.*,

2001; Betts *et al.*, 2009) have examined the response of interleukin-6 (IL-6). There was no effect of protein-CHO intake on IL-6, however, these studies failed to demonstrate a benefit on any indirect markers of EIMD thus limiting the conclusions that can be drawn. Similarly, Miles *et al* (2007) did not observe any attenuation of IL-6 following CHO supplementation for 2 days after high force eccentric exercise. Again there was no impact of CHO on other indirect markers of EIMD, which is in agreement with findings from this thesis. IL-6 may not play a role in muscle protein breakdown as research demonstrated that the limitation of muscle proteolysis with branched chain amino acids supplementation was independent of the IL-6 response (Rohde *et al.*, 1997). This thesis did not find any conclusive evidence linking the benefit of milk ingestion to attenuated IL-6 and C-reactive protein (CRP) responses. Future research is required to investigate this potential theory fully.

PLA_2 leads to the production of prostaglandins and leukotrienes (Duncan, 1988; Armstrong, Warren & Warren, 1991). This pathway has been implicated in the degradation of membrane phospholipids and thus the release of intramuscular proteins into the serum, as inhibiting it protected against the efflux (Duncan & Jackson, 1987). CK has been shown to be significantly related to platelet activating factor, which is synthesised from PLA_2 (Milias *et al.*, 2005). The intake of milk may limit the activation of PLA_2 and thus increases in intramuscular proteins measured in the plasma. Other parts of this pathway include PGE_2 and leukotriene activation via cyclooxygenase-2 (COX2) and lipoxygenase, respectively. Therefore, milk may impact on signalling downstream of PLA_2 activation that could limit membrane damage and thus increases in CK and myoglobin (Mb). COX2 activation is required for muscle recovery as it catalyses the reaction of $PGF_{2\alpha}$, which is involved in protein synthesis (Rodemann & Goldberg, 1982). Therefore, dampening down this pathway may not be beneficial to recovery from EIMD as it is required to increase muscle protein synthesis and thus repair of damaged proteins.

Cycloxygenase activation is thought to be hyperalgesic (Dubois *et al.*, 1998) with PGE_2 production sensitising afferent receptors to stimuli (Smith, 1991). Therefore, muscle soreness may be related to cycloxygenase activity. However, evidence analysing muscle soreness, COX2 and PGE_2 does not support this notion (Kuipers *et al.*, 1985; Croisier *et al.*, 1996; Buford *et al.*, 2009). This thesis found no impact of milk on measures of passive muscle soreness (DOMS) and inconclusive evidence of its impact on active DOMS. It is possible that milk does not impact on this

pathway.   However, as previously discussed the measurement of muscle
soreness is subjective, it is difficult to compare independent groups and some
measurements of active DOMS were conducted without the hamstrings being
isolated. Therefore, this theory should not be discarded.

Reactive oxygen species activation can lead to protein and lipid oxidation, which has
been shown to occur following eccentric exercise (Sacheck *et al.*, 2003; Close *et al.*,
2005a; Liao *et al.*, 2010).   Lipid oxidation would cause damage to the membrane
phospholipids and thus lead to increases in CK and Mb.   Protein oxidation would
cause damage to muscle proteins leading to changes in many of the measured
variables.   Reactive oxygen species activation has also been implicated in activating
the NF-κB pathway (Muller, Rupec & Baeuerle, 1997; Li *et al.*, 2003), which in turn
activates the Ub-P system (Jackman and Kandarian, 2004).   Therefore, increased
reactive oxygen species production may lead to decrements in muscle function. Milk
contains anti-oxidants, including selenium, vitamin E, vitamin A and glutathione (GSH)
(Haug, Hostmark & Harstad, 2007).

Reduced glutathione is synthesised from L-cysteine, L-glutamic acid and glycine.
Whey protein is rich in cysteine, and as cysteine is the limiting factor of glutathione
synthesis, it may represent an effective cysteine delivery system for glutathione
replenishment (Bounous & Molson, 2003).   In its reduced state, glutathione is able to
donate a reducing equivalent (H^+ and e^-) to reactive oxygen species to remove them,
forming oxidised glutathione (GSSH) (Haug, Hostmark & Harstad, 2007).   Therefore,
the intake of milk may limit oxidative stress, thus limiting Ub-P activation, and protein
and lipid oxidation that would subsequently dampen myofibrillar and membrane
damage and, therefore, changes in muscle function and intramuscular proteins
measured in the serum. There is little research investigating the oxidative response to
milk intake. One study in mice showed that lipid oxidation was prevented following a
heavy exercise session when the diet was supplemented with whey protein (Elia *et al.*,
2006).

Oxidised glutathione may also have a role in preserving Ca^{2+} homeostasis,
specifically inhibiting its release by reducing the open status of Ca^{2+} channels and
stimulating its uptake via metabolic Ca^{2+} pumps (Belia *et al.*, 2000).   Prolonged
increases in Ca^{2+} concentration have been implicated in initiating the proteolytic and

lipolytic pathways discussed. Preserving Ca^{2+} homeostasis would limit the activation of these pathways and thus myofibrillar and membrane damage. This would reduce decrements in muscle function and increases in CK and Mb. Milk was most beneficial when consumed immediately following muscle damaging exercise and is unlikely to have impacted on initial increases in Ca^{2+} concentration. However, the secondary influx of Ca^{2+} (Yeung & Allen, 2004; Gissel, 2005) could be altered, which may be another reason why benefits are not observed until 48 - 72 h following muscle damaging exercise.

The intake of milk may impact on a number of processes occurring during the process of EIMD (Figure 8.1). One of the problems with the process of EIMD is that it is still not fully understood (Kendall & Eston, 2002), which adds to the difficulty when postulating what impact milk may be having. The area of research investigating the use of protein-CHO in aiding recovery following EIMD has only become increasingly popular in the last few years, with results equivocal and few studies investigating mechanisms.

Participants were unable to be blinded to the supplements used. Furthermore, participants knew the purpose of the studies. This could have potentially led to a placebo effect, whereby the participants' belief in the efficacy of milk led to enhanced performance. Research has shown that the placebo effect does exist in sport, whereby participants believed they had ingested a supplement, performed better than baseline or controls (Clark *et al.*, 2000; Beedie *et al.*, 2006). Participants may have heard claims regarding milk or had an understanding of the metabolic impact of protein and CHO. This could have influenced their performance psychologically by impacting on motivation and expectancy (Beedie & Foad, 2009). Although the placebo effect was possibly apparent and may have impacted on the results this would be of interest and not concern. Performance was improved following muscle damaging exercise for those participants consuming milk, and for athletes the concern is optimal performance not if the supplement is having a physiological impact. However, for applied scientists it is important to know what physiological impact an intervention has. The improvement in muscle performance may have been due to an interaction between psychological and physiological variables (Beedie & Foad, 2009). However, there was no benefit of milk on subjective measures (muscle soreness and rating of perceived exertion), reactive strength index and countermovement jump height. Therefore, if a placebo

effect existed, positive results in all measured variables would be expected.

The practical implications of the findings presented in this thesis are that it provides athletes with specific advice for type, amount and timing of supplementation to aid recovery following muscle damaging exercise. This information would be of benefit to those experiencing a new exercise stress that is mechanical in nature, and would allow them to train and/or perform closer to optimal levels in the days following exercise. It would also be of benefit to athletes during competition periods.   It should be highlighted that preventing the process of EIMD may not be beneficial to athletes.   It could be argued that in many situations blunting the processes that occur following muscle damaging exercise may hinder recovery and chronic adaptations. There has been limited research investigating the impact of recovery interventions on chronic adaptations, however, it should be considered when working with athletes. One study has investigated the effect of cold water immersion on endurance training effects (Yamane *et al.*, 2006).   It was found that following 6 weeks of endurance cycling training there was a significant improvement in performance time, maximal oxygen uptake and ventilator threshold in the control leg. However, in the leg that was immersed in cold water following each training session there was no significant improvements in maximal oxygen uptake and ventilator threshold, therefore, post-exercise cooling may be adverse for physical training (Yamane *et al.*, 2006).   In the current study, there was some evidence of milk limiting increases in active muscle soreness; however, this was not conclusive as previously discussed. Muscle soreness may provide a protective effect and, therefore, reducing this may alter an athlete's perception of their ability to carry out certain training loads (Barnett, 2006). However, this may only be harmful when there are no concurrent impacts of supplementation on muscle function. The other practical implication of this thesis is that convenient and accessible milk products have been used throughout the studies. This makes it very easy for athletes to use the information presented to their benefit. The findings have also impacted on industry by allowing companies to utilise the results in marketing material and highlighting the role of milk in sport.   This thesis has shown that milk benefits recovery from eccentric muscle actions. Although CHO is beneficial for recovery from glycogen depleting exercise (Tarnapolsky *et al.*, 1997), the first study of this thesis found no benefits for recovery from muscle damaging exercise. Recovery is multi-dimensional, and it is important that the strategy used accounts for this. Milk, due to its combination of CHO and protein, is beneficial for other aspects of athletic performance and recovery (Shirreffs, Watson & Maughan, 2007; Thomas, Morris &

Stevenson, 2009).   Milk will also help in meeting the athletes' CHO needs for successful performance as well as providing many health benefits.

8.2   Directions for Future Investigation

This thesis was applied in its direction and although it has provided some novel research findings that athletes could utilise, it has also raised more questions. Therefore, there are two main directions for future research: further applied research or investigating potential mechanisms. From an applied perspective there is vast potential for other measures of muscle function to be analysed, specifically those that can be applied to real world sport settings. The protocol to initiate EIMD throughout this thesis was novel to all participants mainly because it does not represent movements that would be commonly replicated by exercising individuals during their training. Although this provided an appropriate way to study EIMD, it initiated damage that was likely more pronounced than that which would be experienced during a new exercise stress, during a tournament or following a heavy training session. This may limit its applicability to the real-world setting. Therefore, future research should focus on using an exercise stress that is applied in nature.

Participants throughout this thesis were male who regularly competed in a variety of sports.   Therefore, the findings can be generalised to this group.   Future research should consider utilising different groups of participants including more highly trained individuals and women. Women are rarely used as participants in this area of research. Estrogen levels may affect skeletal muscles response to muscle damage. Tiidus and Enns (2009) argue that gender or estrogen minimses evidence of EIMD based on findings from animal studies   These studies demonstrate that females or higher estrogen levels limit damage to structural muscle proteins (Komulainen *et al.*, 1999), increases in CK (Amelink *et al.*, 1990) and the inflammatory response (St. Pierre Schneider, Correia & Cannon, 1999; Tiidus *et al.*, 2001). However, Hubal and Clarkson (2009) concluded that the majority of data from studies involving humans indicate that gender and estrogen levels do not significantly affect EIMD measured directly (Stupka *et al.*, 2000; Stupka *et al.*, 2001) or indirectly via CK (Sorichter *et al.*, 2001; Sewright *et al.*, 2008), strength recovery (Thompson *et al.*, 1997; Sayers & Clarkson, 2001) and muscle soreness (Rinard *et al.*, 2000; Dannecker *et al.*, 2005; Sewright *et al.*, 2008). Results from humans and animals clearly show different results and although animal studies provide a model that allows for control of confounding variables and participant variability, they are limited as the results are not easily transferred to humans. Although, the majority of

evidence demonstrates there are no gender differences in the response to muscle damage, it cannot be assumed that the findings of this thesis are transferrable to women. A recent investigation demonstrated that milk consumption in combination with resistance exercise lead to gains in muscle mass in women (Josse, et al, 2010). Therefore, milk does have benefits for exercising women, and future investigations should concentrate on milk for recovery in women. Milk may also have additional benefits in recovery. Studies have demonstrated increased perceptions of satiety when milk was consumed in comparison to a fruit drink (Dove *et al.*, 2009) or carbonated beverage (Harper *et al.*, 2007) at breakfast. Dove *et al* (2009) found that this translated into decreased energy intake at lunchtime. Therefore, milk may also suppress appetite and help achieve a negative energy balance, which could aid weight loss or weight maintenance. For many individuals, particularly women, this is one of the main focuses of exercising.

The benefits of milk have been postulated to be due to limiting the breakdown of muscular proteins or enhancing their repair via increased protein synthesis and/or limiting increases in protein breakdown. There has been very little research investigating protein metabolism following EIMD and only one study examining the impact of protein-CHO on these changes (Wojcik *et al.*, 2001). Therefore, future research should consider these types of studies. In conjunction with this it is important to examine the parts of the pathway where milk ingestion may be impacting on, as outlined in Figure 8.1. Studies that have attempted this by measuring cytokines have done so by analysing changes in the blood. This may not reflect changes in the muscle, and therefore, future studies should use muscle biopsies and microdialysis techniques. However, these methods can lead to methodological artefacts and microdialysis cannot be used during exercise.

8.3   Conclusion

In conclusion, 500 mL of milk consumed immediately following eccentric resistance based exercise limits decrements in some facets of muscle function, such as sprinting and isokinetic concentric muscle actions. There is a possible benefit of milk for reducing increases in muscle soreness experienced during activity and intramuscular proteins measured in the serum. There are a number of postulated mechanisms which could lead to these observed benefits. However, until further research is conducted on the mechanisms of EIMD and changes in protein metabolism following muscle damaging exercise it is difficult to conclude with

certainty what these are.

APPENDICES

INFORMED CONSENT FORM

Project Title: The effect of milk supplementation on football performance
following exercise-induced muscle damage

Principal Investigator: Emma Cockburn

Participant Number: _____

please tick
where applicable

I have read and understood the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to take part in this study.	☐
I would like to receive feedback on the overall results of the study at the email address given below. I understand that I will not receive individual feedback on my own performance.	☐

Email address…………………………………………………………………

Signature of participant.. Date.....……………..

(NAME IN BLOCK LETTERS)...………………………

Signature of Parent / Guardian in the case of a minor

....

Signature of researcher.. Date.....……………..

(NAME IN BLOCK LETTERS)...………………………

FOR USE WHEN TISSUE IS BEING REMOVED <u>BUT NOT</u> STORED

Project Title: The effect of milk supplementation on football performance following exericse-induced muscle damage.

Principal Investigator: Emma Cockburn

Participant Number: _____

I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
Blood (plasma)	For analysis of the following: Creatine Kinase, Myoglobin and markers of inflammation	Via venipuncture

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

Signature of participant.. Date.....………………..

Signature of Parent / Guardian in the case of a minor

.... Date.....………………..

Signature of researcher.. Date.....………………..

GENERAL HEALTH QUESTIONNAIRE

Name: ……………………………

Date of Birth: ……………………

Subject number: …………………

As you are participating in exercise within this laboratory, please can you complete the following questionnaire. Your co-operation is greatly appreciated.

All information within this questionnaire is considered confidential.

Where appropriate please circle your selected answer.

1. How would you describe your current level of activity?
   Sedentary / Moderately Active / Highly Active

2. How would you describe your current level of fitness?
   Very Unfit / Moderately Fit / Trained / Highly Trained

3. How would you describe your current body weight?
   Underweight / Ideal / Slightly Overweight / Very Overweight

4. Smoking Habit: -
   Currently a non-smoker   Yes / No
   Previous smoker   Yes / No
   If previous smoker, how long since you stopped? ………Years
   Regular smoker   Yes / No   of …… per day
   Occasional smoker   Yes / No   of …… per day

5. Alcohol Consumption: -
   Do you drink alcohol?   Yes / No
   If yes then do you -   have an occasional drink   Yes / No
                          Have a drink every day?   Yes / No
                          Have more than one drink per day? Yes / No

6. Have you consulted your doctor within the last 6 months?
   Yes / No
   If yes, please give details to the test supervisor

7. Are you currently taking any medication (including anti-inflammatory drugs)?
   Yes / No
   If yes, please give details to the test supervisor

8. Do you, or have you ever suffered from:-
   Diabetes   Yes / No
   Asthma   Yes / No
   Epilepsy   Yes / No
   Bronchitis   Yes / No
   Elevated cholesterol   Yes / No
   High Blood Pressure   Yes / No

9. Do you suffer from, or have you ever suffered from any heart complaint or pains in your chest, either associated with exercise or otherwise?
   Yes / No

10. Is there a history of heart disease in your family?
    Yes / No

11. Do you feel faint or have spells of severe dizziness when undertaking exercise
    or otherwise?
    Yes / No

12. Do you currently have any form of muscle joint injury?
    Yes / No

13. Have you ever suffered from any knee joint injury or thigh injury?
    Yes / No

14. Have you had any reason to suspend your training in the last 2 weeks?
    Yes / No

15. Do you currently take any anti-inflammatory medication?
    Yes / No
    If yes, please give details to the test supervisor

16. Do you currently take any form of nutritional supplement (e.g. creatine, whey
    and casein protein, HMB, etc)?
    Yes / No
    If yes, please give details to the test supervisor

17. Are you able to drink milk?
    Yes / No

18. Is there anything to your knowledge that may prevent you from successfully
    completing the test that has been explained to you?
    Yes / No
    If yes, please give details to the test supervisor

Please provide any further information concerning any condition/complaint that you
suffer from and any medication that you may be taking by prescription or otherwise.
……………………………………………………………………………………………………
………………………………………………………………………………………

Signature of Subject: …………………………
Signature of test supervisor: …………………
Date: ……………………

DIETARY FOOD RECORD INSTRUCTIONS

Name:……………………..

Dates to record:……………………………………………………………………

……………………………………………………………………………………………

- **Please record dietary intake for the following days: day prior to visit 2; 2 days prior to visit 3; visit 3,4 & 5 (day 4 should be same as day prior to visit 2)**

- **Follow the guidelines for recording foods, beverages, and supplements provided.**

INSTRUCTIONS:

- **ALL foods and beverages (INCLUDING WATER)** that are consumed should be recorded.
    - this includes any supplements (e.g., vitamins, minerals)
    - record the date and time of consuming each food, drink or snack
    - record everything at the time of consumption, not from memory at the end of the day
    - start each day on a new page

- Be very specific in your description of the type of food
    - "bread" was it white or wholemeal?
    - "meat" was it streaky bacon or lean beef topside?
    - "milk" was it full fat, semi-skimmed or skimmed?
    - "cheese" was it cheddar or edam?
    - "margarine" was it low fat?
    - "spaghetti Bolognese" how much pasta, how much mince, type of sauce, any vegetables?

- Include the preparation method
    - grilled or fried bacon
    - boiled or roast potatoes
    - bread toasted

- Include anything that was added to the food or drink
    - addition of sugar to tea
    - addition of margarine to bread
    - addition of oil (what type) to cook bacon

- Use nutrient descriptors and brand names to describe foods
    - diet

- low calorie
- reduced fat
- Dairylee
- Cadbury

- Write down how much you have consumed
  - use the scales provided to weigh all food
  - use the labels on foods to help you determine amounts
  - save labels from packages and return them with your food record forms, especially for ready prepared food (this will greatly assist and enhance our analysis of your true nutrient intake)
  - write down the number of sausages, fish fingers, slices of meat, etc
  - describe the size of a piece of food (e.g., small apple, large banana, medium egg)

- Write everything down – don't forget snacks, nibbles, second helpings, sweets

- Do not guess the amounts of food eaten as this makes results inaccurate.

Example of Diary

Day: _____   Date:_____

Time	Food/Drink	Description	Amount Eaten
8am	Cereal	Kelloggs Branflakes (see box)	50g
	Milk	Semi skimmed	100ml
	Orange Juice	Smooth from concentrate (see carton)	500ml
10.30am	Cereal Bar	Brunch bar with hazelnut (see wrapper)	1 bar
12pm	Baked potatoe	Microwave	Large
	Butter	Lurpak spreadable (see carton)	5g
	Cheese	Medium cheddar, grated	10g
	Beans	Heinz baked beans (see label)	100g
	Orange diluting juice	Tesco own with no added sugar (see bottle)	20ml diluting; 150ml water
2.45pm	Water	Tap	500ml
7pm	Chicken	Diced and stir-fryed with extra virgin olive oil	200g Oil: 10ml
	Carrots	2 medium stir-fryed, chopped	30g
	Rice	White boiled rice (see label)	75g
	Brocolli	3 florets stir-fryed	30g
	Mushrooms	Button, stir-fryed	30g
	Water	Tap	200ml
9pm	Toast	1 slice white bread	20g
	Jam	Strawberry with real fruit (see label)	10g
	Milk	Semi-skimmed	100ml

Day: _____   Date:_____

Time	Food/Drink	Description	Amount Eaten

IF MORE SPACE IS REQUIRED WRITE ON THE BACK

Study 2 - Timing of Milk Supplementation

Caloric (kcal) Content of Participants Recorded Diet

	48 h pre	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	2727 ± 731	2330 ± 753	2280 ± 762	2214 ± 928	2203 ± 966
PRE	2442 ± 1004	2700 ± 594	2345 ± 741	2916 ± 673	3054 ± 1429
POST	1748 ± 554	2375 ± 879	1876 ± 422	1824 ± 574	2111 ± 630
TWENTY-FOUR	1930 ± 972	2141 ± 727	2021 ± 653	2790 ± 847	2461 ± 1169

Values are reported as mean ± SD

Carbohydrate (g) Content of Participants Recorded Diet

	48 h pre	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	295 ± 104	311 ± 132	289 ± 100	337 ± 196	281 ± 129
PRE	340 ± 166	384 ± 118	322 ± 107	390 ± 111	429 ± 284
POST	214 ± 86	287 ± 151	243 ± 69	230 ± 123	289 ± 114
TWENTY-FOUR	233 ± 102	288 ± 88	262 ± 88	376 ± 109	354 ± 195

Values are reported as mean ± SD

Protein (g) Content of Participants Recorded Diet

	48 h pre	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	112 ± 25	90 ± 20	102 ± 20	76 ± 33	103 ± 36
PRE	122 ± 76	131 ± 50	108 ± 42	132 ± 24	140 ± 84
POST	77 ± 36	104 ± 48	76 ± 24	91 ± 55	124 ± 39
TWENTY-FOUR	88 ± 47	107 ± 53	94 ± 43	140 ± 72	113 ± 69

Values are reported as mean ± SD

Fat (g) Content of participants Recorded Diet

	48 h pre	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	116 ± 54	78 ± 31	88 ± 45	72 ± 32	82 ± 54
PRE	109 ± 55	80 ± 24	79 ± 32	103± 51	99 ± 41
POST	67 ± 34	98 ± 43	73 ± 29	66 ± 18	88 ± 32
TWENTY-FOUR	58 ± 24	71 ± 36	74 ± 28	91 ± 44	76 ± 27

Values are reported as mean ± SD

Study 3 - Volume of Milk Supplementation

Caloric (kcal) Content of Participants Recorded Diet

	48 h pre	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	2434 ± 937	1811 ± 1398	1968 ± 735	2146 ± 892	2082 ± 1002
FIVE	1893 ± 637	2076 ± 743	2250 ± 962	2125 ± 914	1847 ± 585
LITRE	2137 ± 884	1877 ± 559	1945 ± 640	2113 ± 841	1897 ± 527

Values are reported as mean ± SD

Carbohydrate (g) Content of Participants Recorded Diet

	48 h pre	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	303 ± 155	301 ± 304	238± 103	312± 148	278± 162
FIVE	298 ± 98	283± 61	286± 150	253± 104	229 ± 76
LITRE	287 ± 150	247± 60	211 ± 32	223 ± 87	240± 129

Values are reported as mean ± SD

Protein (g) Content of Participants Recorded Diet

	48 h pre	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	95 ± 75	67 ± 66	95 ± 54	95 ± 64	112± 78
FIVE	75 ± 43	86 ± 41	88 ± 35	98 ± 48	86± 44
LITRE	93 ± 37	93 ± 55	106 ± 57	104 ± 66	93± 21

Values are reported as mean ± SD

Fat (g) Content of Participants Recorded Diet

	48 h pre	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	64 ± 30	46 ± 21	78 ± 38	66 ± 39	65 ± 27
FIVE	53 ± 27	75 ± 48	90 ± 44	87 ± 64	72 ± 47
LITRE	76 ± 24	64 ± 31	81 ± 39	95 ± 64	70 ± 25

Values are reported as mean ± SD

Study 4 - Effects of Milk on Field-Based Team Sport Performance

Caloric (kcal) Content of Participants Recorded Diet

	Week pre	**48 h pre**	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	3598 ± 759	2174 ± 973	2447 ± 1176	2220 ± 580	2322 ± 985	2819 ± 1139
MILK	2123 ± 727	2033 ± 582	2232 ± 918	2454 ± 874	1710 ± 599	2367 ± 828

Values are reported as mean ± SD

Carbohydrate (g) Content of Participants Diet

	Week pre	**48 h pre**	**24 h pre**	**Baseline**	**24 h**	**48 h**
CON	573 ± 131	313 ± 157	349 ± 192	314 ± 102	334 ± 160	456 ± 254
MILK	280 ± 75	260 ± 80	341 ± 139	325 ± 112	240 ± 71	340 ± 103

Values are reported as mean ± SD

Protein (g) Content of Participants Diet

	Week pre	48 h pre	24 h pre	Baseline	24 h	48 h
CON	142 ± 33	96 ± 37	102 ± 25	86 ± 34	115 ± 32	106 ± 48
MILK	101 ± 32	90 ± 19	105 ± 38	109 ± 24	85 ± 29	92 ± 62

Values are reported as mean ± SD

Fat (g) Content of Participants Diet

	Week pre	48 h pre	24 h pre	Baseline	24 h	48 h
CON	98 ± 47	69 ± 41	81 ± 52	78 ± 37	67 ± 41	76 ± 21
MILK	71 ± 35	71 ± 35	59 ± 33	89 ± 46	52 ± 35	80 ± 35

Values are reported as mean ± SD

Protein (g) Content of Participants Diet

Variable	Baseline	24 h	48 h
CK (U/l)	178	351	1178
Peak Torque Dominant (Nm)	159	143	135
Peak Torque Non-dominant (Nm)	136	123	126
Total Work of the Set Dominant(J)	1193	1099	913
Total Work of the Set Non-dominant (J)	1106	986	816

Muscle Pain/Soreness Data Sheet

Subject:_____   Group:_____

Exercise bout: 1   Day: 1 / 2 / 3 / 4

Instructions: Draw a vertical line corresponding to the pain/soreness that you have as a result of the exercise protocol.

General

|———————————————————————————————|

No pain/   Pain/soreness
soreness   as bad as it
                                              could be

Active (right)

|———————————————————————————————|

No pain/   Pain/soreness
soreness   as bad as it
                                              could be

Active (left)

|———————————————————————————————|

No pain/   Pain/soreness
soreness   as bad as it
                                              could be
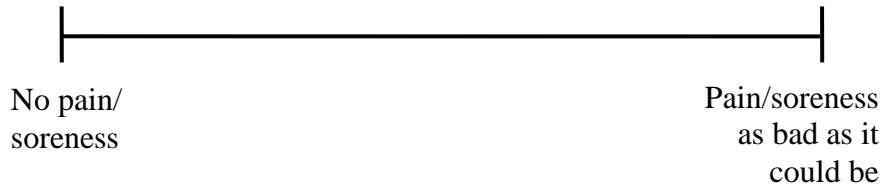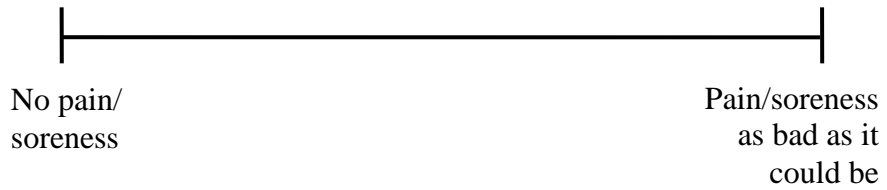
REFERENCES

Abbott, B. C., Bigland, B. & Ritchie, J. M. (1952) 'The physiological cost of negative work', *Journal of Physiology,* 117, pp. 380-390.

Allen, D. G. (2001) 'Eccentric muscle damage: mechanisms of early reduction of force', *Acta Physiologica Scandinavica,* 171, pp. 311-319.

Allen, D. G., Whitehead, N. P. & Yeung, E. W. (2005) 'Mechanisms of stretch-induced muscle damage in normal and dystrophic muscle: role of ionic changes', *Journal of Physiology,* 567 (3), pp. 723-735.

Amelink, G. J., Koot, G. J., Erich, W. B. M., Van Gijn, J. & Bar, P. R. (1990) 'Sex-linked variation in creatine kinase release and its dependence on oestrodiol can be demonstrated in an in-vitro rat skeletal muscle preparation', *Acta Physiologica Scandinavica,* 138, pp. 115-123.

Andreu, A. L. & Schwartz, S. (1995) 'Nutrition, lysosomal proteases, and protein breakdown', *Nutrition,* 11 (4), pp. 382-387.

Appell, H. J., Soares, J. M. & Duarte, J. A. (1992) 'Exercise, muscle damage and fatigue.', *Journal of Sports Medicine,* 13, pp. 108-115.

Armstrong, R. B. (1984) 'Mechanisms of exercise-induced delayed onset muscular soreness: a brief review', *Medicine and Science in Sports and Exercise,* 16 (6), pp. 529-538.

Armstrong, R. B. (1990) 'Initial events in exercise-induced muscular injury', *Medicine and Science in Sports and Exercise,* 22 (4), pp. 429-435.

Armstrong, R. B., Ogilvie, R. W. & Schwane, J. A. (1983) 'Eccentric exercise-induced injury to rat skeletal muscle', *Journal of Applied Physiology,* 54, pp. 80-93.

Armstrong, R. B., Warren, G. L. & Warren, J. A. (1991) 'Mechanisms of Exercise-Induced Muscle Fibre Injury', *Sports Medicine,* 12 (3), pp. 184-207.

Ascensao, A., Rebelo, A., Oliveira, E., Marques, F., Pereira, L. & Magalhaes, J. (2008) 'Biochemical impact of a soccer match - analysis of oxidative stress and muscle damage markers throughout recovery', *Clinical Biochemistry,* 41, pp. 841-851.

Asmussen, E. (1952) 'Positive and negative muscular work', *Acta Physiologica Scandinavica,* 28, pp. 365-382.

Asp, S., Daugaard, J. R., Kristiansen, S., Kiens, B. & Richter, E. A. (1998) 'Exercise metabolism in human skeletal muscle exposed to prior eccentric exercise', *Journal of Physiology,* 509 (1), pp. 305-313.

Asp, S., Daugaard, J. R. & Richter, E. A. (1995) 'Eccentric exercise decreases glucose transporter GLUT4 protein in human skeletal muscle', *Journal of Physiology,* 482 (3), pp. 705-712.

Asp, S., Kristiansen, S. & Richter, E. A. (1995) 'Eccentric muscle damage transiently decreases rat skeletal muscle GLUT-4 protein', *Journal of Applied Physiology,* 79, pp. 1338- 1345.

Asp, S., Rohde, T. & Richter, E. A. (1997) 'Impaired muscle glycogen synthesis after a marathon is not impaired by decreased muscle GLUT-4 content', *Journal of Applied Physiology,* 83, pp. 1482-1485.

Attaix, D. & Taillandier, D. (1998) 'The critical role of the ubiquitin-proteasome pathway in muscle wasting in comparison to lysosomal and Ca^{2+} dependent systems', *Advances in Molecular and Cell Biology,* 27, pp. 235-266.

Ballard, F. J. & Tomas, F. M. (1983) '3-methylhistidine as a measure of skeletal muscle protein breakdown in human subjects: the case for its continued use', *Clinical Science,* 65, pp. 209-215.

Balnave, C. D. & Allen, D. G. (1995) 'Intracellular calcium and force in single mouse muscle fibers following repeated contractions with stretch', *Journal of Physiology,* 488, pp. 25-36.

Baltzopoulos, V. & Gleeson, N. P. (2001) 'Skeletal Muscle Function', in Eston, R. & Reilly, T. (eds.) *Kinanthropometry and Exercise Physiology Laboratory Manual: Tests, Procedures and Data. 2nd Edition, Volume 2: Exercise Physiology.* London: Routledge, pp. 7-35.

Barnett, A. (2006) 'Using recovery modalities between training sessions in elite athletes: does it help?', *Sports Medicine,* 36 (9), pp. 781-796.

Batterham, A. M. & Hopkins, W. G. (2006) 'Making meaningful inferences about magnitudes', *International Journal of Sports Physiology and Performance,* 3 (4), pp. 547-557.

Baty, J. J., Hwang, H., Ding, Z., Bernard, J. R., Wang, B., Kwon, B. & Ivy, J. L. (2007) 'The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage', *Journal of Strength and Conditioning Research,* 21 (2), pp. 321-329.

Beaton, L. J., Tarnapolsky, M. A. & Phillips, S. M. (2002a) 'Contraction-induced muscle damage in humans following calcium channel blocker administration', *Journal of Physiology,* 544 (3), pp. 849-859.

Beaton, L. J., Tarnapolsky, M. A. & Phillips, S. M. (2002b) 'Variability in Estimating Eccentric Contraction-Induced Muscle Damage and Inflammation in Humans', *Canadian Journal of Applied Physiology,* 27 (5), pp. 516-526.

Bechet, D., Tassa, A., Taillandier, D., Combaret, L. & Attaix, D. (2005) 'Lysosomal proteolysis in skeletal muscle', *The International Journal of Biochemistry and Cell Biology,* 37, pp. 2098-2114.

Beedie, C. J. & Foad, A. J. (2009) 'The placebo effect in sports performance: a brief review', *Sports Medicine,* 39 (4), pp. 313-329.

Beedie, C. J., Stuart, E. M., Coleman, D. A. & Foad, A. J. (2006) 'Placebo effects of caffeine in cycling performance', *Medicine and Science in Sports and Exercise,* 38, pp. 2159-2164.

Beelen, M., Koopman, R., Gijsen, A. P., Vandereyt, H., Kies, A. K., Kuipers, H., Saris, W. H.

M. & Van Loon, L. J. C. (2008) 'Protein coingestion stimulates muscle protein synthesis during resistance-type exercise', *American Journal of Physiology,* 295, pp. E70-E77.

Behm, D. G., Baker, K. M., Kelland, R. & Lomond, J. (2001) 'The Effect of Muscle Damage on Strength and Fatigue Deficits', *Journal of Strength and Conditioning Research,* 15 (2), pp. 255-263.

Belcastro, A. N. (1993) 'Skeletal muscle calcium-activated neutral protease (calpain) with exercise', *Journal of Applied Physiology,* 74 (3), pp. 1381-1386.

Belcastro, A. N., Shewchuk, L. D. & Raj, D. A. (1998) 'Exercise-induced muscle injury: A calpain hypothesis', *Molecular and Cellular Biochemistry,* 179, pp. 135-145.

Belia, S., Vecchiet, J., Vecchiet, L. & Fano, G. (2000) 'Modifications of Ca^{2+} transport induced by glutathione in sarcoplasmic reticulum membranes of frog skeletal muscle', *Journal of Muscle Research and Cell Motility,* 21, pp. 279-283.

Beltman, J. G. M., van der Vliet, M. R., Sargent, A. J. & de Haan, A. (2004) 'Metabolic cost of lengthening, isometric and shortening contractions in maximally stimulated rat skeletal muscle', *Acta Physiologica Scandinavica,* 182, pp. 179-180.

Bergstrom, J. & Hultman, E. (1966) 'Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man', *Nature,* 210, pp. 309-310.

Betts, J. A., Toone, R. J., Stokes, K. A. & Thompson, D. (2009) 'Systemic indices of skeletal muscle damage and recovery of muscle function after exercise: effect of combined carbohydrate-protein ingestion', *Applied Physiology, Nutrition and Metabolism,* 34, pp. 773-784.

Bigland-Ritchie, B. & Woods, J. J. (1976) 'Integrated electromyogram and oxygen uptake during positive and negative work', *Journal of Physiology (London),* 260, pp. 267-277.

Biolo, G., Bosutti, A., Iscra, F., Toigo, G., Gullo, A. & Guarnieri, G. (2000) 'Contribution of the Ubiquitin-Proteasom Pathway to Overall Muscle Proteolysis in Hypercatabolic Patients', *Metabolism,* 49 (6), pp. 689-691.

Biolo, G., Fleming, R. Y. D. & Wolfe, R. R. (1995) 'Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle', *Journal of Clinical Investigation,* 95 (2), pp. 811-819.

Biolo, G., Maggi, S. P., Williams, B. D., Tipton, K. D. & Wolfe, R. R. (1995) 'Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans', *American Journal of Physiology,* 268 (31), pp. E514-E520.

Biolo, G., Tipton, K. D., Klein, S. & Wolfe, R. R. (1997) 'An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein', *American Journal of Physiology,* 273 (36), pp. E122-E129.

Biolo, G., Williams, B. D., Declan Fleming, R. Y. & Wolfe, R. R. (1999) 'Insulin Action on Muscle Protein Kinetics and Amino Acid Transport During Recovery After Resistance Exercise', *Diabetes,* 48, pp. 949-957.

Bird, S. P., Tarpenning, K. M. & Marino, F. E. (2006) 'Liquid carbohydrate / essential amino acid ingestion during a short term bout of resistance exercise suppresses myofibrillar protein degradation', *Metabolism,* 55 (5), pp. 570-577.

Blacker, S. D., Williams, N. C., Fallowfield, J. L., Bilzon, J. L. J. & Willems, M. E. T. (2010) 'Carbohydrate vs protein supplementation for recovery of neuromuscular function following prolonged load carriage', *Journal of the International Soceity of Sports Nutrition,* 7 (2).

Boirie, Y., Dangin, M., Gachon, P., Vasson, M. P., Maubois, J. L. & Beaufrere, B. (1997) 'Slow and fast dietary proteins differently modulate postprandial protein accretion', *Proceedings of the National Academy of Sciences of the United States of America,* 94, pp. 14930-14935.

Bondesen, B. A., Mills, S. T., Kegley, K. M. & Pavlath, G. K. (2004) 'The COX-2 pathway is essential during early stages of skeletal muscle regeneration', *American Journal of Physiology,* 287 (2), pp. C475-C483.

Borg, G. (1998) *Borg's perceived exertion and pain scales.* Human Kinetics.

Borsheim, E., Aarsland, A. & Wolfe, R. R. (2004) 'Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise', *International Journal of Sport Nutrition and Exercise Metabolism,* 14, pp. 249-265.

Borsheim, E., Cree, M. G., Tipton, K. D., Elliott, T. A., Aarsland, A. & Wolfe, R. R. (2004) 'Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise', *Journal of Applied Physiology,* 96, pp. 674-678.

Borsheim, E., Tipton, K. D., Wolf, S. E. & Wolfe, R. R. (2002) 'Essential amino acids and muscle protein recovery from resistance exercise', *American Journal of Physiology,* 283, pp. E648-E657.

Bos, C., Gaudichon, C. & Tome, D. (2000) 'Nutritional and physiological criteria in the assessment of milk protein quality for humans', *Journal of the American College of Nutrition,* 19 (2), pp. 191S-205S.

Bounous, G. & Molson, J. H. (2003) 'The antioxidant system', *Anticancer research,* 23, pp. 1411-1416.

Bowers, E. J., Morgan, D. L. & Proske, U. (2004) 'Damage to the human quadriceps muscle from eccentric exercise and the training effect', *Journal of Sports Sciences,* 22 (11-12), pp. 1005-1014.

Brancaccio, P., Maffuli, N. & Limongelli, F. M. (2007) 'Creatine kinase monitoring in sports medicine', *British Medical Bulletin,* 81, pp. 209-230.

Brown, S. J., Child, R. B., Day, S. H. & Donnelly, A. E. (1997) 'Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions', *Journal of Sports Sciences,* 15, pp. 215-222.

Brown, S. J., Child, R. B., Donnelly, A. E., Saxton, J. M. & Day, S. H. (1996) 'Changes in human skeletal muscle contractile function following stimulated eccentric exercise', *European Journal of Applied Physiology and Occupational Physiology,* 72 (5 - 6), pp. 515-521.

Buford, T. W., Cooke, M. B., Shelmadine, B. D., Hudson, G. M., Redd, L. & Willoughby, D. S. (2009) 'Effects of eccentric treadmill exercise on inflammatory gene expression in human skeletal muscle', *Applied Physiology, Nutrition and Metabolism,* 34, pp. 745-753.

Butterfield, T. A., Best, T. M. & Merrick, M. A. (2006) 'The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair', *Journal of Athletic Training,* 41 (4), pp. 457-465.

Byrd, S. K. (1992) 'Alterations in the sarcoplasmic reticulum: a possible link to exercise-induced muscle damage', *Medicine and Science in Sports and Exercise,* 24 (5), pp. 531-536.

Byrne, C. & Eston, R. (2002a) 'The effect of exercise-induced muscle damage on isometric and dynamic knee extensor strength and vertical jump performance', *Journal of Sports Sciences,* 20 (5), pp. 417-425.

Byrne, C. & Eston, R. (2002b) 'Maximal-intensity isometric and dynamic exercise performance after eccentric muscle actions', *Journal of Sports Sciences,* 20 (12), pp. 951-959.

Byrne, C., Twist, C. & Eston, R. (2004) 'Neuromuscular Function After Exercise-Induced Muscle Damage: Theoretical and Applied Implications', *Sports Medicine,* 34 (1), pp. 49-69.

Caiozzo, V. J., Haddad, F., Baker, M. J., Herrick, R. E., Prietto, N. & Baldwin, K. M. (1996) 'Microgravity-induced transformations of myosin isoforms and contractile properties of skeletal muscle', *Journal of Applied Physiology,* 81 (1), pp. 123-132.

Calbet, J. A. L. & MacLean, D. A. (1997) 'Role of caloric content on gastric emptying in humans', *Journal of Physiology,* 498 (2), pp. 553-559.

Cannon, J. G., Meydani, S. N., Fielding, R. A., Fiatarone, M. A., Meydani, M., Farhangmehr, M., Orencole, S. F., Blumberg, J. B. & Evans, W. J. (1991) 'Acute phase response in exercise. II. Associations between vitamin E, cytokines, and muscle proteolysis', *American Journal of Physiology,* 260 (29), pp. R1235-R1240.

Chandler, R. M., Byrne, H. K., Patterson, J. G. & Ivy, J. L. (1994) 'Dietary supplements affect the anabolic hormones after weight-training exercise', *Journal of Applied Physiology,* 76 (2), pp. 839-845.

Chang, J., Musser, J. H. & McGregor, H. (1987) 'Phospholipase A2: Function and Pharmacological Regulation', *Biochemical Pharmacology,* 36 (15), pp. 2429-2436.

Cheung, K., Hume, P. A. & Maxwell, L. (2003) 'Delayed Onset Muscle Soreness: Treatment Strategies and Performance Factors', *Sports Medicine,* 33 (2), pp. 145-164.

Child, R. B., Saxton, J. M. & Donnelly, A. E. (1998) 'Comparison of eccentric knee extensor

muscle action at two muscle lengths on indices of damage and angle-specific force in humans', *Journal of Sports Sciences,* 16, pp. 301-308.

Childs, A., Jacobs, C., Kaminski, T., Halliwell, B. & Leeuwenburgh, C. (2001) 'Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise', *Free Radical Biology and Medicine,* 31 (6), pp. 745-753.

Clark, V. R., Hopkins, W. G., Hawley, J. A. & Burke, L. M. (2000) 'Placebo effect of carbohydrate feeding during a 4-km cycling time trial', *Medicine and Science in Sports and Exercise,* 32, pp. 1642-1647.

Clarkson, P. M., Byrnes, W. C., McCormick, K. M., Turcotte, L. P. & White, J. S. (1986) 'Muscle Soreness and Serum Creatine Kinase Activity Following Isometric, Eccentric, and Concentric Exercise', *International Journal of Sports Medicine,* 7 (3), pp. 152-155.

Clarkson, P. M. & Hubal, M. J. (2001) 'Are women less susceptible to exercise-induced muscle damage?', *Current Opinion in Clinical Nutrition and Metabolic Care,* 4 (6), pp. 527-531.

Clarkson, P. M., Nosaka, K. & Braun, B. (1992) 'Muscle function after exercise-induced muscle damage and rapid adaptation', *Medicine and Science in Sports and Exercise,* 24 (5), pp. 512-520.

Clarkson, P. M. & Sayers, S. P. (1999) 'Etiology of Exercise-Induced Muscle Damage', *Journal of Applied Physiology,* 24 (3), pp. 234-248.

Cleak, M. J. & Eston, R. G. (1992) 'Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise', *British Journal of Sports Medicine,* 26 (4), pp. 267-272.

Close, G. L., Ashton, T., Cable, T., Doran, D., Holloway, C., McArdle, F. & MacLaren, D. P. (2006) 'Ascorbic acid supplementation does not attenuate post-exercise muscle soreness following muscle-damaging exercise but may delay the recovery process', *British Journal of Nutrition,* 95 (5), pp. 976-981.

Close, G. L., Ashton, T., Cable, T., Doran, D. & MacLaren, D. P. (2004) 'Eccentric exercise, isokinetic muscle torque and delayed onset muscle soreness: the role of reactive oxygen species', *European Journal of Applied Physiology,* 91, pp. 615-621.

Close, G. L., Ashton, T., Cable, T., Doran, D., Noyes, C., McArdle, F. & MacLaren, D. P. M. (2005a) 'Effects of dietary carbohydrate on delayed onset muscle soreness and reactive oxygen species after contraction induced muscle damage', *British Journal of Sports Medicine,* 39, pp. 948-953.

Close, G. L., Ashton, T., McArdle, A. & MacLaren, D. P. M. (2005b) 'The emerging role of free radicals in delayed onset muscle soreness and contraction-induced muscle injury', *Comparitive Biochemistry and Physiology,* 142, pp. 257-266.

Cooke, M. (2005) *The effects of nutritional supplementation on regeneration of muscle function after damage.* Victoria University.

Costelli, P., Carbo, N., Tessitore, L., Bagby, G. J., Lopez-Soriano, F. J., Argiles, F. M. & Baccino, J. M. (1993) 'Tumor necrosis factor-alpha mediates changes in tissue

protein turnover in a rat cancer cachexia model', *Journal of Clinical Investigation,* 92, pp. 2783-2789.

Costill, D. L., Pascoe, D. D., Fink, W. J., Robergs, R. A., Barr, S. I. & Pearson, D. (1990) 'Impaired muscle glycogen resynthesis after eccentric exercise', *Journal of Applied Physiology,* 69 (1), pp. 46-50.

Crenshaw, A. G., Thornell, L. E. & Friden, J. (1994) 'Intramuscular pressure, torque and swelling for the exercise-induced sore vastus lateralis muscle', *Acta Physiologica Scandinavica,* 152, pp. 265-277.

Croisier, J. L., Camus, G., Deby-Dupont, G., Bertrand, F., Lhermerout, C., Crielaard, J. M., Juchmes-Ferir, A., Deby, C., Albert, A. & Lamy, M. (1996) 'Myocellular enzyme leakage, polymorphonuclear neutrophil activation and delayed onset muscle soreness induced by isokinetic eccentric exercise', *Archives of Physiology and Biochemistry,* 104 (3), pp. 322-329.

Curtin, N. A. & Davies, R. E. (1973) 'Chemical and mechanical change during stretch of activated frog skeletal muscle', *Symposia on Quantitative Biology XX VII*, pp. 619-626.

Dalton, R. A., Walberg-Ranking, J., Sebolt, D. & Gwazdauskas, F. C. (1999) 'Acute Carbohydrate Consumption Does Not Influence Resistance Exercise Performance During Energy Restriction', *International Journal of Sport Nutrition,* 9 (4), pp. 319-332.

Dannecker, E. A., Hausenblas, H. A., Kaminski, T. W. & Robinson, M. E. (2005) 'Sex differences in delayed onset muscle pain', *Clinical Journal of Pain,* 21, pp. 120-126.

Dartnell, T. J., Rogasch, N. C., Nordstrom, N. A. & 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, pp. 413-423.

Davies, C. D., Rowlands, A. V. & Eston, R. (2009) 'Effect of exercise induced muscle damage on ventilatory and perceived exertion responses to moderate and severe intensity exercise', *European Journal of Applied Physiology,* 107, pp. 11-19.

Davis, J. M., Murphy, E. A., Carmichael, M. D., Zielinski, M. R., Groschwitz, C. M., Brown, A. S., Gangemi, J. D., Ghaffar, A. & Mayer, E. P. (2007) 'Curcumin effects on inflammation and performance recovery following eccentric exercise-induced muscle damage', *American Journal of Physiology,* 292, pp. R2168-R2173.

Denne, S. C., Liechty, E. A., Liu, Y. M., Brechtel, G. & Baron, A. D. (1991) 'Proteolysis in skeletal muscle and whole body in response to euglycemic hyperinsulinemia in normal adults', *American Journal of Physiology,* 261 (24), pp. E809-E814.

Doherty, F. J. & Mayer, R. J. (1992) *Intracellular Protein Degradation.* New York: Oxford University Press, In Focus.

Dousset, E., Avela, J., Ishikawa, M., Kallio, J., Kuitunen, S., Dyrolainen, H., Linnamo, V. & Komi, P. V. (2007) 'Bimodal recovery pattern in human skeletal muscle induced by exhaustive stretch-shortening cycle exercise', *Medicine and Science in Sports and Exercise,* 39 (3), pp. 453-460.

Dove, E. R., Hodgson, J. M., Puddey, I. B., Beilin, L. J., Lee, Y. P. & Mori, T. A. (2009) 'Skim milk compared with a fruit drink acutely reduces appetite and energe intake in overweight men and women', *American Journal of Clinical Nutrition,* 90 (1), pp. 70-75.

Doyle, J. A., Sherman, W. M. & Strauss, R. L. (1993) 'Effects of eccentric and concentric exercise on muscle glycogen replenishment', *Journal of Applied Physiology,* 74 (4), pp. 1848-1855.

Duan, C., Delp, M. D., Hayes, D. A. & Armstrong, R. B. (1990) 'Rat skeletal muscle mitochondrial [Ca2+] and injury from downhill walking', *Journal of Applied Physiology,* 68, pp. 1241-1251.

Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., van de Putte, L. B. A. & Lipsky, P. E. (1998) 'Cyclooxygenase in biology and disease', *FASEB Journal,* 12, pp. 1063-1073.

Duncan, C. J. (1987) 'Role of calcium in triggering rapid ultrastructural damage in muscle: a study with chemically skinned fibres', *Journal of Cell Science,* 87, pp. 581-594.

Duncan, C. J. (1988) 'The role of phospholipase A2 in calcium-induced damage in cardiac and skeletal muscle', *Cell Tissue Research,* 253, pp. 457-462.

Duncan, C. J., Greenaway, H. C. & Smith, J. L. (1980) '2,4-Dinitrophenol, lysosomal breakdown and rapid myofilament degradation in vertebrate skeletal muscle', *Naunyn Schmiedeberg's Archives of Pharmacology,* 315, pp. 77-82.

Duncan, C. J. & Jackson, M. J. (1987) 'Different mechanisms mediate structural changes and intracellular enzyme following damage to skeletal muscle', *Journal of Cell Science,* 87, pp. 183-188.

Duncan, C. J. & Smith, J. L. (1978) 'The action of caffeine in promoting ultrastructural damage in frog skeletal muscle fibres', *Naunyn Schmiedeberg's Archives of Pharmacology,* 305, pp. 159-166.

Duncan, C. J. & Smith, J. L. (1980) 'Action of caffeine in initiating myofilament degradation and subdivision of mitochondria in mammalian skeletal muscle.', *Comparitive Biochemistry and Physiology,* 65C, pp. 143-145.

Ebashi, S. & Endo, M. (1968) 'Calcium ion and muscle contractions', *Progress in Biophysics and Molecular Biology,* 18, pp. 125-183.

Ebbeling, C. B. & Clarkson, P. M. (1989) 'Exercise-induced muscle damage and adaptation', *Sports Medicine,* 7, pp. 207-234.

Elia, D., Stadler, K., Horvath, V. & Jakus, J. (2006) 'Effect of soy- and whey protein-isolate supplemented diet on the redox parameters of trained mice', *European Journal of Nutrition,* 45, pp. 259-266.

Eliasson, J., Elfegoun, T., Nilsson, J., Kohnke, R., Ekblom, B. & Blomstrand, E. (2006) 'Maximal lengthening contractions increase p70 S6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply', *American Journal of Physiology,* 291, pp. E1197-E1205.

Elliot, T. A., Cree, M. G., Sanford, A. P., Wolfe, R. R. & Tipton, K. D. (2006) 'Milk Ingestion Stimulates Net Muscle Protein Synthesis following Resistance Exercise', *Medicine and Science in Sports and Exercise,* 38 (4), pp. 667-674.

Enoka, R. M. (1996) 'Eccentric contractions require unique activation strategies by the nervous sytem', *Journal of Applied Physiology,* 81 (6), pp. 2339-2346.

Erickson, R. H. & Kim, Y. S. (1990) 'Digestion and absorption of dietary protein', *Annual Review of Medicine,* 41, pp. 133-139.

Eston, R. G., Finney, S., Baker, S. & Baltzopoulos, V. (1996) 'Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise', *Journal of Sports Sciences,* 14, pp. 291-299.

Etheridge, T., Philp, A. & Watt, P. W. (2008) 'A single protein meal increases recovery of muscle function following an acute eccentric exercise bout', *Applied Physiology, Nutrition and Metabolism,* 33 (3), pp. 483-488.

Evans, W. J. & Cannon, J. G. (1991) 'The Metabolic Effects of Exercise-Induced Muscle Damage', *Exercise and Sport Sciences Reviews,* 19, pp. 99-125.

Fenn, W. O. (1923) 'A quantitative comparison between the energy liberated and the work performed by the isolated sartorius muscle of the frog', *Journal of Physiology,* 58, pp. 175-203.

Field, A. (2005) *Discovering Statistics Using SPSS*. 2 edn. London: Sage, ISM Introducing Statistical Methods.

Fielding, R. A., Manfredi, T. J., Ding, W., Fiatarone, M. A., Evans, W. J. & Cannon, J. G. (1993) 'Acute phase response in exercise III: Neutrophil and IL-1beta accumulation in skeletal muscle', *American Journal of Physiology,* 265 (34), pp. R166-R172.

Fielding, R. A., Meredith, C. N., O'Reilly, K. P., Frontera, W. R., Cannon, J. G. & Evans, W. J. (1991) 'Enhanced protein breakdown after eccentric exercise in young and older men', *Journal of Applied Physiology,* 71 (2), pp. 674-679.

Fluckey, J. D., Asp, S., Enevoldsen, L. H. & Galbo, H. (2001) 'Insulin action on rates of muscle protein synthesis following eccentric, muscle-damaging contractions', *Acta Physiologica Scandinavica,* 173, pp. 379-384.

Fredsted, A., Clausen, T. & Overgaard, K. (2008) 'Effects of step exercise on muscle damage and muscle Ca^{2+} content in men and women', *Journal of Strength and Conditioning Research,* 22 (4), pp. 1136-1146.

Frenette, J., St, P., M., Cote, C. H., Mylona, E. & Pizza, F. X. (2002) 'Muscle impairment occurs rapidly and preceeds inflammatory cell accumulation after mechanical loading', *American Journal of Physiology,* 282, pp. R351-R357.

Friden, J. & Lieber, R. L. (1992) 'Structural and mechanical basis of exercise-induced muscle injury', *Medicine and Science in Sports and Exercise,* 24 (5), pp. 521-530.

Friden, J. & Lieber, R. L. (1996) 'Ultrastructural evidence for loss of calcium homeostasis in exercised skeletal muscle', *Acta Physiologica Scandinavica,* 158, pp. 381-382.

Friden, J. & Lieber, R. L. (1998) 'Segmental muscle fiber lesions after repetitive eccentric contractions', *Cell Tissue Research,* 293, pp. 165-171.

Friden, J. & Lieber, R. L. (2001) 'Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components', *Acta Physiologica Scandinavica,* 171 (3), pp. 321-326.

Friden, J., Seger, J. & Ekblom, B. (1981) 'Sublethal muscle fibre injuries after high-tension anaerobic exercise', *European Journal of Applied Physiology,* 57, pp. 360-368.

Friden, J., Sjostrom, M. & Ekblom, B. (1981) 'A morphological study of delayed muscle soreness', *Experentia,* 37, pp. 506-507.

Friden, J., Sjostrom, M. & Ekblom, B. (1983) 'Myofibrillar Damage Following Intense Eccentric Exercise in Man', *International Journal of Sports Medicine,* 4 (3), pp. 170-176.

Froiland, K., Koszewski, W., Hingst, J. & Kopecky, L. (2004) 'Nutritional supplement use among college athletes and their sources of information', *International Journal of Sport Nutrition and Exercise Metabolism,* 14, pp. 104-120.

Frost, R. A., Lang, C. H. & Gelato, M. C. (1997) 'Transient exposure of human myoblasts to tumor necrosis factor-alpha inhibits serum and insulin-like growth factor I stimulated protein synthesis', *Endocrinology,* 138, pp. 4153-4159.

Fulks, R. M., Li, J. B. & Goldberg, A. L. (1975) 'Effects of insulin, glucose adn amino acids on protein turnover in rat diaphragm', *Journal of Biological Chemistry,* 250, pp. 290-298.

Furuno, K. & Goldberg, A. L. (1986) 'The activation of protein degradation in muscle by Ca^{2+} or muscle injury does not involve a lysosomal mechanism', *Biochemistry Journal,* 237, pp. 859-864.

Gabbett, T. J. (2006) 'Performance changes following a field conditioned program in junior and senior rugby league players', *Journal of Strength and Conditioning Research,* 20 (1), pp. 215-221.

Gannon, M. C., Nuttall, F. Q., Krezowski, P. A., Billington, C. J. & Parker, S. (1986) 'The serum insulin and plasma glucose responses to milk and fruit products in type 2 (non-insulin-dependent) diabetic patients', *Diabetologia,* 29 (11), pp. 784-791.

Garcia-Lopez, D., Cuevas, M. J., Almar, M., Lima, E., De Paz, J. A. & Gonzalez-Gallego, J. (2007) 'Effects of eccentric exercise on NF-kappaB activation in blood mononuclear cells', *Medicine and Science in Sports and Exercise,* 39 (4), pp. 653-664.

Garcia-Lopez, D., de Paz, J. A., Jimenez-Jimenez, R., Bresciani, G., De Souza-Teixeira, F., Herrero, J. A., Alvear-Ordenes, I. & Gonzalez-Gallego, J. (2006) 'Early explosive force reduction associated with exercise-induced muscle damage', *Journal of Physiology and Biochemistry,* 62 (3), pp. 163-170.

Gary, G. M. (1991) 'Dietary protein processing: intraluminal and enterocyte surface events', in Schultz, S. G., Field, M., Frizzell, R. A. & Rauner, B. B. (eds.) *Handbook of Physiology, section 6, The Gastrointestinal System, vol. IV, Intestinal absorption and secretion.* Marlyland: American Physiological Soceity, pp. 411-420.

Ghosh, G. & May, M. J. (1998) 'NF-kappa B and Rel proteins: evolutionary conserved mediators of immune responses', *Annual Review of Immunology,* 16, pp. 255-260.

Gibala, M. J., Interisano, S. A., Tarnapolsky, M. A., Roy, B. D., MacDonald, J. R., Yarasheski, K. E. & MacDougall, J. D. (2000) 'Myofibrillar disruption following acute concentric and eccentric resistance exercise in strength-trained men', *Canadian Journal of Physiology and Pharmacology,* 78, pp. 656-661.

Gibala, M. J., MacDougall, J. D., Tarnapolsky, M. A., Stauber, W. T. & Elorriaga, A. (1995) 'Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise', *Journal of Applied Physiology,* 78, pp. 702-708.

Gill, N. D., Beaven, C. M. & Cook, C. (2006) 'Effectiveness of post-match recovery strategies in rugby players', *British Journal of Sports Medicine,* 40, pp. 260-263.

Gissel, H. (2000) 'Ca^{2+} accumulation and cell damage in skeletal muscle during low frequency stimulation', *European Journal of Applied Physiology,* 83, pp. 175-180.

Gissel, H. (2005) 'The Role of Ca^{2+} in Muscle Cell Damage', *Annals New York Academy of Sciences,* 1066, pp. 166-180.

Gissel, H. & Clausen, T. (2001) 'Excitation-induced Ca^{2+} influx and skeletal muscle cell damage', *Acta Physiologica Scandinavica,* 171, pp. 327-334.

Goldfarb, A. H., Bloomer, R. J. & McKenzie, M. J. (2005) 'Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise', *Medicine and Science in Sports and Exercise,* 37 (2), pp. 234-239.

Goll, D. E., Neti, G., Mares, S. W. & Thompson, V. F. (2008) 'Myofibrillar protein turnover: the proteasome and the calpains', *Journal of Animal Science,* 86, pp. E19-E35.

Goll, D. E., Thompson, V. F., Li, H., Wei, W. & Cong, J. (2003) 'The calpain system', *Physiological Reviews,* 83, pp. 73 1-801.

Goll, D. E., Thompson, V. F., Taylor, R. G. & Zalewska, T. (1992) 'Is calpain activity

regulated by membranes and autolysis or by calcium and calpastatin?', *Bioessays,* 14, pp. 549- 556.

Gordon, A. M., Huxley, A. F. & Julian, F. J. (1966) 'The variation in isometric tension with sarcomere length in vertebrate muscle fibers', *Journal of Physiology,* 184, pp. 170-192.

Green, H. J. (1997) 'Mechanisms of muscle fatigue in intense exercise', *Journal of Sports Sciences,* 15, pp. 247-256.

Green, M. S., Corono, B. T., Doyle, J. A. & Ingalls, C. P. (2008) 'Carbohydrate-Protein Drinks Do Not Enhance Recovery From Exercise-Induced Muscle Injury', *International Journal of Sport Nutrition and Exercise Metabolism,* 18 (1), pp. 1-18.

Greer, B. K., Woodard, J. L., White, J. P., Arguello, E. M. & Haymes, E. M. (2007) 'Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise', *International Journal of Sport Nutrition and Exercise Metabolism,* 17, pp. 595-607.

Gulick, D. T. & Kimura, I. F. (1996) 'Delayed onset muscle soreness: what is it and how do we treat it?', *Journal of Sports Rehabilitation,* 5, pp. 234-243.

Halliwell, B. & Gutteridge, J. M. C. (1986) 'Oxygen free radicals and iron in relation to biology and medicine', *Archives of Biochemistry and Biophysics,* 246, pp. 501-514.

Hamada, K., Vannier, E., Sacheck, J. M., Witsell, A. L. & Roubenoff, R. (2005) 'Senescence of human skeletal muscle impairs the local inflammatory cytokine response to acute eccentric exercise', *FASEB Journal,* 19 (2), pp. 264-266.

Hamer, P. W., McGeachie, J. M., Davies, M. J. & Grounds, M. D. (2002) 'Evans blue dye as an in vivo marker of myofibre damage: optimising parameters for detecting initial myofibre membrane permeability', *Journal of Anatomy,* 200, pp. 69-79.

Hampton, M. B., Kettle, A. J. & Winterbourn, C. C. (1998) 'Inside the neutrophil phagsome: oxidants, myeloperoxidase, and bacterial killing', *Blood,* 92, pp. 3007-3017.

Hansen, M., Trappe, T. A., Crameri, R. M., Qvortrup, K., Kjaer, M. & Langberg, H. (2009) 'Myofibrillar proteolysis in response to voluntary or electrically stimulated muscle contractions in humans', *Scandinavian Journal of Medicine and Science in Sports,* 19, pp. 75-82.

Harper, A., James, A., Flint, A. & Astrup, A. (2007) 'Increased satiety after intake of a chocolate milk drink compared with a carbonated beverage, but no difference in subsequent *ad libitum* lunch intake', *British Journal of Nutrition,* 97, pp. 579-583.

Harrison, A. J. & Gaffney, S. D. (2004) 'Effects of Muscle Damage on Stretch-Shortening Cycle Function and Muscle Stiffness Control', *Journal of Strength and Conditioning Research,* 18 (4), pp. 771-776.

Haug, A., Hostmark, A. T. & Harstad, O. M. (2007) 'Bovine milk in human nutrition - a review', *Lipids in Health and Disease,* 6 (25).

Herschko, A. & Ciechanover, A. (1992) 'The ubiquitin system for protein degradation', *Annual*

Reviews of Biochemistry, 61, pp. 761-807.

Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J. M. & Suzuki, K. (2004) 'Changes in inflammatory mediators following eccentric exercise of the elbow flexors', *Exercise Immunology Review,* 10, pp. 75-90.

Holm, L., van Hall, G., Rose, A. J., Miller, B. F., Doessing, S., Richter, E. A. & Kjaer, M. (2010) 'Contraction intensity and feeding affect collagen and myofibrillar protein synthesis rates differently in human skeletal muscle', *American Journal of Physiology,* 298, pp. E257-E269.

Hopkins, W. G. (2002) 'Probabilities of Clinical or Practial Significance', *Sportscience,* 6.

Hopkins, W. G. (2003) 'A spreadsheet for analysis of straightforward controlled trialsq', *Sportscience,* 7.

Hopkins, W. G. (2006) 'Analysis of a pre-post controlled trial (Excel spreadsheet)', *newstats.org/xParallelGroupsTrial.xls.*

Horita, T., Komi, P. V., Nicol, C. & Kyrolainen, H. (1999) 'Effect of exhausting stretch-shortening cycle exercise on the time course of mechanical behaviour in the drop jump: possible role of muscle damage', *European Journal of Applied Physiology,* 79 (2), pp. 160-167.

Horowits, R., Kempner, E. S., Bisher, M. E. & Podolsky, R. J. (1986) 'A physiological role for titin and nebulin in skeletal muscle', *Nature,* 323, pp. 160-164.

Hortobagyi, T., Barrier, J., Beard, D., Braspennincx, J., Coens, P., Devita, P., Dempsey, L. & Lambert, J. (1996a) 'Greater initial adaptation to submaximal lengthening than maximal shortening', *Journal of Applied Physiology,* 81, pp. 1677-1682.

Hortobagyi, T., Hill, J. P., Houmard, J. A., Fraser, D. D., Lambert, N. J. & Israel, R. G. (1996b) 'Adaptive response to muscle lengthening and shortening in humans', *Journal of Applied Physiology,* 80, pp. 765-772.

Houston, M. E. (1995) *Biochemistry Primer for Exercise Science.* United States of America: Human Kinetics.

Howatson, G. & Van Someren, K. A. (2008) 'The prevention and treatment of exercise-induced muscle damage', *Sports Medicine,* 38 (6), pp. 483-503.

Howell, J. N., Chleboun, G. & Conatser, R. (1993) 'Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans', *Journal of Physiology,* 464 (1), pp. 183-196.

Hubal, M. J. & Clarkson, P. M. (2009) 'Point: Counterpoint: estrogen and sex do/do not significantly influence post-exercise indexes of muscle damage, inflammation, and repair', *Journal of Applied Physiology,* 106, pp. 1010-1015.

Hughes, K., Antonsson, A. & Grundstrom, T. (1998) 'Calmodulin dependence of NFκB activation', *FEBS Letters,* 441, pp. 132-136.

Huxley, A. F. (1957) 'Muscle structure and theories of contraction', *Progress in Biophysics*

and Biophysical Chemistry, 7, pp. 255-3 18.

Huxley, A. F. (1971) 'The activation of striated muscle and its mechanical response', *Proceedings of the Royal Soceity of London,* 178 (50), pp. 1-27.

Huxley, A. F. & Niedergerke, R. (1954) 'Interference microscopy of living muscle fibres', *Nature,* 173, pp. 971-973.

Huxley, A. F. & Simmons, R. M. (1971) 'Proposed mechanisms of force generation in striated muscle', *Nature,* 233, pp. 533-538.

Huxley, H. E. & Hanson, J. (1954) 'Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation', *Nature,* 173, pp. 973- 976.

Ingalls, C. P., Warren, G. L. & Armstrong, R. B. (1998) 'Dissociation of force production from MHC and actin contents in muscles injured by eccentric contractions', *Journal of Muscle Research and Cell Motility,* 19, pp. 215-224.

Ingalls, C. P., Warren, G. L., Williams, J. H., Ward, C. W. & Armstrong, R. B. (1998) 'E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions', *Journal of Applied Physiology,* 85 (1), pp. 58-67.

Ivy, J. L., Ding, Z., Hwang, H., Cialdella-Kam, L. C. & Morrison, P. J. (2008) 'Post exercise carbohydrate-protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation', *Amino Acids,* 35, pp. 89-97.

Jackman, R. W. & Kandarian, S. C. (2004) 'The molecular basis of skeletal muscle atrophy', *American Journal of Physiology,* 287 (4), pp. C834-C843.

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1984) 'Experimental skeletal muscle damage: the nature of the calcium-activated degenerative processes', *European Journal of Clinical Investigations,* 14, pp. 369-374.

Jagoe, R. & Goldberg, A. (2001) 'What do we really know about the ubiquitin-proteasome pathway in muscle atrophy?', *Current Opinion in Clinical Nutrition and Metabolic Care,* 4, pp. 183-190.

Jansson, E., Hjemdahl, P. & Kaijser, L. (1986) 'Epinephrine-induced changes in muscle carbohydrate metabolism during exercise in male subjects', *Journal of Applied Physiology,* 60, pp. 1466-1470.

Jefferson, L. S., Li, J. B. & Rannels, S. R. (1977) 'Regulation by insulin of amino acid release and protein turnover in the perfused rat hemicorpus', *Journal of Biological Chemistry,* 252, pp. 1476-1483.

Jones, D., Round, J. & de Haan, A. (2004) *Skeletal muscle from molecules to movement: A textbook of muscle physiology for sport, exercise, physiotherapy and medicine.* London: Churchill Livingstone.

Jones, D. A., Jackson, M. J., McPhail, G. & Edwards, R. H. T. (1984) 'Experimental mouse muscle damage: the importance of external calcium', *Clinical Science,* 66, pp. 317- 322.

Jones, D. A., Newham, D. J. & Clarkson, P. M. (1987) 'Skeletal muscle stiffness and pain following eccentric exercice of the elbow flexors', *Pain,* 30 (22), pp. 233-242.

Jones, D. A. & Round, J. M. (1990) 'Skeletal Muscle in Health and Disease, A Textbook of Muscle Physiology', *Medicine and Science in Sports and Exercise.*

Julian, F. J. & Morgan, D. L. (1979) 'The effect of tension of non-uniform distribution of length changes applied to frog muscle fibres', *Journal of Physiology,* 293, pp. 379-392.

Karlsson, H. K. R., Nilsson, P., Nilsson, J., Chibalin, A. V., Zierath, J. R. & Blomstrand, E. (2004) 'Branched-chain amino acids increase p70^{S6k} phosphorylation in human skeletal muscle after resistance exercise', *American Journal of Physiology,* 287, pp. E1-E7.

Karp, J. R., Johnston, J. D., Tecklenburg, S., Mickleborough, T. D., Fly, A. D. & Stager, J. M. (2006) 'Chocolate Milk as a Post-Exercise Recovery Aid', *International Journal of Sport Nutrition and Exercise Metabolism,* 16 (1), pp. 78-91.

Kasperek, G. J., Conway, G. R., Krayeski, D. S. & Lohne, J. J. (1992) 'A reexamination of the effect of exercise on rate of muscle protein degradation', *American Journal of Physiology,* 263 (26), pp. E1144-E1150.

Kasperek, G. J. & Snider, R. D. (1985) 'Increased protein degradation after eccentric exercise', *European Journal of Applied Physiology,* 54, pp. 30-34.

Kendall, B. & Eston, R. (2002) 'Exercise-induced muscle damage and the potential protective role of estrogen', *Sports Medicine,* 32 (2), pp. 103-123.

Keogh, J. W. L., Weber, C. L. & Dalton, C. T. (2003) 'Evaluation of anthropometric, physiological, and skill-related tests for talent identification in female field hockey', *Canadian Journal of Applied Physiology,* 28 (3), pp. 397-409.

Kettlehut, I. C., Wing, S. S. & Goldberg, A. L. (1988) 'Endocrine regulation of protein breakdown in skeletal muscle', *Diabetes Metabolism Review,* 4, pp. 751-772.

Kimball, S. R., Farrell, P. A. & Jefferson, L. S. (2002) 'Invited review: role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise', *Journal of Applied Physiology,* 93, pp. 1168-1180.

Kirwan, J. P. & Del Aguila, L. F. (2003) 'Insulin signalling, exercise and cellular integrity', *Biochemical Soceity Transactions,* 31, pp. 1281-1285.

Komi, P. V. (2000) 'Stretch-shortening cycle: a powerful model to study normal and fatigued muscle', *Journal of Biomechanics,* 33, pp. 1197-1206.

Komi, P. V. & Viitasalo, J. T. (1977) 'Changes in motor unit activity and metabolism in human skeletal muscle during and after repeated eccentric and concentric contractions', *Acta Physiologica Scandinavica,* 100, pp. 246-254.

Komulainen, J., Koskinen, S., Kalliokoski, R., Takala, T. & Vihko, V. (1999) 'Gender differences in skeletal muscle fibre damage after eccentrically biased downhill running in rats.', *Acta Physiologica Scandinavica,* 165, pp. 57-63.

Koopman, R., Wagenmakers, A. J. M., Manders, R. J. F., Zorenc, A. H. G., Senden, J. M. G., Gorselink, M., Keizer, H. A. & Van Loon, L. J. C. (2005) 'Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects', *American Journal of Physiology - Endocrinology and Metabolism,* 288, pp. E645-E653.

Korge, P., Byrd, S. K. & Campbell, K. B. (1993) 'Functional coupling between sarcoplasmic-reticulum-bound creatine kinase and Ca^{2+}-ATPase', *European Journal of Biochemistry,* 21 (3), pp. 973-980.

Kramer, H. F. & Goodyear, L. J. (2007) 'Exercise, MAPK, and NF-κB signaling in skeletal muscle', *Journal of Applied Physiology,* 103, pp. 388-395.

Kreider, R. B., Earnest, C. P., Lundberg, J., Rasmussen, C., Greenwood, M., Cowan, P. & Almada, A. L. (2007) 'Effects of ingesting protein with various forms of carbohydrate following resistance-exercise on substrate availability and markers of anabolism, catabolism, and immunity.', *Journal of the International Soceity of Sports Nutrition,* 4 (1), pp. 18-28.

Kristiansen, M., Levy-Milne, R., Barr, S. & Flint, A. (2005) 'Dietary supplement use by varsity athletes at a Canadian university', *International Journal of Sport Nutrition and Exercise Metabolism,* 15, pp. 195-210.

Kristiansen, S., Asp, S. & Richter, E. A. (1996) 'Decreased muscle GLUT-4 and contraction-induced glucose transport after eccentric contractions', *American Journal of Physiology,* 271 (40), pp. R477-R482.

Kristiansen, S., Jones, J., Handberg, A., Dohm, G. L. & Richter, E. A. (1997) 'Eccentric contractions decrease glucose transporter transcription rate, mRNA, and protein in skeletal muscle', *Amercian Journal of Physiology,* 272 (41), pp. C1734-C1738.

Kuipers, H., Keizer, H. A., Verstappen, F. T. J. & Costill, D. L. (1985) 'Influence of a prostaglandin-inhibiting drug on muscle soreness after eccentric work', *International Journal of Sports Medicine,* 6 (6), pp. 336-339.

Kumar, V., Atherton, P., Smith, K. & Rennie, M. J. (2009) 'Human muscle protein synthesis and breakdown during and after exercise', *Journal of Applied Physiology,* 106, pp. 2026-2039.

Lapointe, B. M., Frenette, J. & Cote, C. H. (2002) 'Lengthening contraction-induced inflammation is linked to secondary damage but devoid of neutrophil invasion', *Journal of Applied Physiology,* 92, pp. 1995-2004.

Lauritzen, F., Paulsen, G., Raastad, T., Bergersen, L. H. & Owe, S. G. (2009) 'Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans', *Journal of Applied Physiology,* 107, pp. 1923-1934.

Lazarides, E. (1980) 'Intermediate filaments as mechanical integrators of cellular space', *Nature*, pp. 238-249.

Lee, J. & Clarkson, P. M. (2003) 'Plasma Creatine Kinase Activity and Glutathione after

Eccentric Exercise', *Medicine and Science in Sports and Exercise,* 35 (6), pp. 930-936.

Lee, J., Goldfarb, A. H., Rescino, M. H., Hegde, S., Patrick, S. & Apperson, K. (2002) 'Eccentric exercise effect on blood oxidative-stress markers and delayed onset of muscle soreness', *Medicine and Science in Sports and Exercise,* 34 (3), pp. 443-448.

Levenhagen, D. K., Gresham, J. D., Carlson, M. G., Maron, D. J., Borel, M. J. & Flakoll, P. J. (2001) 'Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis', *American Journal of Physiology,* 280, pp. E982-E993.

Li, V. P., Atkins, C. M., Sweatt, M. B. & Reid, J. D. (1999) 'Mitochondria mediate tumor necrosis factor-α/NF-KB signalling in skeletal muscle myotubes', *Antioxidants and redox signalling,* 1, pp. 97-104.

Li, V. P., Chen, V. L., John, J., Moylan, J., Jin, B. W., Mann, D. L. & Reid, M. B. (2005) 'TNFalpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle', *FASEB Journal,* 19, pp. 362-370.

Li, V. P., Chen, V. L., Li, A. S. & Reid, M. B. (2003) 'Hydrogen peroxide stimulates ubiquitin-conjugating activity and expression of genes for specific E2 and E3 proteins in skeletal muscle myotubes', *American Journal of Physiology,* 285, pp. C806-C812.

Liao, P., Zhou, J., Ji, L. L. & Zhang, V. (2010) 'Eccentric contraction induces inflammatory responses in rat skeletal muscle: role of tumor necrosis factor-α', *American Journal of Physiology,* 298, pp. R599-R607.

Lieber, R. L. & Friden, J. (1993) 'Muscle damage is not a function of muscle force but active muscle strain', *Journal of Applied Physiology,* 74 (2), pp. 520-526.

Lieber, R. L. & Friden, J. (1999) 'Mechanisms of Muscle Injury after Eccentric Contraction', *Journal of Science and Medicine in Sport,* 2 (3), pp. 253-265.

Lieber, R. L. & Friden, J. (2002) 'Morphological and Mechanical Basis of Delayed-Onset Muscle Soreness', *Journal of American Orthopeadic Surgery,* 10 (1), pp. 67-73.

Lieber, R. L., Schmitz, M. C., Mishra, D. K. & Friden, J. (1994) 'Contractile and cellular remodelling in rabbit skeletal muscle after cyclic eccentric contractions', *Journal of Applied Physiology,* 77 (4), pp. 1926-1934.

Lieber, R. L., Thornell, L. E. & Friden, J. (1996) 'Muscle cytoskeletal disruption occurs within the first 15 min of cyclic eccentric contraction', *Journal of Applied Physiology,* 80 (1), pp. 278-284.

Lieber, R. L., Woodburn, T. M. & Friden, J. (1991) 'Muscle damage induced by eccentric contractions of 25% strain', *Journal of Applied Physiology,* 70 (6), pp. 2498-2507.

Lieper, J. B., Broad, N. P. & Maughan, R. J. (2001) 'Effects of intermittent high-intensity exercise on gastric emptying in man', *Medicine and Science in Sports and Exercise,* 33 (8), pp. 1270-1278.

Lindstedt, S. L., LaStayo, P. C. & Reich, T. E. (2001) 'When active muscles lengthen: properties

and consequences of eccentric contractions', *News in Physiological Sciences,* 16, pp. 256-261.

Lowe, D. A., Warren, G. L., Ingalls, C. P., Boorstein, D. B. & Armstrong, R. B. (1995) 'Muscle function and protein metabolism after initiation of eccentric contraction-induced injury', *Journal of Applied Physiology,* 79 (4), pp. 1260-1270.

Luden, N. D., Saunders, M. J. & Todd, M. K. (2007) 'Postexercise Carbohydrate-Protein-Antioxidant Ingestion Decreases Plasma Creatine Kinase and Muscle Soreness', *International Journal of Sport Nutrition and Exercise Metabolism,* 17 (1), pp. 109-123.

Lynch, G. S., Fary, C. J. & Williams, D. A. (1997) 'Quantitative measurement of resting skeletal muscle [Ca2+]i following acute and long-term downhill running exercise in mice', *Cell Calcium,* 22, pp. 373-383.

MacIntyre, D. L., Reid, W. D. & McKenzie, D. C. (1995) 'Delayed Muscle Soreness: The Inflammatory Response to Muscle Injury and its Clinical Implications', *Sports Medicine,* 20 (1), pp. 24-40.

MacIntyre, D. L., Sorichter, S., Mair, J., Berg, A. & McKenzie, D. C. (2001) 'Markers of inflammation and myofibrillar proteins following eccentric exercise in humans', *European Journal of Applied Physiology,* 84 (3), pp. 180-186.

MacKey, A. L., Bojsen-Moller, J., Qvortrup, K., Langberg, H., Suetta, C., Kalliokoski, K. K., Kjaer, M. & Magnusson, S. P. (2008) 'Evidence of skeletal muscle damage following electrically stimulated isometric muscle contractions in humans', *Journal of Applied Physiology,* 105, pp. 1620-1627.

Magid, A. & Law, D. J. (1985) 'Myofibrils bear most of the resting tension in frog skeletal muscle', *Science,* 230, pp. 1280-1282.

Malm, C. (2001) 'Exercise-induced muscle damage and inflammation: fact or fiction?', *Acta Physiologica Scandinavica,* 171, pp. 233-239.

Malm, C., Lenkei, R. & Sjodin, B. (1999) 'Effects of eccentric exercise on the immune system in men', *Journal of Applied Physiology,* 86 (2), pp. 461-468.

Malm, C., Nyberg, P., Engstrom, M., Sjodin, B., Lenkei, R., Ekblom, B. & Lundberg, I. E. (2000) 'Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies', *Journal of Physiology,* 529 (1), pp. 243-262.

Mann, R. A., Moran, G. T. & Dougherty, S. E. (1986) 'Comparative electromyography of the lower extremity in jogging, running, and sprinting', *The American Journal of Sports Medicine,* 14 (6), pp. 501-510.

Marcora, S. M. & Bosio, A. (2007) 'Effect of exercise induced muscle damage on endurance running performance in humans', *Scandinavian Journal of Medicine and Science in Sports,* 17, pp. 662-671.

Marginson, V., Rowlands, A. V., Gleeson, N. P. & Eston, R. G. (2005) 'A comparison of the symptoms of exercise-induced muscle damage following an initial and repeated bout of plyometric exercise in men and boys', *Journal of Applied Physiology,* 99, pp. 1174-

1181.

Marsolais, D., Cote, C. H. & Frenette, J. (2001) 'Neutrophils and macrophages accumulate sequentially following achilles tendon injury', *Journal of Orthopaedic Research,* 19, pp. 1203-1209.

McArdle, A., van der Meulen, J. H., Catapano, M., Symons, M. C. R., Faulkner, J. A. & Jackson, M. J. (1999) 'Free radical activity following contraction-induced injury to the extensor digitorum longus muscles of rats', *Free Radical Biology and Medicine,* 26, pp. 1085-1091.

McBride, T. A., Stockert, B. W., Gorin, F. A. & Carlsen, R. C. (2000) 'Stretch-activated ion channels contribute to membrane depolarization after eccentric contractions', *Journal of Applied Physiology,* 88, pp. 91-101.

McComas, A. J. (1996) *Skeletal muscle: form and function*. Champaign: Human Kinetics.

McCully, K. K. & Faulkner, J. A. (1986) 'Characteristics of lengthening contractions associated with injury to skeletal muscle fibers', *Journal of Applied Physiology,* 61, pp. 293-299.

McHugh, M. P. (2003) 'Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise', *Scandinavian Journal of Medicine and Science in Sports,* 13, pp. 88-97.

McLennan, I. S. (1996) 'Degenerating and regenerating skeletal muscles contain several subpopulations of macrophages with distinct spatial and temporal distributions', *Journal of Anatomy,* 188 (1), pp. 17-28.

McLoughlin, T. J., Mylona, E., Hornberger, T. A., Esser, K. A. & Pizza, F. X. (2003) 'Inflammatory cells in rat skeletal muscle are elevated after electrically stimulated contractions', *Journal of Applied Physiology,* 94 (3), pp. 876-882.

Mero, A. & Komi, P. V. (1987) 'Electromyographic activity in sprinting at speeds ranging from sub-maximal to supra-maximal.', *Medicine and Science in Sports and Exercise,* 19, pp. 266-274.

Miles, M., Pearson, S. D., Andring, J. M., Kidd, J. R. & Volpe, S. L. (2007) 'Effect of carbohydrate intake during recovery from eccentric exercise on interleukin-6 and muscle-damage markers', *International Journal of Sport Nutrition and Exercise Metabolism,* 17, pp. 507-520.

Miles, M. P., Andring, J. M., Pearson, S. D., Gordon, L. K., Kasper, C., Depner, C. M. & Kidd, J.R. (2008) 'Diurnal variation, response to eccentric exercise, and association of inflammatory mediators with muscle damage variables', *Journal of Applied Physiology,* 104, pp. 451-458.

Milias, G. A., Nomikos, T., Fragopoulou, E., Athanasopoulos, S. & Antonopoulou, S. (2005) 'Effects of eccentric exercise-induced muscle injury on blood levels of platelet activating factor (PAF) and other inflammatory markers', *European Journal of Applied Physiology,* 95, pp. 504-513.

Millard-Stafford, M., Warren, G. L., Thomas, L. M., Doyle, J. A., Snow, T. & Hitchcock, K. (2005) 'Recovery from Run Training: Efficacy of a Carbohydrate-Protein Beverage?',

International Journal of Sport Nutrition and Exercise Metabolism, 15 (6), pp. 610-624.

Miller, S. L., Chinkes, D. L., MacLean, D. A., Gore, D. & Wolfe, R. R. (2004) 'In vivo muscle amino acid transport involves two distinct processes', *American Journal of Physiology,* 287, pp. E136-E141.

Miller, S. L., Tipton, K. D., Chinkes, D. L., Wolf, S. E. & Wolfe, R. R. (2003) 'Independent and Combined Effects of Amino Acids and Glucose after Resistance Exercise', *Medicine and Science in Sports and Exercise,* 35 (3), pp. 449-455.

Mitch, W. E. & Goldberg, A. L. (1996) 'Mechanisms of muscle wasting: The role of the ubiquitin-proteasome pathway', *The New England Journal of Medicine,* 335, pp. 1897-1905.

Moore, D. R., Phillips, S. M., Babraj, J. A., Smith, K. & Rennie, M. J. (2005) 'Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions', *American Journal of Physiology,* 288, pp. E1153-E1159.

Moore, D. R., Robinson, M. J., Fry, J. L., Tang, J. E., Glover, E. I., Wilkinson, S. B., Prior, T., Tarnapolsky, M. A. & Phillips, S. M. (2009a) 'Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men', *American Journal of Clinical Nutrition,* 89 (1), pp. 161-168.

Moore, D. R., Tang, J. E., Burd, N. A., Rerecich, T., Tarnapolsky, M. A. & Phillips, S. M. (2009b) 'Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise', *Journal of Physiology,* 587 (4), pp. 897-904.

Morgan, D. L. (1990) 'New insights into the behaviour of muscle during active lengthening.', *Biophysical Journal,* 57, pp. 209-221.

Morgan, D. L. & Allen, D. G. (1999) 'Early events in stretch-induced muscle damage', *Journal of Applied Physiology,* 87 (6), pp. 2007-2015.

Morgan, D. L., Claflin, D. R. & Julian, F. J. (1996) 'The effects of repeated active stretches on tension generation and myoplasmic calcium in frog single fibres', *Journal of Physiology (London),* 497, pp. 665-674.

Morgan, D. L. & Proske, U. (2004) 'Popping sarcomere hypothesis explains stretch-induced muscle damage', *Clinical and Experimental Pharmacology and Physiology,* 31, pp. 541-545.

Mortimore, G. A., Ward, W. F. & Schworer, C. M. (1978) 'Lysosomal processing of intracellular proteins in rat liver and its general regulation by amino acids and insulin', in Segal, H. L. & Dole, D. J. (eds.) *Protein Turnover and Lysosome Function.* New York: Academic Press, pp. 67-87.

Mujika, I., Santisteban, J., Impellizzeri, F. M. & Castagna, C. (2009) 'Fitness determinants of success in men's and women's football', *Journal of Sports Sciences,* 27 (2), pp. 107-114.

Muller, J. M., Rupec, R. A. & Baeuerle, P. A. (1997) 'Study of gene regulation by NF-kappa B and AP-1 in response to reactive oxygen intermediates', *Methods,* 11, pp. 301-312.

Murphy, R. M., Goodman, C. A., McKenna, M. J., Bennie, J., Leikis, M. & Lamb, G. D. (2007) 'Calpain-3 is autolyzed and hence activated in human skeletal muscle 24 h following a single bout of eccentric exercise', *Journal of Applied Physiology,* 103, pp. 926-931.

Murton, A. J., Constantin, D. & Greenhaff, P. L. (2008) 'The involvement of the ubiquitin proteasome system in human skeletal muscle remodelling and atrophy', *Biochemica et Biophysica Acta,* 1782, pp. 730-743.

Nair, K. S. (1995) 'Muscle protein turnover: methodological issues and the effect of aging', *Journal of Gerentology and Medical Sciences,* 50A, pp. 107-112.

Negro, M., Giardina, S., Marzani, B. & Marzatico, F. (2008) 'Branched-chain amino acid supplementation does not enhance athletic performance but affects muscle recovery and the immune system', *Journal of Sports Medicine and Physical Fitness,* 48 (3), pp. 347-351.

Nevill, A. & Lane, A. (2007) 'Why self-report "Likert" scale data should not be log-transformed', *Journal of Sports Sciences,* 25 (1), pp. 1-2.

Newham, D. J., Jones, D. A. & Clarkson, P. M. (1987) 'Repeated high-force eccentric exercise: effects on muscle pain and damage', *Journal of Applied Physiology,* 63, pp. 1381-1386.

Newham, D. J., Jones, D. A., Ghosh, G. & Aurora, P. (1988) 'Muscle fatigue and pain after eccentric contractions at long and short lengths', *Clinical Science,* 74, pp. 553-557.

Newham, D. J., McPhail, G., Mills, K. R. & Edwards, R. H. T. (1983) 'Ultrastructural changes after concentric and eccentric contractions of human muscle', *Journal of Neurological Sciences,* 61 (1), pp. 109-122.

Nicholas, C. W., Nuttall, F. E. & Williams, C. (2000) 'The Loughborough Intermittent Shuttle Test: A field test that simulates the activity pattern of soccer', *Journal of Sports Sciences,* 18, pp. 97-104.

Nicholas, C. W., Tsintzas, K., Boobis, L. H. & Williams, C. (1999) 'Carbohydrate-electrolyte ingestion during intermittent high-intensity running', *Medicine and Science in Sports and Exercise,* 31 (9), pp. 1280-1286.

Nikolaidis, M. G., Jamurtas, A. Z., Paschalis, V., Gatouros, I. G., Koutedakis, Y. & Kouretas, D. (2008) 'The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: magnitude and time-course considerations', *Sports Medicine,* 38 (7), pp. 579-606.

Nikolaidis, M. G., Paschalis, V., Giakas, G., Fatouros, I. G., Koutedakis, Y., Kouretas, D. & Jamurtas, A. Z. (2007) 'Decreased blood oxidative stress after repeated muscle-damaging exercise', *Medicine and Science in Sports and Exercise,* 39 (7), pp. 1080-1089.

Nosaka, K., Chapman, D., Newton, M. & Sacco, P. (2006) 'Is isometric strength loss immediately after eccentric exercise related to changes in indirect markers of

muscle damage?', *Applied Physiology, Nutrition and Metabolism,* 31, pp. 313-319.

Nosaka, K. & Clarkson, P. M. (1996) 'Changes in indicators of inflammation after eccentric exercise of the elbow flexors', *Medicine and Science in Sports and Exercise,* 28 (8), pp. 953-961.

Nosaka, K., Newton, M. & Sacco, P. (2002) 'Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage', *Scandinavian Journal of Medicine and Science in Sports,* 12 (6), pp. 337-346.

Nottle, C. & Nosaka, K. (2007) 'Changes in power assessed by the wingate anaerobic test following downhill running', *Journal of Strength and Conditioning Research,* 21 (1), pp. 145-150.

Nurenberg, P., Giddings, C. J., Stray-Gundersen, J., Fleckenstein, J. L., Gonyea, W. J. & Peshock, R. M. (1992) 'MR imaging-guided muscle biopsy for correlation of increased signal intensity with ultrastructural change and delayed-onset muscle soreness after exercise', *Radiology,* 184, pp. 865-869.

O'Reilly, K. P., Warhol, M. J., Fielding, R. A., Frontera, W. R., Meredith, C. N. & Evans, W. J. (1987) 'Eccentric exercise-induced muscle damage impairs muscle glycogen repletion', *Journal of Applied Physiology,* 63 (1), pp. 252-256.

Overgaard, K., Fredsted, A., Hyldal, A., Ingemann-Hansen, T., Gissel, H. & Clausen, T. (2004) 'Effect of running distance and training on Ca^{2+} content and damage in human muscle', *Medicine and Science in Sports and Exercise,* 36 (5), pp. 821-829.

Overgaard, K., Lindstrom, T., Ingemann-Hansen, T. & Clausen, T. (2002) 'Membrane leakage and increased content of Na^+-K^+ pumps and Ca^{2+} in human muscle after a 100-km run', *Journal of Applied Physiology,* 92 (5), pp. 1891-1898.

Page, S. G. & Huxley, H. E. (1963) 'Filament lengths in striated muscle', *Journal of Cell Biology,* 19, pp. 369-390.

Pardo, J. V., Siliciano, J. D. & Craig, S. W. (1983) 'A vinculin-containing cortical lattice in skeletal muscle: transverse lattice elements ("costameres") mark sites of attachment between myofibrils and sarcolemma', *Proceedings of the National Academy of Sciences of the United States of America,* 80 (4), pp. 1008-1012.

Patel, T. J. & Lieber, R. L. (1997) 'Force transmission in skeletal muscle: from actomysosin to external tendons', *Exercise and Sport Sciences Reviews,* 25, pp. 321-363.

Paulsen, G., Crameri, R. M., Benestad, H. B., Fjeld, J. G., Morkrid, L., Hallen, J. & Raastad, T. (2010) 'Time course of leukocyte accumulation in human muscle after eccentric exercise', *Medicine and Science in Sports and Exercise,* 42 (1), pp. 75-85.

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G. J., Nosaka, K. & Coombes, J. S. (2005) 'Plasma cytokine changes in relation to exercise intensity and muscle damage', *European Journal of Applied Physiology,* 95, pp. 514-521.

Peterson, A. M. W. & Pederson, B. K. (2005) 'The anti-inflammatory effect of exercise', *Journal of Applied Physiology,* 98, pp. 1154-1162.

Philippou, A., Bogdanis, G. C., Maridaki, M., Halapas, A., Sourla, A. & Koutsilieris, M. (2009) 'Systemic cytokine response following exercise-induced muscle damage in humans', *Clinical Chemistry and Laboratory Medicine,* 47 (6), pp. 777-782.

Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E. & Wolfe, R. R. (1997) 'Mixed muscle protein synthesis and breakdown after resistance exercise in humans', *American Journal of Physiology,* 273 (36), pp. E99-E107.

Pizza, F. X., Koh, T. J., McGregor, S. J. & Brooks, S. V. (2002) 'Muscle inflammatory cells after passive stretches, isometric contractions and lengthening contractions', *Journal of Applied Physiology,* 92 (5), pp. 1873-1878.

Pizza, F. X., Mitchell, J. B., Davis, B. H., Starling, R. D., Holtz, R. W. & Bigelow, N. (1995) 'Exercise-induced muscle damage: effect on circulating leukocyte and lymphocyte subsets', *Medicine and Science in Sports and Exercise,* 27, pp. 363-370.

Plante, P. D. & Houston, M. E. (1984) 'Effects of concentric and eccentric exercise on protein catabolism in man', *International Journal of Sports Medicine,* 5, pp. 174-178.

Proske, U. & Allen, T. J. (2005) 'Damage to Skeletal Muscle from Eccentric Exercise', *Exercise and Sport Sciences Reviews,* 33 (2), pp. 98-104.

Proske, U. & Morgan, D. L. (2001) 'Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications', *Journal of Physiology,* 537 (2), pp. 333-345.

Publicover, S. J., Duncan, C. J. & Smith, J. L. (1978) 'The use of A23187 to demonstrate the role of intracellular calcium in causing ultrastructural damage in mammalian muscle', *Journal of Neuropathology and Experimental Neurology,* 37, pp. 544-557.

Pyne, D. B. (1994) 'Exercise-induced muscle damage and inflammation: A review', *The Australian Journal of Science and Medicine in Sport,* 26 (3/4), pp. 49-58.

Raastad, T., Owe, S. G., Paulsen, G., Enns, D., Overgaard, K., Crameri, R. M., Kiil, S., Belcastro, A. N., Bergersen, L. & Hallen, J. (2010) 'Changes in calpain activity, muscle structure, and function after eccentric exercise', *Medicine and Science in Sports and Exercise,* 42 (1), pp. 86-95.

Raj, D. A., Booker, T. S. & Belcastro, A. N. (1998) 'Striated muscle calcium-stimulated cysteine protease (calpain-like) activity promotes myeloperoxidase activity with exercise.', *Pflugers Arch - European Journal of Physiology,* 435 (6), pp. 804-809.

Ramsbottom, R., Brewer, J. & Williams, C. (1988) 'A progressive shuttle run test to estimate oxygen uptake', *British Journal of Sports Medicine,* 22, pp. 141-144.

Rasmussen, B. B., Tipton, K. D., Miller, S. L., Wolf, S. E. & Wolfe, R. R. (2000) 'An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise', *Journal of Applied Physiology,* 88, pp. 386-392.

Reddy, M. K., Etlinger, J. D., Rabinowitz, M., Fischman, D. A. & Zak, R. (1975) 'Removal of Z- Lines and α-Actinen from Isolated Myofibrils by a Calcium-activated Neutral Protease', *The Journal of Biological Chemistry,* 250 (11).

Reichsman, F., Scordilis, S. P., Clarkson, P. M. & Evans, W. J. (1991) 'Muscle protein changes following eccentric exercise in humans', *European Journal of Applied Physiology,* 62, pp. 245-250.

Reid, M. B. (2005) 'Response of the ubiquitin-proteasome pathway to changes in muscle activity', *American Journal of Physiology,* 288, pp. R1423-R1431.

Reilly, T., Bangsbo, J. & Franks, A. (2000) 'Anthropometric and physiological predispositions for elite soccer', *Journal of Sports Sciences,* 18, pp. 669-683.

Reilly, T. & Ekblom, B. (2005) 'The use of recovery methods post-exercise', *Journal of Sports Sciences,* 23 (6), pp. 619-627.

Reilly, T., Williams, A. G., Nevill, A. & Franks, A. (2000) 'A multi-disciplinary approach to talent identification in soccer', *Journal of Sports Sciences,* 18, pp. 695-702.

Rennie, M. J. & Millward, D. J. (1983) '3-methylhistidine excretion and the urinary 3-methylhistidine/creatinine ratio are poor indicators of skeletal muscle protein breakdown', *Clinical Science,* 65, pp. 217-225.

Rennie, M. J. & Tipton, K. D. (2000) 'Protein and Amino Acid Metabolism During and After Exercise and the Effects of Nutrition', *Annual Review of Nutrition,* 20, pp. 457-483.

Rinard, J., Clarkson, P. M., Smith, L. L. & Grossman, M. (2000) 'Response of males and females to high-force eccentric exercise', *Journal of Sports Science,* 18, pp. 229-236.

Rodemann, H. P. & Goldberg, A. L. (1982) 'Arachidonic acid, prostaglandin E2 and F2 influence rates of protein turnover in skeletal and cardiac muscle', *Journal of Biological Chemistry,* 257, pp. 1632-1638.

Roden burg, J. B., Bar, P. R. & De Boer, R. W. (1993) 'Relations between muscle soreness and biochemical and functional outcomes of eccentric exercise', *Journal of Applied Physiology,* 74 (6), pp. 2976-2983.

Rohde, T., MacLean, D. A., Richter, E. A., Kiens, B. & Pederson, B. K. (1997) 'Prolonged submaximal eccentric exercise is associated with increased levels of plasma IL-6', *American Journal of Physiology,* 273 (36), pp. E85-E91.

Romano-Ely, B. C., Todd, M. K., Saunders, M. J. & St. Laurent, T. (2006) 'Effect of an Isocaloric Carbohydrate-Protein-Antioxidant Drink on Cycling Performance', *Medicine and Science in Sports and Exercise,* 38 (9), pp. 1608-1616.

Rooyackers, J. M., Berkeljen, D. A. & Folgering, H. T. (2003) 'Eccentric exercise training in patients with obstructive pulmonary disease', *International Journal of Rehabilitation Research,* 26, pp. 47-49.

Rossi, F. (1986) 'The O2 forming NADPH oxidase of the phagocytes: nature, mechanisms of activation and function', *Biochemica et Biophysica Acta,* 853 (1), pp. 65-89.

Rowlands, D. S., Rossler, K., Thorp, R. M., Graham, D. F., Timmons, B. W., Stannard, S. R. & Tarnapolsky, M. A. (2008) 'Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men', *Applied Physiology,*

Nutrition and Metabolism, 33 (1), pp. 39-51.

Roy, B. D. (2008) 'Milk: the new sports drink? A Review', *Journal of the International Soceity of Sports Nutrition,* 5 (15).

Roy, B. D., Fowles, J. R., Hill, R. & Tarnapolsky, M. A. (2000) 'Macronutrient intake and whole body protein metabolism following resistance exercise', *Medicine and Science in Sports and Exercise,* 32 (8), pp. 1412-1418.

Roy, B. D., Tarnapolsky, M. A., MacDougall, J. D., Fowles, J. R. & Yarasheski, K. E. (1997) 'Effect of glucose supplement timing on protein metabolism after resistance training', *Journal of Applied Physiology,* 82 (6), pp. 1882-1888.

Sacheck, J. M., Milbury, P. E., Cannon, J. G., Roubenoff, R. & Blumberg, J. B. (2003) 'Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men', *Free Radical Biology and Medicine,* 34 (12), pp. 1575-1588.

Saido, T. C., Sorimachi, H. & Suzuki, K. (1994) 'Calpain: new perspectives in molecular diversity and physiological-pathological involvement', *FASEB Journal,* 8, pp. 814-822.

Salazar, J. J., Michele, D. E. & Brooks, S. V. (2010) 'Inhibition of calpain prevents muscle weakness and disruption of sarcomere structure during hindlimb suspension', *Journal of Applied Physiology,* 108, pp. 120-127.

Sanobe, T., Inagaki, T., Poole, D. C. & Kano, Y. (2008) 'Intracellular calcium accumulation following eccentric contractions in rat skeletal muscle in vivo: role of stretch-activated channels', *American Journal of Physiology,* 294, pp. R1329-R1337.

Saunders, M. J. (2005) 'Creatine phosphokinase and muscle damage: Response', *Medicine and Science in Sports and Exercise,* 37 (1), p. 167.

Saunders, M. J., Kane, M. D. & Todd, M. K. (2004) 'Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage.', *Medicine and Science in Sports and Exercise,* 36 (7), pp. 1233-1238.

Saunders, M. J., Luden, N. D. & Herrick, J. E. (2007) 'Consumption of an Oral Carbohydrate-Protein Gel Improves Cycling Endurance and Prevents Postexercise Muscle Damage', *Journal of Strength and Conditioning Research,* 21 (3), pp. 678-684.

Sayers, S. P. & Clarkson, P. M. (2001) 'Force recovery after eccentric exercise in males and females', *European Journal of Applied Physiology,* 84 (1-2), pp. 122-126.

Sayers, S. P., Clarkson, P. M. & Lee, J. (2000a) 'Activity and immobilization after eccentric exercise: I. Recovery of muscle function', *Medicine and Science in Sports and Exercise,* 32 (9), pp. 1587-1592.

Sayers, S. P., Clarkson, P. M. & Lee, J. (2000b) 'Activity and immobilization after eccentric exercise: II. Serum CK', *Medicine and Science in Sports and Exercise,* 32 (9), pp. 1593-1597.

Scott, A., Khan, K. M., Roberts, C. R., Cook, J. L. & Duronio, V. (2004) 'What do we mean by the term "inflammation"? A contemporary basic science update for sports

medicine', *British Journal of Sports Medicine,* 38, pp. 372-380.

Seifert, J. G., Kipp, R. W., Amann, M. & Gazal, O. (2005) 'Muscle Damage, Fluid Ingestion, and Energy Supplementation During Recreational Alpine Skiing', *International Journal of Sport Nutrition and Exercise Metabolism,* 15 (5), pp. 528-536.

Semark, A., Noakes, T. D., St. Clair Gibson, A. & Lambert, M. I. (1999) 'The effect of a prophylactic dose of flurbiprofen on muscle soreness and sprinting performance in trained subjects', *Journal of Sports Sciences,* 17 (3), pp. 197-203.

Sewright, K. A., Hubal, M. J., Kearns, A., Holbrook, M. & Clarkson, P. M. (2008) 'Sex differences in response to maximal eccentric exercise', *Medicine and Science in Sports and Exercise,* 40, pp. 242-251.

Shahbazpour, N., Carroll, T. J., Riek, S. & Carson, R. G. (2004) 'Early alterations in serum creatine kinase and total cholestrol following high intensity eccentric muscle actions', *Journal of Sports Medicine and Physical Fitness,* 44 (2), pp. 193-199.

Shirreffs, S. M., Watson, P. & Maughan, R. J. (2007) 'Milk as an effective post-exercise rehydration drink', *British Journal of Nutrition,* 98 (1), pp. 173-180.

Shoelson, S. E., Lee, J. & Goldfine, A. B. (2006) 'Inflammation and insulin resistance', *Journal of Clinical Investigation,* 116, pp. 1793-1801.

Skurvydas, A., Sipaviciene, S., Krutulyte, G., Gailiuniene, A., Stasiulis, A., Mamkus, G. & Stanislovaitis, A. (2006a) 'Dynamics of indirect symptoms of skeletal muscle damage after stretch-shortening exercise', *Journal of Electromyography and Kinesiology,* 16, pp. 629-636.

Skurvydas, A., Streckis, V., Mickeviciene, D., Kamandulis, S., Stanislovaitis, A. & Mamkus, G. (2006b) 'Effect of age on metabolic fatigue and on indirect symptoms of skeletal muscle damage after stretch-shortening exercise', *Journal of Sports Medicine and Physical Fitness,* 46 (3), pp. 431-441.

Small, J. V., Furst, D. O. & Thornell, L. E. (1992) 'The cytoskeletal lattice of muscle cells', *European Journal of Biochemistry,* 208, pp. 559-572.

Smith, C., Kruger, M. J., Smith, R. M. & Myburgh, K. H. (2008) 'The inflammatory response to skeletal muscle injury: illuminating complexities', *Sports Medicine,* 38 (11), pp. 947-969.

Smith, L. L. (1991) 'Acute inflammation: the underlying mechanism in delayed onset muscle soreness?', *Medicine and Science in Sports and Exercise,* 23 (5), pp. 542-551.

Snow, R. (2003) 'Skeletal Muscle', in Hargreaves, M. & Hawley, J. (eds.) *Physiological bases of sports performance.* Sydney: McGraw-Hill, pp. 9-26.

Solomon, V. & Goldberg, A. L. (1996) 'Importance of the ATP-ubiquitin-proteasome pathway in degradation of soluble and myofibrillar proteins in rabbit muscle extracts', *Journal of Biological Chemistry,* 271, pp. 26690-26697.

Sorensen, M. M., Coelho, H. S. & Reuben, J. P. (1986) 'Caffeine inhibition of calcium accumulation by the sarcoplasmic reticulum in mammalian skinned fibers', *The*

Journal of Membrane Biology, 90 (3), pp. 219-230.

Sorichter, S., Mair, J., Koller, A., Calzolari, C., Huonker, M., Pau, B. & Puschendorf, B. (2001) 'Release of muscle proteins after downhill running in male and female subjects', *Scandinavian Journal of Medicine and Science in Sports,* 11 (1), pp. 28-32.

Sorichter, S., Puschendorf, B. & Mair, J. (1999) 'Skeletal Muscle Injury Induced by Eccentric Muscle Action: Muscle Proteins as Markers of Muscle Fiber Injury', *Exercise Immunology Review,* 5, pp. 5-21.

Spencer, M., Bishop, D., Dawson, B. & Goodman, C. (2005) 'Physiological and metabolic responses of repeated-sprint activities specific to field-based team sports', *Sports Medicine,* 35 (12), pp. 1025-1044.

St. Pierre Schneider, B., Correia, L. A. & Cannon, J. G. (1999) 'Sex differences in leukocyte invasion in injured murine skeletal muscle', *Research in Nursing and Health,* 22, pp. 243-250.

St. Pierre Schneider, B., Fine, J. P. & Tiidus, P. M. (2005) 'Indices of leukocyte infiltration and muscle recovery after eccentric contraction-induced injury in young and adult male mice', *Orthopaedic Nursing,* 24 (6), pp. 399-405.

St. Pierre Schneider, B. & Tiidus, P. M. (2007) 'Neutrophil Infiltration in Exercise-Injured Skeletal Muscle: How do we resolve the controversy?', *Sports Medicine,* 37 (10), pp. 837-856.

Starkie, R., Ostrowski, S. R., Jauffred, S., Febbraio, M. & Pederson, B. K. (2003) 'Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α production in humans ', *FASEB Journal.*

Stephens, J. M. & Pekala, P. H. (1991) 'Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumour necrosis factor-α', *Journal of Biological Chemistry,* 266, pp. 21839-21845.

Sterne, J. A. C. & Smith, G. D. (2001) 'Sifting the evidence - what's wrong with significance tests?', *British Medical Journal,* 322 (7280), pp. 226-261.

Stolen, T., Chamari, K., Castagna, C. & Wisloff, U. (2005) 'Physiology of Soccer: An update', *Sports Medicine,* 35, pp. 501-536.

Straub, V., Rafael, J. A., Chamberlain, J. S. & Campbell, K. P. (1997) 'Animal models for muscular dystrophy show different patterns of sarcolemmal disruption', *Journal of Cell Biology,* 139, pp. 375-385.

Stupka, N., Lowther, S., Chorneyko, K., Bourgeois, J. M., Hogben, C. & Tarnopolsky, M. A. (2000) 'Gender differences in muscle inflammation after eccentric exercise', *Journal of Applied Physiology,* 89, pp. 2325-2332.

Stupka, N., Tarnapolsky, M. A., Yardley, N. J. & Phillips, S. M. (2001) 'Cellular adaption to repeated eccentric exercise-induced muscle damage', *Journal of Applied Physiology,* 91, pp. 1669-1678.

References

Suzuki, M., Umeda, T., Nakaji, S., Shimoyama, T., Mashiko, T. & Sugawara, K. (2004) 'Effect of incorporating low intensity exercise into the recovery period after a rugby match', *British Journal of Sports Medicine,* 38, pp. 436-440.

Taivassalo, T., Fu, K., Johns, T., Arnold, D., Karpati, G. & Shoubridge, E. A. (1999) 'Gene shifting: a novel therapy for mitochondrial myopathy', *Human Molecular Genetics,* 8, pp. 1047-1052.

Takekura, H., Fujinami, N., Nishizawa, T., Ogasawara, H. & Kasuga, N. (2001) 'Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle', *Journal of Physiology,* 533 (2), pp. 571-583.

Talbot, J. A. & Morgan, D. L. (1996) 'Quantitative analysis of sarcomere non-uniformities in active muscle following a stretch', *Journal of Muscle Research and Cell Motility,* 17, pp. 261-268.

Talbot, J. A. & Morgan, D. L. (1998) 'The effects of stretch parameters on eccentric exercise induced damage to toad skeletal muscle', *Journal of Muscle Research and Cell Motility,* 19, pp. 237-245.

Tang, J. E., Manolakos, J. J., Kujbida, G. W., Lysecki, P. J., Moore, D. R. & Phillips, S. M. (2007) 'Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men', *Applied Physiology, Nutrition and Metabolism,* 32, pp. 1132-1138.

Tang, J. E., Moore, D. R., Kujbida, G. W., Tarnapolsky, M. A. & Phillips, S. M. (2009) 'Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men', *Journal of Applied Physiology,* 107, pp. 987-992.

Tarnapolsky, M. A., Bosman, M., MacDonald, J. R., Vandeputte, D., Martin, J. & Roy, B. D. (1997) 'Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women', *Journal of Applied Physiology,* 83 (6), pp. 1877-1883.

Tee, J. C., Bosch, A. N. & Lambert, M. I. (2007) 'Metabolic Consequences of Exercise-Induced Muscle Damage', *Sports Medicine,* 37 (10), pp. 827-836.

Thomas, K., Morris, P. & Stevenson, E. (2009) 'Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sports drinks', *Applied Physiology, Nutrition and Metabolism,* 34 (1), pp. 78-82.

Thompson, H. S., Hyatt, J. P., De Souza, M. J. & Clarkson, P. M. (1997) 'The effects of oral contraceptives on delayed onset muscle soreness following exercise', *Contraception,* 56, pp. 59-65.

Thompson, D., Nicholas, C. W. & Williams, C. (1999) 'Muscular soreness following prolonged intermittent high-intensity shuttle running', *Journal of Sports Sciences,* 17, pp. 387-395.

Thompson, H. S. & Scordilis, S. P. (1994) 'Ubiquitin changes in human biceps muscle following exercise-induced damage', *Biochemical and Biophysical Research*

Communications, 204 (3), pp. 1193-1198.

Thyfault, J. P., Carper, M. J., Richmond, S. R., Hulver, M. W. & Potteiger, J. A. (2004) 'Effects of Liquid Carbohydrate Ingestion on Markers of Anabolism Following High-Intensity Resistance Exercise', *Journal of Strength and Conditioning Research,* 18 (1), pp. 174- 179.

Tidball, J. G. (2005) 'Inflammatory processes in muscle injury and repair', *American Journal of Physiology,* 288, pp. R345-R353.

Tidball, J. G., Berchenko, E. & Frenette, J. (1999) 'macrophage invastion does not contribute to muscle membrane injury during inflammation', *Journal of Leukocyte Biology,* 65 (4), pp. 492-498.

Tiidus, P. M. & Enns, D. L. (2009) 'Point: Counterpoint: estrogen and sex do/do not influence post-exercise indexes of muscle damage, inflammation, and repair', *Journal of Applied Physiology,* 106, pp. 1010-1015.

Tiidus, P. M., Holden, D., Bombardier, E., Zachowski, S., Enns, D. & Belcastro, A. N. (2001) 'Estrogen effects on post-exercise skeletal muscle neutrophil infiltration and calpain activity', *Canadian Journal of Physiology and Pharmacology,* 79, pp. 400-406.

Tipton, K. D. (2009) 'Protein and Gains in Muscle Mass', *Sport Nutrition Conference.* Munich.

Tipton, K. D., Borsheim, E., Wolf, S. E., Sanford, A. P. & Wolfe, R. R. (2003) 'Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion', *American Journal of Physiology,* 284, pp. E76-E89.

Tipton, K. D., Elliot, T. A., Cree, M. G., Aarsland, A., Sanford, A. P. & Wolfe, R. R. (2007) 'Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise', *American Journal of Physiology,* 292 (1), pp. E71-E76.

Tipton, K. D., Elliott, T. A., Cree, M. G., Wolf, S. E., Sanford, A. P. & Wolfe, R. R. (2004) 'Ingestion of Casein and Whey Proteins Result in Muscle Anabolism after Resistance Exercise', *Medicine and Science in Sports and Exercise,* 36 (12), pp. 2073-2081.

Tipton, K. D., Ferrando, A. A., Phillips, S. M., Doyle Jr, D. & Wolfe, R. R. (1999a) 'Postexercise net protein synthesis in human muscle from orally administered amino acids', *American Journal of Physiology,* 276 (39), pp. E628-E634.

Tipton, K. D., Gurkin, B. E., Matin, S. & Wolfe, R. R. (1999b) 'Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers', *Journal of Nutritional Biochemistry,* 10, pp. 89-95.

Tipton, K. D., Rasmussen, B. B., Miller, S. L., Wolf, S. E., Owens-Stovall, S. K., Petrini, B. E. & Wolfe, R. R. (2001) 'Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise', *American Journal of Physiology,* 281 (2), pp. E197-E206.

Tortora, G.J. & Grabowski, S.R. (2004) *Introduction to the Human Body: The essentials of anatomy and physiology.* USA: John Wiley & Sons, Inc.

Trappe, T. A., Carrithers, J. A., White, F., Lambert, C. P., Evans, W. J. & Dennis, R. A.

(2002a) 'Titin and nebulin content in human skeletal muscle following eccentric resistance exercise', *Muscle and Nerve,* 25, pp. 569-579.

Trappe, T. A., Fluckey, J. D., White, F., Lambert, C. P. & Evans, W. J. (2001) 'Skeletal muscle PGF2α and PGE2 in response to eccentric resistance exercise: influence of ibuprofen and acetaminophen', *The Journal of Clinical Endocrinology and Metabolism,* 86 (10), pp. 5067-5070.

Trappe, T. A., White, F., Lambert, C. P., Cesar, D., Hellerstein, M. & Evans, W. J. (2002b) 'Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis', *American Journal of Physiology - Endocrinology and Metabolism,* 282, pp. E551-E556.

Trenell, M. I., Rooney, K. B., Sue, C. M. & Thompson, C. H. (2006) 'Compression garments and recovery from eccentric exercise: a ^{31}P-MRS study', *Journal of Sports Science and Medicine,* 5, pp. 106-114.

Tsivitse, S. K., McLoughlin, T. J., Peterson, J. M., Mylona, E., McGregor, S. J. & Pizza, F. X. (2003) 'Downhill running in rats: influence on neutrophils, macrophages, and MyoD+ cells in skeletal muscle', *European Journal of Applied Physiology,* 90 (5-6), pp. 633-638.

Twist, C. & Eston, R. (2005) 'The effects of exercise-induced muscle damage on maximal intensity intermittent exercise performance', *European Journal of Applied Physiology,* 94 (5-6), pp. 652-658.

Twist, C. & Eston, R. (2009) 'The effect of exercise-induced muscle damage on perceived exertion and cycling endurance performance', *European Journal of Applied Physiology,* 105, pp. 559-567.

Twist, C., Gleeson, N. P. & Eston, R. (2008) 'The effects of plyometric exercise on unilateral balance performance', *Journal of Sports Sciences,* 26 (10), pp. 1073-1080.

Valentine, R. J., Saunders, M. J., Kent Todd, M. & St. Laurent, T. (2008) 'Influence of Carbohydrate-Protein Beverage on Cycling Endurance and Indices of Muscle Disruption', *International Journal of Sport Nutrition and Exercise Metabolism,* 18 (4), pp. 363-378.

Van Hall, G., Shirreffs, S. M. & Calbet, J. A. L. (2000) 'Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion', *Journal of Applied Physiology,* 88, pp. 163 1-1636.

Verburg, E., Murphy, R. M., Stephenson, D. G. & Lamb, D. G. (2005) 'Disruption of excitation-contraction coupling and titin by endogenous Ca^{2+} activated proteases in toad muscle fibers', *Journal of Physiology,* 564, pp. 775-790.

Vijayan, K., Thompson, J. L., Norenberg, K. M., Fitts, R. H. & Riley, D. A. (2001) 'Fiber-type susceptibility to eccentric contraction-induced damage of hindlimb-unloaded rat AL muscles', *Journal of Applied Physiology,* 90, pp. 770-776.

Vissing, K., Overgaard, K., Nedergaard, A., Fredsted, A. & Schjerling, P. (2008) 'Effects of concentric and repeated eccentric exercise on muscle damage and calpain-calpastatin gene expression in human skeletal muscle', *European Journal of Applied*

Physiology, 103, pp. 323-332.

Volek, J. S. (2004) 'Influence of Nutrition on Responses to Resistance Training', *Medicine and Science in Sports and Exercise,* 36 (4), pp. 689-696.

Wang, K. & Ramirez-Mitchell, R. (1983) 'A network of transverse and longitudinal intermediate filaments is associated with sarcomeres of adult vertebrate skeletal muscle', *Journal of Cell Biology,* 96, pp. 562-570.

Warren, G. L., Ingalls, C. P., Lowe, D. A. & Armstrong, R. B. (2001) 'Excitation-Contraction Uncoupling: Major Role in Contraction-Induced Muscle Injury', *Exercise and Sport Sciences Reviews,* 29 (2), pp. 82-87.

Warren, G. L., Ingalls, C. P., Lowe, D. A. & Armstrong, R. B. (2002) 'What Mechanisms Contribute to the Strength Loss That Occurs During and in the Recovery from Skeletal Muscle Injury?', *Journal of Orthopaedic and Sports Physical Therapy,* 32 (2), pp. 58-64.

Warren, G. L., Lowe, D. A., Hayes, D. A., Farmer, M. A. & Armstrong, R. B. (1995) 'Redistribution of cell membrane probes following contraction-induced injury of mouse soleus muscle', *Journal of Physiology (London),* 468, pp. 487-499.

Warren, G. L., Lowe, D. A., Hayes, D. A., Karwoski, C. J., Prior, B. M. & Armstrong, R. B. (1993) 'Excitation Failure in Eccentric Contraction-Induced Injury of Mouse Soleus Muscle', *Journal of Physiology,* 468, pp. 487-499.

Waterlow, J. C. (2006) *Protein Turnover.* UK: CABI.

Watson, R. T. & Pessin, J. E. (2001) 'Intracellular organisation of insulin signalling and GLUT4 translocation', *Recent Progress in Hormone Research,* 56, pp. 175-194.

Weerakkody, N. S., Whitehead, N. P., Canny, B. J., Gregory, J. E. & Proske, U. (2001) 'Large- Fiber Mechanoreceptors Contribute to Muscle Soreness After Eccentric Exercise', *The Journal of Pain,* 2 (4), pp. 209-219.

Weinheimer, E. M., Jemiolo, B., Carroll, C. C., Harber, M. P., Haus, J. M., Burd, N. A., LeMoine, J. K., Trappe, S. W. & Trappe, T. A. (2007) 'Resistance exercise and cyclooxygenase (COX) expression in human skeletal muscle: implications for COX-inhibiting drugs and protein synthesis', *American Journal of Physiology,* 292, pp. R2241-R2248.

Wellen, K. E. & Hotamisligil, G. S. (2005) 'Inflammation, stress, diabetes', *Journal of Clinical Investigation,* 115, pp. 1111-1119.

White, J. P., Wilson, J. M., Austin, K. G., Greer, B. K., St. John, N. & Panton, L. B. (2008) 'Effect of carbohydrate-protein supplement timing on acute exercise-induced muscle damage', *Journal of the International Soceity of Sports Nutrition,* 5 (5).

Widmaier, E. P., Raff, H. & Strang, K. T. (2004) *Vander, Sherman, Luciano's Human Physiology: The Mechanisms of Body Function.* 9 edn. New York: McGraw-Hill.

Wilkie, D. R. (1985) 'Muscle function: a personal view', *Journal of Experimental Biology,* 115, pp. 1-13.

Willoughby, D. S., Rosene, J. & Myers, J. (2003) 'HSP-72 and ubiquitin expression and caspase-3 activity after a single bout of eccentric exercise', *Journal of Exercise Physiology,* 6 (2), pp. 96-104.

Willoughby, D. S., Taylor, M. & Taylor, L. (2003) 'Glucocorticoid Receptor and Ubiquitin Expression after Repeated Eccentric Exercise', *Medicine and Science in Sports and Exercise,* 35 (12), pp. 2023-2031.

Wisloff, U., Castagna, C., Helgerud, J., Jones, R. & Hoff, J. (2004) 'Maximal squat strength is strongly correlated to sprint-performance and vertical jump height in elite soccer players.', *British Journal of Sports Medicine,* 38, pp. 285-288.

Wojcik, J. R., Walberg-Ranking, J., Smith, L. L. & Gwazdauskas, F. C. (2001) 'Comparison of a Carbohydrate and Milk-Based Beverages on Muscle Damage and Glycogen Following Exercise.', *International Journal of Sport Nutrition and Exercise,* 11 (4), pp. 406-419.

Woledge, R. C., Curtin, N. A. & Homsher, E. (1985) *Energetic aspects of muscle contraction.* London: Academic Press Inc`.

Wolfe, R. R. & Miller, S. L. (1999) 'Amino acid availability controls muscle protein metabolism', *Diabetes, Nutrition and Metabolism,* 12, pp. 322-328.

Yamane, M., Teruya, H., Nakano, M., Ogai, R., Ohnishi, N. & Kosaka, M. (2006) 'Post-exercise leg and forearm flexor muscle cooling in humans attenuates endurance and resistance training effects on muscle performance and on circulatory adaptation', *European Journal of Applied Physiology,* 96 (5), pp. 572-580.

Yasuda, T., Sakamoto, K., Nosaka, K., Wada, M. & Katsuta, S. (1997) 'Loss of sarcoplasmic reticulum membrane integrity after eccentric contractions', *Acta Physiologica Scandinavica,* 161, pp. 581-582.

Yeung, E. W. & Allen, D. G. (2004) 'Stretch-activated channels in stretch-induced muscle damage: role in muscular dystrophy', *Clinical and Experimental Pharmacology and Physiology,* 31, pp. 551-556.

Yeung, E. W., Head, S. I. & Allen, D. G. (2003) 'Gadolinium reduces short-term stretch-induced muscle damage in isolated mdx mouse muscle fibres', *Journal of Physiology,* 552, pp. 449-458.

Yeung, E. W., Whitehead, N. P., Suchyna, T. M., Gottlieb, P. A., Sachs, F. & Allen, D. G. (2005) 'Effects of stretch-activated channel blockers on [Ca^{2+}]i and muscle damage in the *mdx* mouse', *Journal of Physiology,* 562, pp. 367-380.

Young, W. (1995) 'Laboratory strength assessment of athletes', *New Studies in Athletics,* 10 (1), pp. 89-96.

Yu, J., Furst, D. O. & Thornell, L. E. (2003) 'The mode of myofibrillar remodelling in human skeletal muscle affected by DOMS induced by eccentric contractions', *Histochemistry and Cell Biology,* 119, pp. 383-393.

Yu, J., Malm, C. & Thornell, L. E. (2002) 'Eccentric contractions leading to DOMS do not cause loss of desmin nor fibre necrosis in human muscle', *Histochemistry and Cell Biology,* 118, pp. 29-34.

Yu, J. & Thornell, L. E. (2001) 'The cytoskeleton is not primarily affected upon delayed muscle soreness', *Medicine and Science in Sports and Exercise,* 33, pp. S41.

Yu, J. & Thornell, L. E. (2002) 'Desmin and actin alterations in human muscles affected by delayed onset muscle soreness: a high resolution immunocytochemical study', *Histochemistry and Cell Biology,* 118, pp. 171-179.

Zehender, M., Muelli, M., Buchli, R., Kuehne, G. & Boutellier, U. (2004) 'Further glycogen decrease during early recovery after eccentric exercise despite high carbohydrate intake', *European Journal of Nutrition,* 43, pp. 148-159.

Zerba, E., Komorowski, T. E. & Faulkner, J. A. (1990) 'Free radical injury to skeletal muscles of young, adult, and old mice', *American Journal of Physiology,* 258, pp. C429-C435.

Zhang, B. T., Veung, S. S., Allen, D. G., Qin, L. & Veung, E. W. (2008) 'Role of the calcium-calpain pathway in cytoskeletal damage after eccentric contractions', *Journal of Applied Physiology,* 105, pp. 352-357.