The Effect of Milk on Recovery From Exercise in Females

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Abstract

It is well established that strenuous physical activity places both mechanical and metabolic stress on participants, resulting in fatigue, increased muscle soreness, reduced muscle function and performance. Post-exercise nutritional interventions aim to minimise the negative effects of exercise participation while maximising recovery. Milk has become a popular post-exercise drink in this context, though exploration of milk's effect on recovery from varied types of exercise has been minimal. Furthermore there is a dearth of research examining the effects of post-exercise nutritional interventions on female athletes. Therefore the overarching aim of this thesis is to explore the effect of the post-exercise consumption of 500ml of milk on recovery from a range of exercise activities in female athletes. These were an isolated eccentric hamstring protocol (Study 1), an isolated concentric cycling protocol designed to mimic the metabolic demands of team sport (Study 2), repeated jumping and sprinting (Study 3) and a repeated simulated team game (STG) circuit which was repeated following 48h recovery (Study 4).

Generally for the protocols with a high eccentric loading (Study 1, 3, 4) milk had a beneficial effect in attenuating losses in peak torque, sprint performance and perceptions of stress, though the likelihood and magnitude of benefit varied. Effects on other variables were less conclusive. The effects of milk on oxidative stress are unknown (Study 2 and Study 4), though this is because oxidative stress was not observed. Milk also had no clear effect on inflammation and CK and minimal impact on muscle soreness and tiredness. When the protocol had no eccentric loading, little effect of milk was observed (Study 2). Interestingly, in study 4 while milk benefited recovery, analysis of STG variables were trivial (HR, CMJ) and unclear (5m sprint, 15m sprint, lap-time, RPE). Of interest, in study 4 the second STG had little impact on variables measures.

Overall the findings of this thesis suggest that milk is a beneficial recovery drink for female athletes. The greatest benefits are following exercise with eccentric loading. Further investigation is warranted to determine if these benefits are carried through to subsequent exercise performance.

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List of Abbreviations

Ach	Acetylcholine
ADP	Adenosine diphosphate
Akt	Protein kinase B
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BCAA	Branched chain amino acid
BM	Body mass
BMI	Body mass index
BS	Blood sample
BV	Biological value
Ca^{2+}	Calcium
CANP	Calcium activated neutral protease
CAT	Catalase
CHO	Carbohydrate
CI	Confidence interval
CISP	Cycling intermittent sprint protocol
CK	Creatine Kinase
CMJ	Countermovement jump
CRP	C-reative protein
CV	Coeffiecient of variation
DALDA	Daily analysis of life demands
DIAAS	Digestible indispensable amino acid score
DIAAS	Digestible indispensible amino acid score
DJ	Drop jump
DOMS	Delayed onset muscle soreness
DT-MRI	Diffusion-tensor magnetic resonance imaging
eEF2	Eukaryotic elongation factor 2
EIMD	Exercise induced muscle damage
ERK1/2	Extracellular signal-regulated kinase ½
ES	Effect size
FFM	Fat free milk
FOX 1	Ferrous oxidation of xylenol orange 1
FSR	Fractional synthetic rate

GATOR	GAP activity towards Rags
GPx	Glutathione peroxidise
GSH	Glutathione
GSSG	Oxidised glutathione
H	Hydrogen
H_2O_2	Hydrogen peroxide
HMM	Heavey meromyosin
HR	Heart-rate
HRP	Horseradish peroxidise
hsCRP	High sensitivity C-reactive protein
HSP	Heat shock protein
IIMD	Impact-induced muscle damage
IF	Intermediate filaments
IL	Interleukin
LDH	Lactate dehydrogenase
LMM	Light meromyosin
LOOH	Lipid hydroperoxides
Mb	Myoglobin
MBI	Magnitude based inference
MDA	Malondialdehyde
mGPDH	Mitochondrial membrane-bound glycerol 3-phosphate dehydrogenase
MnSOD	Mnaganese superoxide dismutase
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
mTORC	Mechanistic target of rapamycin complex
MuRF-1	Muscle ring finger-1
MVIC	Maximal voluntary isometric contraction
Nm	Newton metres
NO	Nitric Oxide
OH	Hydroxyl radical
PC	Protein carbonyls
PDCAAS	Protein digestibility corrected amino acid score
Pi	Inorganic phosphate

PLA	Phospholipase
PLA2	Phospholipase A2
PLD	Phospholipase D
PP	Peak power
RBE	Repeated bout effect
RFD	Rate of force development
ROS	Reactive oxygen species
RPE	Rate of perceived exertion
rpS6	Ribosomal protein S6
RSI	Reactive strength index
RSI	Reactive strength index
RyR	Ryanodine receptor
S6K1	Ribosomal protein S6 kinase beta-1
SAC	Stretch activated channel
SD	Standard deviation
SJ	Squat jump
SOD	Superoxide dismutase
SR	Sacroplasmic reticulum
SR	Sarcoplasmic reticulum
SSC	Stretch shortening cycle
STG	Simulated team game
sTnI	Skeletal troponin-I
T2	Transverse relaxation time
TAS	Total antioxidant status
TBARS	Thiobarbituric acid reactive substances
TMB	Tetramethylbenzidine
Tn	Troponin
TNF-α	Tumor necrosis factor- α
TTE	Time to exhaustion
Ub	Ubiquitin
Ub-P	Ubiquitin-proteasome
VA	Voluntary activation
VAS	Visual analogue scale
WM	Whole milk

Chapter 1: Introduction

1.1 Introduction

Participation in exercise places significant mechanical loads and metabolic demands on complex musculoskeletal, physiological, metabolic and biochemical processes that can result in fatigue, reduced muscle function, increased muscle soreness and reduced ability to perform (Howatson and Milak, 2009; Brownstein *et al.,* 2017). Once a training bout or exercise session is complete, the athlete commences a recovery phase in which fuel is restored (metabolic recovery) and damage of the musculoskeletal system is repaired (mechanical recovery) (Heaton *et al.,* 2017). Furthermore, recovery is considered to be multi-faceted (both physiological and psychological), and is thus an umbrella term characterised by both physiological regeneration modalities and psychological recovery strategies (Kellmann *et al.,* 2018). Accordingly, recovery after exercise is clearly a complex challenge to the athlete (Burke, 1997) and typically involves a systematic approach for maintainting athletes' physical and mental readiness to perform in the next training session or competition (Heaton *et al.,* 2017). This recovery process is further confounded by varying lengths of the inter-bout recovery periods. High training loads can reduce the recovery time to less than 8h and during intense competition periods athletes may be competing daily or have as little as 48h between competitions (Fatouros *et al.,* 2010; Carling *et al*., 2015). Optimal recovery places an athlete in a better position to maintain their performance or indeed enhance their performance in a subsequent exercise bout, which over time may optimise adaptation to repeated bouts of exercise (Moore, 2015). It is thus no surprise to see the amount of attention given to recovery interventions and strategies, both in the literature and popular media (Heaton *et al*., 2017).

Regardless of the recovery intervention (lifestyle, physiological, nutritional or pharmacological), it is vital that the intervention is cognisant of the nature of exercise performed (Minett and Costello, 2015; Howatson *et al*., 2016). Exercise with a substantial mechanical load, such as isolated eccentric exercise, results in impaired muscle function and increased soreness and serum markers of muscle damage (Highton *et al.,* 2009; Kanda *et al*., 2013). Exercise that is non-eccentric in nature, such as cycling, involves increased metabolic load characterised by increased energy flux, heart-rate, lactate and oxidative stress and varying levels of muscle damage and reduced performance (Mujika and Padilla, 2001; Bell *et al*., 2014). Most

exercise activities reside somewhere between isolated mechanical exercise and isolated metabolic exercise, inducing both mechanical and metabolic stress to varying degrees (Fatouros *et al*., 2010; Howatson and Milak, 2009). Examining the effects of specific interventions on recovery from these types of exercise should allow the identification of effective recovery strategies that match the demands of the bout, resulting in enhanced recovery, subsequent performance and adaptation.

Nutritional intervention is recognised as a key component of optimal sporting performance (Beck *et al.,* 2015) and also a vital component of recovery (Moore, 2015). Traditionally, recovery nutrition has focused on macronutrient intake for muscle regeneration (Phillips *et al*., 2011) and glycogen resynthesis (Burke *et al*., 2011), with more recent attention given to micronutrients and supplements that support and accelerate recovery (Rawson *et al*., 2018; Burke, 2017). Consumption of protein and carbohydrate is of importance in relation to muscle regeneration during the recovery period. Skeletal muscle protein turnover is an ongoing process and is the sum of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) (Morton *et al*., 2015). Exercise is a stimulus for muscle protein synthesis via activation of the mammalian target of rapamycin complex (mTORC) pathway (Egan and Zierath, 2013) but protein synthesis is also stimulated following the consumption of protein and protein with carbohydrate (Fluckley *et al.,* 2006) which is possible via direct activation of mTOR (Phillips *et al*., 2011). The effect of carbohydrate on protein synthesis is negligible but it likely decreases protein breakdown through its insulinogenic effect (Chow *et al*., 2006; Børsheim *et al*., 2004). The combination of exercise and nutrition, therefore, has a positive effect on protein balance resulting from increased protein synthesis (Børsheim *et al*., 2004; Tipton *et al*., 1999) and an attenuation of the rise in muscle protein breakdown (Biolo *et al*., 1997). Research has focused on the source, timing and dose of protein and carbohydrate intake in order to facilitate optimal recovery (Jeukendrup, 2017; Moore *et al.,* 2015).

Milk has become a popular post-exercise recovery drink, particularly with many athletes recently adopting a “food first” approach to their nutritional planning (Close *et al*., 2016). Milk is a source of both whey and casein, providing 36g protein per litre and has a Digestible Indispensible Amino Acid Score (DIAAS) of 1.14. This scoring system is based on ileal digestibility of protein and utilises a test protein which can give values greater than 1.0 (Phillips, 2017) and confirms that milk is readily

absorbed by the body, promoting protein synthesis and tissue repair and provides all essential amino acids. Of significance is that milk contains high concentrations of essential amino acids and branched chain amino acids (Haug *et al*., 2007) including leucine, a key trigger for muscle protein synthesis. Milk also has a carbohydrate concentration that is comparable to many sports drinks which may benefit post-exercise glycogen resynthesis while attenuating protein breakdown (Thomas *et al*., 2009; Ferguson-Stegall *et al.*, 2011) and has a high concentration of electrolytes which supports post-exercise rehydration (Shirreffs *et al*., 2007; Desbrow *et al*., 2014). Moreover, milk has many important nutrients including calcium, Vitamin A and Vitamin E. The whey content of milk is rich in cysteine which is a precursor for the production of glutathione (Madureira *et al*., 2007), an important antioxidant. From a practical perspective, milk is easily accessed and obtained, inexpensive and highly convenient; factors that determine athletes' nutritional choices.

Many investigations examining the effect of milk on recovery from exercise have focused on endurance exercise and chocolate milk consumption. In addition to the sugars naturally occurring in milk, chocolate milk has added sugars, usually sucrose, which may benefit the restoration of depleted glycogen stores (Karfonta *et al*., 2010; Ferguson-Stegall *et al*., 2011) and subsequent performance (Karp *et al*., 2006; Thomas *et al*., 2009; Lunn *et al*., 2012). Benefits of milk consumption have been observed with resistance training programmes (Rankin *et al*., 2004; Hartman *et al.,* 2007; Josse *et al.,* 2010) and positive protein balance has been noted with consumption of milk following resistance training bouts (Elliot *et al.,* 2006; Wilkinson *et al.,* 2007). The effect of milk on recovery from exercise-induced muscle damage (EIMD) has been logically investigated by Cockburn and colleagues in a landmark sequence of studies (Cockburn *et al*., 2008, 2010, 2012, 2013) and it is apparent that 500ml milk following muscle damaging exercise can attenuate decreases in muscle functional capacity in males including peak torque, reactive strength index (RSI) and sprint performance. However, the nature of the exercise protocols utilised in these studies (isolated eccentric protocols) is quite unlike the exercise stress experienced by most exercising athletes, and hence, the role of milk in the recovery from other, ecologically valid forms of exercise is necessary to inform the practical application of this knowledge. It is also unknown if milk can impact on recovery from exercise that does not include an eccentric bias or if milk can play a role in recovery from repeated bouts of exercise.

Furthermore, all of the above investigations included male participants only and it would be naive to assume that the guidelines for the use of milk in recovery generated from studies involving all males can be identically applied to a female cohort. An investigation examining female participation in sports medicine research found that females comprised only 39% of participants in 1382 investigations published across three peer-reviewed journals between 2011 and 2013 (Costello *et al*., 2014). A slightly more recent survey of articles published in two journals in the first 5 months of 2015 reported that females made up 42% of participants across 188 studies. Shockingly, when reviewing sports performance investigations (with one study removed due to the nature of the data), the percentage of female participants was only 3% (Brookshire, 2016). The startling lack of inclusion of females in this sphere of research is partly historical, sociological and cultural (Sheel, 2016) and has led to a poor understanding of basic sex differences (Schilaty, 2017). Sex comparison investigations into responses to exercise are equivocal in their findings; some have reported that females require longer periods to recover from exercise (Flores *et al.*, 2011) but others stated that females are less fatiguable than males and demonstrate a faster recovery (Sayers and Clarkson, 2001). Oestrogen may be an influencing factor in recovery, acting as an antioxidant against lipid peroxidation of the cell membrane (Tiidus, 1995, 2003) thereby protecting the membranes from damage (Kendall and Eston, 2002) during exercise though this does not always have a positive effect on post-exercise muscle function (Power *et al.,* 2010; Kerksick *et al*., 2008). Oestrogen may also influence the inflammatory cascade, gene expression and muscle metabolism. If oestrogen is a modulating factor of the recovery process then research is needed to identify the specific recovery needs of this cohort.

In light of the available research it is tenable that milk has the potential to enhance recovery from varying types of exercise in females. Consequently the overarching aim of this thesis is to explore the effect of milk on recovery from a range of exercise activities in female athletes. As current recovery nutrition is typically generic and does not consider the severity and type of exercise performed (Jeukendrup, 2017), the outcomes of this thesis should highlight the value of choosing nutritional interventions that are specific to the mode of exercise and desired outcomes of the exercise bout, and also specific to the athletic cohort. Establishing this knowledge can present a foundation for practical recovery strategies for this under-investigated population.

Considering the above, this thesis describes a series of studies which investigated the effectiveness of milk on recovery from varying exercise protocols in a female athletic population. In an attempt to identify sex differences in the response to the consumption of milk following eccentric exercise (mechanical in nature), the first study investigated the effect of milk on recovery from an exercise protocol that induced muscle damage in the hamstring muscles, in both males and females. Recovery of muscle function, soreness and serum markers of muscle damage were assessed over a 72h post-exercise period.

In trying to expand an understanding of how milk might be effective as a recovery beverage, the second study explored the effect of milk on isolated, continuous concentric exercise (primarily metabolic in nature). Changes in muscle function, muscle soreness and serum proteins were monitored over the 72h recovery period following a cycling protocol designed to simulate the metabolic demands of team-sports.

The third study endeavoured to determine if milk can assist recovery from a sport-specific protocol with greater ecological validity and practical significance, inducing both mechanical and metabolic stress. In this investigation participants completed a protocol comprising repeated sprints and repeated jumps, following which 500ml of milk or 500ml of an energy-matched energy drink was consumed, with recovery monitored over 72h.

The final study endeavoured to investigate the effect of milk on recovery from repeated bouts of exercise, separated by a 48h recovery period, thus replicating the training load of many athletes. On two occasions, with a 48h recovery period, all participants completed a circuit that simulates the demands and activties of a team sport, with muscle function, soreness, serum proteins and symptoms of stress monitored over a 96h period.

Chapter 2: Review of Literature

2.1 Introduction

This thesis focuses on the application of milk in recovery from varied exercise protocols in a female team-sport cohort. The following review of literature therefore presents an overview of muscle structure, function, protein synthesis, the impact of protein and specifically milk, on recovery from exercise.

2.2 Structure and Function of Muscle

Given the nature of the methods of these investigations, a review of basic muscle structure and function will provide context to the impact of exercise and underpin an understanding of muscle function during the recovery period.

2.2.1 Structure of muscle

Skeletal muscle is a complex tissue representing 40-45% of total body mass and is comprised multinucleated muscle cells embedded in a connective tissue matrix (Meyer and Lieber, 2012). Muscle is contained within the epimysium, a layer of connective tissue. Within the muscle bundles of muscle fibres, called fascicles, are surrounded by another layer of connective tissue called the perimysium. Each individual muscle fibre is enclosed in a meshlike sheath of collagenous tissue called the endomysium. Muscle fibres are inextricably linked with this complex of connective tissue, which is continuous with the tendons, and therefore this matrix may play an important role in regulating muscle contraction, in addition to providing support and assisting the transfer of force (Lieber, 1992).

The muscle fibre, or muscle cell, is cylindrical in shape with a diameter of 10-100 μm in diameter (Lieber, 1992). Each fibre is enclosed in a plasma membrane called the sarcolemma which provides a barrier between the fibre and the extracellular fluid (McComas, 1996). The muscle fibre is made up of myofibrils, each approximately 1μm in diameter, arranged in parallel (Lieber, 1992). Protein filaments, arranged in repeating units, called sarcomeres, constitute each myofibril. These protein filaments, or myofilaments, are categorised as thick or thin, and it is the active interdigitation of these filaments that produces muscle shortening (Lieber, 1992). The arrangement of the protein filaments and the degree of overlap gives the muscle its striated appearance (Lieber, 1992). The region of the sarcomere containing the thicker

filament, myosin, is termed the A band (anisotropic). The thin filament, actin, forms the I band (isotropic). In the centre of the A band there is a pale region without actin-myosin overlap which is called the H-zone (McComas, 1996). Bisecting the I band is the z-line or z- disk to which filaments are attached. One sarcomere extends from z-disk to z-disk.

The actin filament measures approximately 1.6 μm in length and 8 nm in width (McComas, 1996). It is anchored to the z-disk by α-actinin and is composed of a long α-helical arrangement of actin monomers (Lieber, 1992). The helical arrangement creates a groove along the length of the filament, into which the regulatory protein, tropomyosin fits. Approximately, every seven actin monomers contains the protein troponin (Lieber, 1992). Troponin (Tn) is composed of three subunits: Tn-I, an inhibitory component which prevents actin-myosin interaction; Tn-T, which binds troponin to tropomyosin; Tn-C, which binds calcium during muscle contraction (Farah and Reinach, 1995).

The thick myosin filament , approximately 1.6 μm long and 120 nm wide (Maruyama, 1997), is made up of myosin molecules arranged in an anti-parallel fashion, giving the sarcomere symmetry down the middle (Lieber, 1992). The myosin molecule has a long back bone and a region with two globular heads that extends from the backbone (McComas, 1996). These projections can be seen every 143Å along the length of the myosin filament (Lieber, 1992). The myosin molecule is subdivided into separate components based on the formula weight of each: light meromyosin (LMM) forming the backbone of myosin and heavy meromyosin (HMM) making up the projections. The HMM has two further subfragments: subfragment 1 (S-1) which is the globular head of myosin and subfragment 2 (S-2) which is the part of HMM that projects from the LMM backbone (Lieber, 1992). S-1 and S-2 form the ‘cross-bridge’ between the myosin backbone and the actin filament during force production. Part of the S-1 component contains a pocket for binding and hydrolysing ATP (McComas, 1996), in addition to sites for binding to actin located on the opposite side of the S-1 head (Rayment *et al.,* 1993).

After actin and myosin, titin (or connectin) is the most abundant muscle protein (Kontrogianni-Konstantopoulos *et al.,* 2009). Titin is attached to the z-disk and spans half the sarcomere anchoring in the M-band (Small *et al.,* 1992; Maruyama, 1997).

The z-disk portion of titin has binding sites for other proteins inclduing α-actinin, obscurin and nebulin, therefore, playing an essential role in sarcomere stability and maintenance (Kontrogianni-Konstantopoulos *et al*., 2009). The central I-band region of titin, unlike other regions, gradually extends, meaning that it functions in a spring-like manner assisting in the maintenance of a constant resting sarcomere length even after stretch or contraction (Small *et al*., 1992; Maruyama, 1997; Lieber *et al*., 2002; Kontrogianni-Konstantopoulos *et al*., 2009) and the production of force during contraction (Rode *et al.,* 2009). In addition, and of significance in the context of muscle damage, it seems that titin has binding sites for calpain proteases (Kontrogianni-Konstantopoulos *et al.,* 2009).

Nebulin is another 'giant' protein. An inextensible protein, it is anchored in the z-disk (thereby regulating z-disk width (Labeit *et al*., 2011)) and binds along the length of the actin filament (Small *et al.,* 1992; Kontrogianni-Konstantopoulos *et al*., 2009) with two nebulin strands per thin filament winding around the outer edge of the actin helix (Yadavalli *et al*., 2009). Nebulin is required for stabilising the thin filaments (Kontrogianni-Konstantopoulos *et al.,* 2009), maintaining thin filament length (Labeit *et al*., 2011) and enhancing force production (Witt *et al.,* 2006). It has been suggested that nebulin may stiffen the thin filament against impact and promote the healing of severed actin filaments (Yadavalli *et al*., 2009).

In addtion to the contractile filaments and the giant proteins, muscle has an extensive cytoskeletal network comprising intermediate filaments (IF) that connect the cell surface with the nucleus and provide cells with important mechanical properties (Goldman *et al*., 2008). IF proteins are 10-12 nm in diameter (Toivola *et al*., 2005) and all are similar in structure with a central α-helical rod flanked by non- α-helical 'head' and 'tail' domains (Goldman *et al.,* 2008; Herrmann *et al.,* 2007). Mature muscle is abundant with the IF desmin (Capetanaki *et al*., 2007) but other IFs found in muscle include vimetin, synemin, desmuslin, paranemin, vinculin and syncoilin. Reportedly, IFs function mechanically in force transmission and transduction of mechanical perturbations, but also play a role in maintaining cell integrity, signalling, stabilisation and tethering of cell organelles (Milner *et al*., 1996; Helfand *et al*., 2003; Capetanaki *et al*., 2007; Goldman *et al*., 2008).

Desmin is the major muscle-specific IF protein, consituting 0.35% of total muscle protein (Paulin and Li, 2004). A 10 nm filament (Costa *et al*., 2004), desmin links myofibrils laterally by forming a three-dimensional scaffold around the z-disk (Paulin and Li, 2004). Desmin has a clear role in providing and maintaining strength and mechanical integrity for the cell (Milner *et al*., 1996; Paulin and Li, 2004) and for optimal excitation-contraction coupling (Paulin and Li, 2004). Of particular interest to this study is how the amount of structural damage following eccentric exercise correlates with the rapid disappearance of desmin from muscle (Lieber *et al*., 1996; Lieber *et al*., 2002). Furthermore, animal research demonstrates that lack of desmin results in degeneration and disorganisation of the cell, with reduced muscle force production and increased fatigue (Milner *et al*., 1996; Paulin and Li, 2004). Of final relevance is that desmin is a substrate for proteolysis by muscle-specific calpain (Goll *et al*., 2003).

2.2.2 Excitation-contraction coupling and cross-bridge cycling

The term excitation-contraction coupling refers to the process by which neural activation results in contraction of the muscle (Lieber, 1992). The depolarisation of the peripheral nerve axon generates an action potential that propagates down the nerve to reach the neuromuscular junction. Here, the neurotransmitter acetylcholine (Ach) is relased into the synaptic cleft and binds to an Ach receptor which is integrated into the muscle membrane. This binding results in depolarisation of the sarcolemma, and the resulting action potential is quickly conducted deep into the muscle fibre by the T-tubules which ultimately results in the release of calcium from the terminal cisternae of the sarcoplasmic reticulum (SR) (Melzer *et al.,* 1995).

Under resting conditions, the TnI subunit of the troponin complex inhibits an otherwise strong and favourable interaction between actin and myosin (Farah and Reinach, 1995). Following the release of calcium from the SR, the binding of calcium to specific sites on the TnC subunit, displacing magnesium (Melzer *et al.,* 1995), is the initial event in the removal of inhibition of the actomyosin interaction (Farah and Reinach, 1995). It is likely that the removal of this inhibition is via a shift in tropomyosin's position, lifting it away from the actin filament (McComas, 1996).

Movement in muscle is then generated by the myosin cross-bridges which interact cyclically with the actin filaments, drawing them past the myosin thick filaments (Geeves and Holmes, 1999). This 'sliding filament' mechanism was proposed and developed by AF Huxley and HE Huxley in the 1950's. Work by Huxley and Nidedergerke (1954) proposed that small projections from the myosin filaments, termed 'cross bridges' could briefly attach themselves to the actin filament drawing the thin filaments towards the centre of the sarcomere (Huxley, 1974). More recently, protein crystallography of the cross bridge has allowed for greater understanding of this process (Geeves and Holmes, 1999), including the role of ATP in the powering of cross-bridge cycling. The 'swinging cross bridge hypothesis' described clearly by the Lymn-Taylor cycle (Lymn and Taylor, 1971) explains the process. With the myosin head in a 45° conformation ('rigor'), ATP enters the pocket in the upper domain of the myosin head, opening a cleft that splits the heavy chain into two distinct components, termed the upper 50K and lower 50K domains (Holmes *et al.,* 2004). This results in a dissociation of the actin-myosin complex and the myosin head moves 5 nm farther on (McComas, 1996). ATP is then hydrolysed, with ADP and Pi remaining in the myosin head. Reassociation of actin and myosin allows the cleft between the upper and lower domains to close, resulting in Pi being expelled from the myosin head (Fisher *et al.,* 1995). Subsequently the ATP pocket opens, the cross-bridge undergoes a conformational change, with the release of ADP leading to the 'power stroke' (Holmes, 1997) or 'force-generating event' (Geeves, 1991), and the actin filament is moved 5 nm onwards (McComas, 1996). The repeated attachment and detachment between actin and mosin for each ATP hydrolysed leads to the shortening of the sarcomere (Geeves, 1991). A single cross-bridge cycle lasts approximately 50 ms, with the actomyosin complex formed for only 2 ms (McComas, 1996). Of note is the role of magnesium (Mg) in this process. Mg reduces the rate of Ca^2 binding to troponin, as troponin can also bind Mg (Potter *et al.,* 1981). The Mg complex with adenosine triphosphate (MgATP) is the substrate for the enzymatic reactions of the sliding filament mechanism. MgATP reacts with the myosin globular heads, resulting in actomyosin dissociation; thus as MgATP concentration increases, relaxation occurs (Melzer *et al.,* 1995).

Altenative explanations for muscle contraction have been hypothesised. These include a 'swinging-lever arm' process (Geeves and Holmes, 1999; Holmes *et al.,* 2004), a 'flipping-neck' mechanism (Yanagida *et al.,* 1985) or an electrostatic

process (Yu *et al.,* 1970). However, review of such models is beyond the scope of this thesis.

2.2.3 Eccentric muscle action

Although most descriptions of muscle contraction refer to a shortening of the sarcomere, force is also produced when an external force exceeds that produced by the muscle and the muscle lengthens (Enoka, 1996; LaStayo *et al*., 2003). It is likely that the actin-myosin interactions in an eccentric action are similar to a concentric contraction (shortening of the muscle) except that the opposing actin filaments are pulled away from each other in the sarcomeres (McComas, 1996). In this case, the actomyosin bonds are probably disrupted mechanically rather than undergoing an ATP-dependent detachment and absorption of energy during lengthening is converted to mechanical work during subsequent shortening (Constable *et al*., 1997; Lindstedt *et al.,* 2001). It is recognised that an eccentric contraction generates more force than a concentric contraction (Lindstedt *et al.,* 2001; Herzog, 2013) perhaps due to a greater number of cross bridges formed at any given time during lengthening. Unequal lengthening of individual sarcomeres (weaker sarcomeres being stretched considerably more than stronger sarcomeres), giving a dispersion in saromere length, may result in a higher force than if the sarcomeres lengthened uniformly (Morgan, 1990; Koppes *et al*., 2013). Furthermore, it appears that eccentric contractions require unique activation strategies by the nervous system (Enoka, 1996; Duchateau and Baudry, 2013) and it has been suggested that titin may play a greater role than previously considered (Herzog *et al.,* 2016). Readers are directed to Hessel *et al.,* (2017) for a thorough review. Of significance is that eccentric muscle actions may result in disruption of the sarcomeres and sarcolemma and sarcoplasmic reticulum damage (Morgan, 1990) which in turn leads to an increase in intracellular calcium concentration leading to further degredation. This mechanism will be discussed in 2.4.1.

2.3 Protein Synthesis and Breakdown

Skeletal muscle protein turnover is an ongoing process and is the sum of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), with 1-2% of proteins synthesised and broken down every day (Welle *et al.,* 1994) at a rate of 0.06 h^{-1}

(Morton *et al*., 2015). Positive protein balance occurs when the rate of MPS exceeds the rate of MPB. Key stimuli for muscle protein synthesis include resistance exercise and provision of amino acids, particularly, essential amino acids.

2.3.1 Muscle protein synthesis

Muscle protein synthesis involves gene transcription and translation, the latter regulated by mTOR (mammalian target of rapamycin) (Drummond *et al*., 2009). mTOR is a large protein found in two complexes: mTORC1 and mTORC 2 (Ham *et al*., 2014). It is thought that mTORC2 is not involved in the regulation of translation initiation and elongation (Drummond *et al.,* 2009). p70 ribosomal S6 kinase 1 (S6K1) is a downstream target of mTORC1 and a key influence in translation initiation and elongation via the phosphorylation of 40S ribosomal protein S6 (rpS6) and eIF4B (Egan and Zierath, 2013). S6K1 phosphorylation has been found to have a positive association with muscle mass in humans undertaking a 12 week resistance training programme (Terzis *et al*., 2008). This effect may be via S6K1 phosphorylation of ribosomal protein S6 (rpS6) and eukaryotic elongation factor 2 (eEF2) kinase (Drummond *et al.,* 2009), among others. Upstream, mTORC1 is activated by Akt (protein kinase B), but also by amino acids and muscle contraction (see following sections). The regulation of translation initiation and elongation also involves the ERK 1/2 (extracellular signal-regulated kinase 1/2). Interaction between mTORC1 and ERK1/2 has been identified, but it is possible for ERK 1/2 to enhance protein synthesis independent of mTORC1 (Fluckley *et al*., 2006).

2.3.2 Contractile regulation of muscle protein synthesis

Early research indicated that muscle protein synthesis was decreased during exercise (Rennie *et al.,* 1980). It is now well known that this is reversed following exercise, with an increase in protein synthesis observed during the recovery period. It has also been established that there is an increase in muscle protein breakdown during this post-exercise period (Phillips *et al.,* 1997) and greater post-exercise protein synthesis ultimately leads to positive protein balance. The outcome of the increased protein synthesis is driven by the nature of the exercise stimulus. Primarily, muscle hypertrophy is observed with resistance training and mitochondrial biogenesis is observed with endurance exercise. Muscle contraction results in disruption to the sarcolemma, causing an increase in the concentration of membrane

phospholipid phosphatidic acid (PA) through activation of phospholipase D (PLD) (Egan and Zierath, 2013). The subsequent mechanism for enhanced muscle protein synthesis involves the activation of the mTORC1 pathway (Dreyer *et al*., 2006). The observed increase in protein synthesis following resistance training remains elevated for up to 24h in trained individuals and perhaps up to 48h in untrained (MacDougall *et al.,* 1995). This is indicated by increased S6K1 phosphorylation above baseline following a resistance exercise session (Deldicque *et al.,* 2008). Furthermore, Akt phosphorylation has been observed during the recovery period following resistance exercise (Dreyer *et al.,* 2006) confirming the activation of the mTORC1 pathway during this time period.

A number of studies have investigated signalling pathways following eccentrically biased exercise. It has been reported that PKB and p70S6K activation is greater in response to lengthening actions compared to shortening actions in rat muscle (Nader and Esser, 2001) though similar results have not been observed in humans following eccentric exercise (Phillips *et al*.,1997; Cuthbertson *et al*., 2006). In this latter study, no differences in the magnitude or time course of the activation of signalling proteins, or on rates of myofibrillar protein synthesis were observed when eccentric actions were compared to concentric contractions. The different results observed in rats may be due to the electrical stimulation of rat muscle as opposed to voluntary contractions in the human studies. However, in humans, given that eccentric actions are more energy efficient than concentric actions it has been noted that if work is matched between lengthening and shortening actions, greater MPS is observed with lengthening actions (Moore *et al*., 2005). Eliasson *et al*., (2006) compared maximal eccentric actions to maximal concentric actions in untrained males and found an increased activation of p70S6K with eccentric actions, but no change in the phosphorylation of Akt or mTOR, suggesting an Akt/mTOR-independent pathway in the activation of p70S6K following eccentric exercise, and in the absence of nutritional intake. It is likely, therefore, that eccentric actions alone, via both force production and elongation (Eliasson *et al.,* 2006) are a powerful stimulus for translation initiation and protein synthesis, which is relevant to the investigations of this thesis.

There is a lack of consensus on the effect of metabolic stress on protein synthesis. Increased mixed muscle protein synthesis occurs following endurance exercise

(Harber *et al.,* 2010; Mascher *et al*., 2011) and an increase in mitochondrial protein synthesis has been reported following continuous cycling (Wilkinson *et al*., 2008; Dumke *et al*., 2009), probably as a result of increases in PGC-1α and Sirt-mRNA (Dumke *et al*., 2009). The same effect is seen following high intensity interval exercise that induces high levels of metabolic stress (Little *et al*., 2010), again via an increase in PGC-1 α. Both AMPK and mTOR increase after a bout of cycling (Benziane *et al*., 2008; Mascher *et al.,* 2007) with the rates of both myofibrillar and mitochondrial protein synthesis being influenced by exercise intensity (Di Donato *et al.,* 2014). Following sprint interval training, males demonstrate higher mixed muscle and mitochondrial protein synthesis (Scalzo *et al*., 2014).

2.3.3 Nutritional regulation of muscle protein synthesis

The ingestion of essential amino acids along with carbohydrate has been shown to be associated with an increase in protein synthesis via mTOR activation (Fluckey *et al*., 2006). Fujita *et al*., (2007) reported an increase in phosphorylation of Akt, mTOR and S6K1 following the ingestion of a leucine-enriched amino acid and carbohydrate solution. Leucine has been identified as a 'metabolic trigger' for muscle protein synthesis (Crozier *et al.,* 2005; Wilkinson *et al*., 2013). A breakthrough study by Buse and Reid (1975) on rat muscle found that leucine played a key role in stimulating protein synthesis and reducing protein breakdown in the fed or fasted state, either with or without insulin, compared to other amino acids. This was followed by a number of animal studies that similarly reported increased protein synthesis with leucine (Garlick *et al.,* 1988; Anthony *et al*., 2000; Crozier *et al*., 2005). Studies in humans have confirmed the anabolic effect of leucine (Rieu *et al*., 2006; Wilkinson *et al.,* 2013) and research has shown that suboptimal intake of protein can be maximally effective if leucine is added (Churchward-Venne *et al.,* 2012). This suggests that foods rich in leucine are more effective in enhancing MPS than those with low leucine content (Katsanos *et al*., 2006) though a leucine 'threshold' may be specific to age (Katsanos *et al.,* 2006) and activity levels (Glover *et al*., 2009). It is also tenable that leucine may impact muscle protein breakdown. Nair *et al.,* (1992) reported decreased muscle protein breakdown with leucine infusion; this may be as a result of an insulinogenic effect as it is known that only a small increase in insulin concentration can reduce protein breakdown (Wilkes *et al.,* 2009).

Leucine's effect on protein synthesis is via its ability to activate mTOR (Anthony *et al.,* 2000). It is also possible that essential amino acids may directly activate mTOR (rather than through Akt or other targets) via other kinases (hVps34 or MAP4K3) (Byfield *et al.,* 2005; Phillips *et al*., 2011). It has been demonstrated that small GTPases (the Rags), activated by an increase in amino acid abundance, can activate mTORC1 perhaps by changing the subcellular location of mTORC1 to the lysosomal membrane (Efeyan *et al*., 2012; Jewell *et al.,* 2013). When cells are deprived of amino acids, mTORC1 is diffused throughout the cell, but an increase in amino acids results in a rapid translocation of mTORC1 to the lysosomal surface (Bar-Peled and Sabatini, 2014). This translocation permits interaction with Rheb (ras homologous protein-enriched in the brain), a small GTPase located at the lysosomal surface which is an activator of mTORC1 (Phillips *et al.,* 2011) and has been termed the 'ignition key' for the kinase activity of the complex (Efeyan *et al.,* 2012). Further regulation of mTORC1 involves a number of other complexes including Ragulator and GATOR (GAP activity towards Rags) (Bar-Peled and Sabatini, 2014). Readers are referred to Bar-Peled and Sabatini (2014), Jewell *et al*., (2013) and Efeyan *et al.,* (2012) for comprehensive reviews.

There is considerable agreement that carbohydrate ingestion decreases protein breakdown through its insulinogenic effect (Chow *et al*., 2006; Borsheim *et al*., 2004). Circulating insulin plays an important role in the regulation of protein metabolism (Koopman *et al.,* 2005) though its role in promoting post-exercise muscle protein synthesis is controversial (Figuerideo and Cameron-Smith, 2013). In-vitro studies have shown that the presence of insulin increases muscle protein synthesis (Nobukuni *et al.,* 2005; Byfield *et al*., 2005), though these studies compared synthesis rates either with or without insulin. In-vivo conditions without insulin do not exist; overnight fasting results in low, but easily measured, concentrations of insulin (Figuerideo and Cameron-Smith, 2013). Hyperinsulinemia (without exercise) following insulin infusion or carbohydrate ingestion may result in protein synthesis (Bell *et al*., 2005) and reduced protein breakdown (Gelfand *et al.,* 1987; Denne *et al*., 1991; Chow *et al*., 2006) but not all studies have found this (Fujita *et al*., 2006; Glynn *et al*., 2013). It is possible that despite reaching an insulin 'threshold' of >200pmol/l^{-1}, the threshold concentration must be maintained for a minimum amount of time in order to significantly affect muscle protein breakdown (Glynn *et al*., 2013) thus, explaining the disparate results in the aforementioned investigations. Glynn *et al*.,

(2013) reported similar fractional synthetic rate, muscle protein synthesis, muscle protein breakdown and nitrogen balance following the ingestion of essential amino acids or amino acids with carbohydrate (30g sucrose). Nonetheless, it is possible that carbohydrate may stimulate MPS if protein intake is suboptimal (Phillips, 2014).

2.4 Exercise Stress

It is hypothesised that recovery from exercise is affected by mechanical and metabolic stress (Armstrong, 1990; Byrd, 1992; Pyne, 1994). Mechanical stress arises from direct mechanical loading on the muscle fibre, with greater damage resulting from eccentric muscle contractions compared to concentric or isometric contractions (Armstrong *et al.,* 1991). Metabolic stress may be a result of metabolic insufficiencies in contracting muscles (Armstrong *et al.,* 1991). Most exercise incorporates elements of both mechanical stress and metabolic stress resulting in increased muscle damage, inflammation and oxidative damage. For the purpose of this review these aspects are reviewed separately. However, it is important that the reader is cognisant of the simultaneous occurance of all during most forms of exercise.

2.4.1 Mechanical stress

The mechanisms and processes involved in mechanical stress, muscle damage and regeneration are not fully understood. Sarcomeres are considered unstable on the descending limb of the length-tension curve (Allen, 2005). Excessive strain placed on individual sarcomeres during eccentric contractions may initiate muscle injury. When active muscle is stretched beyond optimum length on the descending limb, sarcomeres will exceed their yield point (point of contact between actin and the myosin head) (Morgan, 1990). Subsequently, there is non-uniform lengthening of sarcomeres (Morgan, 1990). When a sarcomere is stretched beyond optimal length it is weakened; the longer the sarcomere the weaker that sarcomere becomes. This results in a more rapid stretching, and even greater weakness until rising passive tension compensates for falling active tension (Morgan and Proske, 2004). This increased length of sarcomeres corresponding to lengths beyond filament overlap results in ‘popping’ (Morgan, 1990; Proske and Allen, 2005). This lengthening of sarcomeres does not occur regularly along the muscle myofibril, therefore, there is a

shearing of sarcomeres predisposing the myofilaments and cellular structures to damage (Morgan and Proske, 2004). The cytoskeleton is disrupted (Lieber *et al*., 1996; Koh and Escobedo, 2004; Choi and Widrick, 2010) and the t-tubules, sarcoplasmic reticulum and sarcolemma are damaged (McNeil and Khakee, 1992; Takekura *et al.,* 2001) impairing activation of the contractile proteins (Balnave and Allen, 1995; Ingalls *et al.*, 1998). Damage to the extracellular matrix (epimysium, perimysium and endomysium) may also occur as a result of sheer stress (Gao *et al*., 2008). Failure of some of the excessively lengthened sarcomeres to re-interdigitate following repeated eccentric contractions further affects sarcomere function (Talbot and Morgan, 1996; McPherson *et al.,* 1997; Morgan and Allen, 1999), though not all studies support this (Panchangam and Herzog, 2011). Failure of re-interdigitation puts extra load on adjacent sarcomeres through transverse connections between myofibrils, increasing the likelihood of those sarcomeres popping in a subsequent contraction, leading to further disruption (Morgan and Allen, 1999). Extensive sarcomere disruption may lead to damage to the sarcolemma or the sarcoplasmic reticulum, in turn increasing intracellular Ca^{2+} concentration, activation of proteolytic enzymes and further damage and losses of function (Zhang *et al*., 2008).

Factors affecting the amount of mechanical stress

Repeated eccentric contractions leads to a greater number of disrupted sarcomeres compared to fewer contractions (McCully and Faulkner, 1986; Talbot and Morgan, 1996) resulting in the large regions of sarcomere damage reported (Armstrong *et al.,* 1983; Friden and Lieber, 2001), though this relationship is nonlinear with further increases in the number of eccentric contractions resulting in smaller increases in damage (McCully and Faulkner, 1986; Talbot and Morgan, 1998). Muscle damage has been observed following sub-maximal eccentric actions (Lieber and Friden, 1993; Talbot and Morgan, 1998; Paschalis *et al.,* 2005), indicating that the magnitude of strain on saromeres is a more significant factor in inducing muscle damage than the amount of force produced (Fridén and Lieber, 1992). Eccentric actions at longer muscle lengths results in greater damage than contractions at shorter muscle lengths (Child *et al*., 1998; Newham *et al*., 1998; Nosaka and Sakamoto, 2001) with an increase in damage as the sarcomere length moves onto the descending limb of the length-tension curve (Morgan and Allen, 1999). It has been reported that the velocity of the eccentric contraction is not a major determinant of muscle damage (McCully

and Faulkner, 1986; Talbot and Morgan, 1998; Barroso *et al*., 2010), though greater z-disk streaming has been reported after fast (210°:s^{-1}) eccentric contractions compared to slow (20-30°:s^{-1}) contractions (Shepstone *et al.,* 2005; Chapman *et al*., 2006, 2008). Conversely, Paddon-Jones *et al*., (2005) found greater damage with slow (30°:s^{-1}) rather than fast (180°:s^{-1}) eccentric contractions.

Importantly, while muscle damage depends on the muscles activated and sarcomere length outlined above, there is a general consensus that fast-twitch muscle fibres are more susceptible to eccentrically induced muscle damage than slow-twitch fibres. Much of this evidence comes from animal studies (Lieber and Friden, 1993, Vijayan *et al.,* 2001) and not all studies have drawn similar conclusions (Moens *et al.,*1993; Consolino and Brooks, 2004) probably because of methodological difficulties. The greater tensions generated by fast-twich fibres may make them more susceptible to muscle damage (Appell *et al*., 1992). Friden and Lieber (1992) have suggested that an increase in stiffness in fast-twich fibres early in exercise, because of their low oxidative capacity and thus increased metabolic stress, leads to a greater liklihood of muscle damage on subsequent eccentric actions. A contributing factor may be structural differences between fast- and slow- twitch fibres, where fast- twitch fibres have narrower z-disks, with less actin and myosin attachments, resulting in weaker sarcomere structure to begin with (Friden and Lieber, 1992). Choi and Widrick (2010) recently found that fibres coexpressing type IIa and IIx isoforms showed exaggerated susceptibility to muscle damage and that this susceptibility was directly related to the amount of the type IIx isoform expressed in the hybrid fibre, confirming that susceptibiity of the cytoskeleton to eccentric muscle damage differs between fibre types.

Finally, prior exposure to eccentric exercise resulting in muscle damage and reduced muscle function, reduces the degree of damage experienced following a subsequent bout of similar exercise performed within several weeks. This is known as the Repeated Bout Effect (RBE) (McHugh, 2003) and has been observed following eccentric loading of the elbow flexors (Howatson *et al*., 2007), knee extensors (Kamandulis *et al.,* 2010) and drop jumping (Bridgeman *et al*., 2017) and may be a result of neuromuscular adaptations, muscle-tendon behaviours, extracellular matrix remodelling or a modified inflammatory response (Hyldahl *et al*., 2017).

2.4.2 Metabolic stress

There appears to be no clear consensus on the definition of metabolic stress but for the most part it is recognised as occurring during exercise when energy is low, leading to an accumulation of lactate, inorganic phosphate and hydrogen ions, impacting hormonal release, inflammation and the production of reactive oxygen species (de Freitas *et al.,* 2017). The increased ATP hydrolysis and glycolytic flux during exercise leads to a metabolic disturbance including increased AMP and metabolites, and possible hypoxia, and increased ROS, triggering the activation of processes leading to mitochondrial biogenesis (de Freitas *et al.,* 2017). Such stress has been observed with resistance exercise and may play a key role in maximising adaptation (Schoenfeld, 2013). Sprint cycling (Kristoffersen *et al*., 2018), cycling (Decroix *et al.,* 2017), and combined resistance and cycling sessions (Bessa *et al.,* 2016) have also been shown to induce metabolic stress, though not all markers increase in all exercise circumstances (Spanidis *et al*., 2017). The impact of metabolic stress on muscle damage, inflammation and oxidative damage is discussed in the next sections.

2.4.3 Autogenic phase

Muscle contraction is triggered by a transient increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), from resting a concentration under 100nM (Armstrong *et al.,* 1991), which facilitates the development of tension. In non-fatigued, non-damaged muscle $[Ca^{2+}]_i$ decreases as the muscle relaxes, through the action of specialised membrane pumps, thus maintaining cytoplasmic Ca^{2+} at around 10^{-7}M (Carafoli, 2004). Following eccentric muscle actions in mice and rats, $[Ca^{2+}]_i$ has been observed to increase 1.5-3 times resting values (Duan *et al.,* 1990; Gissel and Clausen, 1999; Lynch *et al.,* 1997; Sonobe *et al.,* 2008). High Ca^{2+} concentration within the muscle has been associated with cell dysfunction and necrosis. As a result, it has been proposed that Ca^{2+} is a mediator of exercise-induced myocyte damage and subsequent remodelling following eccentric exercise (Armstrong, 1990).

Increases in $[Ca^{2+}]_i$ have been observed following isolated eccentric actions (Armstrong *et al.,* 1983), downhill running (Duan *et al*., 1990; Lynch *et al,* 1997), isometric contractions (Gissel and Clausen, 1999; Mikkelsen *et al*., 2004) and prolonged running (Overgaard *et al.,* 2002, 2004), and is thus a consequence of both

mechanical and metabolic stress. An increase in $[Ca^{2+}]_i$ may be due to a number of reasons (for review see Allen *et al*., 2005; Kano *et al.,* 2012). Firstly, damage to the sarcolemma of the muscle cell because of the mechanical nature of eccentric exercise would allow an influx of Ca^{2+} from extracellular spaces, where $[Ca^{2+}]$ is ≥10,000-fold greater than $[Ca^{2+}]_i$ (Sonobe *et al*., 2008), due to increased membrane permeability (Armstrong *et al*., 1991; McNeil and Khakee, 1992; Gissel, 2000; Allen *et al.,* 2005). Evidence of membrane damage immediately following eccentric exercise has been reported (McNeil and Khakee, 1992; Takekura *et al.,* 2001; Hamer *et al.,* 2002) supporting this hypothesis.

Secondly, damage to the SR following eccentric exercise would result in increased leakage of Ca^{2+} from the SR contributing to the increased $[Ca^{2+}]_i$ observed (Yasuda *et al*., 1997; McNeil and Khakee, 1992; Takekura *et al*., 2001; Gissel and Clausen, 2001).

Thirdly, an inability of the SR to reuptake Ca^{2+} would prolong $[Ca^{2+}]_i$ .Reduced SR Ca^{2+}-ATPase pump activity would negatively affect the muscle's capacity to buffer changes in $[Ca^{2+}]_i$ (Belcastro *et al*., 1981; Carroll *et al*., 1999). Reduced functioning of the SR Ca^{2+}-ATPase pump may be because of over activity during exercise resulting in conformational changes in the binding sites of the SR Ca^{2+}-ATPase and subsequent degredation by proteolytic enzymes (Belcastro *et al*., 1981). Reactive Oxygen Species produced as a consequence of both mechanical and metabolic stress have also been implicated in reduced SR Ca^{2+}-ATPase activity (Favero *et al*., 1998).

Finally, it is postulated that mechanical strain on the muscle fibres during eccentric exercise involves stretch activated channels (SACs) in the sarcolemma resulting in increased influx of Ca^{2+} into the cell (Lieber *et al*., 1996). Blocking these channels has resulted in a reduced increase in $[Ca^{2+}]_i$ highlighting their role in elevated $[Ca^{2+}]_i$ (Yeung *et al.,* 2003; Yeung and Allen, 2004; Sonobe *et al*., 2008). It is possible that initial damage to the cytoskeleton of the cell influences opening of SACs (Allen *et al*., 2005) though a full understanding of this mechanism is elusive.

The increase in $[Ca^{2+}]_i$ observed is seen as a key event in the progression of muscle damage. The initial increase in $[Ca^{2+}]_i$ as a result of membrane and/or SR damage or

activation of SACs leads to the activation of proteolytic enzymes such as calpain, resulting in further damage to the sarcolemma and sarcoplasmic reticulum and further accumulation of Ca^{2+}. This has been termed a 'viscious cyle of cell degredation' (Gissel and Clausen, 2001).

Calpain

Protein degredation during exercise has been linked to both lysosomal and non-lysosomal proteases (Belcastro *et al*., 1998). While it has been found that prolonged exercise causes an increase in lysosomal proteases such as cathepsins (Salminen and Vihko, 1981), the extent of their contribution to protein degradation is likely to be small (Kasperek and Snider, 1989) and non-lysosomal proteases such as calpains are of greater significance in terms of myofibrillar degredation following exercise (Belcastro *et al.,* 1998).

High Ca^{2+} concentration, as mentioned in the previous section, activate calpain, or calcium activated neutral protease (CANP, Croall and Demartino, 1991), a Ca^{2+}-sensitive protesase which has been shown to degrade proteins. Calpains are found throughout the muscle cell (Belcastro *et al.,* 1998) with concentration at the z-disk (Spencer and Tidball, 1992), smaller amounts in the I-Band and a little in the A-band (Goll *et al*., 2003). There are three identified isosymes of calpain, differing in the amount of Ca^{2+} required for activation; µ-calpain requiring low (µM) concentration of Ca^{2+} and m-calpain requiring high (mM) concentration of Ca^{2+} (Belcastro *et al*., 1998), sometimes referred to as 'ubiquitous' or 'typical' calpains (Goll *et al*., 2003). The third calpain, n-calpain (p94, calpain-3) has been identified as being muscle specific (Spencer *et al.,* 1995; Sorimachi *et al*., 1997) and linked to proteolytic activity in the cytosol (Sorimachi *et al.,* 1990). Given that µ-calpain and m-calpain are not activated under normal physiological conditions, suggests that perhaps calpain-3 may have a significant role to play in muscle damage (Branca *et al*., 1999). Furthermore, and of relevance to this study, Murphy *et al*., (2007) found that eccentric exercise is the only physiological circumstance shown to result in the in-vivo actiation of calpain-3, probably resulting from prolonged exposure to Ca^{2+} rather than the lengthening contractions themselves (Murphy and Lamb, 2009).

The role of the calpain system in muscle is in the remodelling of cytoskeletal proteins (Croall and Demartino, 1991; Goll *et al*., 2003). The exact role of calpains is difficult

to study as their response to Ca^{2+} results in almost immediate autolysis (Goll *et al*., 2003). It it thought that damaged skeletal proteins can be disassembled by calpains to prepare for restoration of the cytoskeleton (Mellgren *et al*., 2007) via the involvement of the calcium binding proteins dysferlin and mini-dysferlin $_{C72}$ (Lek *et al*., 2013) and the participation of other proteases in the ubiquitine system (Goll *et al*., 2003; Raastad *et al.,* 2010).

Animal studies investigating muscle damage following eccentric exercise have reported increased calcium, with subsequent increases in calpain activity related to loss of structural proteins (Belcastro *et al*., 1993; Arthur *et al.,* 1994; Raj *et al*., 1998; Zhang *et al.,* 2008; Kanzaki *et al*., 2010; Kanzaki *et al.,* 2014). Furthermore Zhang *et al*., (2008) and Salazar *et al.,* (2010) showed that inhibition of calpain activity minimises cytoskeleton damage after eccentric exercise. Studies with humans have also demonstrated increases in calpain activity following eccentric isokinetic exercise (Feasson *et al*., 2002; Murphy *et al.,* 2007; Raastad *et al.,* 2010) and eccentric stepping (Vissing *et al*., 2008) with a substantial portion of calpain-3, not μ-calpain, activated after such eccentric exericse (Murphy, 2009).

The role of calpain in the regulation of protein breakdown following non-eccentric exercise is not well documented. A number of animal studies have demonstrated an increase in calpain activity following endurance running (Belcastro, 1993; Arthur *et al.,* 1999). However, it has also been shown that calpain-2 increased following 60min of cycling in humans (Harber *et al*., 2010) and thus is relevant in the context of this thesis. The increase in calpain following non-eccentric exercise is likely to be as a result of elevated Ca^{2+} consequential to increased ROS (Powers and Jackson, 2008). Calpain activity is regulated by the binding of Ca^{2+} to specific sites on the calpain molecule, with the Ca^{2+} required for autolysis only slightly higher than that required for proteolytic activity (Belcastro *et al*., 1998). Increased concentration of $[Ca^{2+}]_i$ resulting from muscle damage activate calpain, resulting in degredation of cell proteins. While investigations have found that calpains degrade actin and myosin very slowly, if at all (Goll *et al*., 2003), others have shown that calpain rapidly cleaves talin, vincluin and filamin (Goll *et al.,* 2003), and the intermediate filament proteins such as desmin and dystrophin (Zhang *et al*., 2008). Titin and nebulin (Verburg *et al*., 2005; Zhang *et al*., 2008) are quickly broken down close to the z-disk releasing proteins from the z-disk and resulting in loss of z-disk form. Tropomyosin, troponin I

and troponin T are also rapidly degraded (Goll *et al*., 2002). Calpain is also key in the appearance of neutrophils in the muscle via activation of cytokines (Belcastro *et al*., 1998).

Ubiquitin-proteasome pathway

The vast majority of intracellular proteins are degraded by the ubiquitin-proteasome pathway (Lecker *et al*., 2006), including the degradation of myofibrillar proteins in skeletal muscle (Jagoe and Goldberg, 2001). As the ubiquitin-proteasome degradation system cannot degrade intact myofibrils (Jagoe and Goldberg, 2001), the initial disruption is initiated by calpain activity, as described above.

Following this initial breakdown by calpains, proteins to be degraded are linked by enzymes to the 26S proteasome that degrades the proteins to small peptides, thus resulting in the rapid removal of proteins (Reid, 2005). The process by which ubiquitin (Ub) attaches to a protein is ubiquitination (Taillandier *et al.,* (2004)). The initial step in this degredation process is the activation of Ub by the Ub-activating enzyme E1. Once activated, Ub is transferred to the Ub-conjugating enzyme, E2, a small Ub carrier protein. E3s then anchor Ub to the target protein, in effect creating a scaffold between the E2 and the target protein (Lecker *et al.,* 2006). This process is repeated until a minimum of 4 Ub units are attached to the target protein (Murton *et al*., 2008) which is then a signal for degradation (Taillandier *et al*., 2004). A key E3 in this process is muscle ring finger-1 (MuRF-1). The actual degredation of the target protein is carried out by the 26S proteasome which is a barrel-shaped 20S proteasome comprised of 4 stacked hollow rings, with a 19S regulatory particle at either or both of its ends (Voges *et al*., 2000). The polyubiquitin chain binds to the 19S component and is unfolded which allows its entry to the 20S proteasome central chamber (Benaroudj *et al.,* 2003). It is here that the poypeptide is cleaved into small peptides which are then released by the proteasome. In the cytosol they are quickly digested into amino acids which can be reutilised to synthesise new proteins (Lecker *et al.,* 2006).

Evidence for the activtiy of the Ub-P degradation pathway following eccentric exercise comes from a number of investigations observing increased protein expression of free Ub and components of the 20S proteasome following eccentric exercise, with increased Ub conjugated proteins 48h post-exercise (Thompson and Scordilis, 1994; Stupka *et al.,* 2001; Willoughby *et al.,* 2003a; Willoughby *et al*.,

2003b). These investigations suggest the importance of the Ub-P pathway in the initiation of skeletal muscle myofilament remodelling following exercise (Murton *et al*., 2008). Evidence of activation of the Ub-P pathway following other exercise modes is limited, though Sandri *et al.,* (1995, 1997) reported increased Ub in the muscles of mice after approximately 16h of spontaneous running and increased proteasome activity has been noted during ultraendurance running (Kim *et al.,* 2011; Jamart *et al*., 2012). While evidence from varied exercise modes in humans is lacking, it seems that eccentric exercise produces the largest increases in ubiquitin conjugation to muscle proteins (Reid, 2005).

Lysosomal and PLA2 pathways

Myofibrillar proteins can also be degraded by proteases found in lysosomes (Armstrong, 1990). Filamin C, a protein located at the z-disk has been has been identified as a target of lysosomal proteases (Schiaffino *et al*., 2013). Alternatively, this pathway may be responsible for the degredation of other cellular components such as mitochondria (Lecker *et al*., 2006) or membrane proteins (Tipton *et al.,* 2018). Increased intracellular calcium can activate phospholipases (PLA), including PLA2 which has a calcium binding site (Armstrong, 1990). This is of significance as activation of PLA2, which is located in the sarcolemma, may result in the degredation of membrane phospholipids (Jackson *et al*., 1984), leading to increased sarcolemma permeability (Gissel and Clausen, 2001). This would not only result in the leakage of cell contents into the extracellular environment and circulation, but also allows for the possibility of Ca^{2}+ to enter the cell triggering further degradation (Armstrong, 1990).

Oxidative stress

Oxidative stress is defined as ‘an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signalling and control and/or molecular damage’ (Sies and Jones, 2007). Oxidative stress typically results from increased production of Reactive Oxygen Species (ROS) and Nitric Oxide (NO) which can happen during and after exercise and/or from reduced concentrations of antioxidants such as essential vitamins (Gohil *et al*., 1986). Free radicals are reactive molecules with one or more unpaired electrons in the valence shells (Sen, 2001) and are capable of independent existence (Powers and Jackson, 2008). The main free radicals produced in cells are superoxide ($O_2^{\bullet -}$), generated through incomplete reduction of oxygen or as a product of enzymatic systems and nitric oxide which is

generated by a series of enzymes (Powers and Jackson, 2008), but also peroxide and the hydroxyl radicals, singlet oxygen and hypochlorous acid (Steinbacher and Eckl, 2015). When these atoms have no unpaired electrons in their outer shell, they have a strong attraction for electrons; the attraction is considerably greater when there is an unpaired electron (Buresh and Berg, 2015).

Superoxide is produced by the addition of a single electron to ground state oxygen (Powers *et al.,* 2011). It is a commonly produced radical in mitochondria and because of a relatively long half-life, superoxide has a great number of targets in the cell (Powers and Jackson, 2008). It is the precursor to many other ROS that can result in cellular damage. Dismutation of superoxide by manganese superoxide dismutase (MnSOD) results in its partial neutralisation through the formation of hydrogen peroxide (H_2O_2) (Powers and Jackson, 2008; Buresh and Berg, 2015). Hydrogen peroxide, also produced by a number of enzymes (Powers and Jackson, 2008), does not oxidise lipids or DNA directly as it does not have an unpaired electron, but generates hydroxyl radicals (OH) through the Fenton reaction and thus, is still considered a ROS by some (Buresh and Berg, 2015). The stability of hydrogen peroxide means that it can serve as a signal as to the redox state of the muscle (Buresh and Berg, 2015). Hydroxyl radicals are highly reactive and have a damaging effect on molecules near where they are produced (Powers and Jackson, 2008), initiating lipid peroxidation, stealing a H and forming a carbon centred lipid radical. Nitric oxide, produced from l-arginine (Powers and Jackson, 2008) reacts very quickly with superoxide to produce peroxynitrate, which damages DNA and proteins (Powers and Jackson, 2008).

Lipid peroxidation and protein oxidation

The lipid bilayer of the cell membrane is made up of lipids, including polyunsaturated fatty acids, and protein (Powers and Jackson, 2008). Lipid peroxidation is initiated by hydroxyl radicals with the polyunsaturated fatty acids being a target for the removal of a hydrogen atom as their double bonds are more susceptible for oxidation (Halliwell and Chirico, 1993) which leads to more ROS being produced and further damage, the ‘chain reaction’ typically described in the literature (Urso and Clarkson, 2003). Lipid peroxidation of the membrane means that proteins in the membrane are attacked more easily, which impairs membrane function (Powers and Jackson, 2008). ROS production may also lead to protein oxidation of contractile proteins such

as nebulin, titin, myosin and troponin thus leading to reduced contractile function (Steinbacher and Eckl, 2015). Furthermore, ROS readily react with all types of biological molecules and thus cause damage to many cellular and extracellular constituents, including DNA, proteins and lipids (Brieger *et al*., 2012).

Oxidative stress and exercise

Increases in oxidative stress have been observed following isometric exercise (Alessio *et al*, 1999) sprinting (Marzatico *et al.,* 1997) and endurance exercise (Marzatico *et al.,* 1997; Dufaux *et al.,* 1997; Child *et al.,* 2000), suggesting that increased oxygen consumption is not the sole mechanism for oxidative stress following exercise (Alessio *et al.,* 1999). An increased level of ROS has been linked to decreased muscular force (Reid *et al*., 1993) and to muscular fatigue, perhaps by modifying SR calcium handling, or influencing myofilament structure and function through influencing the calcium sensitivity of myofilaments (Powers *et al.,* 2011). Oxidative stress may also activate the ubiquitin pathway, thus increasing structural damage to contractile proteins. All of these outcomes would be significant for athletes involved in regular training and competition.

Oxidant production in muscle

The level of ROS produced in muscle is dependent on the nature of exercise, i.e. whether the exercise is primarily aerobic or anaerobic, intensity, duration, volume of oxygen consumption and degree of mechanical stress (Fisher-Wellman and Bloomer, 2009).

Mitochondria have historically been considered the main source of ROS in skeletal muscle, with superoxide produced in complexes I (NADH dehydrogenase) and III (coenzyme Q and cytochrome C oxidoreductase) of the electron transport chain (Muller *et al*., 2004) as a result of elevated oxygen consumption during exercise to meet increased energy demands (Vollaard *et al*., 2005). The ‘leak’ in the respiratory chain has been considered to be the most significant source of ROS (Vollaard *et al*., 2005; Sachdev and Davies, 2008). It has been suggested that 2-5% of mitochondrial oxygen consumption undergoes reduction subsequently generating superoxide (Boveris *et al.,* 1972; Urso and Clarkson, 2003; Muller *et al*., 2004), though contrasting research has estimated that less than 0.15% of oxygen consumed actually generates superoxide (St-Pierre *et al*., 2002), suggesting that mitochondria

are not the main source of ROS generation during exercise (Vollaard *et al*., 2005; Jackson *et al*., 2007; Powers *et al.,* 2011).

ROS may also be generated from the transfer of electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to oxygen by NADPH oxidases (NOXs) (Powers *et al.,* 2011), increased phospholipase A2 (PLA2) activity (Powers *et al.,* 2011; Steinbacher and Eckl, 2015), mitochondrial membrane-bound glycerol 3-phosphate dehydrogenase (mGPDH), increased production of xanthine oxidase (Gomez-Cabrera *et al.,* 2010; Vollaard *et al*., 2005; Judge and Dodd, 2004) and neutrophil burst (Powers and Jackson, 2008). It is likely that several mechanisms together may result in the generation of ROS during exercise, or that different forms of exercise involve different mechanisms of generation (Vollaard *et al*., 2005).

Effects of ROS on muscle

When muscle is in an unfatigued state, ROS are essential for the generation of force (Steinbacher and Eckl, 2015). Chronic increases in ROS with moderate exercise intensities play a part in muscular training adaptations. However, a single bout of intense exercise results in oxidative damage which can result in muscle contractile dysfunction and fatigue (Vollard *et al*., 2005, Powers *et al.,* 2011). Reid *et al.,* (1993) proposed a model to explain the effects of redox balance on muscle force production. This model identifies that there is an optimal redox state that produces maximal force production; an increase in ROS or decrease in antioxidants results in a reduced production of force. Research on mice has found that exposure to low concentration of H_2O_2 resulted in a 27% increase in force production, while longer exposure resulted in a decrease in force production (Andrade *et al.,* 1998).

ROS oxidation of the ryanodine receptor (RyR), the Ca^{2+} release channel of the sarcoplasmic reticulum, may lead to muscle fatigue (Cherednichenko *et al.,* 2004). Animal research has suggested that exposure of the sacoplasmic reticulum to ROS may increase (Anzai *et al*., 2000) or decrease (Posterino *et al.,* 2003) calcium release, may reduce calcium reuptake (Powers and Jackson, 2008), but has no effect on excitation-contraction coupling (Posterino *et al.,* 2003). It is also possible that ROS has an effect on the functioning of myofilaments. While mechanisms are as yet unclear, it is possible that ROS may affect troponin I (Steinbacher and Eckl, 2015), troponin C (Kaneko *et al.,* 1992), myosin heavy chain proteins (Coirault *et al*.,

2007), or the S1 fragment of the myosin head (Steinbacher and Eckl, 2015), thus affecting muscle contractility. ROS may also result in protein degradation of titin, nebulin, troponin and myosin, through the activation of MuRF1 (Muscle Really interesting new gene Finger protein 1), which would affect muscle contraction (Steinbacher and Eckl, 2015). Finally, an increase in ROS may inhibit Ca^{2+}-ATPase activity which would lead to a decrease in the removal of Ca^{2+} from the cell. This increase in cellular Ca^{2+} would amplify calpain activity and result in damage to muscle proteins (Kourie, 1998).

Measurement of oxidative stress

Because of the short half-life of ROS (10-5s for superoxide and 10-9s for hydroxyl radical), it is very difficult to measure ROS directly (Powers and Jackson, 2008; Fisher-Wellman and Bloomer, 2009). Indirect assessment of oxidative stress is possible via the measurement of molecular products that are produced as a result of ROS reactions because of their greater stability (Fisher-Wellman and Bloomer, 2009). These include products of lipid peroxidation such as isoprostanes, lipid hydroperoxides, malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), products of protein oxidation including protein carbonyls, individual oxidised amino acids, and oxidised DNA bases. The investigations in this thesis measured protein carbonyls (PC) and lipid hydroperoxides (LOOH) and the glutathione:oxidised glutathione ratio (GSH:GSSG).

Because proteins are a major target for ROS, measurement of PC is a commonly used method for measuring protein oxidation in relation to exercise. PC are an indirect by-product of oxidative stress and are advantageous as a marker of stress due to their early formation in the process and their subsequent stability (Dalle-Donne *et al*., 2003). Protein oxidation can lead to degradation of enzymes and contractile or structural protein damage (Levine and Stadtman, 2001). They have been utilised to determine the degree of post-exercise oxidative stress in a number of investigations (Bowtell *et al*., 2011; Howatson *et al*., 2010). However, it is argued that PC has limited use as a marker due to interference with oxidation products of lipids and carbohydrates (Griffiths, 2000).

Determination of lipid hydroperoxides (LOOH) is considered a suitable measure of lipid peroxidation and quantification is reliably determined using the FOX 1 method

(Ferrous Oxidation of Xylenol orange 1). There is limited concentration of these molecules in cells, even during oxidative stress, making the measurement difficult (Powers and Jackson, 2008).

Many investigations measure antioxidant enzyme activity, including superoxide dismutase (SOD), glutathione peroxidise (GPx) and catalase (CAT) though responses to exercise are very varied (Aguilo *et al*., 2000; Rokitzki *et al.,* 1994) and these measures were thus not selected for the investigations in this thesis. It is also possible to measure alterations of the antioxidant defence system. Changes in circulating concentration of vitamin E and vitamin C can be used as an indicator of antioxidant activity and thus, the level of oxidative stress experienced (Fisher-Wellman and Bloomer, 2009). However, Study 2 in this thesis measured the GSH:GSSG ratio. Glutathione (GSH), is a major non-enzymatic antioxidant, a decrease in which (with a concomitant increase in oxidised glutathione (GSSH)) is an indicator of oxidative stress (Fisher-Wellman and Bloomer, 2009). Changes in GSH, or the GSH:GSSH ratio (decrease) is commonly used as an oxidative stress marker (Urso and Clarkson, 2003), and an indicator of cellular redox balance (Powers and Jackson, 2008).

Overall, a greater picture of the level of oxidative stress can be elucidated with more than one measure (Powers and Jackson, 2008). Even so, it is relevant that plasma measures of these markers are not necessarily fully representative of the state of stress in the muscle cell.

Cytokines and inflammation

Degeneration and regeneration of muscle tissue following exercise involves the immune system (Peake *et al.,* 2005; Tidball, 2005). Animal studies indicate a relationship between eccentric exercise and inflammation (Tomiya *et al.,* 2004; Pizza *et al.,* 2005) though results from human investigations are less conclusive, with many indicating that muscle soreness and other markers of muscle damage can be experienced without any signs of inflammation (Malm *et al.,* 2000; Malm *et al.,* 2004). Furthermore, in human studies, the mode of exercise (resistance exercise, downhill running, maximal eccentric exercise) influences the degree of leucocyte accumulation in the exercised muscle (Paulsen *et al*., 2012), with results further confounded by exercise intensity (Nieman *et al.,* 2012), amount of muscle mass

recruited during exercise (Peake *et al*., 2005) and individual variation (Nieman *et al.,* 2012; Paulsen *et al.,* 2012).

Leukocyte infiltration commences immediately after exercise, with increases observed following both aerobic exercise (Tvede *et al.,* 1989) and resistance exercise (Simonson and Jackson, 2004). The appearance of neutrophils in the muscle is noted within hours post-exercise and they remain for up to 24h (Beaton *et al.,* 2002; Malm *et al*., 2000) as they release agents that aid in the digestion of adjacent dead tissue cells (Smith, 1991). Neutrophils may exacerbate the damage to myofibrils but could also facilitate recovery of muscle tissue (Pizza *et al*., 2005). They are phagocytic, but also may release proteases that can degrade debris that has resulted from muscle damage (Tidball, 2005). However, Pizza *et al.,* (2005) demonstrated the destructive effect of neutrophils in mice following lengthening contractions and concluded that neutrophils exacerbate damage and impair the restoration of muscle structure and function following such exercise. This is thought to either be by direct damage to myofibres or via the release of cytokines which can lead to further damage (Pizza *et al.,* 2005).

Macrophages are typically detected 24-48h post-exercise and may still be present up to 14 days later as they dispose of necrotic tissue (Beaton *et al*., 2002; Fielding *et al.,* 1993; Hamada *et al.,* 2005). As mentioned, numerous investigations refute a relationship between muscle damage and inflammation due to the lack of neutrophils but also macrophage infiltration during the post-exercise period both in rats (Armstrong *et al.,* 1983) and humans (Schwane *et al*., 1983; Bobbert *et al.,* 1986; Friden *et al.,* 1983). Both neutrophils and macrophages may produce pro-inflammatory cytokines (Peake *et al*., 2005). It has been suggested that macrophages play the most crucial role in the repair process (Kharraz *et al.,* 2013), infiltrating the damaged area to phagacytose myofiber debris, and it is possible that macrophages directly promote muscle repair (Tidball, 2005). Whatever the specific role, the influence of macrophages is complex given that they are a source of cytokines and free radicals (Tidball, 2005).

Cytokines are proteins involved in the regulation of immune and inflammatory responses (Kanda *et al.,* 2013). They are termed ‘messenger molecules’ and strenuous exercise stimulates an increase in the concentration of circulating

cytokines (Paulsen *et al.,* 2012; Sugama *et al*., 2012). Tumor Necrosis factor-α (TNF-α), interleukin 1-β (IL-1β) and interleukin-6 (IL-6) are the cytokines most often measured following muscle damage with the production of IL-6 resulting from increased concentration of TNF-α and IL-1β (Miles *et al.,* 2008). IL-6 is produced in larger amounts (up to 100-fold) than any other cytokine in response to exercise (Fischer, 2006). It has been shown to increase following varying forms of exercise (Ostrowski *et al.,* 1999; Peake *et al.,* 2005a; Buford *et al.,* 2009; Philippou *et al*., 2009; Nieman *et al.,* 2003; Philippou *et al*., 2009; Nieman *et al.,* 2012) and has been identified as being involved in muscle damage (Bruunsgaard *et al.,* 1997). IL-6 may be directly involved in increased protein degradation following exercise but it also induces the proliferation of satellite cells resulting in muscle regeneration (Cantini *et al.,* 1995). Tomiya *et al.,* (2004) reported a relationship between IL-6 and muscle damage in mice, detecting IL-6 in the cytoplasm until 12h post-exercise and subsequently in the inflammatory cells and satellite cells. C-reactive protein (CRP), an often-used marker of inflammation, is an inflammatory protein produced in the liver in response to increases in IL-6 and other inflammatory mediators (Edward, 2005) and is an indicator of an acute-phase, systemic inflammatory response (Kerasioti *et al.,* 2013). It is frequently used as a measure of post-exercise inflammation (Dousset *et al.,* 2007; Chatzinikolaou *et al*., 2010) indicating that IL-6 produced at the tissue level is resulting in an acute-phase, systemic inflammatory response (Kerasioti *et al.,* 2013). It is possible that exercise intensity, and not eccentric loading, may be the most influencing factor in relation to increases in cytokines. Indicative of this, Peake *et al.,* (2005b) found greater increases in inflammatory cytokines following high-intensity running compared to downhill running, though the participants in this study were trained runners. Untrained athletes are likely to experience a greater cytokine response to unaccustomed downhill running (Peake *et al.,* 2005b). Nieman *et al.,* (2012) investigated the inflammatory response to 1.75h at 60% $watts_{max}$, followed by a 10km time trial and reported the greatest increases in cytokines in the athletes cycling at the highest intensity over the full protocol, regardless of body composition, fitness or training volume. Malm and Yu (2012) found no indication of inflammation following 45 min of downhill running. Nevertheless, greater increases in IL-6, IL-8, IL-10 and CRP have recently been observed following similar workloads of running compared to cycling suggesting the influence of eccentric loading on the inflammatory response (Nieman *et al.,* 2014). Given the aforementioned disparate results, it is possible that even when increases

in serum cytokines are not observed, they may act at a localised tissue level (Pyne *et al.,* 1984).

Dousset *et al.,* (2007) noted increased CRP following a series of drop jumps in a sitting position on a sledge apparatus, with peak CRP occuring at the same time as peak CK (48h post exercise). In contrast, an earlier peak in CRP (and IL-6 and IL-1 β) was noted following intense plyometric jumping (Chatzinikolaou *et al.,* 2010). To further confound the exercise-cytokine relationship, Bessa *et al.,* (2016) reported a 50% increase in leukocytes (70% increase in circulating neutrophils) 3h following an exercise protocol that involved 6 sets of squats and bench press, followed by 1h of cycling, but found no increase in TNF-α, IL-6 or CRP. The authors suggested that the results observed may be indicative of a localized, rather than systemic inflammatory response. Similar results were reported (31% increase in leukocyte count; 44% increase in circulating neutrophils, with no increases in cytokines) following single-leg calf raises in untrained males (Kanda *et al.,* 2013).

2.4.4 Markers of exercise stress

Direct markers of mechanical stress can be identified using electron microscopy or muscle biopsy techniques and are discussed in the following section. However, for validity and practical reasons many researchers have focused on indirect markers of stress which can reflect both initial structural damage and the secondary phase of damage. These include measures of muscle function, soreness and serum indicators. The inherent difficulty in the utilisation of these markers is the variation in the time-frame of the manifestation of these markers and thus the lack of correlation between them (Clarkson and Hubal, 2002).

Evidence of structural damage

Measures of muscle damage are generally conducted using light or electron microscopy on muscle biopsy samples and most evidence arises from studies of eccentrically biased exercise. The first evidence of structural damage following eccentric exercise was reported by Friden *et al.,* (1981). Muscle biopsies taken from human muscle following repeated running down flights of stairs showed no tissue necrosis but considerable disturbance to the Z-band, including broadening, streaming and in some places, total disruption. Similar morphological changes have been

reported in other studies following electrically stimulated isometric muscle contractions in humans (Mackey *et al.,* 2008) and eccentric exercise in animals and humans, including widening of the Z-lines (Hortobágyi *et al*., 1998), disruption of normal striation (Armstrong *et al.,* 1983; McCully and Faulkner, 1985; Appell *et al.,* 1992; Friden and Lieber, 1998), focal disruptions of the A-band (Ogilvie *et al.,* 1985; Friden and Lieber, 1998) and extensive sarcomere disruption (Newham *et al.,* 1983; Lauritzen *et al.,* 2009), with initial damage evident immediately post-exercise (Friden *et al.,* 1983) and further damage evident 1-3 days after the exercise (Newham *et al.,* 1983; Friden and Lieber, 1992; Clarkson and Sayers, 1999). It has been suggested that the z-disk is the weakest link in myofibrillar contraction (Friden *et al.,* 1981; Friden *et al.,* 1988) thus, localising the damage to this structure and associated proteins during repeated eccentric contractions though loss of actin and myosin heavy chain have also been observed (Ingalls *et al.,* 1998).

Sarcomere disruption may involve the elastic protein, titin (Proske and Morgan, 2001). Damage to the titin lattice of the sarcomere has been observed (Friden and Lieber, 1998; Trappe *et al.,* 2002).Titin plays an essential role in sarcomere stability and maintenance (Kontrogianni-Konstantopoulos *et al.,* 2009) and in the re-interdigitation of sarcomeres post stretch (Lieber *et al*., 2002; Kontrogianni-Konstantopoulos *et al*., 2009). Failure of reinterdigitation following eccentric exercise could be related to titin damage (Morgan and Allen, 1999).

Lieber *et al.,* (1996) reported early loss of the cytoskeleton protein desmin after 30min of eccentric exercise of the rabbit dorsiflexors and showed a linear relationship between desmin loss and force decreases in the muscle. Animal research demonstates that lack of desmin results in degeneration and disorganisation of the cell, with reduced muscle force production and increased fatigue (Lieber *et al.,* 1994; Milner *et al.,* 1996; Paulin and Li, 2004). Similarly, Barash *et al*., (2002) observed a rapid loss of the cytoskeleton protein desmin following repeated eccentric contractions in rats with a subsequent threefold increase in desmin content 168h post exercise suggesting the importance of desmin in the remodelling of the IF system post eccentric exercise. However, a number of investigations, while observing irregularities in the desmin cytoskeleton post eccentric exercise in humans, found no loss of desmin (Yu *et al*., 2002; Yu and Thornell, 2002; Yu *et al.,* 2004; Crameri *et al.,* 2007) concluding that structural damage and protein losses may follow different

patterns in animals and humans (Yu *et al*., 2002), and that desmin alterations reflect remodelling and adaptation following eccentric exercise (Yu and Thornell, 2002). More recently, Malm and Yu (2012) utilised proteomics to examine muscle tissue post-downhill running and reported no tissue necrosis or loss of desmin. Rather, they recorded increased expression of desmin and actin, suggesting remodelling rather than tissue degeneration. However, it is possible that the lack of desmin loss reported was due to the nature of the exercise, which was considerably less extreme than the repeated eccentric exercise of other studies.

Further conflicting results have been reported on the effect of eccentric exercise on the integrity of the sarcolemma. A small number of studies have reported minor damage to the muscle membrane (Stauber *et al*., 1990; Crenshaw *et al.,* 1993). However, many have noted no damage (Malm *et al*., 2000; Yu *et al.,* 2002; Crameri *et al.,* 2007; Costa *et al.,* 2009; Yu *et al.,* 2013), perhaps reflecting the modes of exercise utilised in the damage protocol.

Lovering and De Deyne (2004) noted that the loss of the cytoskeleton protein dystrophin following a single eccentric contraction of the tibialis anterior in rats was far greater than that of desmin. Dystrophin is a large protein primarily located at the z-disk and M-line and is important for the transmission of force (Patel and Lieber, 1997), and thus loss of dystrophin may be critical in the loss of muscle force prodcution after eccentric exercise (Lovering and De Deyne, 2004).

Muscle biopsies require surgical intervention, and are therefore not likely representative of the whole muscle (Kano *et al.,* 2008; Cermak *et al.,* 2012a), may overestimate or underestimate actual damage (Clarkson and Hubal, 2002), and may even induce tissue damage (Malm *et al*., 2000). Therefore, other techniques have been used to determine the amount of structural damage present following eccentric exercise. Numerous investigations found an increase in transverse relaxation time (T2) measured by magnetic resonance imaging (MRI) following eccentric exercise (Takahashi *et al.,* 1994; Sorichter *et al.,* 1997; Foley *et al.,* 1999; Jayaraman *et al.,* 2004; Sesto *et al.,* 2005; Larsen *et al.,* 2007; Kubota *et al.,* 2007; Maeo *et al.,* 2017a, b). Others have reported changes in fibre geometry with diffusion-tensor magnetic resonance imaging (DT-MRI), (Cermak *et al.,* 2012a) and MRI measurements (Sorichter *et al.,*1997; Cermak *et al*., 2012a).

Quantification of structural damage in muscle tissue post-eccentric exercise is difficult due to influencing factors discussed above and limitations associated with the representation of muscle biopsies. Animal studies have reported damage as low as 5% in muscle fibres (Armstrong *et al.,* 1993; Barash *et al.,* 2002) and as high as 54% (Lovering and DeDeyne, 2004), with others finding structural disruption in 25-35% of fibres (McCully and Faulkner, 1986; Lieber *et al.,* 1996; Lovering *et al.,* 2009). Studies of human muscle are also mixed, reporting 8% of fibres with very extensive damage (Newham *et al*., 1983) and 16-32% with damage (Newham *et al.,* 1983; Friden *et al.,* 1983). More recently, the extent of damage has been expressed as disrupted z-bands per fibre (0.075) or disrupted z-bands per area of muscle measured (2.2/mm^2) (Cermak *et al.,* 2012) which is more specific than giving a percentage value.

Excitation-contraction coupling

A significant indication of damage in skeletal muscle is failure or disruption in excitation-contraction coupling. Histological evidence for structural damage described above is associated with deficits in force prodcution. However, such deficits may be due to an inability to activate contractile proteins because of disruption to the excitation-contraction coupling process, which may be the primary event in muscle damage (Warren *et al*., 2001). Initial evidence for this hypothesis came from Warren *et al.,* (1993) when he showed that the decrease in maximal isometric tetanic force post-eccentric exercise in mice could be avoided with caffeine (caffeine releases Ca^{2+} from the SR), suggesting that reduced force production resulted from a failure of E-C coupling at a stage prior to Ca^{2+} release from the SR. Similar results have been observed in other studies, though not when the eccentric load was more severe (Belnave and Allen, 1995) which could suggest that reduced force of muscle following eccentric exercise may be as a result of interference to both the E-C coupling system and mechanical damage, with as much as 75% of the force loss due to E-C failure (Warren *et al*., 2001). It has been suggested that when sarcomeres are over-stretched it can result in direct damage to the SR, or in its connections with the t-tubules, thus resulting in E-C coupling failure (Allen, 2001). Disorder of the t-tubule network, including an increase in longitudinal segments and changes in direction of triads has been observed in animal studies following downhill running (Takekura *et al.,* 2001), with both the SR and t-tubules appearing rounded and grainy post-eccentric loading (Friden and Lieber, 1996). Thus, damage and possible sealing off

of t-tubules, would lead to inactivation of some sarcomeres (Proske and Morgan, 2001). However, it seems more likely that initial sarcomere disruption with subsequent t-tubule damage, rather than the other way around, may be a more likely sequence of events (Proske and Morgan, 2001).

Muscle function

In the context of athletic recovery, the assessment of post-exercise muscle function is of primary relevance and importance. Warren *et al.,* (1999) stated that the measurement of maximal voluntary contraction torque is the best method for quantifying muscle damage as it is accurate and reliable. While other measures are now also commonly used, changes in peak torque are consistently used as indicators of fatigue and recovery (Delecroix *et al.,* 2017; Ives *et al*., 2017). A number of investigations have reported a decrease in maximal isometric voluntary contraction following exercise with high levels of mechanical stress (Howatson and Milak, 2009; Howatson *et al*., 2012; Brown *et al.,* 2015; Marginson *et al*., 2005), with losses in strength dependent on the nature and intensity of the exercise performed (Clarkson and Hubal, 2002) and peak decrements between 24 and 72h (Cleak and Eston, 1992). Decreases in MVIC have also been observed following marathon running (Clifford *et al.,* 2016; Howatson *et al.,* 2009), an activity associated with high levels of metabolic stress, though values had returned to baseline 24-48h post-exercise. Given that exercise rarely involves isolated isometric contraction, other investigators have examined isokinetic peak torque following eccentric exercise, again reporting significant reductions lasting at least 48h (Cockburn *et al.,* 2008, 2010, 2012, 2013). The typically utilised lower-velocity measures are considered more reliable than faster speeds (Greig *et al.,* 2008) though these are significanly lower than the joint velocities reported in actual activity (Baltzopoulos and Gleeson, 2001), perhaps questioning their validity.

Rate of Force Development (RFD) is defined as the slope of the force-time curve obtained under isometric contractions (Aagaard *et al*., 2002) and is considered to be a measure of the ability of the neuromuscular system to generate rapid force at the start of a contraction (Blazevich *et al*., 2008). It has been reported as being a more specific and sensitive marker of eccentrically induced muscle damage than MVC (Peñailillo *et al*., 2015). Determinants of RFD include both neural determinants (motor unit recruitment and discharge rate, muscle activation) and muscle

determinants (muscle size and architecture, muscle fibre type and myofibrillar mechanisms) (Maffiuletti *et al*., 2016). RFD has been found to decrease following eccentric exercise, returning to baseline values within 48h (Molina and Denadai, 2012), though no investigations have examined RFD following exercise with greater metabolic stress.

The dynamic nature of movements in exercise activites has led to the use of jumping and sprinting, both of which involve the stretch shortening cycle, for the monitoring of recovery post-exercise. Jumping occurs in many sporting situations and measures of countermovement jump (CMJ), squat jump (SJ) and drop jump (DJ) have been shown to be impacted by intense exercise (Byrne and Eston, 2002; Marginson *et al.,* 2005; Ormsbee *et al.,* 2015), with SJ being more affected than either CMJ or DJ (Byrne and Eston, 2002). Decreased CMJ performance has also been reported following metabolic stress induced by running (Clifford *et al.*, 2016). Because the measurement of CMJ does not take into account the duration of the eccentric and concentric phase (Cormack *et al.,* 2008), the Reactive Strength Index (RSI) is often used as a measure of neuromuscular performance due to the increased eccentric and SSC demand (Hamilton, 2009). RSI is considered to be a reliable representation of the SSC and the ability to change quickly from an eccentric to a concentric contraction (Beattie and Flanagan, 2015). RSI, determined from DJ, is also negatively affected by both mechanical (Cockburn *et al*., 2012) and metabolic stress (Clifford *et al*., 2016) though the magnitude of decrease is greater following mechanical exercise. It has been reported as the biggest differentiator between fast and slow team sport athletes across a range of tests (Lockie *et al.*, 2011) with fast athletes having shorter ground contact times. RSI also correlates with a players change of direction speed performance (Young *et al.,* 2015). Decreases in RSI across tournament play in youth soccer players have been observed suggesting its value as a tool in monitoring fatigue (Hamilton, 2009). Furthermore, RSI is sensitive to change over a playing season and is more representative of fatigue than countermovement jump alone in Australian Rules players (Cormack *et al.,* 2008). 20m sprint is considered to be an ecologically valid measurement of sprint ability for team sport athletes (Nedelec *et al*., 2012). Few investigations have examined the effect of metabolic stress on sprint perfomance. Following mechanical stress the effect on sprint peformance is somewhat mixed, with some reporting no negative effect (Semark *et al.,* 1999) and others reporting reduced performance (Keane *et al.,*

2015; Highton *et al.,* 2009; Twist and Eston, 2005), with performance remaining below baseline for up to 48h (Twist and Eston, 2005). Variation in recovery time may be due to differences in the exercise protocols.

Muscle soreness

Muscle soreness is probably the most often reported effect following exercise with high mechanical stress, peaking 24-48h post-exercise and decreasing towards baseline in the subsequent 4-6 days (Armstrong, 1990; Tee *et al.,* 2007; Brown *et al.,* 2015). Soreness is also reported following exercise with high metabolic stress (Clifford *et al.,* 2016; Howatson *et al.,* 2009) including non-eccentric exercise such as cycling (Hall et al., 2013; Thomson et al., 2011), though the soreness is usually lower than those following eccentrically biased exercise. The mechanisms responsible for increased soreness and tenderness are not fully understood. It has been hypothesised that soreness is related to inflammatory response as increases in some inflammatory markers (eg IL-6) have been found alongside increases in muscle soreness (MacIntryre *et al.,* 1995), though not all have found this (Malm, 2001). Malm *et al.,* (2004) reported no evidence of inflammation 48h post either uphill or downhill running, despite increased muscle soreness and serum CK. The authors concluded that the soreness experienced is confined to immunological reactions in the muscle epimysium and not in muscle tissue. Any previous investigations reporting this relationship may have been a result of an inflammatory response consequential to the muscle biopsy performed rather than the actual exercise protocol. Extrapolating this further, it has been shown that mechanical hyperalgesia occurs in rat muscle following eccentric exercise without any increases in markers of muscle damage or inflammation (Hayashi *et al*., 2017).

The manifestation of soreness may be exacerbated by increased histamines and bradykinins that sensitise nocicptors and increase the sensation of pain (Malm, 2001). Furthermore, oedema may exert increased osmotic pressure within the fibres further sensitising nociceptors (Clarkson and Hubal, 2002; Malm, 2001). Importantly, muscle soreness does not correlate well with other markers of muscle damage (Warren *et al.,* 1999) including ultrastructural damage (Nurenberg *et al.,* 1992), which means that caution should be exerted in using measures of muscle soreness as a reflection of muscle damage. Still, perceptions of soreness may be reflective of

overall athlete wellbeing and psychological readiness for participation in exercise and thus should form part of the monitoring strategy for athletes.

Serum markers

A number of serum markers are used to assess the degree of muscle damage post-exercise, based on the premise that leakage of these proteins into circulation would not occur if the sarcolemma is not damaged (Lee *et al.,* 2002). This damage may be as a result of the stress placed on the membrane during the exercise bout, or perhaps by lipid peroxidation of the membrane by reactive oxygen species produced during exercise (Powers and Jackson, 2008). The most commonly used serum marker of muscle damage is creatine kinase (CK). Creatine kinase is the enzyme that catalyzes the exchange of high-energy phosphate bonds between phosphocreatine and ADP to form ATP and creatine (Brancaccio *et al*., 2007). The appearance of CK in the blood is generally taken to be an indicator of metabolic and/or mechanical stress within the muscle and the integrity of the cell membrane (Brancaccio *et al.,* 2007). More specifically, serum CK is an indicator of sarcolemma damage, and reflects both relative amounts of CK released and the rate of clearance from the serum (Baird et al., 2012). CK values typically increase within hours of exercise cessation; the slight delayed response in increase is likely due to the size of the CK molecule, which is relatively large and does not easily permeate damaged membranes. Peak CK is usually observed 24-48h following exercise and remain elevated for 72h, or longer, after the initial exercise bout (Mougios, 2007). Post-exercise values can be slightly above normal or be in the tens of thousands $IU.L^{-1}$ (Baird *et al*., 2012), depending on the nature and intensity of the exercise (Baird *et al.,* 2012) and values can remain elevated for several days post-exercise (Russell *et al.,* 2015; Rodrigues *et al*., 2010; Malm *et al.,* 2004). Increased CK has been observed following downhill running (Sorichter *et al.,* 2001), isolated eccentric actions (Cockburn *et al.,* 2008, 2012), sprinting (Keane *et al.,* 2015), and jumping (Harrison and Gaffney, 2004) and also following running (Clifford *et al.,* 2016; Howatson *et al.,* 2010) and cycling (Bell *et al.,* 2014). The increases following the latter, more metabolic exercise, tend to be smaller, returning to baseline within 24-48h. However, it is important to be cognisant of the individual variability of the CK response to exercise (Baird *et al.,* 2012). Genetic factors such as percentage of fast twitch fibres and permeability of the cell membrane may be highly influential (Wiewelhove *et al.,* 2015), contributing to the possibility of responders and non-responders (Brancaccio

et al., 2007). Furthermore, many athletes, because of frequent training, have CK values that are outside normative values which makes it difficult to accurately measure change as a result of exercise (Nedelec *et al.,* 2012). Of importance, is the observation that post-exercise increases in CK do not correspond to post-exercises losses in muscle function (Johnston *et al*., 2016; Doeven *et al*., 2018).

Other serum markers of mechanical stress include myoglobin, which increases following exercise, peaking as late as 72h post exercise (Cockburn *et al.,* 2013). sTnI, a potential marker for myofibrillar damage (Sorichter *et al.,* 1997), has not been utilised as often, probably due to the large post-exercise variability in response. sTnI is a regulatory protein only expressed in striated skeletal muscle (Sorichter *et al.*, 1997). Given its absolute skeletal muscle specificity it is considered a marker of myofibrillar disruption and is a valuable measure during the post-exercise period (Sorichter *et al.,* 1997). sTnI exists in two isoforms, slow (ssTnI) and fast (fsTnI) corresponding to slow- and fast- twitch muscles, respectively (Rebalka and Hawke, 2014). Increases in sTnI have been reported following downhill treadmill running (Sorichter *et al.,* 2001; Mickleborough *et al*., 2015) and eccentric exercise of the elbow flexors (Chapman *et al.,* 2013). Furthermore, sTnI increases are greater with eccentric exercise compared to concentric exercise (Willoughby *et al.,* 2003c).
In conclusion, the most insightful approach to athletic recovery is likely to provide a range of markers reflecting all aspects of the recovery process, including biochemical measures, measures of muscle function, and perceptual measures of recovery.

2.5 Mixed Mechanical and Metabolic Stress – Focus on Team Sport

Previous sections have focused on the processes involved in mechanical and metabolic exercise. However, a key aspect of this thesis is team sport, and all participants were drawn from team sport populations. Multi-sprint sports such as Gaelic football, camogie, rugby, hockey and soccer are prolonged in nature, are characterised by periods of high-intensity work interspersed with periods of low-intensity effort or rest and include numerous accelerations, decelerations and jumping. They are known to result in substrate depletion, muscle damage, oxidative stress, inflammation and fatigue (Ascensao *et al.,* 2008; Magalhaes, 2010), thus, inducing both metabolic and mechanical stress. The purpose of this section is to review the demands of team sports to give context to the nature and specificity of

recovery and post-exercise nutritional intervention, with reference to female athletes where possible. The majority of participants in the investigations of this thesis were drawn from the sports of Gaelic football and camogie, with smaller numbers from soccer and rugby union. The vast amount of research in team sport originates from investigation in soccer which provides a foundation for our understanding of the demands of other team sports.

2.5.1 Team sports

Despite their popularity and playing numbers there is a dearth of research into Gaelic games. The physiological demands of camogie have never been investigated, though may be similar to hurling. Reilly (2000) considered the demands of hurling to be comparable to those of Gaelic football, though the greater distance the ball can be struck results in play changing more quickly from end to end. Analysis of hurling indicates that the ball is in play 28-32min of a 70min match (Young, 2003; Rochford, 2004) with 65% of players' time spent walking, jogging and 15% running and sprinting. Specific intense activity bouts last 5-82s in hurling (Young, 2003) which is shorter than the 2-136s in Gaelic football (Hennessy, 2006), probably due to the speed in which the ball is released in hurling. In hurling, 70% of accelerations are under 10steps (<15m) (Young, 2002). Data on female Gaelic games is sparse, though McErlean ***et al's*** (1998) study of ladies Gaelic football suggests that female players spend more time standing still and less time in high-intensity running than males. With advances in time-motion methods, a number of investigations have recently been conducted to explore the running patterns in men's Gaelic football. Distance covered is reported as 8160-9222m (Malone *et al*., 2016a, 2016b; Malone *et al*., 2017a), with 1731m of high-intensity effort and 445-524m of sprinting (Malone *et al.,* 2016a; Ryan *et al.,* 2017). This is slightly lower than soccer (Bangso *et al.,* 2006) and Australian Rules football (Burgess *et al.,* 2006) but is similar to hockey, (Gabbett *et al.,* 2010; Sunderland and Edwards, 2017). It is worth noting that both soccer and Australian Rules football matches are longer in duration. Similar to other field sports, midfield players cover significantly more than players in other positions (Malone *et al.,* 2016a; 2016b; 2017b). As with other sports, a reduction in distance covered is noted from first to second half (Malone *et al.,* 2016a; Malone *et al.,* 2017b). Of relevance, given the eccentric load associated with deceleration and the effect that subsequent muscle damage may have on recovery, is the 166

accelerations reported in elite Gaelic football match play, totalling 267m of acceleration distance (Ryan *et al.,* 2017). This is considerably less than that reported in soccer, although this is a result of different categorisation of acceleration speeds. While no data is presented on deceleration, it is likely to be similar. In a comprehensive investigation, Russell *et al.,* (2016) reported a total of 656 accelerations and 612 decelerations during soccer match play, with a significant decrease in both in the final 15min of play. There is a lack of comparable data in female players, but Mara *et al*., (2016) noted a total of 423 accelerations and 430 decelerations per match in 12 elite players across seven competitive matches. Analysis of mean heart-rates and activity patterns in Gaelic football (Reilly & Keane, 2002) suggests a predominance of aerobic metabolism during performance, a metabolic loading that is similar to other field games. Of relevance to this investigation, in particular study 4, is the effect that muscle damage may have on glycogen storage and thus, subsequent exercise performance which is glycogen-dependent. To this end, Asp et al., (1998) noted reduced glycogen content (~402 mmol/kg) in damaged muscle compared to control tissue (~515 mmol/kg) with a predominant effect on fast-twitch muscle fibres.

2.5.2 Recovery from team sports

Many researchers have examined the effect of team sport participation on markers of fatigue and muscle damage, reporting that symptoms and/or markers of muscle damage and fatigue may persist for up to 72h post-exercise (Andersson *et al.,* 2008; Ispirlidis *et al.,* 2008; Ascensão *et al.,* 2008; McLellan *et al.,* 2011). Recovery is considered to be complete when the athlete is able to reach or exceed their benchmark performance in a particular activity (Bishop *et al*., 2008). The persistence of fatigue and markers of muscle damage is not surprising given the high aerobic and anaerobic demand of these team sports and the repeated eccentric loading during sprinting, jumping, accelerating, decelerating and changing direction (Nedelec *et al*., 2012). Correlations between high speed running, accelerations and decelerations have been reported in soccer (de Hoya *et al.,* 2016) and in Australian Rules (Young *et al.,* 2012). Contributing to muscle damage are the contact events that occur throughout phases of play in team sports, including tackling, contact with the ball or bat/stick, and through contact with the playing surface (Smart *et al.,* 2008). This Impact-Induced Muscle damage (IIMD) has only recently been investigated in animal

studies and the magnitude and time-course is poorly understood (Naughton *et al.,* 2017). With a different mechanism of injury when compared to EIMD, leading to a disruption of capillary networks and intramuscular bleeding (Elmer *et al.,* 2012), IIMD is likely to result in greater secondary damage and subsequent inflammation. In addition, little is know on on how best to manage IIMD in the recovery period (Naughton *et al.,* 2017).

A summary of the responses to participation in team sport can be seen in Table 2.1. While researchers have examined the impact of competitive matches in American Football (Kraemer *et al*., 2009), basketball (Montgomery *et al.,* 2008; Pliauga *et al.,* 2015; Gentle *et al*., 2014) and handball (Marin *et al*., 2011) on markers of muscle damage, only sports relevant to the current investigations are included in this section. Furthermore, a number of investigations have only looked at immediate post-exercise measurement of the response to match play (Thorlund *et al.,* 2009; Oliver *et al*., 2008; Greig *et al.,* 2008) and these are not included in the table below.

A number of investigations have reported decreases in peak torque measurements (up to 36%) following soccer match play (Rampinini *et al.,* Ascensão *et al*., 2008, Magalhães *et al.,* 2010; Andersson *et al.,* 2008), regardless of the mode of movement (concentric or eccentric) or speed of exercise. Sprint performance is typically reduced by approximately 2-9% immediately following match play though the recovery of sprint performance varies significantly from 5h (Andersson *et al.,* 2008) to 96h (Ispirlidis *et al.,* 2008). Decreases in countermovement jump performance are even greater (up to 12%, Magalhães *et al.,* 2010) and again can take more than 72h to return to baseline values (Andersson *et al.,* 2008; Magalhães *et al*., 2010). In a recent thorough investigation, recovery following a soccer match was investigated (Brownstein *et al.,* 2017). Reductions in maximum voluntary contraction (-14%), CMJ (-5%), 20m sprint performance (-4%) and RSI (-17%) were observed from pre- to post- match with return to baseline achieved 48-72h post-match. Of interest, is the additional measures of neuromuscular function including voluntary activation (VA) and muscle contraction function (potentiated twitch force) which were substantially reduced following the match and confirms CNS impairments which also take days to return to normal (Brownstein *et al.,* 2017). Peak CK from rugby investigations is typically higher than from other sports, suggesting that the

amount of physical contact may influence the degree of muscle damage as indicated by an increase in circulating CK (Gill *et al.,* 2006).

Table 2. 1 Effect of match play on recovery measures

Reference	Participants	Sport	Markers of Recovery
Rampinini *et al*., (2011)	N=20 M; professional	Soccer	↓ MVC, Sprint perf ↑DOMS
Ascensão *et al.* (2008)	N=16 M; semi-elite	Soccer	↑Mb, CK, DOMS ↓isokinetic strength, 30m sprint
Cunniffe *et al.,* (2010)	N=10 M; elite	Rugby Union	↑CK, IL-6, CRP
McLellan *et al.,* (2010)	N= 17 M; elite	Rugby League	↑CK; ↓PP during CMJ
Rowsell *et al.,* (2009)	N=20 M; junior	Soccer – successive matches	↑CK, LDH, Mb ↓12x20m sprint speed, CMJ
Smart *et al.,* (2008)	N=23 M; elite	Rugby Union	↑CK
Fatouros *et al.,* (2010)	N=30 M; semi-elite	Soccer	↑CK, DOMS ↑TBARS, PC ↓GSH/GSSG ↓20m sprint, CMJ
Takarada (2003)	N=15 M; amateur	Rugby Union	↑CK, LDH, Mb
Magalhães *et al.,* (2010)	N=16 M; trained	Soccer	↑CK, Mb ↓Isok strength, 20m sprint, CMJ
Ispirlidis *et al.,* (2008)	N=14 M; elite	Soccer	↓ sprint performance CK, LDH, DOMS ↑TBARS, PC ↓GSH/GSSG
Thorpe and Sunderland (2012)	N=7 M; semi-professional	Soccer	↑CK, Mb
Gravina *et al.,* (2011)	N=28 F; elite and subelite	Soccer	↑CK, LDH ↑TAS
Andersson *et al.,* (2008)	N=17 F; elite	Soccer	↑CK, soreness ↓ Sprint, CMJ, Peak Tq flexion and extension
Hoffmann et al., (2003)	N=19 F; subelite	Soccer	↓ PP during CMJ, PP during SJ, PF during CMJ
Gill *et al.,* (2006)	N=23 M; elite	Rugby Union	↑CK
Cormack *et al*., (2008)	N=22M; elite	Australian Rules	↓CMJ
de Hoyha et al., (2016)	N=15; elite youths	Soccer	↓CMJ, ↑CK
Brownstein et al., (2017)	N= 16M, semi-professional	Soccer	↓ MVC, ↓20m sprint, ↓CMJ, ↓RSI, ↓VA, ↓$Q_{tw,\ pot}$

MVC Maximum Voluntary Contraction, DOMS Delayed Onset Muscle Soreness, Mb Myoglobin, CK Creatine Kinase, IL-6 Interleukin 6, CRP C-reactive protein, CMJ Countermovement jump, LDH Lactate Dehydrogenase, TBARS Thiobarbituric Acid Reactive Species, PC Protein Carbonyls, TAS Total Antioxidant Status, PP Peak Power, SJ Squat jump, RSI Reactive Strength Index, VA voluntary activation, Qtw, pot Potentiated quadriceps twitch force

A direct comparison of all of the above investigations is difficult given the varying methods employed to measure muscle function and different time points for measurement during the recovery period. Also significant is the use of actual match performance as the exercise protocol, given variations, not only in the sports listed, but playing conditions, environmental conditions and the uncontrollable performance of the opposition. One observation is that the vast majority of these investigations were conducted with male athlete participants. However, Gravina *et al.,* (2011) explored the effects of soccer match play on markers of muscle damage and oxidative stress over an 18h recovery period in female soccer players. Similar to results obtained from studies utilising male soccer players, they reported increased CK (332 ± 302 U/l), LDH (342 ± 43 U/l) and Total Antioxidant Status (TAS) (0.81 ± 0.1 mmol/l^{-1}) though no measurements of muscle function were considered. In addition to increased CK (+152%), Andersson *et al*., (2008) reported a decrease in sprint performance (-3%), CMJ (-4%), peak torque for knee extension (-7.1%) and knee flexion (-9.4) following participation in a soccer match with female athletes, slightly lower losses in muscle function than those observed in male players (Ascencão *et al.,* 2008; Fatouros *et al*., 2010; Magalhães *et al.,* 2010).

It is clear from the reviewed investigations that participation in intermittent team sports challenges both the anaerobic and aerobic systems, with increased HR, oxygen consumption and blood lactate concentrations. Furthermore, significant oxidative stress, inflammation and muscle damage is experienced following both acute and simulated match play, arising from the eccentric component of activities in team sports. Additionally, the number of sprints completed in a match correlates with CK (Thorpe and Sunderland, 2012) and the number of impacts sustained in a match may also contribute to muscle damage (Gravina *et al.,* 2011).

2.6 Sex Differences and the Role of Oestrogen in Response to Exercise Stress

In investigating the effect that milk may have on recovery from exercise in females, it is relevant to consider sex differences both in the response to exercise and in the repair process. It is suggested that oestrogen may have a protective effect on skeletal muscle in terms of damage and inflammation, thus resulting in lower magnitudes of muscle damage among females. Oestrogens are corticosteroid

molecules that are secreted primarily by the ovaries in females, but also by the adrenals. In males, small amounts of oestrogens are produced in the testes and the adrenals (Bunt, 1990). Three similarly structured oestrogens are found in the human body: estradiol-17ß (E2), estrone (E1) and estriol (E3), of which E2 is the primary and most abundant form (Kendall and Eston, 2002). Oestrogen has been found to affect inflammation, damage and repair in skin, neural and hepatic tissue (Enns and Tiidus, 2010). The mechanisms by which oestrogen may offer protection to skeletal muscle during exercise are largely unknown but a number of possibilities have been suggested and it is likely that there may be interaction between these factors (Kendall and Eston, 2002).

Tiidus (1995, 2003) proposed that oestrogen may act as an antioxidant against lipid peroxidation of the cell membrane during exercise. Oestrogens possess a hydroxyl group on their A (phenolic) ring in a similar structure to tocopherol (Vitamin E) which is a known antioxidant (Ayres *et al.,* 1998; Persky *et al*., 2000). Acting in a similar way to tocopherol, oestrogen may donate hydrogen atoms leading to a termination of peroxidation chain reactions (Tiidus, 1995; Ayres *et al*., 1998; Persky *et al.,* 2000). Studies involving female rats show that exercise tocopherol concentration is maintained, unlike in their male counterpart and immature female rats, suggesting that oestrogen may provide antioxidant defences while sparing tocopherol (Tiidus, 1995).

Consequently, it is possible that oestrogen may enhance cell membrane stability and decrease membrane fluidity (Wiseman and Quinn, 1994), thereby protecting the membranes from damage (Kendall and Eston, 2002) by interacting with membrane phospholipids in a similar way to tocopherol and cholesterol (Tiidus, 1995; Persky *et al*., 2000). Oestrogen may intercalate into the bilayer of the cell plasma membrane which could result in an alteration of the fluidity and function of the membrane (Kendall and Eston, 2002).

Oestrogen could play a role in gene regulation. Because of its antioxidant properties (Tiidus, 1995; Persky *et al.,* 2000) oestrogen may perhaps have an effect on the expression of adhesion molecules and thus play a protective role in the progression of inflammation and a decrease in neutrophil infiltration (Kendall and Eston, 2002).

Muscle Heat Shock Protein (HSP) expression may be increased by oestrogen. HSPs are involved in protein assembly and maintenance of protein structure when challenged by stressors, including exercise (Milne and Noble, 2008). Exercise, heat or trauma result in a rapid release of HSP72 which functions to attenuate further protein disruption. An attenuation of HSP72 by oestrogen has been observed which would in theory minimise muscle damage (Bombardier *et al*., 2009).

Finally, oestrogen may reduce secondary muscle damage by increasing nitric oxide synthase (NOS) (Tiidus, 2011). This would lead to increased concentration of nitric oxide (NO) which may influence calcium concentration in the cell by stabilising calcium channels in the sarcolemma and affecting calcium uptake by sarcoplasmic reticular pumps (Murphy and Steenburgen, 2007). A stabilisation of calcium concentration may reduce activation of lysosomal and non-lysosomal enzymes with minimized leukocyte infiltration (Tiidus, 2011). However, support for this mechanism, including an attenuation of calpain, comes from animal studies (Tiidus *et al.,* 2001; Stupka and Tiidus, 2001; Enns and Tiidus, 2008; Enns *et al*., 2008) and it is difficult to apply to human protocols. The following sections will examine sex differences in muscle protein synthesis, and direct and indirect markers of exercise stress.

2.6.1 Muscle protein synthesis

Though oestrogen has been proposed to influence recovery from muscle damage, there are no differences in muscle protein synthesis rates in males and females. This lack of sex difference has been observed in young and middle-aged males and females in a rested state (Volpi *et al*., 1998; Fujita *et al.,* 2006; Smith *et al*., 2009), following aerobic exercise (Short *et al.,* 2004) and resistance exercise (West *et al.,* 2012). Furthermore, no differences in myofibrillar or connective tissue protein synthesis have been found across the menstrual cycle phases following kicking exercise (Miller *et al*., 2006).

2.6.2 Direct measures of exercise stress

Results from numerous animal studies suggest that oestrogen may attenuate some of the effects of muscle damage (Amelink and Bar, 1986; Bar *et al*., 1988; Komulainen *et al.,* 1999; Stupka and Tiidus, 2001) but studies from humans are far

more inconclusive. Komulainen *et al.,* (1999) demonstrated that female rats showed less structural damage than male rats following 96h of downhill running. The male rats had higher concentration of ß-glucuronidase (a marker of muscle damage), and also greater losses of desmin and dystrophin than female rats. Similar results have been found when ovarisectomised rats compared to ovariesectomised rats supplemented with oestrogen (Enns and Tiidus, 2008; Enns *et al.,* 2008), suggesting that early muscle damage following eccentrically biased exercise is attenuated by oestrogen, perhaps through its ability to stabilise the muscle membrane (Enns and Tiidus, 2008; Whiting *et al.,* 2000). On the contrary, Van der Meulen *et al.,* (1991) found no sex differences in muscle damage of rats on markers such as multiple central nuclei, multiple vacuoles and hyaline aspect. Fewer human studies have examined sex differences in direct measures of muscle damage. Though no male-female comparison was performed, Staron *et al.,* (1992) reported that muscle biopsies of both males and females taken during eight weeks of high intensity resistance training, showed evidence of atrophic and degenerating fibres with z-line streaming. Similar z-disk disruption was observed by Stupka *et al*., (2000) following unilateral eccentric leg exercise, with no difference between males and females.

2.6.3 Indirect measures of exercise stress

Serum proteins

The appearance of creatine kinase (CK) in the blood is commonly used as an indicator of muscle membrane disruption, though its interindividual variability has led to criticisms of the use of this marker (Warren *et al.,* 1999). Animal studies have consistently demonstrated lower CK in females compared to males following exercise designed to induce muscle injury (Amelink and Bar, 1986; Bar *et al.,* 1988; Amelink *et al.,* 1990). These differences have been ascribed to oestrogen concentration, highlighted by studies that compared ovariectomised female rats with male rats and ovariectomised female rats supplemented with oestrogen. The rats supplemented with oestrogen consistently showed lower CK than ovariectomised rats (Feng *et al.,* 2004; Bar *et al.,* 1988; Amelink *et al*., 1990; Tiidus *et al.,* 2001). Lower CK has been observed in human females compared to males at rest (Meltzer *et al.,* 1971; Miles *et al.,* 1994), following cycling (Shumate *et al*., 1979), distance running (Berg and Keul, 1981; Rogers *et al.,* 1985), downhill running (Webber *et al.,* 1989) and eccentric exercise (Stupka *et al*., 2000; Hicks *et al.,* 2016) though not all

studies show this (Miles *et al.,* 1994; Sorichter *et al.,* 2001). Lower observed concentration of CK in females may be due to faster CK clearance rates (Hyatt and Clarkson, 1998; Warren *et al*., 2006) though Amelink et al., (1990) observed an in-vitro direct inverse relationship between oestrogen supplementation and CK release in animals, suggesting that lower circulating CK in females may be due to less muscle membrane damage (Amelink *et al.,* 1986; Bar *et al.,* 1988).

In trying to establish whether oestrogen has a protective effect following eccentric exercise, some investigations have examined the responses of menarchial females with post-menopausal females but results are mixed. Buckley-Bleiler *et al*., (1989) found no differences in CK of subjects while Arnett *et al*., (2000) unexpectedly found highest CK in menarchial females compared to post-menopausal and pre-menarchial females following unaccustomed eccentric hamstring exercise, leading the authors to conclude that oestrogen had no significant effect on CK after strenuous eccentric exercise. Hormone replacement therapy (HRT), resulting in increased oestrogen concentration, has been found to have no protective effect on skeletal muscle following intense exercise (Dobridge and Hackney, 2004). The diverse results from these investigations may be due to a lack of control of pre-experimental activity and the protocols used to induce muscle damage.

Muscle function

Males and females exhibit similar isometric and eccentric fatigue, work done and rates of torque development and relaxation during eccentric exercise (Hubal *et al*., 2008). Surprisingly few studies examining sex differences following exercise stress have measured aspects of muscle function. While a small number of investigations have reported greater losses of force production in females (Sayers and Clarkson, 2001; Sewright *et al.,* 2008), most have found no differences in relative force loss for males and females following eccentric exercise (MacIntyre *et al.,* 2000; Borsa and Sauers, 2000, Rinard *et al.,* 2000; Sayers and Clarkson, 2001; Kerksick *et al.,* 2008; Hubal *et al*., 2008, 2009; Power *et al.,* 2010) though different patterns of loss have been observed with females showing greater loss at 24h post compared to men (MacIntyre *et al.,* 2000) and a higher incidence of extreme strength loss (>70%) in females with faster recovery than males (Sayers and Clarkson, 2001), perhaps due to an attenuation of the inflammatory response (Sayers and Clarkson, 2001). On the contrary, other studies have reported faster recovery in males, though with greater

deficits for peak rate of force production (Borsa and Sauers, 2000). Recently, Hicks et al. (2016) found no sex differences in recovery of muscle torque losses following eccentric exercise.

Examining muscle function in the early follicular and mid luteal phases in female soccer players, Julian *et al.* (2017) reported a reduction in endurance performance in the latter phase, but noted no difference in sprint performance and countermovement jump height, suggesting that some performance variables may be affected by menstrual cycle phase, whicle others may not. Recent research has shown that recovery of muscle strength following exercise-induced muscle damage is influenced by the stage of the menstrual cycle during which the damage occurred (Sipavičienė *et al.,* 2013), with recovery over 72h faster in the ovulatory phase compared to the follicular phase, which may have affected sex comparisons in other studies. The same authors, however, noted no differences in CK or muscle soreness over the same 72h. Nevertheless, Markofski and Braun (2014), while concluding that menstrual cycle phase has little impact on recovery from eccentric exercise, found that recovery of muscle strength was faster in the follicular phase compared to the luteal phase, suggesting that the oestrogen concentration in the days following exercise (increasing in the follicular group, decreasing in the luteal group) may play a more crucial role than oestrogen concentration at the time of the bout.

Muscle soreness

Reviews of investigations comparing pain and soreness in males and females are conflicting (Le Resche, 2011; Racine *et al*., 2012). Oestrogen may have an effect on the perception of pain and level of muscle soreness experienced following damaging exercise (Kendall and Eston, 2002). Some studies have focused on oestrogen concentration throughout the menstrual cycle with low oestrogen associated with a high pain threshold (Riley *et al.,* 1999), or comparisons between females with and without oral contraceptive use showing high oestrogen with less soreness (Kendall and Eston, 2002) though similar studies show no difference in soreness with a variation in oestrogen concentration (Buckley-Bleiler *et al.,* 1989; Carter *et al.,* 2001).

There are conflicting results from studies that have compared soreness in males and females. MacIntyre *et al.,* (2000) reported different patterns of soreness in males and females with females reporting a progressive increase in soreness following eccentric

exercise of the quadriceps, compared to a rapid increase and plateau in males over the same timeframe. However, this study examined soreness over a 24h period only. Following a similar protocol, Kerksick *et al.,* (2008) stated that males reported higher muscle soreness at 24h, 48h and 72h after exercise. Dannecker *et al.,* (2012) also reported higher soreness in males following eccentric contractions of the elbow extensors. Other investigations have reported no sex differences in muscle soreness following eccentric exercise (Rinard *et al.,* 2000; Nie *et al.,* 2005; Dannecker *et al.,* 2005, 2008; Sewright *et al*., 2008; Hicks *et al.,* 2016).

While there is no clearly identified mechanism for oestrogen's effect on perception of pain, it is possible that oestrogen may play a role in the binding of opioids to receptors in the brain and spinal cord, thereby modifying the transmission of pain (Kendall and Eston, 2002).

Oxidative stress

It has been found that oestrogen can limit lipid peroxidation of the cell membrane (Sugioka *et al.,* 1987; Persky *et al.,* 2000), and some animal investigations have demonstrated this effect after muscle damaging exercise (Stupka *et al.,* 2001; Feng *et al*., 2004), though not all studies have found this (Tiidus *et al.,* 1995). There are few human studies that have examined sex differences in the oxidative stress response to damaging exercise. Previous research found that F_2-isoprostanes were elevated in both males and females immediately following a 50km ultramarathon (Mastaloudis *et al*., 2004). Values for males fell slightly after two hours but remained elevated above placebo for six days post-race. In contrast, the values for females returned to pre-race concentration by 2h post-race, and did not differ from the placebo group for the remaining 6 days of the investigation (Mastaloudis *et al.,* 2004). A lower level of oxidative stress in females is possibly due to a higher metabolic rate in males because of larger muscle mass, thus leading to increased mitochondrial flux and greater ROS production, or perhaps the antioxidant properties of oestrogen (Ide *et al.,* 2002).

Heat shock proteins and calpains

Oestrogen may diminish increases in HSPs through its antioxidant membrane stabilising properties (Enns and Tiidus, 2010). Animal studies comparing female and oestrogen supplemented ovariectomised female rats have shown attenuated HSP

concentration after eccentric exercise. To the author's knowledge, there are no studies that have examined this response in humans.

As it is postulated that oestrogen has membrane stabilising properties, it is possible that it may limit membrane disruption and calcium increase, thus subsequently limiting calpain activation and subsequent damage (Enns and Tiidus, 2010). There are no studies that have examined sex differences in this relationship.

Inflammatory response

Numerous studies have shown that female rats, male rats supplemented with oestrogen, and ovariectomised rats supplemented with oestrogen have decreased neutrophil infiltration (Tiidus and Bombardier, 1999; Stupka *et al.,* 2001; Enns *et al.,* 2008; Tiidus *et al.,* 2005) and that female mice (St Pierre Schnieider *et al*., 1999) and oestrogen supplemented rats (Enns *et al.,* 2008) show attenuated macrophage infiltration following eccentric exercise. Studies on humans have been less conclusive. Stupka *et al*., (2000) found lower leukocyte concentration in females compared to males following eccentric leg exercise despite the same degree of z-disk streaming, though MacIntyre *et al.,* (2000) found females showed a greater presence of neutrophils in the muscle than males. These differences may be explained by differences in subjects or protocols, with MacIntyre's subjects completing a greater volume of eccentric work.

It is possible that reduced leukocyte infiltration may originate from oestrogen's non-receptor mediated effect on membrane stability (Enns and Tiidus, 2010; Kendall and Eston, 2002), attenuating calcium increase and thereby reducing the activation of calpain which would decrease proteolysis and neutrophil chemotaxis (Tiidus *et al.,* 2001; Enns and Tiidus, 2010). The reduced leukocyte infiltration in female animals may be because oestrogen averts permeation by reducing the availability of endothelial adhesion molecules that are required for leukocyte binding (St Pierre Schneider *et al.,* 1999). Oestrogen increases nitric oxide synthase which can reduce leukocyte adhesion to damaged tissue (Enns and Tiidus, 2010).

Of relevance, a number of investigations have reported sex differences in leucocyte infiltration into muscle tissue, with greater neutrophil (MacIntyre *et al.,* 2000; Stupka *et al.,* 2001) and macrophage (Stupka *et al.,* 2001) infiltration observed in females

during the post-exercise period, perhaps due to sex differences in muscle membrane permeability following exercise (Peake *et al.,* 2005a), though not all studies have found this (Stupka *et al.,* 2000). The disparity may reflect different assessment techniques though sex differences in muscle membrane permeability may also influence leukocyte infiltration (Peake *et al.,* 2005c).

The previous sections have focused on the processes involved in exercise stress responses and how these responses can be measured. WhileThe following sections will examine the impact of post-exercise nutrition on these processes and measures, culminating with a review of the research that is specific to milk and the recovery process.

2.7 Exercise and Nutrition and Recovery

The impact of exercise and nutrition on protein balance and muscle protein synthesis was reviewed in Section 2.3.2 and 2.3.3. The following sections examine the effect of protein and protein-carbohydrate intake on recovery from both metabolic and mechanical stress.

2.7.1 Protein and recovery from metabolic exercise

Metabolic exercise is characterised by increases in oxygen consumption over long-duration periods, but exercise incorporating shorter-duration and higher intensities are also dependent on the aerobic energy system (Bogdanis *et al.,* 1985) and thus, result in metabolic stress. Such activities, including team sports, depend primarily on carbohydrate as an energy source (vanLoon *et al.,* 2001). The exercise protocols undertaken by participants in study 2 and study 4 of this thesis involved aerobic exercise and therefore, consideration of the role of protein in the recovery from this type of exercise is relevant to the overall discussion. The overall purpose of including protein in post-endurance exercise is to i) increase the rate of muscle glycogen repletion following glycogen depleting exercise which should enhance performance in subsequent exercise sessions; ii) reduce rates of protein degradation post-exercise to reduce muscle soreness and attenuate losses in muscle power; iii) enhance muscle synthesis, leading to enhanced muscle function and strength (McLellan *et al.,* 2014). For the purpose of specificity, this section will focus on protein and

carbohydrate-protein consumption in the immediate post-exercise period and on the effects on the attenuation of losses in muscle function and reducing muscle soreness.

A number of studies have examined the effect of carbohydrate-protein intake on recovery from endurance cycling, subsequent performance and markers of muscle damage (Saunders *et al.,* 2004, 2007; Hall *et al.,* 2013). Saunders *et al.,* (2004) reported an 83% reduction in CK and a 40% increase in subsequent time to exhaustion when a carbohydrate-protein beverage was consumed during and after an initial bout of cycling to exhaustion, though the format of both cycling bouts are not representative of typical competitive structures. Baba et al., (2014) demonstrated that the consumption of whey protein before, during and after 60min of running resulted in a suppression of the elevation of creatine kinase and myoglobin. Similar results have been observed with female cyclists (Saunders *et al.,* 2007). It is relevant to note that the beverage (Saunders *et al.,* 2004) and the gel (Saunders *et al.,* 2007) consumed in these studies were carbohydrate-matched, and not energy-matched, thus there was a 25% lower caloric intake in the participants in the carbohydrate-only condition. Valentine *et al.,* (2008) compared energy matched carbohydrate and carbohydrate-protein beverages and reported an attenuation of CK and Mb increase, and greater leg extension performance 24h post-exercise, although no other performance measures were included. Thomson *et al.,* (2011) examined the effect of leucine-protein supplemented feeding on repeat sprint cycling performance. Trained cyclists completed 3 consecutive days of high-intensity interval training followed immediately by consumption of the protein supplement. A decrease in CK was observed following the ingestion of the protein supplement compared to a control. It is clear from the aforementioned investigations that the consumption of protein following exercise typically associated with increased metabolic stress, results in an attenuation of markers of stress during the recovery period.

2.7.2 Protein and recovery from mechanical stress - resistance exercise

The following sections will review the literature that has investigated the effects of post-exercise protein and carbohydrate intake on recovery from exercise with a high mechanical load - resistance exercise and isolated eccentric exercise.

Amount of protein intake

Witard *et al.,* (2009) noted a 20% increase in post-resistance exercise muscle protein synthesis when exercise was completed in a fed state. As discussed in Section 2.3.3, the post-exercise consumption of amino acids increases muscle protein synthesis (Moore *et al.,* 2009; Børsheim *et al.,* 2004; Tipton et al., 1999) and attenuates the rise in muscle protein breakdown (Biolo *et al.,* 1997; MacLean *et al.,* 1994). This could lead to an enhanced recovery from resistance-type exercise. The amount of protein required to stimulate an increase in muscle protein synthesis has been investigated thoroughly and the summative conclusions from these studies has indicated that muscle protein synthesis increases in a dose-dependent manner (Tipton *et al.,* 1999; Børsheim *et al.,* 2002; Miller *et al.,* 2003, *Moore et al.,* 2009). In a key study, Moore *et al.,* (2009) investigated young men who consumed either 0g, 5g, 10g, 20g or 40g of whole egg protein following a session of lower limb resistance exercise. It was demonstrated that muscle protein synthesis displayed a dose response to dietary protein ingestion with a 20g protein dose maximally stimulating muscle protein synthesis. Any protein consumption in excess of this amount is likely to be oxidised (Moore *et al.,* 2009). The authors also suggested that chronic protein consumption in excess of 20g could lead to a dampening of the protein synthetic response when the intake is less than 20g. The outcomes of this study have been replicated with whey protein intake in young men (Witard *et al.,* 2014) and have formed the basis for post-exercise protein recommendations for much of the past decade (Morton *et al.,* 2015; Witard *et al.,* 2016), with up to 40g recommended for the elderly (Yang *et al.,* 2012) and possibly cachexic patients (Breen and Phillips, 2012). However, it is relevant to note that the exercise completed by participants in both the Moore and Witard studies was lower limb only and may not be reflective of the resistance training sessions of many athletes. In consideration of this, Macnaughton *et al.,* (2016) investigated the effect of 20g and 40g of whey protein following whole-body resistance exercise and found that 40g of protein resulted in a greater rate of muscle protein synthesis (0.059 $\%.h^{-1}$) compared to 20g (0.02$\%.h^{-1}$) over a 300min recovery period. They concluded that the activation of a greater muscle mass, along with a subsequent increased blood flow in the post-exercise period facilitating the delivery of amino acids to the muscle, may explain the observed results. Further research is required to extrapolate this hypothesis.

Timing of protein intake

Immediate post-exercise consumption of protein has been found to be of greater benefit compared to delayed intake in terms of net protein synthesis rate (Levenhagen *et al.,* 2001) and increasing muscle mass over a longer period of time (Esmarck *et al.,* 2001), even though the post-exercise protein intake in both of the aforementioned studies was only 10g, and not all studies have found similar results (Rasmussen *et al.,* 2000). Whether pre- or post- exercise protein intake is more beneficial has been investigated (Tipton *et al.,* 2001, 2007) with contradictory results. Tipton *et al.,* (2001) reported greater protein synthesis with pre-exercise ingestion of protein possibly due to increased amino acid availability as a result of elevated blood flow, while Tipton *et al.,* (2007) found no difference in protein synthesis when comparing pre- and post- exercise whey protein intake. The disparate results may be related to protein versus amino acid intake (Shimomura *et al.,* 2012). Furthermore, rapid aminoacidemia following resistance exercise results in greater muscle protein synthesis compared to small amounts of protein consumed over a longer period of time (West *et al.,* 2011). In this investigation, 8 males consumed either a single bolus of whey protein (25g) immediately following exercise or 10 'pulsed' drinks of 2.5g. The provision of amino acids immediately after exercise resulted in an increase in acute phosphorylation of anabolic signalling proteins ($PRAS40^{THR246}$, $S6K1^{Thr389}$, $rpS6^{Ser235/6}$) that regulate translation initiation and thus, was more beneficial to support muscle accretion. It is likely that the smaller dose of protein was too small to maximally stimulate MPS, and did not reach the leucince 'threshold' for stimulation of the mTOR pathway. The enhanced MPS response with immediate protein feeding has relevance to the methodology of the investigations of this thesis.

Type of protein intake

It has been reported that the ingestion of both whey and casein protein give similar increases in net muscle protein balance following resistance exercise (Tipton *et al.,* 2004) though Tang *et al.,* (2009) concluded that stimulation of muscle protein synthesis following whey consumption is greater than casein. This greater increase in muscle protein synthesis may be because of the faster availability of amino acids with the consumption of whey, or the greater leucine concentration (Tang *et al.,* 2009). Whey is a watery and thin liquid that remains after the casein curd separates from the milk (Smithers, 2008) and is a rich source of essential amino acids (>20% ww). Most investigations use whey protein concentrates (35-80% protein), whey

protein isolates (>90% protein) or whey protein hydrolysates (resulting from the enzymatic breakdown of protein into peptides and free amino acids), (Sharma and Sha, 2010). Whey protein is water soluble and is digested quickly resulting in a rapid aminoacidemia following consumption. Contrastingly, casein is water insoluble resulting in a slower and more sustained appearance of amino acids in the blood stream.

Training studies have produced contradictory results with regard to the effect of casein and whey. Wilborn *et al.,* (2013) reported similar effects while Cribb *et al.,* (2006) reported greater gains in strength and lean body mass in male bodybuilders with the consumption of whey versus casein over a 10 week training period. Volek *et al.,* (2013) found whey protein to have greater benefits than soy. Of greater relevance, Hoffman *et al.,* (2010) investigated the effect of a pre- and post- exercise protein blend supplement containing 42g protein, compared to a maltodextrin placebo on recovery from a resistance training session and performance in subsequent session. Results showed that those consuming the post-exercise protein supplement performed more repetitions in subsequent sessions at 24h and 48h post-exercise compared to those who consumed a placebo, which interestingly, correlates well with CK during this time frame.

Protein plus carbohydrate intake

Børsheim *et al*., (2004) observed that supplementation with 100g of carbohydrate alone post-resistance exercise resulted in reduced protein breakdown rather than increased protein synthesis, although other studies have not found this (Staples *et al.,* 2011). The addition of carbohydrate to protein intake post-resistance exercise has potential to further increase post-exercise muscle protein synthesis (Rasmussen *et al.,* 2000; Miller *et al*., 2003; Børsheim *et al*., 2004; Tang *et al.,* 2007), although not all studies have found this (Greenhaff *et al.,* 2008; Staples *et al.,* 2011; Koopman *et al*., 2007; Gorissen *et al.,* 2014), especially if protein intake is sufficient to stimulate muscle protein synthesis maximally (Staples *et al.,* 2011). In the latter study, participants consumed 25g of whey protein or 25g of whey protein plus 50g maltodextrin following unilateral knee extension trials. Results indicated an increase in muscle protein synthesis but no difference in either muscle protein synthesis or muscle protein breakdown between conditions as indicated by the phosphorylation of markers of muscle protein synthesis including 4EBP1, p70S6K and eEF2. The

authors suggested that the plasma aminoacidemia and insulinemia resulting from the ingestion of protein alone was sufficient to maximise muscle protein synthesis, with a minimal plasma insulin concentration of ~11μU.ml^{-1} sufficient to inhibit muscle protein breakdown. The findings of these studies suggest that an intake of protein following resistance exercise is important for maximising muscle protein synthesis and that the inclusion of carbohydrate with protein does not seem to enhance the rate of muscle protein synthesis or decrease the rate of breakdown. Nevertheless, Lynch (2013), despite not measuring markers of protein synthesis or breakdown, reported that the consumption of a protein-carbohydrate supplement immediately following a whole-body bout of high intensity resistance training, resulted in an attenuated reduction in subsequent performance of agility and sprint tests when compared to a high-carbohydrate drink. However, it is relevant to note that the post-exercise recovery period was only 2h in duration.

Sex differences, protein intake and protein synthesis

Most studies examining protein intake and resistance exercise have been conducted with young males (Hoffman *et al.,* 2010; Cribb and Hayes, 2006) and it is likely that a greater protein intake is required to maximally stimulate protein synthesis in males. Post-absorptive muscle protein synthesis (Smith *et al*., 2009) and post-exercise muscle protein synthesis (Dreyer *et al.,* 2010) are not different between sexs. Few studies have specifically compared the muscle protein synthesis response post-exercise in males and females. In an informative study, West *et al.,* (2012) explored this area, with 8 men and 8 women consuming 25g of whey protein immediately following a bout of resistance exercise. They reported similar rates of muscle protein synthesis and anabolic signalling responses in males and females in both the early recovery period (1-5h) and late recovery period (24-48h). Further sex comparison research and female-only investigations will be beneficial in enhancing our understanding in this area.

2.7.3 Protein and recovery from mechanical stress – eccentric exercise and muscle damage

Numerous investigations have examined the effect of protein intake on recovery from isolated eccentric exercise. Acute unaccustomed exercise, particularly exercise with an eccentric bias, results in muscle damage, muscle soreness and reduced muscle

function that can persist for several days (Clarkson *et al.,* 1992). When post-damage nutrition is not provided, degradation rates can exceed synthesis rates and a negative protein balance results (Levenhagen *et al*., 2002). Consumption of protein during the post-exercise period promotes muscle protein synthesis (Rasmussen *et al*., 2000) and therefore, it is logical that the consumption of protein post-eccentric exercise may enhance protein synthesis or decrease protein breakdown, preserving muscle fibre integrity and attenuating losses in muscle function and performance. Readers are directed to Sousa *et al.,* (2014) and Pasiakos *et al.,* (2014) for reviews. A number of studies have examined the effect of protein, primarily branched-chain amino acids, on recovery from isolated eccentric exercise resulting in EIMD, implementing a variety of supplementation protocols. The consumption of protein can reduce muscle damage as measured by creatine kinase (Howatson *et al.,* 2012; Matsumoto *et al.,* 2009; Nosaka *et al.,* 2006; Shimomura *et al.,* 2006; Sharp and Pearson, 2010) and muscle soreness (Shimomura *et al.,* 2006; Shimomura *et al.,* 2010; Coombes *et al.,* 2000) though the mechanism underlying the latter observations has never been elucidated. Jackman *et al.,* (2010) reported no differences in CK, Mb, IL-6 and maximal isometric strength with the ingestion of a branched-chain amino acid solution before, during and after eccentric exercise, though muscle soreness was reduced by 64%, suggesting that branched-chain amino acids can modulate soreness independent of biochemical markers. Other studies have not found a similar effect of branched-chain amino acids on muscle soreness (Nosaka *et al*., 2006; White *et al.,* 2008) although the longer supplementation period in the Jackman study may have resulted in the observed positive effect on soreness. Dahlstrom Burnley *et al.,* (2010) investigated the effect of protein (0.4g/kg) compared to carbohydrate (0.4g/kg) following eccentric exercise and found no differences between groups in CK, muscle soreness 24h and 48h post recovery. However, a significant limitation in the experimental design of this study was a three-way crossover trial, with a two week recovery time between trials. A trial-effect was observed with significantly greater damage following the first trial compared to the subsequent trials, known as the ‘repeated-bout’ effect. Still, the lack of effect of protein on the markers of muscle damage may be due to the relatively mild level of muscle damage induced (Dahlstrom Burnley *et al*., 2010).

Supplementation with whey protein has produced equivocal results in relation to recovery from EIMD. White *et al.,* (2008) reported no effect when whey protein was

consumed after 50 eccentric contractions of the quadriceps, while both Buckley *et al*., (2010) and Cooke *et al.,* (2010) found improved muscle strength recovery, though both of the latter investigations supplemented with hydrolyzed whey protein for the duration of the recovery period compared to White's single post-exercise bolus of non-hydrolysed whey. The hydrolysed form of whey may have increased the plasma concentrations of amino acids compared to non-hydrolysed proteins (Morifuji *et al.,* 2010) which may help to explain the observed results.

While the vast majority of EIMD investigation utilise specific eccentrically biased exercise either with isokinetic protocols or downhill running, a small few have utilised more ecologically valid protocols. A number of investigations have induced muscle damage utilising a drop jump protocol (Kirby *et al*., 2012, Howatson *et al.,* 2012). Kirby *et al.,* (2012) investigated the effect of leucine on recovery from EIMD following drop jumps and eccentric-only leg presses and found that the 250mg/kg dose ingested before, during and after exercise attenuated the loss in isometric force production but had no effect on other markers of muscle damage (CK, Mb, soreness). However, the practical application of isometric force production is limited and therefore, of little value when interpreting the results from this thesis. On the contrary, Howatson *et al.,* (2012) demonstrated a reduction in both CK and muscle soreness when branched-chain amino acids were consumed for 7 days prior to, and 4 days following 100 depth jumps. Similar to Kirby *et al.,* (2012) the recovery of maximum isometric voluntary contraction was greater with BCAA ingestion. The longer supplementation period in the Howatson study may explain the disparate findings for CK and muscle soreness. The availability of BCAA during the secondary phase of muscle damage may have facilitated an increased uptake of protein resulting in enhanced cell signalling and muscle remodelling (Howatson *et al.,* 2012), resulting in a reduced loss of muscle function. Readers are directed to da Luz *et al.,* (2011) for a short review on the effects of BCAA supplementation on EIMD.

Recently, Brown *et al.,* (2017) reported a positive effect of whey protein hydrolysate consumption on recovery from EIMD. Of particular relevance was the protocol used to induce muscle damage (repeated sprinting) and the cohort (female). An attenuation of losses in RSI and flexibility along with reduced increases in CK were observed. However, the supplementation protocol in this instance was 2 x 20g bolus amounts per day for 4 days, commencing immediately upon completion of the

exercise protocol and recovery benefits may be as a result of the repeated intake in the days of recovery post-exercise (Brown *et al.,* 2017).

2.7.4 Protein and recovery - mixed mechanical and metabolic stress

Recently a number of investigations have examined the effect of protein on recovery from exercise involving both mechanical and metabolic stress. In a well-controlled trial, Eddens *et al.,* (2017) noted that the consumption of 20g of protein had no effect on recovery from an exercise protocol designed to simulate the concurrent nature of typical exercise programmes (high-intensity cycling and 100 drop jumps). No effect was observed on CK, CRP, countermovement jump performance and maximal voluntary contractions. This is despite previous concurrent exercise research indicating that consumption of protein following concurrent exercise (resistance exercise and cycling) resulted in increased mTOR phosphorylation and enhanced protein synthesis along with an attenuation of markers of muscle catabolism compared to a placebo (Camera *et al.,* 2015). The authors cite a number of possible explanations for the observed results including low levels of EIMD and a resultant smaller window for an intervention to display efficacy (Eddens *et al.,* 2017). Nevertheless, it is likely that the greatest influencing variable was the highly controlled diet for the duration of the investigation which may have over-ridden the possible benefits of the supplemented protein (Eddens *et al.,* 2017), a factor which is of relevance to this research. Of greater relevance to this thesis are studies that have investigated protein and protein-carbohydrate intake following team-sport exercise. Naclerio *et al.,* (2013, 2014) have reported reduced markers of muscle damage, but no performance benefit, with the consumption of a multi-ingredient protein-carbohydrate beverage before, during and after a 90min intermittent repeat sprint test. However, the recovery period was only 24h and no muscle function variables were measured during this time. Poulios *et al.* (2018) conducted a comprehensive investigation into the effects of protein intake on recovery from 2 soccer games separated by a 3 day recovery period. Protein consumption resulted in improved locomoter activity in the second game and attenuation of losses in peak torque measurements. However, the protein intake prescribed was considerably greater than that in this thesis- 80g of pulsed post-exercise intake plus 20g on each recovery day. In contrast, Roberts *et al.,* (2011) reported no effect of a carbohydrate-protein supplement on recovery from an exercise protocol designed to simulate the demands

of rugby union, despite the beverage being consumed before, during and after the protocol. The recovery period was only 24h, though it is possible that the lack of effect was because the participants were habituated to the type of activity performed (Roberts *et al.,* 2011). Based on the aforementioned studies, it is possible that protein consumption can have positive effect on recovery from exercise that involves both mechanical and metabolic stress, though given the varied exercise protocols and nutritional strategies employed, it is difficult to draw definite conclusions.

2.8 Milk

2.8.1 Composition of milk

Bovine milk is a complex food comprised of many components. While there is some seasonal and product variation in the nutritional composition of milk, a general breakdown of semi-skimmed milk can be seen in Table 2.2.

Table 2. 2 Nutritional composition of semi-skimmed milk

	Per 100ml
Energy (kcal)	47
Energy (kJ)	201
Carbohydrate (g)	4.8
Of which sugars	4.8
Protein (g)	3.6
Fat (g)	1.8
Of which saturates	1.2
monounsaturates	0.4
polyunsaturates	trace
Trans fatty acids	0.1
Dietary fibre (g)	0
Thiamin (mg)	0.03
Riboflavin (mg)	0.25
Niacin (mg)	0.1
Niacin from Tryptophan (mg)	0.6
Vitamin B6 (mg)	0.06
Vitamin B12 (µg)	0.9
Folate (µg)	9
Pantothenate (mg)	0.70
Biotin (µg)	3.1
Vitamin C (mg)	2
Retinol (µg)	20
Carotene (µg)	Trace
Vitamin D (µg)	Trace
Vitamin E (mg)	0.04
Sodium (mg)	11

	Per 100ml
Potassium (mg)	161
Calcium (mg)	124
Magnesium (mg)	11
Phosphorous (mg)	97
Iron (mg)	0.02
Copper (mg)	Trace
Zinc (mg)	0.4
Chloride (mg)	90
Manganese (mg)	Trace
Selenium (µg)	1
Iodine (µg)	31

Source: The Nutritional Composition of Dairy Products, The Dairy Council (2018)

Bovine milk contains approximately 36g of protein per litre. There are a number of scales that determine protein quality. The Biological Value (BV) of a protein determines how efficiently exogenous protein leads to protein synthesis in body tissues once absorbed. It is determined by calculating the nitrogen used for tissue formation and dividing by the nitrogen absorbed from food; this value is then multiplied by 100 and expressed as a percentage of nitrogen utilised. BV therefore has a maximum score of 100 (Hoffman and Falvo, 2004). The Protein Digestibility Corrected Amino Acid Score (PDCAAS) ranks protein sources based on the completeness of their essential amino acid content, utilising egg protein as the reference. It is determined by expressing the content of the first limiting essential amino acid of the protein as a percentage of the content of the same amino acid content in a reference pattern. It has a maximum score of 1.0. There are a number of limitations with the use of PDCAAS, including inaccurately elevated values due to the use of fecal protein digestibility as the basis of the scoring and the limit of 1.0 which may not account for proteins of very high quality (Phillips, 2017). Therefore, the use of a new scoring system is replacing the PDCAAS. This is the digestible indispensable amino acid score (DIAAS) and utilises a theoretical best protein as the reference. This scoring system is based on ileal digestibility of protein and utilises a different test protein which can give values greater than 1.0 (Phillips, 2017).

Overall bovine milk has a BV of 91, PDCAAS of 1.00 and a DIAAS of 1.14, indicating that it is readily absorbed by the body, promoting protein synthesis and tissue repair and provides all essential amino acids. The protein components of bovine milk, whey (20%) and casein (80%) have different BV and PDCAAS scores. Casein's BV and PDCAAS are 77 and 1.00 respectively, compared to 104 and 1.00 for whey (Hoffman

and Falvo, 2004), indicating the rapid availability of amino acids following the consumption of whey protein. Thus, casein is considered a ‘slow’ protein, with a slow and prolonged appearance of amino acids in circulation following consumption (Tipton *et al.,* 2004). With its rapid digestion and appearance in the blood, whey is considered a ‘fast’ protein though this increase is short-lived (Tipton *et al.,* 2004). The consumption of both proteins following exercise results in a net muscle protein synthesis (Tipton et al., 2004). Milk has high concentrations of essential amino acids and branched chain amino acids (Haug *et al.,* 2007), including leucine, which is of relevance to the current investigations as leucine is a key trigger for muscle protein synthesis.

While the antioxidant capacity of milk is somewhat dependent on the method of analysis (Chen *et al*., 2003), it has been demonstrated that the antioxidant capacity is related to the fat content of milk, with the greatest antioxidant capacity in whole milk (Ryan and Petit, 2010). Reducing the fat content of milk reduces the concentration of fat-soluble antioxidants including tocopherols, carotenoids and retinols (Ryan and Petit, 2010). Zulueta *et al*., (2009) conducted a comprehensive analysis of varying brands of milk, both low fat and whole, utilising the oxygen radical absorbance capacity (ORAC) method and noted decreased ORAC values in whole milk compared to low fat milk.

Milk contains whey protein which is a source of cysteine and methionine amino acids that are converted to glutathione, a key antioxidant, thus enhancing the anti-oxidant defense system (Madureira *et al.,* 2007; Elia *et al*., 2006). GSH is a tripeptide of glutamate, cysteine and glycine (McPherson and Hardy, 2011). The majority of GSH must be synthesised, primarily in the liver (McPherson and Hardy, 2011). Reduced glutathione donates an electron to reactive oxygen species forming oxidised glutathione, an important antioxidant (Haug *et al.,* 2007). Whey protein is known to raise GSH concentration (McPherson and Hardy, 2011) and Choi *et al.,* (2015) have reported increased cerebral glutathione concentrations with increased dairy intake in older adults. It is possible that milk’s calcium and riboflavin content may contribute to glutathione (Pascoe and Reed, 1989) though it is more likely that it is the cysteine content of the whey component that influences glutathione concentrations (Choi *et al*., 2015). Of interest, Zavorsky *et al.,* (2007) noted a 24% increase in glutathione concentrations in young adults who consumed whey protein bars.

The casein subunit of milk also has antioxidant properties, with a capacity to inhibit lipid peroxidation. Zulueta *et al.,* (2009) found significantly greater ORAC values in milk compared to whey protein, concluding that casein contributes to the main antioxidant capacity in milk, due to the greater tyrosine, tryptophan, lysine and methionine in casein compared to whey. Furthermore, peptides resulting from the digestion of casein have free radical scavenging ability (Rival *et al.,* 2001). In other words, the structure of casein itself determines it's scavenging ability, though no studies have specifically examined casein's effect on exercise-induced oxidative stress.

Milk also has valuable concentrations of Vitamin E, an antioxidant. Milk has a Vitamin E concentration of approximately 0.6mg/l, with the main form (>85%) being alpha-tocopherol (Haug *et al.,* 2007). A number of studies have reported decreases in markers of oxidative stress with Vitamin E supplementation (Goldfarb *et al.,* 1994; Kanter *et al.,* 1993). However, a recent review concluded that while chronic Vitamin E supplementation appears to have a positive effect on performance at altitude, it has a negative effect at sea level (Braakguis and Hopkins, 2015).

Milk is also carbohydrate-rich. A number of studies have investigated the effects of carbohydrate intake on the development of oxidative stress. Because carbohydrate intake maintains blood glucose concentration, it results in a decrease in the release of ACTH, cortisol and epinephrine which could reduce oxidative stress (McAnulty *et al.,* 2003). It is possible that catecholamines may undergo autooxidation to ROS though this may not contribute significantly to overall oxidative stress (McAnulty *et al.,* 2003). Furthermore, increases in cortisol are linked to increased mobilization of neutrophils and inflammatory responses.

Milk also contains a myriad of bioactive compounds which have been shown to have health benefits (Hill and Newburg, 2015; Ebringer *et al.,* 2008). These compounds include lactose and oligosaccharides, growth factors and cytokines, and immunoglobulins, among others (Park and Nam, 2015). Some of these compounds are naturally occurring in milk; others emerge during digestion (Ebringer *et al,* 2008). Positive effects on gastrointestinal activity and function, immunological function and microbial activity (Park and Nam, 2015) are likely to contribute to overall athlete and individual wellbeing, and may possible contribute to recovery processes following

exercise. Bioactive peptides released from αs-casein have free radical-scavenging activity and prevent lipid peroxidation (Park and Nam, 2015). Immunomodulatory peptides influence lymphocyte proliferation and cytokine regulation (Park and Nam, 2015), and thus may influence the post-exercise inflammatory response. Opportunity exists to expolore the effects of these bioactive compounds, individually and collectively, on recovery from specific forms of exercise.

In light of the nutritional composition of milk, it is tenable that milk has the potential to enhance recovery from varying types of exercise. The following sections review the existing evidence that supports milk as a recovery beverage.

2.8.2 Milk and recovery from metabolic exercise

Recovery from exercise that induces a metabolic stress is a complex process involving rehydration, muscle repair and fuel replenishment (Saunders, 2011). There is limited research on the effect of milk on recovery from metabolic stress, with the vast majority of these investigations focusing on endurance exercise and chocolate milk (Table 2.3), which has been reported to be an effective recovery drink (Pritchett and Pritchett, 2012). This is not surprising given that in addition to the sugars naturally occurring in milk, chocolate milk has added sugars, usually sucrose, which can contribute to glycogen replenishment post-exercise. Nevertheless, in the context of this investigation, a brief review of these investigations is appropriate.

A small number of studies have reported increased post-exercise glycogen replenishment with the consumption of chocolate milk compared to an isocaloric carbohydrate drink (Karfonta *et al*., 2010; Ferguson-Stegall *et al.,* 2011). However, the latter study reported no significant difference in this enhanced rate of replenishment (30.6μmol.g^{-1} v 23.6 μmol.g^{-1}, for chocolate milk versus carbohydrate, respectively).

Increased mixed muscle fractional synthetic rate (FSR) and lower whole-body proteolysis was reported when chocolate milk was consumed following 45min of endurance exercise (Lunn *et al.,* 2012). The elevated FSR rates are similar to those reported in previous studies investigating carbohydrate-protein beverages (Howarth *et al.,* 2009) and the reduced protein breakdown is possibly as a result of an 83%

higher insulin concentration following the consumption of the chocolate milk (Lunn *et al*., 2012). Nonetheless, Ferguson-Stegall *et al.,* (2011) reported no differences in markers of muscle damage (including plasma CK and Mb) between chocolate milk and an isocaloric carbohydrate beverage, in contrast to Pritchett *et al.,* (2009) who reported a greater increase in CK in participants who consumed a carbohydrate beverage (211.9 ± 192.5 U/L^{-1}) compared to chocolate milk (+27.9 ± 134.8 U/L^{-1}). The post-exercise consumption of chocolate milk seems to be as effective as carbohydrate and carbohydrate-protein drinks on subsequent exercise performance (Lunn *et al.,* 2012; Karp *et al.,* 2006; Thomas *et al.,* 2009) though these investigations utilised an exercise time to exhaustion which has limited relevance in the context of this thesis.

2.8.3 Milk and recovery from mechanical stress- resistance exercise

While acknowledging the demonstrated benefits of milk consumed during resistance training programmes (Rankin *et al.,* 2004; Hartman *et al.,* 2007; Josse *et al.,* 2010), the acute recovery period is of greater relevance to the series of investigations of this thesis.

A number of studies have examined the immediate effects of milk consumption on the post-exercise period (Elliott *et al*., 2006; Wilkinson *et al.,* 2007). The first investigation to examine the effect of a complete food on muscle protein balance following exercise was Elliott *et al.,* (2006). Looking at the consumption of fat-free milk and whole milk following a legs-only resistance training session with males and females, the ingestion of milk stimulated uptake of phenylalanine and threonine and a change from negative to positive muscle protein balance. The authors concluded that this was probably due to both an increase in protein synthesis (due to provision of essential amino acids) and a decrease in protein breakdown (due to a milk-induced insulin response), despite a small intake of protein in the drinks (8.8g for fat-free milk and 8.0g for whole milk). Interestingly, the authors reported a 2.8 fold greater net amino acid uptake for threonine ($p<0.05$) and an 80% greater uptake of phenylalanine ($p>0.05$) when whole milk was compared to fat-free milk, though the reasons for this difference are not clear. Similar results were reported by Wilkinson *et al.,* (2007). In a randomised cross-over trial, 8 males consumed either 500ml skim milk or an isoenergetic and isonitrogenous soy beverage following a standardized leg

workout. Results indicated that milk promoted a more moderate rise and a sustained elevation in blood amino acid concentrations, and a more sustained net positive protein balance following the exercise bout than the soy protein, clearly suggesting that differences in the delivery and pattern of change in amino acids are key influencing factors in subsequent rates of muscle protein synthesis.

While these investigations did not measure any aspect of muscle function or other markers of recovery, it is likely that the consumption of milk post-exercise creates a positive environment for enhancing muscle protein synthesis which could logically have positive effects on those outcomes.

2.8.4 Milk and recovery from mechanical stress- eccentric exercise and muscle damage

The effect of milk on recovery from eccentrically biased exercise has received considerable attention in the literature (Table 2.3). Jimenez-Florez *et al*., (2012) reported that consumption of a milk product resulted in enhanced anaerobic capacity following damage-inducing simulated mountain hikes and activities in army personnel, but little effect on other measures including CK. However, this was not an independent groups design and there was a repeated bout effect with lower levels of damage reported following the second bout which may have influenced the results. Hatchett *et al.,* (2016) explored the effect of chocolate milk versus raw milk with honey on DOMS following 4 sets of 10 repetitions of back squats. Despite higher soreness in the early recovery period, raw milk and honey reduced reported muscle soreness more than chocolate milk over the 72h period. However, a very small amount of each supplement was ingested (240ml) and no other measures of muscle damage were conducted. Consequently, and particularly because of the subjective nature of the measurement of muscle soreness, little explanation and practical advice can be deducted from the study. Again, focusing on chocolate milk and with small participant numbers, but with excellent ecological validity, Gilson *et al*., (2010) explored the effect of chocolate milk versus an isocaloric carbohydrate beverage during 4 days of increased soccer training duration (>25% during 4 consecutive days of training). Overall, the beverages provided similar effects on markers of post-exercise recovery, though at the conclusion of the 4day training period, CK concentration was lower with the chocolate milk supplement (~310 $U.L^{-1}$) compared

to carbohydrate (~430 $U.L^{-1}$). The lack of effect on sport-specific effects (T-drill, vertical jump) is surprising considering the chocolate milk supplement contained 28g protein which could enhance protein synthesis and reduce protein degradation and this has a positive effect on muscle function. It is tenable that the nature of the training sessions and the modest increase in training volume over the duration of the investigation may not have been an adequate stimulus to impair recovery.

The vast amount of our knowledge regarding milk and recovery from isolated eccentric exercise arises from a sequence of well-designed investigations by Cockburn and colleagues. Cockburn *et al.,* (2008, 2010, 2012, 2013) reported an attenuation of the negative effects of EIMD when milk was consumed following maximal eccentric hamstring exercise with male participants. In these studies, male participants completed 6 sets of 10 repetitions of unilateral eccentric-concentric knee flexions at 1.05 $rad.s^{-1}$ on an isokinetic dynamometer. The initial investigation (Cockburn *et al*., 2008) reported an attenuation of peak torque, total work of the set, CK and Mb 48h following the exercise bout. Investigating the importance of timing of nutrient intake, Cockburn *et al*., (2010) examined the effects of consuming 1000ml of milk either pre, immediately post, or 24h post- muscle damaging exercise. Milk consumed immediately post-exercise resulted in reduced increases in muscle soreness, peak torque and RSI over the 48h post-exercise period. The authors deduced that these benefits were probably a result of increased protein synthesis and reduced protein degradation, leading to a reduction of contractile protein loss during this time frame. While protein balance was not measured in this study, previous research has indicated that consumption of milk post-resistance exercise creates a positive protein balance following resistance exercise (Elliott *et al.,* 2006). Furthermore, the consumption of milk post-exercise is likely to be of greatest benefit due to maximising the benefits of milk during the phase of secondary muscle damage (Cockburn *et al.,* 2010). Interestingly, the results of this study found that pre-exercise consumption of milk was beneficial compared to a control over the 72h recovery period, though given that most athletes train more frequently than this limits the practical application of this outcome (Cockburn *et al.,* 2010). Utilising the same muscle-damaging protocol as previous investigations, Cockburn *et al.,* (2012) found that the post-exercise consumption of 500ml of milk was as beneficial as 1000ml in attenuating the negative effects of EIMD. Despite the 500ml containing only 17g protein, less than the 20g ‘threshold’ for maximal stimulation of protein synthesis

(Moore *et al.,* 2009), an attenuation of decrements in peak torque at 48h when 1000ml (-3%) was consumed compared to water (-10%), with no clear benefit of 1000ml versus 500ml (-3%), was observed. Similarly, a 6.7 fold increase in CK was observed with the consumption of water, compared to a 0.5 fold increase for 500ml milk and 0.1 fold increase for 1000ml of milk, a very likely benefit of milk, with no clear benefit of 500ml v 1000ml. These results indicate that consuming 1000ml of milk, containing 34 g protein, does not provide extra amino acids that can be incorporated into new proteins to preserve or synthesise myofibrillar and membrane proteins (Cockburn *et al.,* 2012), and thus, 500ml of milk was the volume chosen for the series of investigations of this thesis.

Finally, in the study, with the greatest practical application (Cockburn *et al.,* 2013), 14 male soccer players consumed either 500ml of milk or water following muscle-damaging exercise. Baseline data included the Loughborough Intermittent Shuttle Test which was also performed 48h post-exercise. Results indicated a possible benefit of milk at 48h for 10m sprint time (1.7% v 5.0% increases in time for milk versus water) and at 72h for 15m sprint time (2.6% v 5.9%, milk versus water). Additionally, a benefit for milk was also seen at 72h for limiting increases in agility times (0.7% v 4.8%, milk versus water), though there were no clear benefits of milk for the other variables including RSI, CMJ, CK, Mb or soreness.

The contribution of the aforementioned investigations to an understanding of how milk can assist recovery from exercise is significant; however, the nature of the exercise protocol utilised in these studies is quite unlike the exercise stress experienced by team-sport athletes during and following participation in their team sport and thus, the role of milk in the recovery of other, more ecologically valid, forms of exercise are necessary to inform the practical application of this knowledge. Furthermore, all of the above investigations included male participants only and it would be naive to assume that the guidelines for the use of milk in recovery generated from studies involving all males can be identically applied to a female cohort. Kirk *et al.,* (2017), in examining the effect of 500ml milk and A2 milk on recovery, have addressed the issue of the protocol used to induce muscle damage, with 21 male team sport athletes taking part in 15 x 30m sprints with a rapid deceleration on completion of each repetition. Decrements in both CMJ and 20m sprint time were minimised in both milk conditions compared to placebo, though no

effect on soreness was noted. Exploration of the responses of females in a similar experimental design would be useful.

Table 2. 3 Summary of trials investigating the effect of milk on recovery from exercise

Reference	Participants	Exercise model	Group treatments	Key outcomes
Endurance exercise				
Lunn *et al.,* (2012)	N=8 male Age: 23.7 ± 1.6y BM: 76.0 ± 3.8kg	Randomised crossover design 45 min treadmill run at 65% VO2max; 3h recovery period	Post exercise: 480ml Chocolate milk (296kcal, 58g cho, 16g protein v 480ml sweetened grape drink (296kcal, 74g cho)	CHOCOLATE MILK: • Increased mixed muscle FSR (38%) and decreased whole-body proteolysis (-2.6 fold) • No effect on muscle glycogen • Increased TTE (23%)
Ferguson-Stegall *et al.,* (2011)	N=10 (5 male, 5 female) trained cyclists and triathletes Age: 31.8 ± 1.6y BM: 67.8 ± 2.6kg	Randomised crossover design 1.5h at 70% VO_{2max} plus 10 min intervals at 45% and 90% VO_{2max} 4h recovery followed by 40km timetrial	Immediately post-exercise plus 2h post-exercise: Volume according to BM (63.6kg=500ml; 63.6-77.2kg=600ml; >77.2kg=700ml) Chocolate milk (per 100ml 79.05kcal, 3.67g pro, 11.48g cho) v Cho (79.05 kcal, 0g pro, 15.15g cho) v Placebo	CHOCOLATE MILK: • Improved TT performance • Higher muscle glycogen re-synthesis compared to placebo • Greater mTOR activation
Pritchett *et al.,* (2009)	N=10 trained cyclists and triathletes Age: 27.1 ± 7.9 y BM: 72.1 ± 6.7 kg	Randomised crossover trial 50 min high intensity cycling interval training (3 x 10 maximal sprints with 50s recovery), repeated 6 times 15-18h recovery followed by TTE at 85% VO_{2max}	Immediately post-exercise plus 2h post-exercise Volume according to BM (1.0g/kg) Chocolate milk (409.7kcal, 72.1g cho, 18.7g pro) v Cho (384.3 kcal, 72.1g cho, 19.2g pro)	CHOCOLATE MILK: • Lower CK post-exercise • No difference in TTE

Reference	Participants	Exercise model	Group treatments	Key outcomes
Thomas *et al.*, (2009)	N=9 trained cyclists Age: 25.4 ± 8.0y BM: 72.8 ± 8.4kg	Randomised crossover trial Glycogen depletion (2min intervals at 60-90% Pmax, recovery at 50% Pmax) 4h recovery followed by TTE at 70% Pmax	Immediately post-exercise plus 2h post-exercise Volume 1g cho/kg Chocolate milk (459.2 ml, 394.9 kcal) 62.9g cho, 14.2g pro) v Cho (526.3 ml, 394.9 kcal, 72.5g cho, 18.9 g pro) v Fluid (526.3 ml, 109.5 kcal, 0g cho, 0g pro)	CHOCOLATE MILK: • Longer TTE
Resistance Exercise				
Josse *et al.,* (2010)	N=20 female Age: 22.8 ± 2.6y BMI: 25.7 ± 4.0	Independent groups design 12 week, 5d/wk whole body resistance training programme 2- 4 sets x 12-4 reps; 80% (week 1-10) and 90% (week 11-12) IRM	Immediately and 60min post-exercise: 500ml FFM (670kJ, 18g pro, 24g cho) v 500ml cho (9% maltodextrin solution)	MILK: • Increased lean body mass • Increased strength • Increased fat mass loss
Wilkinson *et al.,* (2007)	N=8 male Regular resistance exercisers Age: 21.6 ± 0.3y BM: 81.7 ± 5.9kg	Randomised crossover design Unilateral lower body resistance bout (leg press, hamstring curl, knee extension); 4 sets x 10 reps (final set to exhaustion) at 80% 1RM; 3h recovery period	Post exercise: 500ml non fat milk v 500ml isonitrogenous, isoenergetic soy-protein beverage (745kJ, 18.2g protein, 23g cho)	MILK: • Greater uptake of amino acids across the leg • Greater positive net protein balance • Greater FSR

Reference	Participants	Exercise model	Group treatments	Key outcomes
Hartman *et al.,* (2007)	N=56 male Age: 18-30y	Independent groups design 12 week, 5d/wk whole body resistance training programme 2- 4 sets x 12-4 reps; 80% (week 1-10) and 90% (week 11-12) IRM	Immediately and 60min post-exercise: 500ml FFM (735kJ, 17.5g pro, 25.7g cho) v 500ml soy (isoenergetic, isonitrogenous, macronutrient matched) v 500ml cho (9% maltodextrin solution)	MILK: • Increased lean mass • Decreased body fat • Increased in Type II muscle fiber area
Elliot *et al.,* (2006)	N= 24 male and female Age: 25.9 ± 1.7 BM: 73.8 ± 6.0	Independent groups design 10 sets x 8 repetitions of knee extension	60min post-exercise: 237g FFM (377 kJ, 8.8g pro, 12.3g cho) v 237g WM (627 kJ, 8.0 g pro, 11.4g cho) v 393g FFM (626 kJ, 14.5 g pro, 20.4g cho)	FFM, WM: • Increased amino acid uptake WM: • Greater amino acid uptake, representative of increased net muscle protein synthesis
Sporting activities				
Papacosta et al., (2015)	N=12 male judo athletes Age: 19 ± 4 BM: 77.4 ± 7.9	Crossover field study 5 days intensive training, simulated competition on Day 6	Immediately post-exercise: 1000ml chocolate milk (870 kcal, 107g cho, 35g pro) v 1000ml water	CHOCOLATE MILK: • Lower soreness • Lower salivary cortisol • Higher judo-specific performance
Gilson *et al.,* (2010)	N=13 male soccer players	4 days of increased training duration (25%); soccer specific, strength, sprint, aerobic, recovery training	Immediately post-exercise: 672ml chocolate milk (504 kcal, 28g pro, 84g cho) v 672ml 18.6% cho (504 kcal, 0g pro, 122g cho)	CHOCOLATE MILK: • Reduced CK increases
Rankin *et al.,* (2004)	N=19 untrained males	Independent groups design	Immediately post-exercise: Milk v cho	MILK: • Tended to increase body mass and fat-free soft tissue

Reference	Participants	Exercise model	Group treatments	Key outcomes
	Age: 18-25y	10 week resistance training programme		• No difference in strength gains
Eccentric Exercise				
Kirk *et al*., (2017)	N=21 male team sport athletes Age: 22.6 ± 1.7y BM: 79.3 ± 10.3 kg	Independent groups design 15 x 30m sprints with 10m deceleration zone; 60s between sprints	Immediately post-exercise: Milk (500ml, 250 kcal, 18g pro, 24g cho) v A2 milk (500ml, 250 kcal, 18g pro, 24g cho) v Placebo (500ml, 220 kcal, 0g pro, 50g cho)	MILK and A2 MILK: • Reduced losses in CMJ and sprint performance
Hatchett *et al.,* (2016)	N= 20 male football players Age: 20.0 ± 0.85 BM: 79.4 ± 1.5kg	Independent groups design 4 sets x 10 reps barbell back squats	Immediately post exercise: 240ml chocolate milk v 240ml raw milk with honey	RAW MILK: • Lower muscle soreness ratings
Cockburn *et al.,* (2013)	N=14 male soccer players Age: 24 ± 4y BM: 79.9 ± 8.4kg	Independent groups design 6 sets x 10 reps maximal eccentric-concentric knee flexions 72h recovery period	Immediately post-exercise: 500ml milk v 500ml water	MILK: • Possible benefit in limiting increases in sprint performance and agility
Cockburn *et al.,* (2012)	N=24 active males Age: 21 ± 3y BM: 79.7 ± 9.3 kg	Independent groups design 6 sets x 10 reps maximal eccentric-concentric knee flexions 72h recovery period	Immediately post-exercise: 500ml milk (240 kcal, 17g pro, 24.5g cho) v 1000ml milk (480 kcal, 34g pro, 49g cho) v 1000ml water	MILK: • Limited decrements in peak torque • Reduced increases in CK • No differences in 500ml v 1000ml

Reference	Participants	Exercise model	Group treatments	Key outcomes
Cockburn *et al.,* (2010)	N=32 active males Age: 20 ± 2y BM: 78.5 ±9.0kg	Independent groups design 6 sets x 10 reps maximal eccentric-concentric knee flexions 72h recovery period	1000ml milk-based drink (707 kcal, 33.4g pro, 118.2g cho) Before exercise v Immediately post-exercise v 24h post-exercise v Control (water)	COMPARED TO CONTROL: • Immediately post- and 24h post-exercise limited changes in DOMS, peak torque and RSI • Pre-exercise reduced increases in CK
Cockburn *et al.,* (2008)	N=24 male team sport athletes Age: 21 ± 3y BM: 80.2 ± 9.1 kg	Independent groups design 6 sets x 10 reps maximal eccentric-concentric knee flexions 72h recovery period	Immediately post-exercise: 500ml plus 500ml within 2h Milk-based chocolate shake (706.8kcal, 33.4g pro, 118.2g cho) v milk (480kcal, 34g pro, 49g cho) v cho (280kcal, trace pro, 64g cho) v water	MILK and MILK-BASED CHO: • Reduced losses in peak torque • Reduced increases in CK and Mb

BM, Body mass; BMI, body mass index; WM, whole milk; FFM, fat-free milk; TT, timetrial; TTE, time to exhaustion; FSR, fractional synthetic rate; CK, creatine kinase, CHO, carbohydrate, Pmax, power at VO_{2max}

2.9 Conclusion

The mechanical and metabolic demands associated with exercise participation clearly result in muscle damage, inflammation, oxidative stress and reduced muscle function. The effects of protein intake on recovery from exercise have received considerable attention in the literature. Alongside this, reasonable elucidation of the mechanisms by which protein consumption influences post-exercise protein balance, synthesis and breakdown, has contributed to our understanding of why protein constitutes an effective recovery strategy. In this context, the majority of evidence is indicative that milk has potential as a post-exercise recovery drink. However, to date, investigations have been limited to recovery from specific exercise protocols, some of which do not represent authentic stresses of exercise participation. The investigations in the subsequent chapters aim to determine if milk can influence recovery from varied forms of exercise, thus enhancing the application of milk in recovery strategies. Furthermore, a simultaneous focus of these investigations is to determine if milk can influence the recovery of female athletes who are under-represented in the literature to date.

Chapter 3: General Methods

3.1 Introduction

This thesis is comprised of four sequential studies designed to investigate the effect of milk on recovery from various exercise protocols in female team-sport athletes. Methods that were identical and repeated are described in this chapter. Ethics approval was gained for each study as follows: Study 1 – Northumbria University; Studies 2, 3, 4 – Middlesex University. Ethics approval was also received from Institute of Technology Carlow, where data collection took place.

3.2 Participants

The target population for these investigations were male (Study 1) and female (Study 1, 2, 3, 4) team-sport athletes. The majority of these participants were drawn from the sports of Gaelic football, hurling and camogie, though a small number of participants in Study 1 played soccer or rugby and some participants participated competitively in more than one sport. Participants were recruited from the student population at the Institute of Technology, Carlow by email and oral presentations to Institute teams and class groups. Individuals who expressed an interest in participating in the investigation were sent a Participant Information sheet that provided details on the nature of the study, the demands involved in terms of the exercise protocol and the time commitment required (See Appendix A for a sample of the Information Sheet). Prospective participants were then screened according to the inclusion and exclusion criteria (See Table 3.1).

Table 3. 1 Inclusion and exclusion criteria across all investigations

	Study 1	**Study 2, 3, 4**
Inclusion	Male and Female	Female
	Team sport athletes	Team sport athletes
	Aged 18-35	Aged 18-35
	Currently competing	Currently competing
	Training at least 2x per week	Training at least 2x per week
Exclusion	Current or recent use of nutritional supplements	
	Intolerance to dairy or lactose products	
	Lower limb or back injury in previous 3 months	
	Surgery in previous 6 months	
	Known coronary disease	
	Uncontrolled metabolic disorder or respiratory disease	
	Pregnancy/postpartum	

Following this, screened participants completed an Informed Consent (Appendix A) and a Health Questionnaire (Appendix B).

3.3 Experimental Design

The experimental design varied depending on the study. Studies 1, 3 and 4 followed an independent-group design in order to avoid a Repeated Bout Effect (RBE) whereby participation in an exercise bout with a high eccentric load results in reduced muscle damage in the next subsequent bout of exercise (McHugh, 2003). Because Study 2 was designed to examine the effects of milk on recovery from a non-eccentric exercise bout, a crossover design was utilised. For Study 1 and Study 2, all female participants completed the exercise trials during the follicular phase of their menstrual cycle as in previous research utilising female participants (Dannecker *et al*., 2013). No participants in Study 1 and Study 2 were hormonal contraceptive users. Self-reported menstrual function identified eumenhorreic participants (13 participants in Study 1; 9 participants in Study 2). The remaining participants identified themselves as oligomenhorreic. These participants informed the researcher when Day 1 of their cycle commenced and participation in the investigation was organised immediately. Control of menstrual cycle was not possible in Study 3 and 4 due to the restricted availability of participants. No details of menstrual cycle history or contraceptive use were recorded in these investigations. However, it has been reported that oestrogen concentration may have limited effect on the symptoms of EIMD in exercising females (Markofski and Braun, 2014), and Hicks et al., (2017), reported no differences in muscle torque and soreness of oral contraceptive users and non-oral contraceptive users following eccentric exercise.

For all studies, complete familiarisation with all testing procedures took place no more than 5 days prior to the exercise bout. Immediately prior to baseline measures, with footwear removed and wearing light clothing, stature was measured to the nearest 0.1 cm using a fixed stadiometer (Seca 264 Digital Stadiometer, Seca Ltd, Germany) and bodymass was determined to the nearest 0.05 kg using a digital column scale (Seca 704, Seca Ltd, Germany).

For Study 1, baseline measures were recorded immediately prior to the exercise bout. For Study 2, 3 and 4, participants completed baseline testing no more than 3

days prior to the exercise protocol. Subsequently, participants attended the laboratory on 4 (Study 1, 2, 3) or 5 (Study 4) consecutive days with the exercise protocol on day 1 and repetition of the baseline measures 24h, 48h, 72h (Study 1, 2, 3, 4) and 96h (Study 4) post-exercise. The measurement of dependent variables (see Table 3.2) took place in the same order each day as follows: measurement of soreness and tiredness, completion of the DALDA questionnaire, blood sample, peak torque, isometric RFD, CMJ, RSI and sprint performance. The number and nature of variables were selected so as not to affect the initial recovery process following the experimental condition (i.e. the exercise protocol) or by inducing cumulative fatigue (Nedelec *et al*., 2012).

All participants were requested to refrain from the consumption of caffeine and alcohol for 24h prior to the exercise bout. Participants were also requested to refrain from any strenuous activity for the duration of the study and from treating symptoms of muscle soreness and tiredness with interventions such as massage, cryotherapy, supplements and non-steroidal anti-inflammatory drugs. All participants were tested at the same time each day (±30min) to minimise the effect of time of day on the variables measured.

Table 3. 2 Dependent variables for each study

Variable (in order of testing)	Study 1	Study 2	Study 3	Study 4
Muscle soreness (squat)	✓	✓	✓	✓
Muscle soreness (general)	✓	✓	✓	✓
Muscle Tiredness	-	✓	✓	✓
DALDA	-	-	✓	✓
CK	✓	✓	✓	✓
sTnI	✓	-	-	-
hsCRP	-	✓	✓	✓
PC	-	✓	-	✓
GSH:GSSG	-	✓	-	-
LOOH	-	-	-	✓
Peak Torque 60/s	✓	✓	✓	✓
Peak Torque 180/s	✓	✓	✓	✓
Isometric RFD	-	✓	✓	✓
CMJ	✓	✓	✓	✓
RSI	-	-	✓	✓
5m Sprint performance	-	-	✓	✓
10m Sprint performance	-	-	✓	✓
20m Sprint performance	✓	✓	✓	✓

DALDA: Daily analysis of life demands of athletes; CK: Creatine kinase, sTni: skeletal troponin I; hsCRP: high sensitivity C-reactive protein; PC: Protein carbonyls; GSH:

glutathione; GSSG: Oxidised gluthathione; LOOH: Lipid hydroperoxides; RFD: Rate of force development; CMJ: Countermovement jump; RSI: Reactive strength index

3.4 Nutritional Intervention and Dietary Control

Immediately upon completion of the exercise protocol participants consumed either 500ml of milk (Avonmore 1% Light, Glanbia, Kilkenny, Ireland) or 500ml of an energy-matched carbohydrate solution. The consumption of carbohydrate drinks post-exercise is common practice and previous research has demonstrated that the administration of carbohydrate alone has no effect in lessening signs of EIMD (Howatson *et al.,* 2008). A volume of 500ml of milk was chosen based on previous research (Cockburn *et al*., 2010, 2012) showing that 500ml of milk is effective in attenuating the negative effects of muscle damage in males. From an applied perspective, it was felt that 500ml was an easily consumed volume and that consumption of larger volumes may lead to stomach fullness and discomfort. Macronutrient composition (per 500ml) of milk was: Energy 910kJ/215kcal, Protein 17.0g, Carbohydrate 25.5g, Fat 5.0g. The energy-matched carbohydrate solution consisted of glucose (52.6g) mixed with water and a commercially available orange flavoured cordial (Nutritional information per 100ml of cordial: Energy 37kJ/9kcal, Protein 0.2g, Carbohydrate 0.8g, Sodium Trace; MiWadi, Dublin, Ireland).

In order to maintain dietary control, participants were requested to maintain their habitual diet and to complete a food diary for 24h prior to the exercise bout and for the subsequent 3 days (Study 1, 2, 3) or 4 days (Study 4) of the recovery period. Each participant was provided with a weighing scale and measuring jug for the duration of the study and was instructed on how to record food and fluid intake (Appendix C). The food diaries were subsequently analysed using dietary analysis software (Nutritics Professional Diet Analysis, Nutritics Ltd., Dublin, Ireland). Analysis using Magnitude Based Inference indicated that there were no clear differences in energy, carbohydrate, protein or fat intake between groups (Study 1, 3, 4). In study 2 the carbohydrate intake over the MILK trial was likely higher than the CHO trial (Appendix D).

3.5 Exercise Protocols

The exercise protocol utilised varied depending on the study. Study 1 employed an eccentric exercise protocol on the hamstring muscle group using isokinetic dynamometry, as described in previous research (Cockburn *et al*., 2008, 2010, 2012). Study 2 used a modified version of the Cycling Intermittent Sprint protocol (Hayes *et al.,* 2013) which simulated the metabolic demands of a team sport without the eccentric loading of muscle groups. The exercise protocol in Study 3 aimed to incorporate commonly executed exercise activities and was a combined protocol of repeated sprints (15 x 20m) with rapid deceleration followed by 8 sets of 10 plyometric jumps. The protocol in Study 4 was a modified version of the team sport circuit described by Bishop *et al.,* (2001). This protocol was completed twice with 48h recovery between. Details of each protocol are provided in Chapters 4, 5, 6 and 7.

3.6 Measurement of Muscle Soreness and Tiredness

Muscle soreness is frequently reported following participation in exercise (Close *et al.,* 2004; Cockburn *et al*., 2008, 2010; Brown *et al.,* 2015) and the use of a Visual Analogue Scale has been found to be reliable (Bijur *et al.,* 2001). Passive muscle soreness was measured on a VAS while standing. Active muscle soreness was measured during squatting to approximately 90° knee flexion and during isokinetic testing of knee extension at 60°/s, with participants rating their level of soreness on a scale of 0 (no soreness) to 10 (as bad as could be). A similar VAS was used to measure passive muscle tiredness, with 0 indicating no tiredness and 10 indicating as tired as could be.

3.7 Measurement of the Symptoms of Stress

Symptoms of stress were monitored at the same time each day using the validated Daily Analysis of Life Demands (DALDA) questionnaire (Rushall, 1990) (Appendix E). This self-report inventory was developed to determine the nature of an athlete's response to training and training load as well as identifying the factors outside of sport that may interfere with the training response (Rushdall, 1990). It has an advantage over other measures as athletes compare their current well-being to 'normal' (Saw *et al*., 2015). DALDA has been used in numerous investigations across a variety of sports in this regard (Halson *et al*., 2002; Coutts and Reaburn, 2008;

Wadley *et al.,* 2015; Tanner and Day, 2017) and is sensitive for monitoring psychophysiological stress response in response to variations in training load (Milanez *et al*., 2014). Recently, DALDA has been used to monitor the effect of a nutritional intervention on symptoms of stress following exercise (Sciberras *et al.,* 2015). The questionnaire is comprised two parts: Part A represents the sources of stress and Part B assesses the manifestation of this stress in the form of symptoms. Each day participants were provided with a copy of the full questionnaire and marked each statement as being either 'normal', 'worse than normal', or 'better than normal'. Data for Part B is presented in this study where the number of 'worse than normal' answers was quantified and used for analysis.

3.8 Blood Sampling

Venous blood samples were collected from a vein in the anti-cubital fossa using venepuncture at baseline and 2h, 24h, 48h, 52h (Study 4), 72h and 96h (Study 4) post-exercise. Samples were collected in 10ml ethylenediaminetetraacetic acid (EDTA) and 8.5ml serum separator (SST) tubes. Tubes were centrifuged at 3000 $r.min^{-1}$ for 15 min before the supernatant was removed and immediately stored in aliquots at -80°C for later analysis.

3.8.1 CK

In all studies total serum CK was analysed using high sensitivity procedures (Roche Cobas 6000 chemistry module c501, Hoffmann-La Roche, Basel, Switzerland). Coefficients of variation for this system are reported as 0.7 - 2.3%.

3.8.2 sTnI

sTnI was determined in Study 1 by a sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (2BScientific, Oxfordshire, UK). With this kit the microtiter plate is pre-coated with an antibody specific to TNNI2 (Troponin I, Type 2). Standards and samples were added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to TNNI2. Then, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After tetramethylbenzidine (TMB) substrate was added, only those wells that contain TNNI2, biotin-conjugated antibody and enzyme-conjugated Avidin exhibited a change

in colour. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450nm±10nm. The concentration was then determined by comparing the OD of the samples to the standard curve. The intra-assay and inter-assay coefficients of variation for this kit are reported as <10% and <12%, respectively.

3.8.3 hsCRP

Total serum hsCRP was determined in Study 2, 3 and 4 using high sensitivity procedures (Roche Cobas 6000 chemistry module c501, Hoffmann-La Roche, Basel, Switzerland). Coefficients of variation for this system are reported as 0.7-2.3%.

3.8.4 Protein carbonyls

PC were determined for Study 2 and Study 4 using a commercially available kit (Abcam, Cambridge, UK) utilising a methodology based on that described by Levine *et al.,* (1990). Briefly, samples were derivatised initially with dinitrophenylhydrazine (DNPH) producing functional groups into the oxidized protein which were detected by spectrophotemetry. The samples were washed to remove excess DNPH and the oxidised carbonyls were solubilised with guanidine prior to their detection by measuring absorbance at 375 nm. Finally, the protein content in each sample was measured so that the carbonyl content was expressed in terms of nmol/mg of total protein. The intra- and inter- assay variation for this kit is <7% and <10%, respectively.

3.8.5 GSH:GSSG

For the determination of GSH:GSSG using a commercially available kit (Abcam, Cambridge, UK) samples were first deproteinised by centrifugation through a 10kDa spin column. Thiol Green Indicator Reaction mix was then added which becomes strongly fluorescent upon reacting directly with glutathione. Samples were incubated prior to the measurement of fluorescence at 520 nm using a fluorescence microplate reader. Intra- and inter- assay CVs for this procedure are <15%.

3.8.6 LOOH

LOOH was determined using the ferrous oxide oxidation of xylenol orange method (FOX 1), using a modification of the methods of Wolff (1994) and Nourooz-Zadeh *et al*., (1994). Absorbance was read spectrophotometrically (U-2001, Hitachi, England) at 560 nm against a linear standard curve (range 0-5μmol.L^{-1}). The inter- assay and intra- assay coefficients of variation were <4% and <2%, respectively.

3.9 Measurement of Peak Torque

For the determination of peak torque participants completed three bilateral maximal effort knee extension and flexion repetitions at 60°/s and 180°/s, with 60s seated recovery between speeds on a Biodex System 3 Isokinetic dynamometer (Biodex Medical System, NY, USA). The isolation of muscle groups during maximal voluntary assessment reduces the validity in regard to the performance of multi-joint movements but increases the reliability of the assessment (Nedelec *et al*., 2012). Interclass correlations for this protocol at IT Carlow are 0.83 - 0.94 and coefficients of variation have been reported as 4.5-4.9% (Cockburn *et al.,* 2010).

For the initial set-up participants were seated on the dynamometer so that the femoral epicondyle was aligned with the dynamometer's axis of rotation and the ankle strap position 5 cm proximal to the medial malleolus. Straps were placed across the chest, hips and the leg to be tested in order to minimise extraneous joint movement and isolate the muscle groups. Participants were requested to grip the handles at the side of the seat during each repetition in order to further stabilise the pelvis (Rampinini *et al.,* 2012). Following a standardised warm-up, participants were instructed to apply maximal resistance to the lever arm during the concentric phase of each extension-flexion repetition, aiming for full range of movement (as previously determined) on each repetition. Verbal encouragement was given throughout.

3.10 Measurement of the Rate of Force Development

In Study 2 and 3, RFD was determined over the first 200ms of an isometric contraction, according to previous studies (Aagard *et al*., 2002). Briefly, two maximal 5s isometric contractions of the dominant leg quadriceps were performed on the same isokinetic dynamometer used for isokinetic peak torque measurements, with

the knee fixed at an angle of 70° (0° = full extension). Participants were instructed to contract and 'push away as fast and forcefully as possible'. Any repetitions that showed a countermovement (a visible drop in the force signal) were excluded; otherwise RFD was determined from the repetition with the highest torque measurement. RFD was calculated over the time interval of 0 – 200 ms (Δtorque/Δtime) relative to the onset of contraction, which was defined as the time point when the torque generated exceeded the baseline by >7.5Nm (Aagard *et al.,* 2002). For Study 4, RFD was determined over the time interval of 100 – 200 ms relative to the onset of contraction. This was following research by Peñailillo *et al*., (2015) suggesting that RFD over this time interval may be a more sensitive marker than the period 0 -100 ms following exercise because of different underlying mechanisms. It was postulated that the early phase of the RFD (0 -100 ms) is greatly influenced by the series elastic component of the muscle, whereas the later phases may be more reflective of muscle contractile potential (Peñailillo *et al*., 2015). Thus measurement of RFD from 100 – 200 ms from the onset of contraction is more reflective of muscle damage.

3.11 Measurement of Countermovement Jump Performance

In these investigations countermovement jump height was determined using an Optojump optical measurement system (Microgate, Bolzano, Italy) employing standard countermovement jump technique. Commencing the movement in an upright position with hands on the hips, participants were required to flex their knees to approximately 90° and then, in one continuous movement, jump vertically for maximum height. Jump height was calculated from flight time and each participant completed three jumps; the highest recorded jump was used for analysis. Interclass correlations from reliability trials at IT Carlow for this protocol are 0.97- 0.98 and coefficients of variation have been previously reported as 3.3% (McLellan *et al.,* 2011), and <4% and <3% for intra-trial and inter-trial CVs, respectively (Brown *et al.*, 2015).

3.12 Measurement of Reactive Strength Index

Reactive Strength Index (RSI) was determined using the Optojump optical measurement system (Microgate, Bolzano, Italy) following the execution of three

maximal effort drop jumps from a height of 45cm as in previous research with female team sport athletes (Werstein and Lund, 2012). Participants were instructed to step forward off the box and minimise ground contact time when landing from the drop height, while maximising jump height. Hands were maintained on the hips for the duration of the jump to eliminate any contribution of arm swing. RSI was calculated by dividing jump height (cm) by ground contact time (ms) (Flanagan *et al.,* 2008), with the highest value used for analysis. Coefficients of variation for RSI from a drop-height of 43cm have been reported as 7.3% (Cockburn *et al.,* 2010), with intra-trial and inter-trial CVs from a drop height of 30cm reported as <12% and <6%, respectively (Brown *et al.,* 2015).

3.13 Measurement of Sprint Performance

All studies measured 20m sprint performance. Study 3 and 4 also determined split 5m and 10m sprint times. Sprint times, from a standing start 20cm behind the start line, were measured using timing gates (Microgate Racetime 2, Bolzano, Italy). Each participant was instructed to sprint through the timing gates as fast as possible, completing three sprints in total, with a rest time of 120s between trials. The fastest sprint time was used for analysis. Interclass correlations from reliability trials at IT Carlow for this protocol are 0.89 - 0.98, with CVs previously reported as <2% (Brown *et al.,* 2015).

3.14 Statistical Analysis

Data for all investigations were analysed by making probabilistic magnitude-based inferences about the true value of outcomes, as described by Batterham and Hopkins (2006). This approach to data analysis is a move away from traditional null hypothesis testing and has become more common in sport and exercise research, including nutrition intervention investigations (Rowlands *et al*., 2008; Highton *et al.,* 2013; Braakhuis *et al*., 2013; D'Lugos *et al*., 2016; Ormsbee *et al*., 2016). There is a growing feeling that null hypothesis testing is flawed given that a p value cannot measure the probability that the hypothesis is true and cannot measure the size of an effect or the importance of a result (Bernards *et al*., 2017). The term 'significant' has become synonymous with 'important' (Cumming, 2014) when, in fact, a significant statistic (P<0.05) could represent an effect that is clinically or practically irrelevant

(Batterham and Hopkins, 2006). Furthermore, the application of an arbitrary p value which focuses on absolute effect versus non-effect is inconsistent considering that with any investigation there is never 'no effect' (Batterham and Hopkins, 2006). By defining the smallest practical or biological effect, the probability of a worthwhile effect with inferential descriptions is possible. Thus, magnitude based inference is a solution to traditional null-hypothesis testing (Wilkinson, 2014). Despite it being shown that MBI outperforms null hypothesis testing in terms of Type I and Type II error rates (Hopkins and Batterham, 2016), there has been recent criticism of MBI with regard to error rates (Sainani, 2018). In particular, Sainani (2018) criticised the MBI definitions of Type I and Type II errors, and stated that the error rates may be 2- or 3- times those of null hypothesis significance testing, depending on the sample size. In response, Hopkins and Batterham (2018), commenting on simulations for the determination of error rates in the use of MBI, reinforced the lower error rates with this statistical method. Specifically, they descrbed Type I error rates of 2.8% (clinical) and 0.8% (non-clinical), comparable or lower than the reported 2.5% Type I error rate with NHST (2.5%). With Type II errors, when the true effect was harmful the error rate was only 0.2%. They, however, acknowledge the higher error rate with MBI when the true effect was beneficial (12%). Hopkins and Batterham (2018) recognise that MBI can still be improved, particularly in relation to the rules for acceptable uncertainty in both clinicial and non-clinical settings. At this point, the author believes that MBI represents a trustworthy mechanism for representing the uncertainty in effects and provides a useful and practical insight into the outcomes of the studies in this thesis. .

Within-group effects over time and the effect of MILK versus CHO were determined using published spreadsheets (Hopkins, 2006a and 2006b, respectively). In Study 1 male-female comparisons were completed with a spreadsheet for combining outcomes from several subject groups (Hopkins, 2006c). Comparisons were made between baseline and 2h (bloods only) 24h 48h, 72h and 96h (Study 4 only) post-exercise. Data for peak torque, RFD, CMJ, RSI, sprint performance and serum markers were log-transformed to overcome heteroscedastic error (Nevill and Lane 2007). Muscle soreness values and DALDA scores were not log-transformed because of interval scaling (Nevill and Lane, 2007). Means of log-transformed data were then back transformed to provide mean percentage change and percentage SD. Serum markers of muscle damage are, however, reported as factors because of

large percentage changes (Hopkins, 2003). The smallest worthwhile effect for muscle function variables was the smallest Cohen change in the mean: 0.2 times the between-subject SD for the baseline values of all participants (Batterham and Hopkins, 2006). The smallest worthwhile effect was 1.0 for muscle soreness and tiredness and 1.1 for blood variables (Hopkins, 2017). Chances of benefit and harm were assessed qualitatively as follows: <0.5% most unlikely, 0.5-5% very unlikely, 5-25% unlikely, 25-75% possibly, 75-95% likely, 95-99.5% very likely, >99.5% most likely (Hopkins, 2017). Effects with confidence limits across a likely small positive or negative change were classified as unclear (Hopkins *et al*., 2009). Unclear outcomes with an odds ratio greater than 66 are declared clear (Hopkins, 2017). Effect Size (ES) magnitudes were calculated as the difference in means/SD for both groups and were qualified as follows: trivial, 0.0-0.2; small, 0.2-0.6; moderate, 0.6-1.2; large, 1.2-2.0; very large, 2.0-4.0; extremely large, 4.0. (Hopkins *et al.,* 2009). ES thresholds for measures of soreness and tiredness were set at 10%, 30%, 50%, 70% and 90% of the VAS range for small, moderate, large, very large and extremely large, respectively.

Chapter 4: The Effect of Milk on the Attenuation of Exercise-Induced Muscle Damage in Males and Females

4.1 Introduction

Unaccustomed eccentric exercise is known to cause mechanical stress resulting in morphological changes within the muscle (Hortobágyi *et al.,* 1998; Lauritzen *et al*., 2009), with initial damage occurring immediately after the muscle insult (Friden *et al*., 1983) and further damage evident 1-3 days after the exercise stress (Clarkson and Sayers, 1999; Friden and Lieber, 1992; Newham *et al.,* 1983). Furthermore, losses in muscle force (Lavender and Nosaka, 2008) increased muscle soreness (Kanda *et al*., 2013; Nosaka *et al.,* 2006), decreased agility (Highton *et al*., 2009), decreased proprioception (Saxton *et al*., 1995) and increased serum muscle proteins (Tsatalas *et al.,* 2013; Sorichter *et al.,* 2001) have been noted, some of which would impact an athlete's ability to parcitipate in training or competition.

Numerous investigations have examined the effects of nutritional interventions on the attenuation of these symptoms of mechanical stress or Exercise Induced Muscle Damage (EIMD), including protein (Dahlstrom Burnley *et al.,* 2010), whey protein (Buckley *et al.,* 2010), branched chain amino acids (da Luz *et al*., 2011; Howatson *et al.,* 2012), leucine (Kirby *et al,.* 2012), creatine (McKinnon *et al.,* 2012), antioxidants (Avery *et al.,* 2003; Beaton *et al*., 2002) and caffeine (Maridakis *et al*., 2007). Studies examining the effects of carbohydrate-protein consumption on EIMD have presented inconsistent results. While a number of investigations have shown a positive effect (Samadi *et al*., 2012; Baty *et al.,* 2007; Bird *et al*., 2006;), others have not (Glynn *et al.,* 2013; Green *et al*., 2008; White *et al*., 2008; Wojcik *et al*., 2001). Individual variability, especially in changes in creatine kinase, and differences in the exercise protocols used to induce muscle damage are possible reasons for these discrepancies (Sousa *et al*., 2014). More recently, studies have examined the effects of available foods on markers of EIMD including cherry juice (Howatson *et al.,* 2010; Connolly *et al.,* 2006), spinach (Nakhostin-Roohi *et al*., 2012) and blueberries (McLeay *et al.,* 2012). Cockburn *et al*., (2010; 2008) has shown that the consumption of milk immediately post-eccentrically biased exercise enhances recovery from EIMD in male sports participants. Milk ingestion resulted in an attenuation of peak torque losses, reactive strength index losses and Creatine Kinase (CK) and Myoglobin (Mb) increases at 48h and 72h post-muscle damaging exercise. Furthermore, an investigation with male soccer players demonstrated that intake of 500 ml of milk post eccentric exercise resulted in a possible attenuation of the increase in 10 m and 15 m sprint times during the Loughborough Intermittent Shuttle Test and a likely

benefit in limiting decreases in agility performance (Cockburn *et al*., 2013). Given that milk is readily available and inexpensive, it makes it an ideal consideration for recovery nutrition. However, no studies have investigated the effects of milk consumption on markers of muscle damage in females.

Results from numerous animal studies suggest that oestrogen may attenuate some of the effects of muscle damage (Stupka and Tiidus, 2001; Komulainen *et al*., 1999; Amelink and Bar *et al*., 1988; Amelink and Bar, 1986) but studies from humans are far more inconclusive. Neither Staron *et al*., (1992) nor Stupka *et al*., (2000) observed any male-female differences in the damage observed in muscle biopsy samples taken post-eccentric exercise. Studies investigating other markers of muscle damage are inconclusive with some reporting greater force loss in females (Sewright *et al*., 2008; Sayers and Clarkson, 2001) and others reporting no difference (Power *et al.,* 2010; Hubal *et al.,* 2008; Kerksick *et al*., 2008). Similarly with muscle soreness, a number of investigations have found higher soreness in males (Dannecker *et al*., 2012; Kerksick *et al*., 2008), with others reporting no sex difference (Sewright *et al*., 2008; Dannecker *et al.,* 2005; 2008;).

A recent study concluded that female participants are under-represented in sports and exercise research, comprising only 39% of participants across 1382 investigations (Costello *et al*., 2014). Furthermore, sex representation in studies investigating the management of delayed onset of muscle soreness ranged from only 16-36% (Costello *et al.,* 2014). Given that there is conflicting evidence regarding sex differences in the response to eccentrically biased exercise, it is difficult to extrapolate to females the effects that nutritional interventions may have on attenuating the effects of EIMD from investigations with predominantly male participants. Therefore, the aim of this investigation is to examine the effect of milk consumption on muscle damage in male and female team sport participants.

4.2 Methods

4.2.1 Participants

Thirty two team sport players (male n=16; female n=16) participated in the study. Mean (±SD) height, body mass and age for male participants was 177.9 ± 6.2 cm, 80.1 ± 10.8 kg, 23.7 ± 3.4 years, respectively and for females was 165.4 ± 5.5 cm,

63.3 ± 5.8 kg, 21.9 ± 2.1 years, respectively. Volunteers completed a medical health screening questionnaire and were excluded from the study if they met any of the criteria detailed in Section 3.2. Following verbal and written briefings, written informed consent was provided by all participants. Ethics approval was granted by the Faculty of Health and Life Sciences Research Ethics Committee at Northumbria University.

4.2.2 Study design

The study utilised an independent group design that required participants to visit the laboratory on five separate occasions (Figure 4.1). Female participants were tested during the follicular phase of their menstrual cycle as in previous muscle damage investigations utilising females (Stupka *et al*., 2000; Dannecker *et al*., 2012, 2013). The first visit comprised familiarisation of all performance variables, following which participants were randomly but equally divided into four groups: Male Milk (MM), Male Carbohydrate (MC), Female Milk (FM) and Female Carbohydrate (FC). Groups were matched according to peak torque of the dominant leg knee extensors at 60°/s (Male 206.1 ± 48.9 v 206.4 ± 52.9 Nm, trivial; Female 148.7 ± 22.4 v 148.0 ± 28.6 Nm, trivial difference). Visits 2, 3, 4 and 5 were conducted on consecutive days no more than 7 days after familiarisation, with all visits taking place at the same time of day following an overnight fast and 24h abstinence from alcohol, caffeine and exercise. Following baseline measures (serum proteins, peak torque, countermovement jump, 20m sprint and muscle soreness), a bilateral eccentric protocol designed to induce muscle damage on the knee flexors was completed. Participants returned to the laboratory at 24h, 48h and 72h post muscle damaging exercise to repeat baseline measures. Participants were requested to refrain from strenuous activity for the duration of the study and from treating the symptoms of muscle damage and soreness with interventions such as nutritional supplements, massage, cryotherapy and non-steroidal anti-inflammatory drugs.

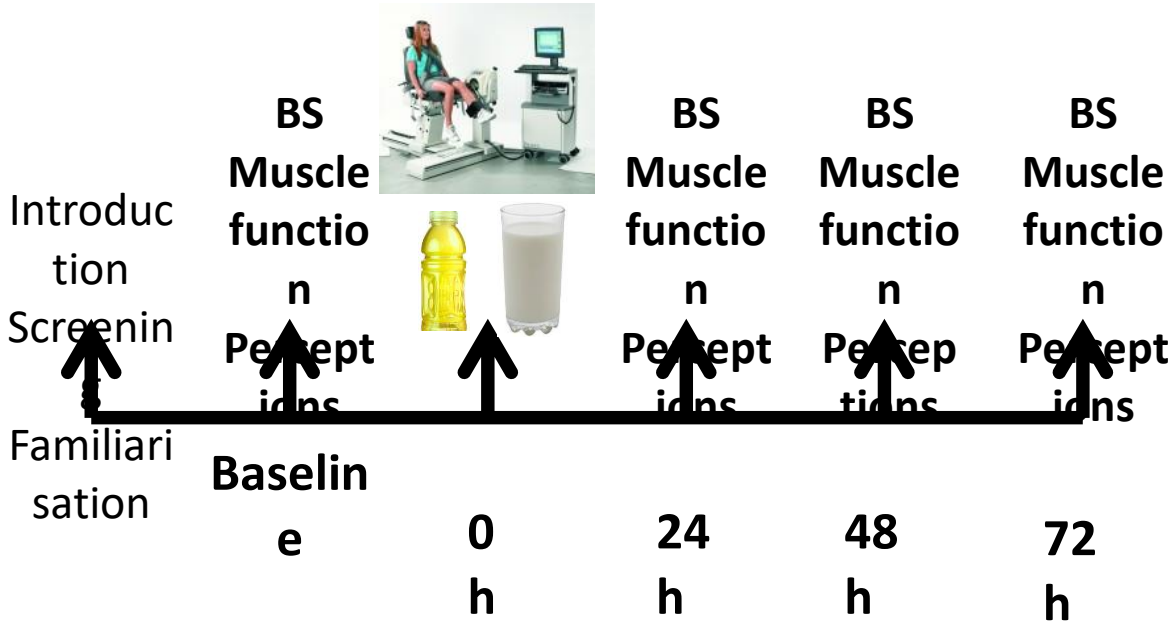

Figure 4. 1 Schematic of Study Design

4.2.3 Nutritional intervention and dietary control

Immediately following the eccentric exercise protocol on Visit 2, participants were provided with either 500ml of milk or 500ml of an energy-matched carbohydrate solution as described in Section 3.4. Food diary analysis revealed unclear outcomes for comparisons of carbohydrate, protein, fat or dairy intake between the male groups and between the female groups over the experimental period.

4.2.4 Muscle damaging exercise

Muscle damage was induced in the hamstrings of both legs through the completion of 6 sets of 10 repetitions of eccentric-concentric contractions at an angular velocity of 60°/s on an Biodex System 3 isokinetic dynamometer (Biodex Medical System, NY, USA), with 90s rest between sets and 4min between legs, during which time participants remained seated on the dynamometer, as previously described and shown to induce EIMD (Cockburn *et al*., 2008, 2010, 2012). Participants were instructed to apply maximal resistance to the lever arm during the eccentric phase of each repetition and to return their leg to the starting position with minimal effort during the concentric phase. Verbal encouragement was given throughout.

4.2.5 Peak torque

Participants completed 3 repetitions of bilateral maximal knee flexion at 60 and 180°/s. Refer to Section 3.9 for more detail.

4.2.6 Muscle soreness

Passive and active muscle soreness was measured on a visual analogue scale (VAS), as detailed in Section 3.6.

4.2.7 20m sprint

20m sprint time from was measured using timing gates as described in Section 3.13.

4.2.8 Countermovement jump height

Countermovement jump height was determined as described in Section 3.11.

4.2.9 Creatine kinase and skeletal troponin I

Skeletal troponin I and creatine kinase were determined from venous blood samples collected from a vein in the anti-cubital fossa using venepuncture. See Section 3.8.1 (CK) and Section 3.8.2 (sTnI) for details.

4.2.10 Statistical analysis

Data was analysed by making probabilistic magnitude-based inferences about the true value of outcomes. Please refer to Section 3.14 for details.

4.3 Results

4.3.1 Within-group effects

Analysis of within-group effects revealed post-exercise decreases in peak torque, CMJ and sprint performance, increases in serum CK, sTnI and in muscle soreness. Mean effects ± 90%CI, with qualitative inferences and magnitude of effect, are presented in Table 4.1.

Table 4. 1 Within-group effects over time for dependent variables

Variable	Timeframe	Mean effect, ± 90% CI or Factor effect x/÷ 90%CI[a]	Qualitative inference[b]
Peak Torque 60°/s Flexion			
MALE MILK	B-24	-7.9, ±8.37	Small decrease*
	B-48	-24.4, ±23.6	Moderate decrease**
	B-72	-18.3, ±26.3	Small decrease**
MALE CHO	B-24	-10.6, ±12.0	Small decrease**
	B-48	-31.6, ±21.2	Moderate decrease**
	B-72	-30.8, ±17.4	Large decrease***
FEMALE MILK	B-24	-10.6, ±9.0	Moderate decrease**
	B-48	-13.2, ±14.7	Moderate decrease**
	B-72	-12.2, ±16.5	Moderate decrease**
FEMALE CHO	B-24	-21.4, ±9.8	Moderate decrease***
	B-48	-37.0, ±14.1	Large decrease***
	B-72	-34.0, ±12.7	Large decrease***
Peak Torque 180°/s Flexion			
MALE MILK	B-24	-6.5, ±12.8	Small decrease*
	B-48	-18.6, ±20.3	Moderate decrease*
	B-72	-15.9, ±19.0	Moderate decrease**
MALE CHO	B-24	-17.0, ±16.7	Moderate decrease**
	B-48	-34.5, ±24.6	Large decrease**
	B-72	-25.9, ±21.0	Large decrease***
FEMALE MILK	B-24	-6.5, ±7.4	Moderate decrease*
	B-48	-4.5, ±16.8	Trivial decrease*
	B-72	-3.4, ±19.7	Unclear
FEMALE CHO	B-24	-12.6, ±7.4	Moderate decrease***
	B-48	-30.7, ±18.4	Moderate decrease***
	B-72	-27.5, ±12.2	Moderate decrease***
CMJ			
MALE MILK	B-24	-1.5, ±2.6	Trivial decrease*
	B-48	0.4, ±3.0	Trivial*
	B-72	1.8, ±2.2	Unclear
MALE CHO	B-24	-1.7, ±3.5	Trivial decrease*
	B-48	-6.7, ±11.8	Small decrease**
	B-72	-2.4, ± 6.0	Small decrease*
FEMALE MILK	B-24	-0.8, ±4.1	Trivial decrease*
	B-48	-0.9, ±3.8	Trivial decrease*
	B-72	0.3, ±4.0	Unclear
FEMALE CHO	B-24	-1.2, ±2.7	Trivial**
	B-48	-2.0, ±4.1	Trivial decrease*
	B-72	-2.5, ±3.5	Small decrease*
20m sprint			
MALE MILK	B-24	2.0, ±1.5	Small decrease**
	B-48	2.4, ±3.3	Moderate decrease**
	B-72	0.9, ±2.2	Small decrease*

Variable	Timeframe	Mean effect, ± 90% CI or Factor effect x/÷ 90%CI[a]	Qualitative inference[b]
MALE CHO	B-24	0.9, ±1.3	Small decrease*
	B-48	3.3, ±3.5	Moderate decrease**
	B-72	2.2, ±2.5	Small decrease**
FEMALE MILK	B-24	1.4, ±1.7	Small decrease**
	B-48	1.5, ±2.2	Small decrease*
	B-72	0.0, ±1.9	Trivial decrease*
FEMALE CHO	B-24	1.9, ±1.9	Small decrease**
	B-48	5.0, ±4.6	Large decrease**
	B-72	2.6, ±2.6	Moderate decrease**
Creatine Kinase			
MALE MILK	B-24	2.6 x/÷ 1.5	Moderate increase***
	B-48	4.1 x/÷ 3.1	Extremely large increase***
	B-72	10.9 x/÷ 4.5	Extremely large increase***
MALE CHO	B-24	3.2 x/÷ 1.6	Large increase****
	B-48	13.6 x/÷ 2.8	Extremely large increase****
	B-72	38.4 x/÷ 2.7	Extremely large increase****
FEMALE MILK	B-24	2.8 x/÷ 1.6	Very large increase****
	B-48	5.6 x/÷ 3.3	Extremely large increase***
	B-72	22.8 x/÷ 2.3	Extremely large increase****
FEMALE CHO	B-24	2.5 x/÷ 2.2	Moderate increase***
	B-48	9.6 x/÷ 4.3	Extremely large increase***
	B-72	47.8 x/÷ 3.7	Extremely large increase ****
sTnI			
MALE MILK	B-24	0.9 x/÷ 2.4	Unclear
	B-48	3.4 x/÷ 5.4	Extremely large increase**
	B-72	15.9 x/÷ 3.2	Extremely large increase****
MALE CHO	B-24	1.1 x/÷ 1.8	Unclear
	B-48	5.6 x/÷ 3.1	Extremely large increase***
	B-72	31.5 x/÷ 2.0	Extremely large increase****
FEMALE MILK	B-24	0.9 x/÷ 2.3	Unclear
	B-48	1.3 x/÷ 1.9	Very large increase*
	B-72	69.6 x/÷ 3.1	Extremely large increase****
FEMALE CHO	B-24	0.4 x/÷ 9.6	Unclear
	B-48	2.8 x/÷ 2.5	Very large increase***
	B-72	66.4 x/÷ 3.8	Extremely large increase****
Passive Soreness			
MALE MILK	B-24	2.0, ±0.6	Small increase****
	B-48	4.1, ±0.8	Moderate increase****
	B-72	3.3, ±1.0	Moderate increase****
MALE CHO	B-24	3.0, ± 0.6	Moderate increase****
	B-48	4.9, ±1.6	Moderate increase****
	B-72	4.9, ±0.9	Moderate increase****
FEMALE MILK	B-24	2.6, ±1.1	Small increase****
	B-48	4.4, ±1.4	Moderate increase****
	B-72	2.9, ±1.4	Smal increase***
FEMALE CHO	B-24	3.3, ±0.7	Moderate increase****
	B-48	6.0, ±0.9	Large increase****

Variable	Timeframe	Mean effect, ± 90% CI or Factor effect x/÷ 90%CI[a]	Qualitative inference[b]
	B-72	4.6, ±0.8	Moderate increase****
Active Soreness (Dom)			
MALE MILK	B-24	2.8, ±0.9	Small increase****
	B-48	4.6, ±1.3	Moderate increase***
	B-72	3.3, ±1.6	Moderate increase***
MALE CHO	B-24	3.5, ±1.1	Moderate increase****
	B-48	5.5, ±1.6	Large increase****
	B-72	5.5, ±1.3	Large increase****
FEMALE MILK	B-24	3.0, ±0.9	Moderate increase****
	B-48	5.1, ±1.5	Large increase****
	B-72	4.3, ±1.4	Moderate increase****
FEMALE CHO	B-24	4.6, ±1.7	Moderate increase****
	B-48	6.0, ±1.3	Large increase****
	B-72	5.0, ±1.6	Large increase****
Active Soreness (Non dom)			
MALE MILK	B-24	2.9, ±0.8	Small increase****
	B-48	4.6, ±0.9	Moderate increase****
	B-72	3.9, ±1.3	Moderate increase****
MALE CHO	B-24	3.6, ±0.9	Moderate increase****
	B-48	5.5, ±1.6	Large increase****
	B-72	5.1, ±1.0	Large increase****
FEMALE MILK	B-24	4.1, ±1.2	Moderate increase****
	B-48	5.4, ±1.7	Large increase****
	B-72	4.0, ±2.1	Moderate increase***
FEMALE CHO	B-24	4.6, ±1.5	Moderate increase****
	B-48	5.6, ±1.4	Large increase****
	B-72	5.5, ±0.9	Large increase****

[a]± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [b]Qualitative Inference represents the likelihood that the true value will have the observed magnitude
**Possible, **Likely, ***Very Likely, ****Most likely*

4.3.2 Between-group effects

Peak torque 60/°s dominant knee flexion

Immediately prior to the muscle damaging exercise, peak torque values for dominant leg flexion at 60/°s for MM and MC were 111.2 ± 25.2 and 122.5 ± 24.9 Nm, respectively. Corresponding values for females, FM and FC, were 77.7 ± 9.7 and 86.9 ± 19.5 Nm. Changes in peak torque at 60/°s for dominant knee flexion can be seen in Figure 4.2.

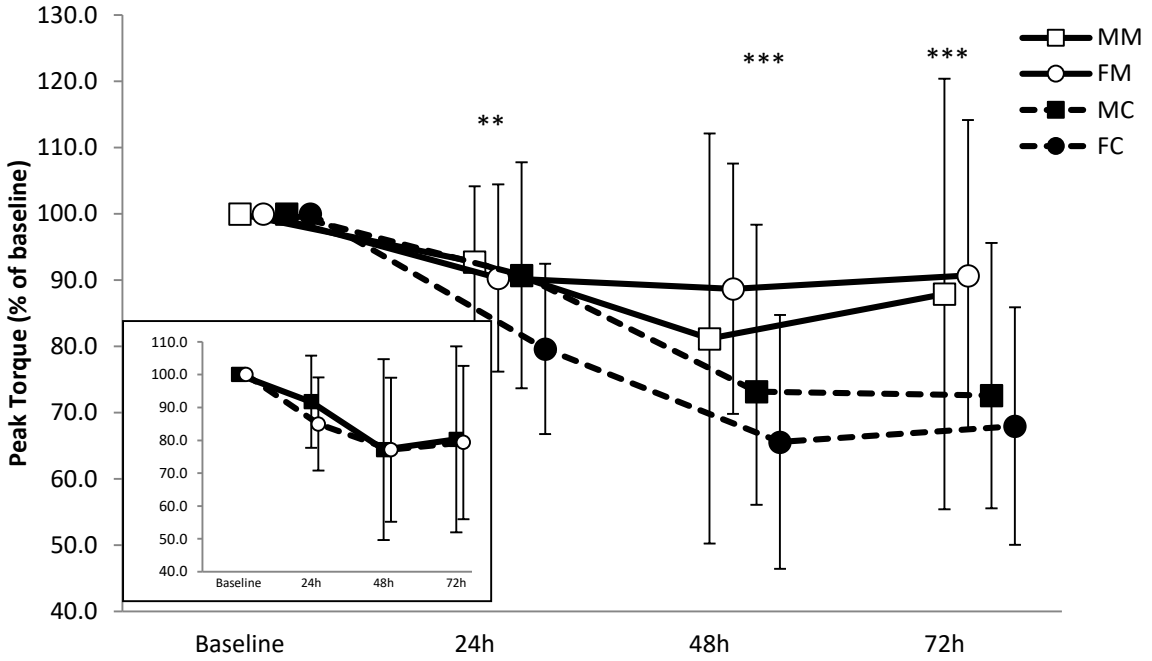

Figure 4. 2 Change in peak torque at 60/°s for dominant knee flexion in response to mechanical stress for MM, MC, FM and FC.

Inset shows Males (■) versus Females (○). Values are presented as means ± SD. **Likely benefit of milk (FM v FC). ***Very likely benefit of milk (FM v FC).

For males, changes in the peak torque of the dominant leg at 60°/s between baseline and 24h of those consuming milk and those consuming carbohydrate were -7.3 ± 11.4 % and -9.3 ± 17.1 %, respectively; an unclear outcome. For females, the changes in peak torque at this time point were -9.7 ± 14.1 and -20.4 ± 12.8 % for those consuming milk and those consuming carbohydrate; a likely large beneficial effect (ES=0.68) of milk consumption. Between baseline and 48h there was an unclear outcome for the consumption of milk for males, with changes in peak torque of -18.8 ± 30.9 % and -26.8 ± 25.2 % for MM and MC, respectively. For females, there was a very likely, very large benefit (ES=1.37) of consuming milk (-11.3 ± 18.9 %) compared to consuming carbohydrate (-34.4 ± 19.2 %). Changes in peak torque between baseline and 72h for males show an unclear outcome for the consumption of milk (-12.1 ± 32.5 % and -22.4 ± 23.0 % for MM and MC, respectively). A very likely large benefit of milk (ES=0.64) was seen for females (-9.3 ± 23.5 %) compared to consuming carbohydrate (-32.0 ± 17.9%). An additional likely large benefit (ES=0.69) of consuming milk compared to consuming carbohydrate was seen for females between 24h and 48h and from 24-72h.

Sex comparisons across all timepoints provided unclear outcomes except between baseline and 24h, where males had a possible small (ES=0.21) attenuated loss of force in comparison to females (-8.3 ± 14.1 % v -13.2 ± 12.8 %) and between 48h and 72h where males demonstrated a likely trivial enhanced recovery (3.1 ± 13.2 % v 2.2 ± 9.5 %).

Peak torque 180°/s dominant knee flexion

At baseline, the peak torque values at 180°/s for the dominant leg of males consuming milk and males consuming carbohydrate were 86.1 ± 17.5 Nm and 97.8 ± 14.4 Nm, respectively. The corresponding values for females consuming milk and females consuming carbohydrate were 57.4 ± 11.7 Nm and 65.1 ± 16.0 Nm. For males, changes in the peak torque of the dominant leg at 180°/s between baseline and 24h of those consuming milk and those consuming carbohydrate were -5.1 ± 16.2 %, and -14.7 ± 18.2 %, respectively; an unclear outcome. Changes in the peak torque of females consuming milk and females consuming carbohydrate were -5.3 ± 15.9 % and -12.0 ± 11.4 %, respectively, indicating a possible small beneficial effect (ES=0.33) for milk consumption. A comparison of MM v MC (-13.5 ± 27.9 % v -28.0 ± 29.8 %) from baseline-48h was unclear. A comparison of FM v FC (-1.8 ± 24.0 % v -27.2 ± 22.6 %) indicated a likely large beneficial effect (ES=1.23) for the consumption of milk. Between baseline and 72h there was an unclear outcome for the consumption of milk for males, with changes in peak torque of -10.7 ± 31.0 % and -21.0 ± 25.2 % for MM and MC, respectively. For females, there was a likely trivial benefit (ES=0.14) of consuming milk (12.5 ± 56.9 %) compared to consuming carbohydrate (-25.8 ± 16.9 %). An additional likely large benefit (ES=0.9) of consuming milk was seen for females from 24-48h (-1.6 ± 13.6 % v -14.1 ± 16.1%) and a very likely large benefit (ES=1.20) from 24-72h (0.4 ± 14.7 v -11.7 ± 15.1). Sex comparisons for all timepoints provided unclear outcomes, except between 24 and 48h, where a possible trivial positive effect was observed for F v M (-7.8 ± 15.8 v -14.6 ± 23.8).

A summary of the statistical analysis for peak torque of the dominant leg at 60/°s and 180/°s can be seen in Table 4.2.

Table 4. 2 Effects on decreases in peak torque of the dominant leg at 60°/s and 180°/s following mechanical stress

Comparison	Time frame	Peak torque 60 Mean effect[a] ± 90% CI[b]	Qualitative Inference[c]	Peak torque 180 Mean effect[a] ± 90% CI[b]	Qualitative Inference[c]
MM v MC	B – 24	3.1, ±14.2	Unclear	12.7, ±23.8	Unclear
	B – 48	10.5, ±42.2	Unclear	24.2, ±50.7	Unclear
	B – 72	18.0, ±41.4	Unclear	13.6, ±39.3	Unclear
FM v FC¥	B – 24	13.6, ±16.5	Large benefit**	7.0, ±14.2	Small benefit*
	B – 48	37.7, ±33.5	Very large benefit***	37.9, ±38.3	Large benefit**
	B – 72	33.0, ±32.1	Large benefit***	42.6, ±43.6	Small benefit**
M v F†	B – 24	6.7, ±8.0	Small benefit*	-1.2, ±8.8	Unclear
	B – 48	-1.1, ±2.4	Unclear	-6.3, ±15.5	Unclear
	B – 72	1.0, ±14.6	Unclear	-1.4, ±15.8	Unclear

*¥Large benefit** also at 24-48h (60°/s and 180°/s), 24-72h (60°/s and 180°/s); †Trivial effect** 48-72h (60°/s), Trivial effect* at 24-48 (180°/s)*

[a] Mean effect refers to the first group minus the second group

[b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference

[c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude

**Possible, **Likely, ***Very likely, ****Most likely*

Countermovement jump performance

Baseline countermovement jump heights prior to the damaging inducing exercise for males consuming milk and males consuming carbohydrate were 39.3 ± 3.9 cm and 40.1 ± 4.4 cm, respectively. The jump heights for females consuming milk and females consuming carbohydrate were 27.5 ± 2.4 cm and 29.8 ± 3.8 cm, respectively. At 24h post muscle damaging exercise a comparison of the changes from baseline for MM v MC (-1.5 ± 3.5 % v -1.6 ± 5.1 %) and FM v FC (-0.7 ± 6.0 % v 1.0 ± 6.2 %) were unclear. Similarly, at baseline-48h male (0.5 ± 4.2 % v -5.6 ± 13.8 %, MM v MC) and female (0.8 ± 5.7 % v -1.6 ± 9.5 %, FM v FC) comparisons were unclear. Between baseline and 72h, there was an unclear outcome for the consumption of milk for males with changes in countermovement jump height of 1.9 ± 4.7 % and -2.1 ± 8.9 % for MM and MC respectively, though a possible small benefit (ES=0.35) for the consumption of milk was found at 24-72h (3.4 ± 4.7 % v -0.4 ± 7.4 %, MM v MC). Between baseline and 72h, an unclear effect was seen for females consuming milk (0.4 ± 5.3 %) compared to females consuming carbohydrate (-2.1 ± 9.4 %). However, a possible small (ES=0.24) and trivial benefit (ES=0.17),

respectively, for milk in attenuating losses in countermovement jump height in females was seen following the consumption of milk at 24-72 h (1.1 ± 2.9 % v -1.1 ± 5.2 %, FM v FC) and 48-72h (1.2 ± 2.9 % v -0.5 ± 2.4 %, FM v FC), compared to the consumption of carbohydrate.

Sex comparisons indicated a very likely trivial effect for females versus males (females demonstrated a smaller reduction in performance) from baseline to 24h (-1.6 ± 4.2 % v -0.9 ± 5.9 %, M v F), and baseline-48h (-2.5 ± 10.4 % v -1.2 ± 7.6 %, M v F) and a most likely trivial effect from 24-48h (-1.0 ± 9.4 % v -0.3 ± 4.3 %, M v F). A likely trivial effect for males versus females was observed at baseline-72h (-0.1 ± 7.2 % v -0.8 ± 7.5%, M v F), 24-72h (1.5 ± 6.3 % v 0.0 ± 4.2 %, M v F) and 48-72h (2.5 ± 7.2 % v 0.4 ± 2.7 %, M v F). A summary of the statistical analysis for countermovement jump can be seen in Table 4.3.

20m sprint performance

Initial 20m sprint times, prior to muscle damaging exercise were 3.19 ± 0.11 s and 3.16 ± 0.11 s for males who consumed milk and males who consumed carbohydrate, respectively. Baseline 20m sprint times for females who consumed milk and females who consumed carbohydrate were 3.62 ± 0.12 s and 3.53 ± 0.13 s, respectively. Changes in 20m sprint performance can be seen in Figure 4.3.

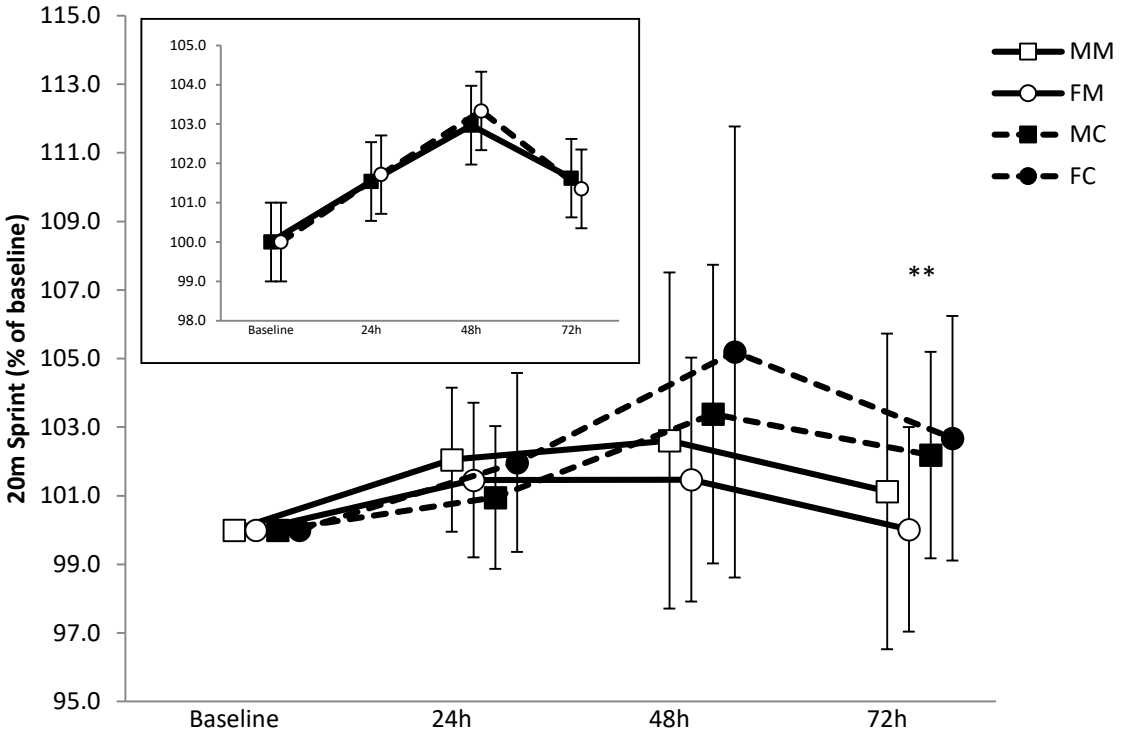

Figure 4. 3 Change in 20m sprint performance in response to mechanical stress for MM, MC, FM and FC.

Inset shows Males (■) versus Females (○). Values are presented as means ± SD. **Likely benefit of milk (FM v FC).

For males, changes in the 20m sprint time between baseline and 24h of those consuming milk and those consuming carbohydrate were 3.3 ± 2.1 % and 7.0 ± 1.9 %, respectively, with an unclear outcome. At this time there was also an unclear outcome for a comparison between females who consumed milk (1.5 ± 2.3 %) and females who consumed carbohydrate (2.0 ± 2.6 %). Changes in 20m sprint times between baseline and 48h for males (4.2 ± 4.9 % and 2.4 ± 38.6 % for MM and MC, respectively) and females (1.5 ± 3.6 % and 5.2 ± 6.6 % for FM and FC, respectively) were unclear. Between baseline and 72h, changes in the 20m sprint time of males consuming milk and males consuming carbohydrate were 3.3 ± 4.6 % and 3.0 ± 6.9 %, respectively; an unclear outcome. A likely moderate benefit (ES=0.65) for females consuming milk (0.0 ± 3.0 %) compared to females consuming carbohydrate (2.7 ± 3.6 %) was indicated over this timeframe. Furthermore, for males consuming milk, a likely moderate beneficial effect (ES=1.05) was seen between 24h and 72h. In addition, for females, a likely moderate and small benefit, respectively, for the consumption of milk in attenuating decreases in sprint performance was observed 24-48h (0.0 ± 3.1% v 3.2 ± 4.6 %, FM v FC, ES=0.82) and 24-72h (-1.4 ± 2.1 % v -0.7 ± 2.1 %, FM v FC, ES=0.54).

Male-female comparisons indicated a trivial effect (males demonstrated a smaller increase in sprint time) from baseline to 24h (1.5 ± 2.0 % v 1.7 ± 2.4 %, M v F). A likely negative effect for males compared to females (9.8 ± 27.6 % v 3.3 ± 5.5 %, M v F) and a possible negative effect for males compared to females (1.3 ± 5.9 % v -0.4 ± 2.3 %, M v F) was observed from baseline-48h and 24-72h, respectively; indicating that females demonstrated a smaller increase in sprint time. A possible trivial effect (2.8 ± 5.9 % v 1.3 ± 3.5 %, M v F) was seen from baseline-72h.

A summary of the statistical analysis for 20m sprint performance can be seen in Table 4.3.

Table 4. 3 Effects on decreases in countermovement jump (CMJ) and 20m sprint performance following mechanical stress

Comparison	Time frame	CMJ Mean effect[a], ± 90% CI[b]	Qualitative Inference[c]	20m Sprint Mean effect[a] ± 90% CI[b]	Qualitative Inference[c]
MM v MC¥	B – 24	0.2, ±4.1	Unclear	1.0, ±1.8	Unclear
	B – 48	7.7, ±13.0	Unclear	-9.3, ±16.0	Unclear
	B – 72	4.4, ±7.0	Unclear	-3.1, ±4.8	Unclear
FM v FC†	B – 24	0.4, ±5.4	Unclear	-0.5, ±2.1	Unclear
	B – 48	1.1, ±6.8	Unclear	-3.4, ±4.2	Unclear
	B – 72	2.8, ±6.9	Unclear	-2.6, ±2.8	Moderate benefit**
M v F‡	B – 24	-0.79, ±2.9	Trivial***	0.1, ±1.2	Trivial**
	B – 48	-1.3, ±2.8	Trivial***	-2.8, ±2.8	Negative**
	B – 72	0.7, ±4.1	Trivial**	-1.2, ±2.5	Trivial*

¥Small benefit 24-72h (CMJ) and moderate benefit** 24-72h (20m sprint); †Small benefit* 24-72h and trivial benefit* 48-72h (CMJ), moderate benefit** 24-24h and small benefit** 24-72h (20m sprint); ‡ possibly negative for males 24-72h (20m sprint)*

[a] Mean effect refers to the first group minus the second group

[b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference

[c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude

Muscle soreness

At 24h post muscle damaging exercise, all groups indicated an increase in passive muscle soreness and active muscle soreness of both the dominant and non-dominant legs. Soreness peaked at 48h for all. By 72h soreness had started to return towards baseline values. Changes in passive soreness can be seen in Figure 4.4.

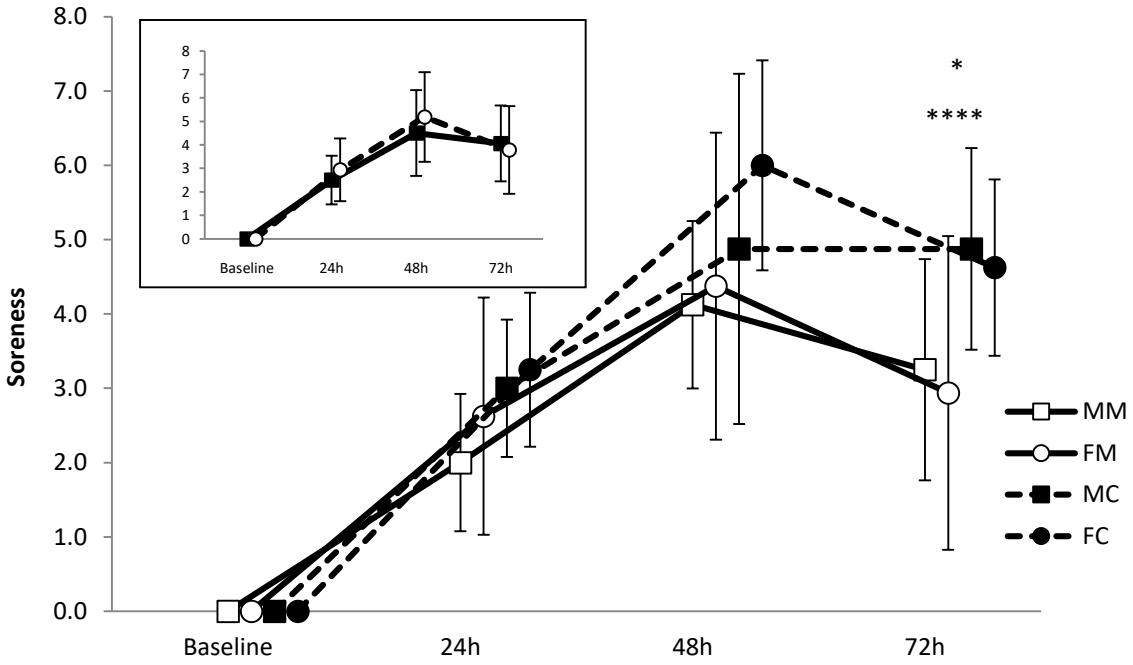

Figure 4. 4 Passive muscle soreness in response to mechanical stress for MM, MC, FM and FC.

Inset shows Males (■) versus Females (○). Values are presented as means ± SD. *Possible benefit of milk (MM v MC). ****Very likely benefit of milk (FM v FC).

Passive soreness

A comparison of changes in passive soreness for the period baseline-24h indicated a likely small benefit of milk for males compared to carbohydrate (20.0 ± 9.3 % v 30.0 ± 9.3 %). An unclear outcome was seen for females consuming milk compared to carbohydrate (26.3 ± 16.0 % v 32.5 ± 10.4 %).Changes in passive soreness from baseline-48h had an unclear outcome for males consuming milk compared to males consuming carbohydrate (41.3 ± 11.3 % v 48.8 ± 23.6 %), though a likely small benefit of milk was seen for females at this time (43.8 ± 20.7 v 60.0 ± 14.1 %). Between baseline and 72h there was a most likely moderate benefit of consuming milk compared to consuming carbohydrate for males (32.5 ± 14.9 % v 48.8 ± 13.6 %). A most likely moderate benefit of milk was observed for females consuming milk compared to females consuming carbohydrate (29.4 ± 21.1 % v 46.3 ± 11.9 %). All other comparisons were unclear.

Sex comparisons indicated unclear outcomes for baseline-24h, baseline-48h and baseline-72h. A most likely trivial positive effect for females (less soreness) and a

very likely small positive effect for females compared to males was observed at 24-72h (15.6 ± 10.9 % v 8.4 ± 20.0 %, M v F) and 48-72h (-4.4 ± 13.1 % v -14.1 ± 13.1 %, M v F).

Active muscle soreness

A comparison of increases in active soreness of the dominant leg from baseline-24h indicated an unclear outcome for males who consumed milk compared to males who consumed carbohydrate (27.5 ± 12.8 % v 35.0 ± 16.9 %). An unclear outcome was also observed for FM v FC (30.0 ± 14.1 % v 40.3 ± 25.6 %). At baseline-48h there were no clear differences between MM and MC (46.3 ± 20.0 % v 55.0 ± 23.3 %) or between FM and FC (51.3 ± 22.3 % v 60.0 ± 20.0 %). Changes in soreness from baseline-72h showed a very likely moderate benefit of milk for the MM group compared to the MC group (32.5 ± 23.8 % v 55.0 ± 19.3 %). A comparison of the soreness of the FM group to the FC group indicated a most likely large benefit of milk (42.5 ± 21.2 % v 50.0 ± 23.9 %). All other comparisons for both sexs were unclear. Sex comparisons at all timepoints were unclear.

Changes in active soreness of the non-dominant leg between baseline and 24h for milk versus carbohydrate for both males (28.8 ± 12.5 % v 36.3 ± 13.0 %) and females (40.6 ± 18.2 % v 46.3 ± 22.0 %) were unclear. From baseline-48h all comparisons were unclear: MM v MC (46.3 ± 13.0 % v 55.0 ± 23.3 %), FM v FC (54.4 ± 24.7 % v 56.3 ± 20.7 %).

Comparisons of changes in soreness from baseline-72h showed a most likely very large beneficial effect of milk for MM compared to MC (38.8 ± 18.9 % v 51.3 ± 14.6 %) and a very likely very large beneficial effect of milk for FM compared to FC (40.0 ± 31.2 % v 55.0 ± 14.1 %). All other comparisons were unclear.

Male-female comparisons provided unclear outcomes from baseline-48h, baseline-72h and 48-72h. A likely moderate negative effect for females (greater soreness) was observed from baseline-24h (32.5 ± 12.9 % v 43.4 ± 19.7 %m M v F). A likely moderate positive effect for females (less soreness) was indicated from 24-48h (18.1 ± 12.8 % v 11.9 ± 13.3 %, M v F) and from 24-72h (-5.6 ± 15.0 % v -7.8 ± 16.2 %, M v F).

A summary of the statistical analysis for passive and active soreness can be seen in Table 4.4.

Table 4. 4 Effects on increases in passive soreness, dominant and non-dominant leg active soreness following mechanical stress

Comparison	Time frame	Passive Soreness Mean effect[a] ± 90% CI[b]	Qualitative Inference[c]	AS DOM Mean effect[a] ± 90% CI[b]	Qualitative Inference[c]	AS NDOM Mean effect[a] ± 90% CI[b]	Qualitative Inference[c]
MM v MC	B – 24	-1.0, ±0.8	Small harm**	-0.8, ± 1.3	Unclear	-0.8, ±1.1	Unclear
	B – 48	-0.8, ±1.7	Unclear	-0.9, ± 1.9	Unclear	-0.9, ±1.7	Unclear
	B – 72	3.3, ±1.3	Moderate benefit*	3.3, ± 2.0	Moderate benefit***	4.3, ±1.5	Very large benefit****
FM v FC	B – 24	-0.6, ±1.2	Unclear	-1.6, ± 1.9	Unclear	-0.6, ±1.8	Unclear
	B – 48	-1.6, ±1.6	Small harm**	-0.9, ± 1.9	Unclear	-0.2, ±2.0	Unclear
	B – 72	4.3, ±1.5	Moderate benefit****	5.3, ± 1.6	Large benefit****	-4.5, ±2.3	Large benefit***
M v F†	B – 24	-0.4, ± 0.7	Unclear	-0.7, ± 1.1	Unclear	-1.0, ±1.0	Small harm**
	B – 48	-0.7, ±1.1	Unclear	-0.5, ± 1.2	Unclear	-0.4, ±1.1	Unclear
	B – 72	0.7, ±1.0	Unclear	-0.2, ± 1.3	Unclear	-0.3, ±1.2	Unclear

*†PS :Trivial effect**** for females 24-72h, small effect*** for females 48-72h; AS NDOM trivial effect** 24-48h, 24-72h; AS DOM = Active Soreness Dominant leg; AS NDOM = Active Soreness Non-Dominant leg*

[a] Mean effect refers to the first group minus the second group

[b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference

[c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude

**Possible, **Likely, ***Very Likely, ****Most likely*

Skeletal Troponin I

Immediately prior to the muscle damaging exercise, serum sTnI values for MM and MC were 783.2 ± 1327.0 pg/ml and 606.6 ± 552.3 pg/ml, respectively. The baseline values for females who consumed milk and females who consumed carbohydrate were 384.5 ± 145.7 and 464.8 ± 299.9 pg/ml, respectively. Percentage change for all groups can be seen in Figure 4.5.

From baseline-24h, a very likely trivial effect (ES=0.18) was observed for MM v MC (0.87 x/÷ 3.27 v 1.07 x/÷ 2.26) and a likely small trivial effect (ES=0.2) for FM v FC (0.86 x/÷ 3.44 v 0.52 x/÷ 3.61). Between baseline and 48h, the outcome was likely trivial (ES=5.90) for MM v MC (3.20 x/÷ 7.81 v 5.40 x/÷ 3.91) and FM v FC (1.34 x/÷ 3.00 v 2.77 x/÷ 2.75, ES=0.56). A likely trivial effect was also observed for MM v MC (15.93 x/÷ 5.53 v 31.50 x/÷ 3.81, ES=0.54) and for FM v FC (27.79 x/÷ 4.39 v 54.55 x/÷ 3.05, ES=7.32) at B-72h. For males, a trivial effect for carbohydrate was seen 24-48h in comparison to milk (3.02 x/÷ 5.17 v 4.97 x/÷ 2.66, MM v MC, ES=5.66) and a trivial effect was observed 24-72h (18.29 x/÷ 3.98 v 29.38 x/÷ 4.3, MM v MC, ES=6.59) . For females, a very likely trivial effect was seen at 48-72h (13.06 x/÷ 2.19 v 18.84 x/÷ 1.54, ES=7.48).

Sex comparisons indicated changes in sTnI values were trivial.

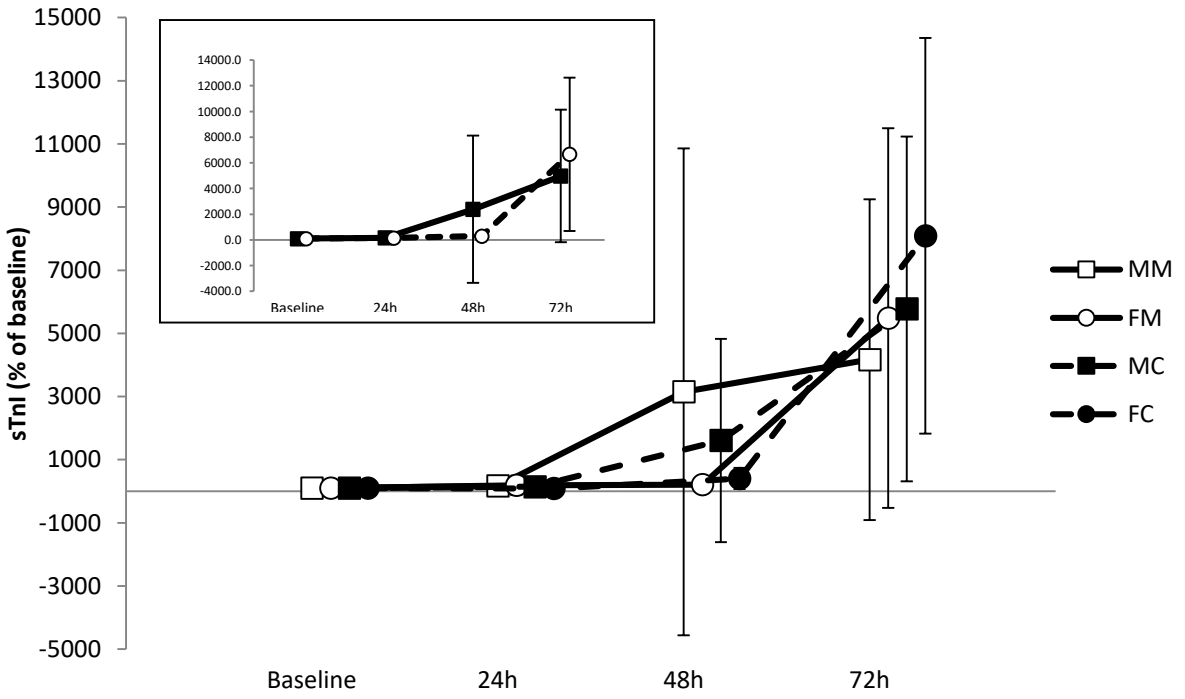

Figure 4. 5 Serum sTnI values in response to mechanical stress for MM, MC, FM and FC.

Inset shows Males (■) versus Females (○). Values are presented as means ± SD.

Creatine kinase

Serum creatine kinase values prior to muscle damaging exercise were 359.5 ± 449.6 U/l and 268.4 ± 346.9 U/l for males who consumed milk and males who consumed carbohydrate, respectively. Baseline CK values for females who consumed milk and females who consumed carbohydrate were 129.3 ± 92.2 U/l and 185.5 ± 176.6 U/l, respectively.

For males, increases in CK between baseline and 24h for those consuming milk and those consuming carbohydrate were 2.25 x/÷ 1.84 and 3.15 x/÷ 1.86, respectively; a most likely trivial outcome (ES=0.41). For females, the increase in CK at this time point was 2.80 x/÷ 2.61 and 2.54 x/÷ 2.27 for those consuming milk and those consuming carbohydrate; a very likely trivial outcome (ES=0.29). Between baseline and 48h, the changes in CK of 4.08 x/÷ 4.85 v 13.60 x/÷ 5.3 for MM and MC, respectively; a possible moderate benefit for milk (ES=1.08). For females, there was a likely trivial effect (ES-4.41) of consuming milk (5.63 x/÷ 8.87) compared to consuming carbohydrate (9.62 x/÷ 6.38). The attenuated increase in CK observed in MM v MC (10.91 x/÷ 7.52 v 38.41 x/÷ 5.85) from baseline to 72h was a possible very large benefit of milk (ES=4.78). A trivial outcome was observed for FM v FC (22.81 x/÷ 11.94 v 47.82 x/÷ 5.13, ES=6.30) at this timepoint.

For males, likely trivial effect was indicated from 24-48h (1.81 x/÷ 3.00 v 4.31 x/÷ 4.49, MM v MC) and from 24-72h (4.85 x/÷ 5.01 v 12.18 x/÷ 5.95, MM v MC). A most likely trivial effect for milk was observed from 48-72h (2.68 x/÷ 2.32 v 2.83 x/÷ 1.85 %, MM v MC). For females, a likely trivial effect was seen at 24-48h (2.01 x/÷ 4.13 v 3.72 x/÷ 4.78, FM v FC). A possible benefit for milk was observed at 24-72h (8.15 x/÷ 5.43 v 24.99 x/÷ 3.82 FM v FC) and a most likely trivial effect for milk was seen from 48-72h (4.05 x/÷ 2.57 v 5.65 x/÷ 1.53).

All sex comparisons for all timepoints were trivial.

Table 4. 5 Effects on increases in sTnI and CK following mechanical stress

Comparison	Time frame	sTnI Mean effect[a] x/÷ 90% CI[b]	Qualitative Inference[c]	CK Mean effect[a] x/÷ 90% CI[b]	Qualitative Inference[c]
MM v MC	B – 24	0.8 x/÷ 2.5	Trivial***	0.7 x/÷ 1.7	Trivial****
	B – 48	0.6 x/÷ 5.4	Trivial**	0.3 x/÷ 4.2	Moderate benefit*
	B – 72	0.5 x/÷ 3.9	Trivial**	0.3 x/÷ 5.4	Very large benefit*
FM v FC	B – 24	1.7 x/÷ 4.8	Trivial**	1.1 x/÷ 2.5	Trivial***
	B – 48	0.5 x/÷ 2.8	Trivial**	0.6 x/÷ 7.0	Trivial**
	B – 72	0.5 x/÷ 4.3	Trivial**	0.5 x/÷ 7.2	Trivial*
M v F	B – 24	1.0 x/÷ 2.6	Trivial****	0.9 x/÷ 1.7	Most likely trivial
	B – 48	6.9 x/÷ 3.4	Trivial**	0.9 x/÷ 3.6	Likely trivial
	B – 72	0.9 x/÷ 1.9	Trivial**	0.8 x/÷ 2.7	Likely trivial

[a] Mean effect refers to the first group minus the second group
[b] x/÷ 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference
[c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude
**Possible, **Likely, ***Very likely, ***Most likely*

4.4 Discussion

The results of this study indicate that the consumption of 500ml of milk post muscle-damaging exercise can attenuate decreases in peak torque and 20m sprint performance and increases in passive and active muscle soreness in female team sport athletes, compared to the consumption of a volume and energy matched carbohydrate drink. Milk consumption had trivial effects on sTnI, and a benefit on CK was only seen for males from B-48h and B-72h. Compared to carbohydrate, consumption of milk by males only had a minimal positive effect on muscle function, though soreness and markers of membrane damage and muscle damage were reduced. Overall, sex comparisons provided many unclear outcomes. However, female participants showed smaller increases in sprint time, passive soreness, active soreness (non-dominant leg) and sTnI values.

For females, the consumption of milk post-muscle damaging exercise was beneficial in attenuating the loss of peak torque over the first 24, 48h and 72h at 60°/s and 180°/s. Milk consumption was also shown to be beneficial in attenuating reductions in 20m sprint performance and, to a lesser extent, countermovement jump performance. The benefit of milk for females is in agreement with previous research

with male athletes (Cockburn *et al*., 2008) and other studies that have looked at the effect of carbohydrate-protein supplements post damaging exercise (Samadi *et al.,* 2012; Baty *et al.,* 2007; Bird *et al*., 2006). Performance of a single bout of resistance training exercise results in a 50-100% increase in muscle protein synthesis which remains elevated for up to 48h post exercise (Phillips *et al*., 1997; Chelsey *et al*., 1992). Previous research has indicated that the consumption of amino acids following resistance training stimulates protein synthesis resulting in increased net protein synthesis (Tipton *et al*., 2004; Miller *et al*., 2003; Børsheim *et al*., 2000). Consuming amino acids and carbohydrate together has been shown to result in an increased rate of protein synthesis that is greater than either nutrient alone (Børsheim *et al*., 2004; Miller *et al*., 2003), though recent studies have not found this (Glynn *et al*., 2013; Staples *et al*., 2011; Greenhaff *et al*., 2008), especially if protein intake is sufficient to stimulate muscle protein synthesis maximally (Staples *et al*., 2011). However, knowing that the consumption of milk results in an increase in insulin (Gannon *et al.,* 1986) and provides protein, milk may be a suitable beverage for stimulating net protein synthesis and preventing protein breakdown. In support of this, the consumption of milk post-resistance training has been shown to result in an increase in amino acid availability, indicative of net muscle protein synthesis (Wilkinson *et a*l., 2007; Elliot *et al*., 2006), which may have been a factor in the current study resulting in the observed attenuation of muscle function losses following eccentric exercise. The provision of amino acids early in the post-exercise period may have enhanced protein synthesis rates, which reach peak values within 2-3h after exercise (van Loon, 2014).

The effect of milk on recovery of muscle function post-eccentric exercise in males was mostly unclear. This may have been due to the amount of protein in the volume of milk provided. The amount of protein provided by the 500ml of milk was 17g. This resulted in a slightly greater intake per kg of body mass for the female participants (0.27g/kg) compared to the male participants (0.22g/kg). The lower relative intake of protein may have been insufficient to maximally stimulate protein synthesis and attenuate muscle function losses, with an overall intake of 17g being close to sufficient for females but suboptimal for males. Previous research has shown that 20g of intact protein is sufficient to maximally stimulate muscle protein synthesis after resistance exercise (Moore *et al*., 2009). Perhaps a greater intake of milk (and thus protein) would have resulted in more positive effects for males. Furthermore, knowing

the positive effect of whey protein on muscle protein synthesis (Tipton *et al*., 2004), and the effect of leucine on protein synthesis via activation of the mTOR pathway (Norton and Layman, 2006), it is possible that 500ml of milk did not provide enough whey protein or leucine to facilitate maximal recovery, especially for male participants.

The consumption of 500ml of milk post-exercise was beneficial in reducing passive soreness in both males (B-24h, B-72h) and females (B-72h). This is in contrast to previous studies (Cockburn *et al*., 2013; 2010; 2008), but in agreement with Cockburn *et al.,* (2012) who reported a positive effect of milk consumption on passive soreness between baseline and 48h following EIMD. A benefit of milk in blunting increases in active soreness for both dominant and non-dominant legs (B-72h) was seen for males, an effect reported by Cockburn *et al.,* (2010), with a similar response for females. The exact mechanism of delayed onset muscle soreness has not been elucidated, though soreness is associated with a series of events following mechanical disruption of the muscle fibres. Following the initial muscle insult there is an increase in intracellular calcium which stimulates degradative pathways resulting in further disruption to the myofibrils (Armstrong, 1990). A subsequent inflammatory response, along with oedema, results in nerve ending potentiation (Lewis *et al*., 2012), which correlates with increased soreness (Proske and Allen, 2005). In the current study, the consumption of milk may have resulted in less muscle damage and thus a reduced inflammatory response and attenuation in soreness. More recently, the role of bradykinin and nerve growth factor have been investigated in relation to muscle soreness (Murase *et al*., 2010), though any effect of milk on this mechanism is unknown.

In the current study, the consumption of milk post exercise had a trivial effect on sTnI for males and females. Despite sTnI being skeletal muscle specific and its early susceptibility to proteolysis making it ideal for early detection of exercise-induced muscle damage (Sorichter *et al*., 2001), few studies have used sTnI as a muscle damage marker. Similar to previous research that found attenuated CK increases with the consumption of carbohydrate-protein solutions (Valentine *et al*., 2008; Baty *et al.,* 2007; Seifert *et al*., 2005; Saunders *et al*., 2004; 2007), the results of this study indicated a beneficial effect of milk for males in attenuating CK increases.

However, for female participants, a benefit of milk was only seen 24-72h and 48-72h, indicating a different pattern in the appearance of CK in the blood.

sTnI and CK showed similar time course changes, with the highest values observed 72h post-muscle insult. The delayed appearance in the bloodstream is probably due to the time taken for the proteins to enter the blood stream via the lymphatic vessels (Lindena *et al*., 1979) and secondary damage as a consequence of calcium increases (Sorichter *et al*., 1997; Armstrong, 1990). It is possible that the consumption of milk reduced myofibrillar protein disruption via the ubiquitin-proteosome system, resulting in a reduced accumulation of CK in the serum for males. Furthermore, milk intake may have attenuated lysosomal breakdown of the non-myofibrillar proteins of the muscle membrane, reducing the leakage of CK into the serum. Still, it is important to note that in all groups CK at 72h was very high, with values for MM, MC and FC greater than 10000 IU/l, indicating significant membrane damage and possible rhabdomyolysis (Kokko, 2000). To this regard, interpreting the effect of milk as being possibly beneficial may be misleading. However, no participant reported visible haematuria and it is possible that severe eccentric exercise can result in significant increases in CK without renal compromise (Clarkson *et al.,* 2006). Further investigation is required to determine why the CK response was different in FM v FC compared to MM v MC.

In agreement with the results of this study, it has been previously suggested that females may experience less muscle damage than males following eccentric exercise perhaps because of a protective effect of oestrogen (Stupka and Tiidus, 2001; Koumulainen *et al.,* 1999; Bar *et al.,* 1988; Amelink and Bar, 1986). This may be because oestrogen can act as an antioxidant (Tiidus *et al.,* 1995, 2003), enhance membrane stability (Kendall and Eston, 2002; Wiseman and Quinn, 1994), increase the expression of heat shock proteins involved in protein assembly and maintenance of protein structure (Bombardier *et al.,* 2009; Milne and Noble, 2008), by increasing nitric oxide synthase (Tiidus *et al.,* 2011) stabilising calcium channels (Murphy and Steenbergen, 2007) or by activating satellite cells (Enns and Tiidus, 2008). Research has shown that female animals demonstrate less muscle damage than males (Tiidus, 2001; Tiidus *et al.,* 2001). However, investigations with human participants have reported equivocal findings. The results of the current study indicate that females experienced a greater rate of recovery across most variables, suggesting that

perhaps sex differences lie in the secondary phase of eccentric muscle injury (Armstrong, 1990).

While a number of studies have indicated no sex differences in loss of muscle force production following eccentric exercise (Power *et al.,* 2010; Hubal *et al.,* 2008; Kerksick *et al.,* 2008; Sayers and Clarkson, 2001; MacIntyre *et al*., 2000; Rinard *et al*., 2000), the results of this study show greater losses of force production at 60°/s from baseline to 24h in females compared to males. This is similar to that reported by Sewright *et al.,* (2008) though in that investigation the sex difference was only observed immediately after the muscle insult and not for the subsequent ten days of the experimental period. In the current study, from 24-48h there were no differences in the pattern of force recovery at 60°/s in males and females, though males recovered more quickly from 48-72h. Previous studies have observed an overall faster recovery both in males (Power *et al*., 2013; Borsa and Sauers, 2000) and in females (Joyce *et al*., 2014; Sayers and Clarkson, 2001; MacIntyre *et al*., 2000) following eccentric exercise, perhaps reflecting different modes of exercise for inducing damage and different recovery period durations.

Sex comparisons of muscle soreness were mostly unclear. Though female participants had slightly higher soreness ratings throughout, they showed a faster rate of recovery from 24-72h and 48-72h for passive soreness and 24-48h and 24-72h for active soreness of the non-dominant leg. Recent reviews have not been in agreement on whether sex differences exist in the experience of pain (Racine *et al*., 2012; Le Resche, 2011). It has been suggested that oestrogen may have an effect on the perception of pain and level of muscle soreness experienced following damaging exercise by playing a role in the binding of opioids to receptors in the brain and spinal cord, thereby modifying the transmission of pain (Kendall and Eston, 2002). Hoeger *et al*., (2008) reported greater muscular pain in females following isometric exercise, though there are a number of investigations that have found that females report lower soreness following eccentric exercise (Kerksick *et al*., 2008; Dannecker *et al*., 2003; 2012). Nevertheless, the majority of studies have reported no sex differences in pain intensity or pain unpleasantness following eccentric exercise (Sewright *et al.,* 2008; Dannecker *et al*., 2005; 2008; Nie *et al*., 2005; Poudevigne *et al.,* 2002; Rinard *et al*., 2000). Methodological variances in these studies (intensity of

eccentric contractions, soreness measurement, participant characteristics) or other influencing variables may account for lack of concurrence.

Both resting CK and CK concentration post-eccentric exercise were higher in males than females, though there were no differences in the rates of recovery. Higher resting reference intervals in males have been described (Mougios, 2007) and higher post-eccentric exercise CK values in males have been reported in previous research (Fernandez Gonzalo *et al*., 2014; Joyce *et al*., 2014; Sewright *et al*., 2008; Webber *et al*., 1989). This contrasts with studies that have found no sex difference in CK (Sorichter *et al.,* 2001; Stupka *et al.,* 2000) or higher values in females (Miles *et al.,* 1994). It is likely that higher values in males may be because of larger muscle mass (Mastaloudis *et al*., 2006).

Similar to Sorichter *et al*., (2001) absolute sTnI values, both prior to and post-damaging exercise, were higher in males than females. However, unlike Sorichter *et al*., (2001) who reported no sex differences in the relative sTnI increases compared to baseline, analysis of the data in the current study found a different pattern of sTnI accumulation in males and females. The increases in sTnI in males occurred at 24h, while the increase in females occurred at 48h, though there was no difference in the overall increase from baseline-72h.

Prior experience of eccentric exercise may have been an influencing factor. While all participants were regularly training for their team sports, details of amount and intensity of resistance training were not recorded. Though the exercise protocol used in this study was novel in terms of the intensity and amount of eccentric exercise performed, prior exposure to eccentric exercise as part of resistance training may have provided a protective effect, resulting in less damage (McHugh, 2003).
The results of this study demonstrate considerable variability in the response of individuals to the muscle damaging exercise, which affects conclusions that can be made. Statistical analysis provided many unclear outcomes despite large differences in the means. A larger number of participants in each group could have provided more certain results. Similar variability has been reported frequently in muscle damage investigations. It has been suggested that susceptibility of some individuals to muscle disruption may be as a result of genetic vulnerability (Baird *et al*., 2012). Individuals with specific genome variations may experience greater muscle

disruption, calcium handling alterations and increased inflammation (Yamin *et al*., 2008; Devaney *et al*., 2007; Clarkson *et al*., 2005). In addition, observed variability in the current study may be as a result of variability in the uptake of amino acids in the milk groups. Previous research has reported variability in response to nutrient ingestion following exercise (Børsheim *et al*., 2004; Tipton *et al*., 2004; Miller *et al.,* 2003) which may be due to natural variability in the partitioning of amino acids between splanchnic and peripheral tissues (Fouillet *et al*., 2002; Bos *et al*., 1999, 2003). Furthermore, the variability in the response to the eccentric exercise protocol may be reflective of the level of fatigue experienced during the protocol (Hody *et al.,* 2013). Recently, Trost *et al*., (2011) reported that following the induction of DOMS, pain-related fear predicted reduced maximal strength production and individual decrements in strength performance. A fear of pain or reinjury in high-fear participants following EIMD may explain some of the variance observed in the current study, though further study in this area, including possible sex differences, is needed before conclusions could be drawn.

The recovery period of 72h in this study was not sufficient for participants to fully recover. A monitored recovery period of 96h or longer may have been adequate for the observation of full recovery or differences between the groups. Moreover, as CK generally peaks at 96h post EIMD (Clarkson and Hubal, 2002), it is likely that peak CK and possibly peak sTNI values, were not seen and group differences may have been noted at this time point. Future investigations should allow for this.
In conclusion, the consumption of 500ml of milk post-eccentric exercise can limit decrements in muscle function and increases in muscle soreness in female athletes, thus hastening recovery, and from a practical perspective, potentially maximising subsequent training and performance. For male athletes, milk was beneficial in attenuating increases in muscle soreness and CK. Sex comparisons showed smaller increases in sprint time, passive soreness, active soreness (non-dominant leg) and sTnI values in females, reinforcing the likelihood that oestrogen may offer a protective effect for females post-eccentric exercise, though further investigation is warranted in this area.

Chapter 5: The Effect of Milk on Recovery From Repeat Sprint Cycling

5. 1 Introduction

Participation in exercise is known to result in both mechanical and metabolic perturbations. Mechanical stress, primarily consequential to eccentric muscle actions, results in morphological changes in the muscle (Lauritzen *et al*., 2009), with early damage occurring immediately following the muscle insult (Friden *et al*., 1983) and further damage evident 1-3 days after the exercise stress (Clarkson and Sayers, 1999). Isolated mechanical stress was the focus of the first study of this thesis. Metabolic stess is evident by increases in metabolic flux through glycolytic and oxidative pathways and increases in blood lactate, inflammation, oxidative stress, aswell as markers of oxidative damage in the post-exercise period (Powers *et al*., 2011). Such stresses lead to a subsequent decline in muscle function and increases in muscle soreness which hinders the ability to perform exercise. In order to facilitate a faster recovery and maintain subsequent training volumes, intensity and performance, athletes employ a range of recovery strategies (Howatson and van Someren, 2008).

Nutritional interventions are one such strategy and there is evidence that the consumption of milk following eccentric exercise can attenuate decreases in muscle function in males (Cockburn *et al*., 2012; 2010; 2008) and females (Rankin *et al.,* 2015, Chapter 4) and limit increases in muscle soreness and creatine kinase, perhaps by enhancing protein synthesis post-exercise or limiting protein degradation. However, these investigations employed an eccentric exercise protocol on an isolated muscle group resulting in greater mechanical stress and less metabolic stress than is normally observed following exercise participation (Silva *et al*., 2013). Metabolic stress leads to diverse physiological processes and may lead to different effects on muscle performance and markers of muscle damage. No studies have examined the effects of milk on recovery from exercise that primarily induces a metabolic rather than mechanical stress.

Milk is a source of carbohydrate and anti-oxidants including Vitamin E, Vitamin A and glutathione (Haug *et al*., 2007) which are effective in reducing lipid peroxidation (Finaud *et al*., 2006). Moreover, milk is a source of whey protein that contains cysteine and methionine amino acids which are necessary for glutathione (GSH) production, thus enhancing the anti-oxidant defence system (Madureira *et al*., 2007; Elia *et al.,* 2006; Marshall, 2004). Previous animal research concluded that

supplementation with whey protein over a six week period prevented oxidative stress induced by heavy exercise (Elia *et al.,* 2006). Still, these studies have examined long term supplementation with whey and not a single post-exercise intake nor its impact on muscle function or soreness.

In addition, unlike many other carbohydrate-protein recovery drinks, milk is an excellent source of casein. It has been suggested that milk's antioxidant capacity comes mainly from its casein fractions (Zulueta *et al*., 2009) due to the high amounts of antioxidant amino acids, including tyrosine, tryptophan, histidine, lysine and methionine present in casein (Rival *et al.,* 2001). However, no studies have specifically examined casein's effects on oxidative stress following exercise. Some previous research has shown that carbohydrate or carbohydrate-protein mixtures do not reduce exercise-induced oxidative stress following endurance exercise (Goldfarb *et al.,* 2009; McAnulty *et al*., 2003; Karolkiewicz *et al*., 2001). However, McAnulty *et al.,* (2007) reported lower oxidative stress when carbohydrate was consumed during both continuous cycling and cycling with intermittent rest periods. Furthermore, Kerasioti *et al*., (2012) reported that the consumption of a carbohydrate-protein cake immediately post-exercise (2h steady-state cycling) and at three further 1-hour intervals before a second exercise bout, reduced lipid peroxidation. The disparate results from these investigations may be because of different exercise modes and varied methods for the determination of oxidative stress and oxidative damage. Furthermore, these investigations focused on immediate recovery (1-24h), and did not investigate the effect of the observed stress on subsequent muscle function and soreness, which is of significance to the training athlete.

Participation in a team sport results in muscle damage, inflammation and oxidative stress (Silva *et al.,* 2013; Marin *et al*., 2011; Fatouros *et al.,* 2010; Ascensão *et al*., 2008) and a subsequent decline in performance (Silva *et al.,* 2013). As previous research has identified milk as being beneficial for recovery from isolated eccentric exercise, investigating the effect of milk on recovery from metabolic stress may provide insight into the mechanisms by which milk might be beneficial for team sport athletes, and also offer an understanding of the relationships between metabolic stress, oxidative stress, inflammation and performance.

Therefore, the aim of this study is to investigate the effect of milk on recovery from metabolic stress in team sport participants utilising a protocol designed to simulate the isolated metabolic demands of team sport.

5.2 Methods

5.2.1 Participants

Ten female team sport (camogie and ladies Gaelic football) athletes (mean age 22.1 ± 1.8 y) were recruited to take part in this study. Mean (±SD) height and mass was 162.7 ± 9.0 cm and 61.9 ± 8.1 kg, respectively. All participants were screened according to the details in Section 3.2. Participants were provided with verbal and written briefings, following which written informed consent was recorded. Ethics Approval was provided by the London Sport Institute Ethics Sub-Committee at Middlesex University and the Institute of Technology Carlow, where the data collection took place.

5.2.2 Study design

The study utilised a randomised cross-over design, with participants attending a familiarisation session plus two blocks of four days of testing. All participants completed the trials during two consecutive follicular phases of their menstrual cycle as in previous investigations utilising female participants (Rankin *et al*., 2015; Dannecker *et al*., 2013). All testing took place at the same time of day following an overnight fast and 24h abstinence from alcohol, caffeine and exercise. Briefly, the first laboratory visits comprised familiarisation with initial measurements of muscle function (peak torque, rate of force development (RFD), countermovement jump height (CMJ), 20m sprint time), and familiarisation with an intermittent cycling protocol (standardised warm-up, five 2-min blocks of the protocol and one 15s maximal sprint) following which participants were randomly assigned to their group order.

Between 3 and 5 days later, participants completed baseline measures of muscle function, soreness and provided a blood sample for analysis of muscle damage (CK), inflammation (hsCRP) and oxidative stress (Protein Carbonyls and GSH:GSSG ratio). This was followed by completion of an intermittent cycling protocol lasting

approximately 60min designed to induce metabolic stress. Immediately upon completion of the protocol participants consumed 500ml of milk (MILK) or 500ml of an energy-matched carbohydrate drink (CHO) and were instructed not to consume any other fluid or food for a period of two hours. Participants returned to the laboratory to repeat the baseline measures at 2h (blood sample only), 24h, 48h and 72h post-exercise (Figure 5.1). Participants were requested to refrain from any other strenuous activity for the duration of the study, and from treating the symptoms of muscle soreness and tiredness with interventions such as massage, cryotherapy, nutritional supplements and non-steroidal anti-inflammatory drugs.

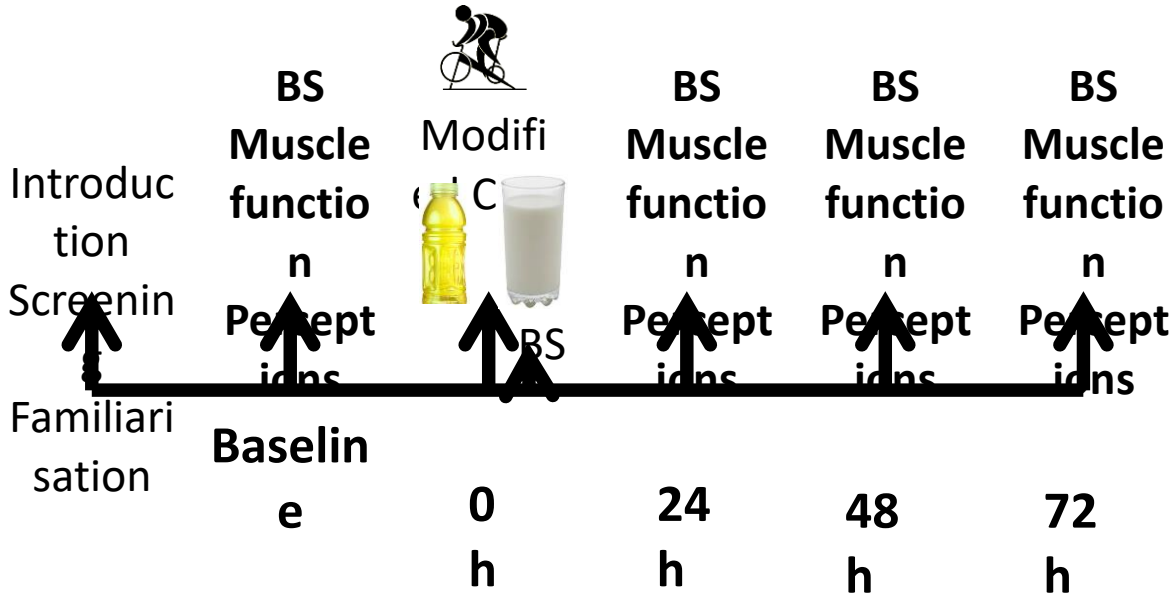

Figure 5. 1 Schematic of the Study Design

5.2.3 Nutritional intervention and dietary control

Immediately following the cycling protocol, participants were provided with either 500ml of milk or 500ml of an energy-matched carbohydrate solution as detailed in Section 3.4. All participants completed a food diary for 24h prior to baseline testing and for the subsequent 3 days and were asked to repeat the same diet for the second block of testing. MBI analysis of participants' food diaries indicated that there were no differences in energy, protein or fat intake between trials. The MILK group had a likely higher mean carbohydrate intake over the experimental period.

5.2.4 Metabolic stress protocol

Metabolic stress was induced by the completion of the Cycling Intermittent Sprint Protocol (CISP, Hayes *et al*., 2013) designed to simulate the metabolic demands of a repeat sprint sport. The protocol was modified slightly to include additional sprints (4x15s) which has been reported to result in oxidative stress (Jówko *et al.,* 2014). Thus, the exercise protocol employed for the current study comprised of a standardised warm-up (5 min at 95 W and two 30-s bouts at 120 W with 30-s rest in between) followed by 2 x (14 x 2min bouts of exercise comprising of 10s of passive rest, 5s of maximal sprinting and 105s of active recovery, with a 15s maximal sprint followed by 1min active recovery taking place after the 7th and 14th 2min bout) (Figure 5.1). The exercise bouts were separated by a 10min 'half-time' period during which the participants were permitted to dismount the ergometer and walk around in order to reduce venous pooling in the lower extremities and minimise feelings of light-headedness and nausea. The total time for each 'half' was 30min and 30s, thereby simulating the ~30min halves of ladies Gaelic football and camogie, sports from which the majority of participants were drawn. Power output, cadence, speed, HR and RPE were recorded throughout the exercise bout. Participants returned to complete the second cycling trial during the follicular phase of their next menstrual cycle, following which the drink not consumed on the first trial was ingested.

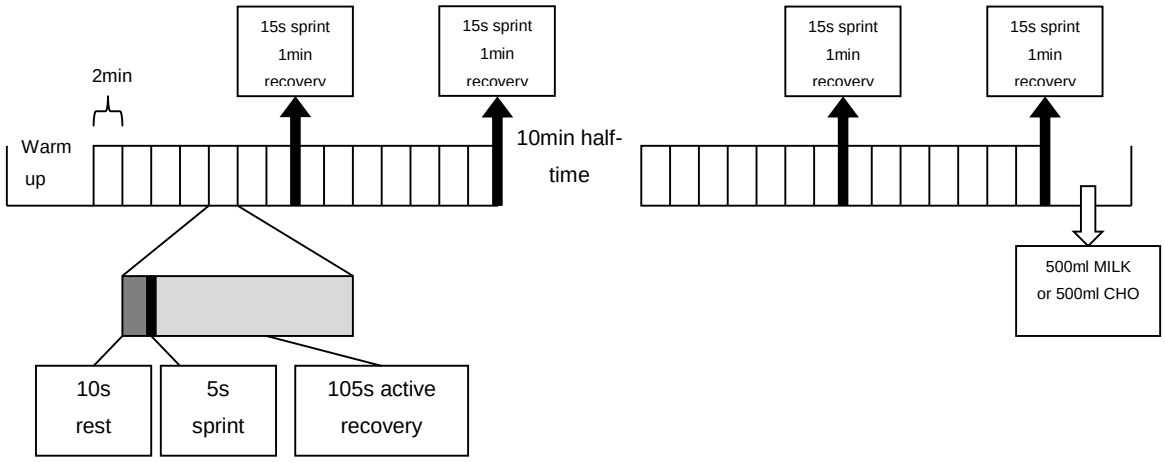

Figure 5. 2 Schematic of the Exercise Protocol

5.2.5 Blood sampling

On each day, prior to any other measures and following a 10min rest, a blood sample was collected by venepuncture from a forearm vein in ethylenediaminetetraacetic acid (EDTA), heparin and serum separator (SST) tubes. The samples were then centrifuged, aliqouted and stored at -80°C for later analysis of creatine kinase (CK), high sensitivity C-reactive protein (hsCRP), protein carbonyls (PC), and GSH:GSSG (oxidized glutathione:reduced glutathione) ratio. A blood sample was also collected 2h post completion of the exercise protocol. Samples were analysed according to the procedures detailed in Section 3.8.1 (CK), 3.8.3 (hsCRP), 3.8.4 (PC) and 3.8.5 (GSH:GSSG).

5.2.6 Muscle soreness and muscle tiredness

Active muscle soreness (during squatting to approximately 90° knee flexion) and tiredness were measured on a visual analogue scale (VAS) as detailed in Section 3.6.

5.2.7 Peak torque

To determine peak torque (Nm), following a standardised warm-up, participants completed three dominant leg maximal effort knee extension repetitions at 60°/s and 180°/s. Refer to section 3.9 for details.

5.2.8 Rate of force development

Rate of force development (RFD) was determined over the first 200ms of an isometric contraction, according to previous studies (Aagard *et al*., 2002). Details are given in Section 3.10.

5.2.9 Countermovement jump height

Countermovement jump height was measured in cm using an Optojump optical measurement system (Microgate, Bolzano, Italy). Refer to Section 3.11 for details.

5.2.10 Sprint performance

Twenty metre sprint performance, from a standing start 20cm behind the start line, was recorded using timing gates (Microgate Racetime 2, Bolzano, Italy). See Section 3.13 for detailed procedures.

5.2.11 Data analysis

Data was analysed by making probabilistic magnitude based inferences about the true values of outcomes as described by Batterham and Hopkins (2006). Details of the analysis are given in Section 3.14.

5.3 Results

5.3.1 Preliminary data

Analysis of the repeat-sprint exercise bouts revealed no clear differences between trials on performance or physiological measures (Table 5.1).

Table 5. 1 Comparison of performance and physiological variables for cycling trials prior to Milk and Carbohydrate consumption.

Variable	1st half		2nd half		Total	
	MILK	CHO	MILK	CHO	MILK	CHO
Average power (W)	117.1 ± 15.7	112.0 ± 14.4	109.3 ± 14.8	105.3 ± 16.1	113.2 ± 14.8	108.7 ± 14.6
Peak power (W)	695.4 ± 135.6	661.3 ± 124.1	638.6 ± 135.4	636.0 ± 129.4	667.0 ± 129.4	648.7 ± 130.3
Power/mass (W/kg)	2.0 ± 0.3	1.8 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.9 ± 0.2	1.8 ± 0.2
Estimated EE (kcal)	333.3 ± 24.2	325.5 ± 25.2	307.7 ± 27.6	308.9 ± 37.3	320.5 ± 24.4	317.2 ± 26.7
Average cadence (rpm)	70.1 ± 3.9	67.7 ± 4.7	68.6 ± 4.2	66.5 ± 4.5	69.4 ± 4.0	67.1 ± 4.1
Peak cadence (rpm)	120.1 ± 12.3	117.8 ± 9.1	118.7 ± 13.0	115.0 ± 9.8	119.4 ± 11.3	116.4 ± 8.8
Rev count	2255.2 ± 124.1	2207.8 ± 128.6	2128.0 ± 136.6	2121.7 ± 215.7	2191.6 ± 112.4	2164.8 ± 130.6
Average speed (kmh)	27.7 ± 1.5	27.3 ± 1.4	27.2 ± 1.5	26.6 ± 1.7	27.5 ± 1.4	27.0 ± 1.4

Variable	1st half		2nd half		Total	
	MILK	CHO	MILK	CHO	MILK	CHO
Distance covered (km)	14.5 ± 0.5	14.5 ± 1.1	14.0 ± 0.8	13.9 ± 0.8	14.2 ± 0.6	14.1 ± 0.9
Average pace (min:ss/km)	1:58 ± 0:06	2:01 ± 0:07	2:00 ± 0:05	2:02 ± 0:08	2:00 ± 0:06	2:01 ± 0:06
HR (bpm)	171.7 ± 10.9	169.9 ± 8.2	168.9 ± 9.7	167.5 ± 5.8	170.3 ± 10.1	168.7 ± 6.5
RPE	11.8 ± 1.6	11.9 ± 2.1	13.8 ± 1.8	14.1 ± 2.8	12.8 ± 1.4	13.0 ± 2.3

5.3.2 Within-group effects

Analysis of within-group effects revealed post-exercise decreases in peak torque, CMJ, sprint performance and RFD, increases in serum CK and hsCRP and in muscle soreness and tiredness. No clear change was observed in PC or GSH:GSSG ratio. Mean effects ± 90%CI, with qualitative inferences, are presented in Table 5.2.

Table 5. 2 Within-group effects over time for dependent variables

Variable	Timeframe	Mean effect, ± or Factor effect x/÷ 90% CI[a]	Qualitative inference[b]
Peak Torque 60°/s Extension			
MILK	B-24	-4.0, ± 2.7	Small decrease*
	B-48	-7.1, ± 7.4	Small decrease**
	B-72	-4.9, ±5.6	Small decrease*
CHO	B-24	-3.2, ± 2.5	Trivial decrease*
	B-48	-4.4, ± 2.5	Small decrease*
	B-72	-2.7, ± 4.7	Small decrease*
Peak Torque 180°/s Extension			
MILK	B-24	-3.4, ± 4.5	Small decrease*
	B-48	-5.8, ± 8.1	Small decrease*
	B-72	-2.5, ± 1.7	Unclear
CHO	B-24	-6.5, ± 4.4	Small decrease*
	B-48	-3.5, ± 5.1	Trivial decrease**
	B-72	-1.1, ±5.3	Unclear
CMJ			
MILK	B-24	-1.7, ± 2.7	Small decrease*
	B-48	-1.5, ± 2.6	Trivial decrease*
	B-72	0.2, ± 2.4	Unclear
CHO	B-24	-2.0, ± 1.8	Small decrease*
	B-48	-2.0, ± 3.2	Trivial decrease*
	B-72	0.1, ± 2.8	Unclear

20m sprint			
MILK	B-24	-1.8, ± 1.2	Small decrease**
	B-48	-0.9, ± 1.6	Trivial decrease*
	B-72	0.2, ± 1.3	Unclear
CHO	B-24	-1.0, ± 0.7	Small decrease**
	B-48	-0.5, ± 1.5	Unclear
	B-72	-0.4, ± 0.7	Unclear
RFD			
MILK	B-24	-8.5, ± 14.6	Small decrease*
	B-48	0.8, ± 8.9	Trivial**
	B-72	-3.9, ± 18.1	Unclear
CHO	B-24	-12.7, ± 11.9	Small decrease**
	B-48	-4.9, ± 12.9	Unclear
	B-72	3.3, ± 15.0	Unclear
Creatine Kinase			
MILK	B-24	1.4 x/÷ 1.3	Moderate increase**
	B-48	1.2 x/÷ 1.4	Unclear
	B-72	1.1 x/÷ 1.3	Unclear
CHO	B-24	1.5 x/÷ 1.3	Moderate increase***
	B-48	1.3 x/÷ 1.3	Small increase**
	B-72	1.1 x/÷ 1.5	Unclear
hsCRP			
MILK	B-24	2.2 x/÷ 1.4	Large increase****
	B-48	1.7 x/÷ 1.3	Large increase****
	B-72	1.5 x/÷ 1.4	Moderate increase**
CHO	B-24	1.7 x/÷ 1.4	Moderate increase***
	B-48	1.4 x/÷ 1.4	Small increase**
	B-72	1.1 x/÷ 1.6	Unclear
Soreness			
MILK	B-24	0.7, ± 0.5	Moderate increase**
	B-48	0.3, ± 0.4	Trivial**
	B-72	0.1, ± 0.2	Trivial****
CHO	B-24	1.2, ± 0.9	Large increase**
	B-48	1.3, ± 1.3	Very large increase**
	B-72	0.8, ± 0.9	Moderate increase*
Tiredness			
MILK	B-24	1.8, ± 0.7	Extremely large increase****
	B-48	1.5, ± 0.8	Very large increase***
	B-72	0.1, ± 0.2	Trivial****
CHO	B-24	2.4, ± 0.9	Extremely large increase*****
	B-48	2.3, ± 1.3	Extremely large increase***
	B-72	0.9, ± 0.8	Moderate increase**
PC			
MILK	B-24	1.1 x/÷ 1.4	Unclear
	B-48	1.1 x/÷ 1.3	Unclear
	B-72	0.8 x/÷ 1.3	Unclear
CHO	B-24	0.9 x/÷ 1.9	Unclear
	B-48	0.8 x/÷ 1.9	Unclear

	B-72	0.7 x/÷ 1.7	Unclear
GSH : GSSG			
MILK	B-24	0.4 x/÷ 1.5	Unclear
	B-48	0.8 x/÷ 2.4	Unclear
	B-72	0.7 x/÷ 3.4	Unclear
CHO	B-24	1.4 x/÷ 3.9	Unclear
	B-48	1.1 x/÷ 8.0	Unclear
	B-72	0.7 x/÷ 5.0	Unclear

[a]± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [b]Qualitative Inference represents the likelihood that the true value will have the observed magnitude
**Possible, **Likely, ***Very Likely, ****Most likely*

5.3.3 Between-group effects

Peak torque

Baseline knee extension peak torque values of the dominant leg at 60°/s for the milk condition and the carbohydrate condition were 143.1 ± 25.5 Nm and 141.1 ± 23.9 Nm, respectively. Percentage changes in peak torque at 60°/s for dominant knee extension can be seen in Figure 5.3. Changes in the peak torque of the dominant leg at 60°/s between baseline and 24h for the milk condition and carbohydrate condition were -3.9 ± 4.5% and -3.1 ± 4.3%, respectively, a trivial effect (ES=0.02). Unclear outcomes for the comparison of MILK versus CHO were found at baseline-48h (-6.4 ± 12.2 % and -4.3 ± 4.4 %) and baseline-72h (-4.4 ± 9.5 % and -2.4 ± 8.5 %).

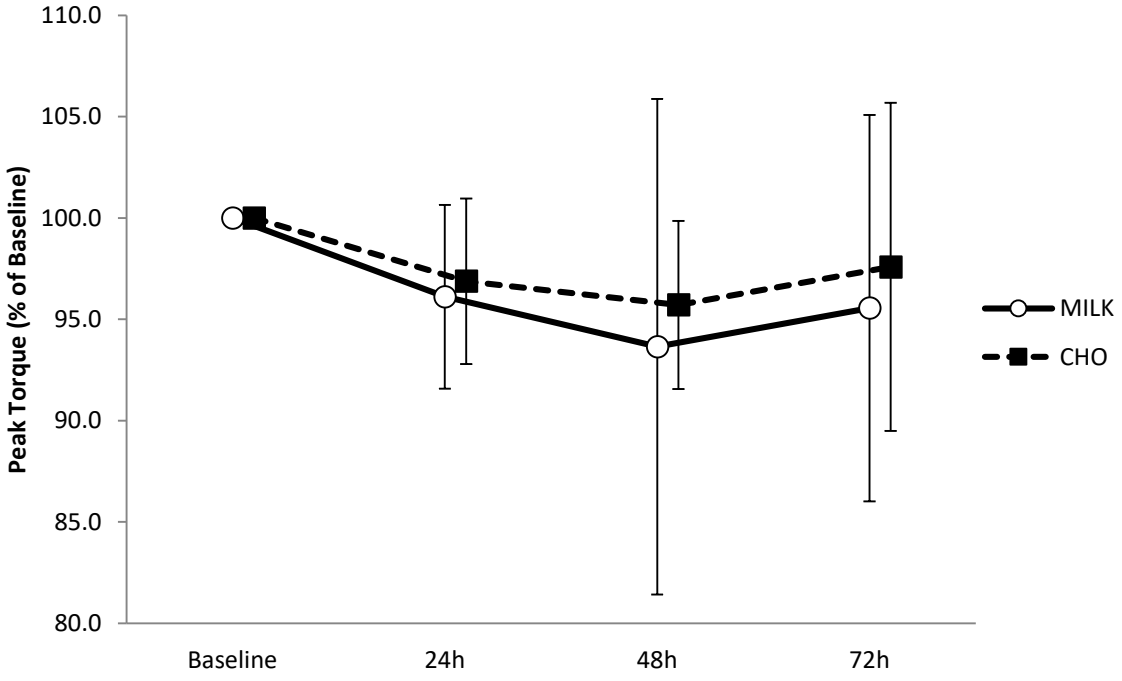

Figure 5. 3 Peak torque at 60°/s for dominant knee extension in response to repeat sprint cycling exercise for MILK and CHO.

Values are presented as means ± SD

At baseline, the peak torque values at 180°/s for the dominant leg of participants for MILK and CHO were 97.7 ± 16.6 Nm and 95.5 ± 16.0 Nm, respectively. Changes in the peak torque of the dominant leg at 180°/s between baseline and 24h for MILK and CHO were -3.2 ± 7.8%, and -6.2 ± 7.5%, respectively, a possible trivial benefit (ES=0.11) of milk. There was an unclear outcome for MILK (-5.0 ± 12.7%) versus CHO (-3.1 ± 8.9%) at baseline-48h. A possible small negative effect (ES=0.28) was observed for MILK (-7.3 ± 12.7%) compared to CHO (-0.8 ± 9.2%) at baseline-72h.

Rate of Force Development

Immediately prior to the repeat-sprint cycling exercise, the mean rate of force development was 433.1 ± 159.1 $Nm.s^{-1}$ and 405.6 ± 106.1 $Nm.s^{-1}$ for the MILK and CHO conditions, respectively. Unclear outcomes for MILK versus CHO were found at baseline-24h (-5.5 ± 25.3% v -10.5 ± 22.0%), baseline-48h (-1.9 ± 16.4% v -2.7 ± 20.3%) and baseline-72h (-0.5 ± 30.7% v 6.3 ± 28.7%).

20m sprint

Baseline 20m sprint times for the milk condition and carbohydrate condition were 3.58 ± 0.12s and 3.59 ± 0.11s, respectively. Unclear outcomes were found for MILK versus CHO at baseline-24h (1.8 ± 2.2% and 1.0 ± 1.3%), baseline-48h (1.0 ± 2.8% and 0.6 ± 2.5%) and baseline-72h (0.2 ± 2.2% and -0.4 ± 1.2%).

Countermovement jump performance

Immediately prior to the cycling exercise, the mean countermovement jump heights of the milk and carbohydrate conditions were 28.9 ± 2.9cm and 28.8 ± 2.4cm, respectively. Unclear outcomes for MILK versus CHO were found at all time points, baseline-24h (-1.6 ± 4.7% v -2.0 ± 3.1%), baseline-48h (-1.4 ± 4.6% and -1.9 ± 5.4%) and baseline-72h (-0.2 ± 4.1% v-0.0 ± 4.7%). A summary of the statistical analysis for Peak Torque, RFD (0-200ms) of the dominant leg, 20m sprint performance and countermovement jump performance can be seen in Table 5.3.

Soreness

The exercise protocol resulted in a moderate-large increase in muscle soreness for both conditions, peaking at 24h for MILK and 48h for CHO (Figure 5.4). By 72h, soreness had almost returned to zero. A comparison of changes in soreness for the period baseline-24h indicated an unclear outcome for MILK (7.0 ± 8.2%) compared to CHO (12.0 ± 15.5%). A possible small benefit of MILK v CHO was seen at baseline-

48h (4.0 ± 7.0% v 14.0 ± 21.7%) and a possible small, trivial benefit baseline-72h (1.0 ± 3.2% v 8.0 ± 10.2%).

Tiredness

Muscle tiredness increased over time for both conditions but by 72h tiredness had almost returned to zero. A comparison of changes in muscle tiredness from baseline to 24h indicated a possible trivial benefit for the consumption of MILK (18.0 ± 11.4 %) compared to the consumption of CHO (24.0 ± 15.8%). An unclear outcome was observed from baseline to 48h (16.0 ± 12.6% versus 24.0 ± 20.7% for MILK and CHO conditions, respectively). Changes in tiredness from baseline-72h showed a possible trivial benefit of MILK (1.0 ± 3.2%) compared to CHO (9.0 ± 13.7%). A summary of the statistical analysis for muscle soreness and muscle tiredness can be seen in Table 5.3.

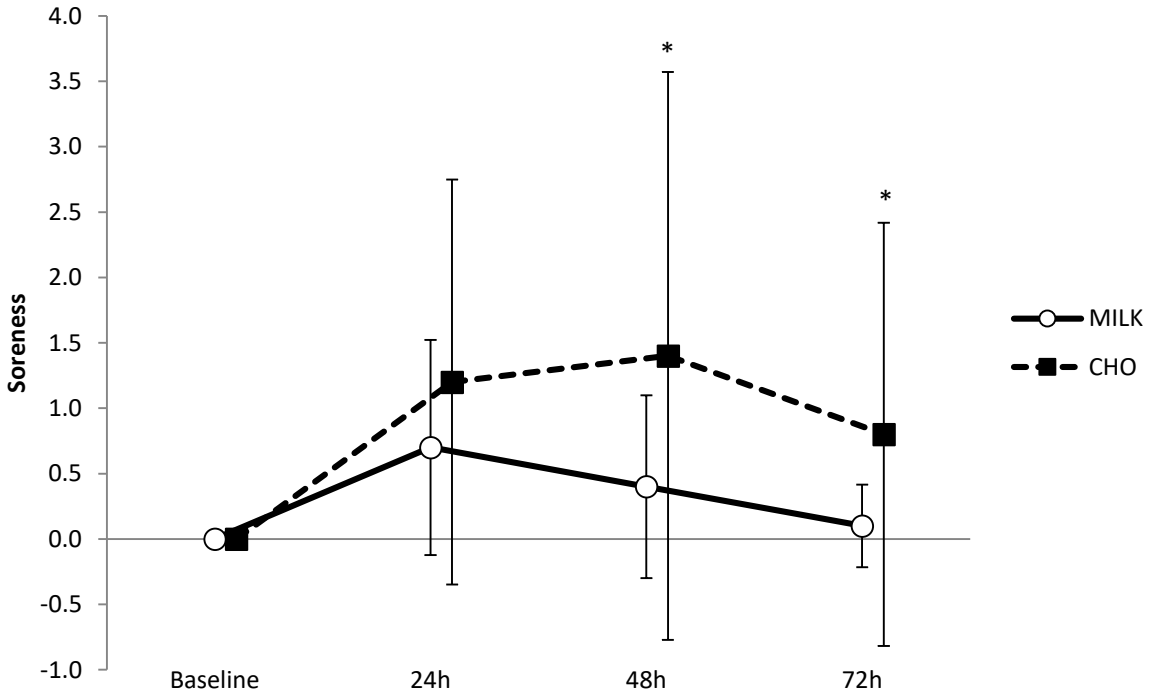

Figure 5. 4 Muscle soreness in response to repeat sprint cycling exercise for MILK and CHO.

Values are presented as means ± SD. *Possible benefit of milk

Table 5. 3 Effects on Peak Torque, RFD, sprint performance, CMJ, soreness, tiredness, CK, hsCRP, PC and GSH:GSSG ratio following repeat sprint cycling exercise

Variable	Time Frame	Mean effect[a], ± 90% CI[b]	Qualitative Inference[c]
Peak Torque 60°/s Dominant leg	B – 24	-0.3, ± 3.2	Trivial**
	B – 48	-2.4, ± 8.0	Unclear
	B – 72	2.0, ± 6.2	Unclear
Peak Torque 180°/s Dominant leg	B – 24	3.3, ± 6.5	Small benefit*
	B – 48	-2.5, ± 9.5	Unclear
	B – 72	-7.1, ± 8.9	Small harm*
RFD (0-200ms) Dominant leg	B – 24	2.0, ± 6.2	Unclear
	B – 48	6.3, ± 16.4	Unclear
	B – 72	-4.4, ± 21.0	Unclear
20m Sprint performance	B – 24	0.8, ± 1.4	Unclear
	B – 48	0.4, ± 2.1	Unclear
	B – 72	0.6, ± 4.0	Unclear
Countermovement jump performance	B – 24	0.3, ± 3.2	Unclear
	B – 48	0.5, ± 4.0	Unclear
	B – 72	-0.1, ± 3.5	Unclear
Muscle soreness	B – 24	-0.4, ± 1.0	Unclear
	B – 48	-1.0, ± 1.3	Small benefit*
	B – 72	-0.7, ± 1.0	Trivial benefit*
Muscle Tiredness	B – 24	-0.6, ± 1.1	Trivial benefit*
	B – 48	-0.8, ± 1.3	Unclear
	B – 72	-0.8, ± 0.8	Trivial benefit*

[a] *Mean effect refers to MILK minus CHO*

[b] *± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference*

[c] *Qualitative Inference represents the likelihood that the true value will have the observed magnitude*

**Possible, **Likely, ***Very likely, ****Most likely*

Creatine kinase

Serum CK values prior to exercise were 155.0 ± 75.4 U/l and 138.0 ± 62.6 U/l for the milk and carbohydrate conditions, respectively. Unclear outcomes for the comparison of MILK versus CHO were found at baseline-2h (1.52 x/÷ 1.27 v 1.41 x/÷ 1.17), baseline-24h (1.30 x/÷ 1.55 v 1.26 x/÷ 1.65), baseline-48h (1.03 x/÷ 1.57 v 1.14 x/÷ 1.57) and baseline-72h (0.99 x/÷ 1.56 and 0.97 x/÷ 1.76). See Figure 5.5.

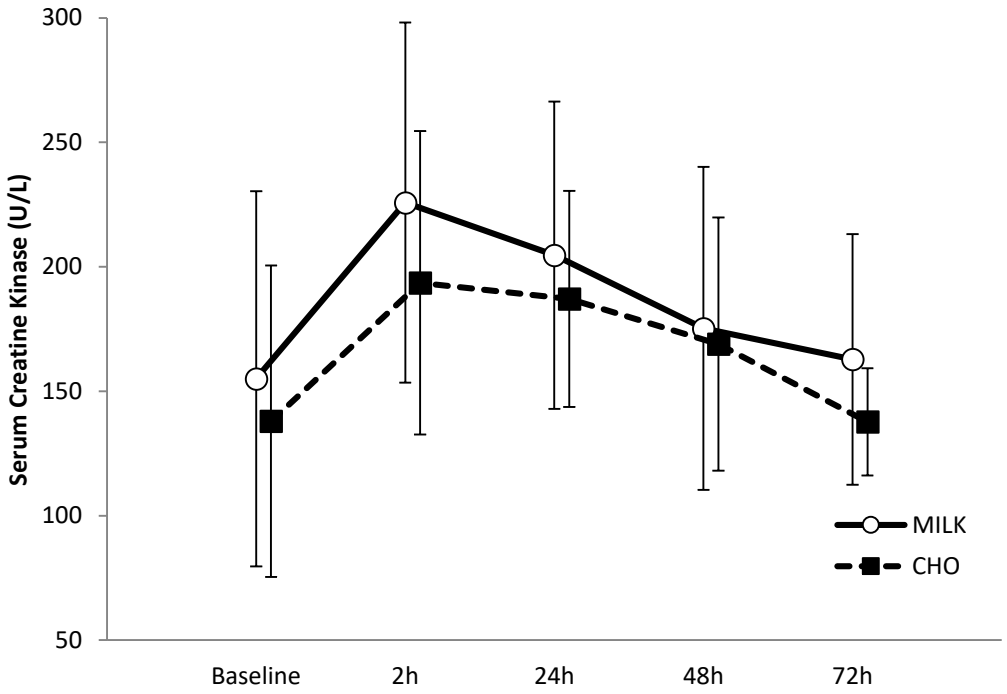

Figure 5. 5 Serum Creatine Kinase in response to repeat sprint cycling exercise for MILK and CHO.

Values are presented as means ± SD

hsCRP

Baseline hsCRP values immediately prior to exercise were 1.16 ± 0.91 mg/l and 1.38 ± 0.99 mg/l for the milk and carbohydrate conditions, respectively. Unclear outcomes were observed for MILK versus CHO at baseline-2h (1.00 x/÷ 1.07 v 1.02 x/÷ 1.17), baseline-24h (2.09 x/÷ 1.75 v 1.42 x/÷ 2.03), baseline-48h (1.59 x/÷ 1.50 v 1.15 x/÷ 1.98) and baseline-72h (1.25 x/÷ 1.73 v 0.925 x/÷ 1.99).

Protein carbonyls (PC)

Protein Carbonyl values prior to exercise were 0.53 ±0.14 nmol/mg/protein for the MILK group and 0.55 ± 0.36 nmol/mg/protein for the CHO group. Changes in PC can be seen in Figure 5.6. Unclear outcomes were observed for MILK versus CHO; baseline-2h (1.10 x/÷ 1.54 v 0.88 x/÷ 2.36), baseline-24h (1.23 x/÷ 1.82 v 0.85 x/÷ 2.72), baseline-48h (1.33 x/÷ 1.23 v 0.76 x/÷ 1.99) and baseline-72h (0.88 x/÷ 1.46 and 0.957 x/÷ 3.04).

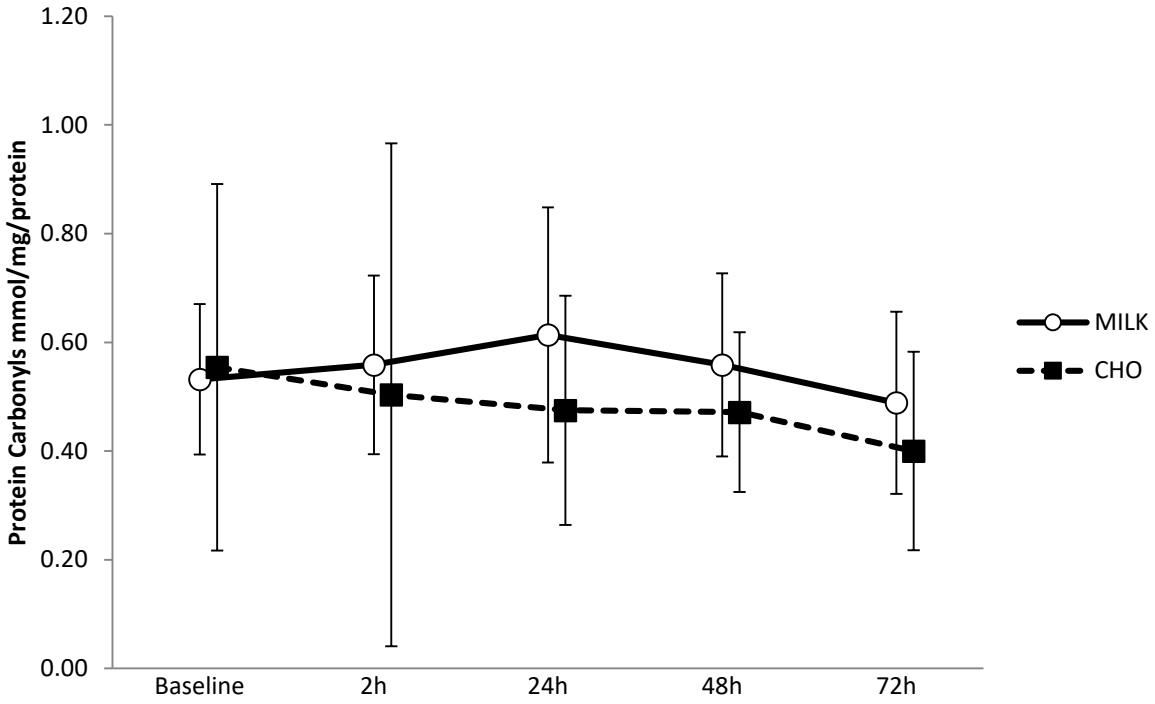

Figure 5. 6 Serum Protein Carbonyls in response to repeat sprint cycling exercise for MILK and CHO.

Values are presented as means ± SD

GSH:GSSG ratio

Prior to the cycling exercise the mean GSH:GSSG ratio of the milk and carbohydrate conditions were 0.57 ± 0.27 and 0.98 ± 0.92, respectively. An unclear outcome for MILK versus CHO was found at baseline-2h (0.871 x/÷ 1.99 v 1.014 x/÷ 8.52). A likely moderate benefit of MILK was observed at baseline-24h (0.369 x/÷ 1.89 v 1.103 x/÷ 3.96). Unclear outcomes were found at other timepoints: baseline-48h (0.827 x/÷ 3.15 and 1.105 x/÷ 8.800) and baseline-72h (0.751 x/÷ 6.39 v 0.686 x/÷ 7.72). A summary of the statistical analysis for Creatine Kinase, hs-CRP, PC and GSH:GSSG ratio can be seen in Table 5.4.

Table 5. 4 Effects on CK, hsCRP, PC and GSH:GSSG ratio following repeat sprint cycling exercise

Variable	Time Frame	Mean effect[a] x/÷ 90% CI[b]	Qualitative Inference[c]
CK	B – 2	1.1 x/÷ 1.2	Unclear
	B – 24	1.0 x/÷ 1.5	Unclear
	B – 48	0.9 x/÷ 1.5	Unclear
	B – 72	1.0 x/÷ 1.6	Unclear
hs-CRP	B – 2	0.9 x/÷ 1.1	Unclear
	B – 24	1.5 x/÷ 1.7	Unclear
	B – 48	1.4 x/÷ 1.6	Unclear
	B – 72	1.4 x/÷ 1.7	Unclear
PC	B – 2	1.2 x/÷ 1.8	Unclear
	B – 24	1.5 x/÷ 2.1	Unclear
	B – 48	1.8 x/÷ 1.9	Unclear
	B – 72	0.9 x/÷ 2.2	Unclear
GSH:GSSG ratio	B – 2	0.9 x/÷ 4.4	Unclear
	B – 24	0.3 x/÷ 3.2	Moderate benefit**
	B – 48	0.8 x/÷ 8.5	Unclear
	B – 72	1.1 x/÷ 5.3	Unclear

[a] *Mean effect refers to MILK minus CHO*
[b] *± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference*
[c]*Qualitative Inference represents the likelihood that the true value will have the observed magnitude*
***Likely*

5.4 Discussion

Repeat-sprint cycling resulted in decrements in muscle function and increases in perceptions of soreness and tiredness, serum markers of muscle damage and inflammation. However, no clear change in oxidative stress was observed. The results indicate that the consumption of 500ml of milk post-intermittent sprint cycling exercise had minimal effect on recovery of muscle function, inflammation and markers of muscle damage. While a possible small benefit of milk was observed for peak torque at 180˚/s at 24h post-exercise, no effect was seen on any other muscle function variable. A possible negative effect for MILK was noted at 72h post-exercise for peak torque at 180˚/s. The consumption of milk attenuated increases in muscle soreness and tiredness over 72h post-exercise, compared to the consumption of a volume and energy matched carbohydrate drink.

A possible benefit for MILK was observed for Peak Torque at 180˚/s at 24h post exercise, but at no other time and for no other muscle function variable, CK or

hsCRP. The lack of effect of milk on muscle function, inflammation and CK is likely because of the nature of the exercise protocol. Previous research has found that the consumption of milk following eccentrically loaded exercise had a beneficial effect in attenuating decreases in peak torque, sprint performance and reactive strength index (Rankin *et al*., 2015; Cockburn *et al*., 2012; 2010; 2008). Cycling employs concentric muscle actions (Bijker *et al*., 2002), resulting in metabolic rather than mechanical stress and may explain the low level of muscle damage observed in this study. This lower level of damage is reflected in the smaller decreases in muscle performance observed across all variables (3.98%) compared to the first study of this thesis (6.95%, Chapter 4, Rankin *et al*., 2015). Serum CK was elevated during the recovery period, peaking 24h post-exercise and returning to baseline values by 72h with no difference between interventions. Mean peak CK values (225.6 ± 72.3 and 193.6 ± 61.0 IU/l for MILK and CHO, respectively) were lower than observed for female athletes in investigations employing eccentric exercise (8873.0 ± 13306.0 and 11697.7 ± 8423.3 IU/l, MILK and CHO, respectively, Rankin *et al*., 2015), though similar to other cycling studies investigating recovery interventions (Bell *et al.,* 2014; Jowko *et al*., 2014). In summary, the observed muscle damage was low, losses in muscle function were small and milk had little effect on minimising these effects.

It is possible that an increase in protein synthesis or a decrease in protein breakdown following the mechanical stress of eccentric loading in previous studies is enhanced with the intake of milk post-exercise, a mechanism not observed following isolated metabolic stress in this study. It is conceivable that the nature of the exercise (cycling) may have stimulated mitochondrial protein synthesis rather than myofibrillar protein synthesis. Wilkinson *et al.,* (2008) noted a 67% increase in myofibrillar FSR following a resistance exercise session but no change following an endurance (cycling) session while Dumke *et al*., (2009) reported stimulation of genes associated with mitochondrial biogenesis following cycling, an effect reported by others following high intensity sprint cycling protocols and training (MacInnis *et al*., 2017; Granata *et al*., 2016; Little *et al*., 2010). In the current study, the measurements of muscle function over the recovery period (peak torque, RFD, CMJ, sprint performance) were not dependent on mitochondrial function and therefore, any benefit of milk intake may not have been apparent from the measures chosen. It is possible that a larger intake of milk may have produced different outcomes. The amount of protein provided by the 500ml of milk was 17g which provided a mean relative intake of 0.27 g/kg, though

given the range in body mass, the range in relative intake was 0.23-0.32 g/kg. The lower relative intake of protein may have been insufficient to maximally stimulate protein synthesis and attenuate muscle function losses. Nevertheless, Cockburn *et al.,* (2012) reported that 500ml of milk consumed post-exercise was as effective as 1000ml in attenuating the negative effects of EIMD. Further research is warranted to extrapolate this further.

Exercise causes a systemic inflammatory response with increases in cytokine and leucocyte activity (Febbraio and Pedersen, 2005). hsCRP is an acute phase protein and its synthesis in the liver is triggered as an inflammatory response following increased cytokine secretion, most notably IL-6. Mean absolute hsCRP values were very similar in both trials, suggesting similar inflammatory responses. Not surprisingly, given that cycling is concentric in nature, the hsCRP response to the exercise protocol was considerably lower than that observed following resistance exercise (Bowtell *et al.,* 2011) and marathon running (Clifford *et al.,* 2016), suggesting that the magnitude of the inflammatory response is significantly influenced by the mode of exercise. The values, however, are comparable to that reported previously in cycling research (Roengrit *et al.,* 2014) with peak hsCRP observed at 24h post-exercise, returning towards baseline values by 72h post-exercise. The magnitude of change was not different between the conditions, indicating that post-exercise consumption of milk did not modulate the inflammatory response after metabolic exercise. There is considerable disagreement in the literature regarding the effects of carbohydrate and carbohydrate-protein intake on inflammatory measures. For example, while Kerasioti *et al*., (2013) observed a decrease in inflammatory markers (IL-6, CRP) following the ingestion of a carbohydrate-protein cake, Cosio-Lima *et al*., (2012) reported no differences in inflammatory responses (TNF-α. IL-6) following the ingestion of carbohydrate versus carbohydrate-protein solutions. Such contradictory results are probably reflective of the variation in exercise protocols employed, the inflammatory markers measured or the specific composition of the nutritional interventions. It is important to note that inhibition of post-exercise inflammation is not always an aim of post-exercise nutritional intervention, as prevention of exercise-induced inflammation may inhibit muscular adaptation to exercise (Mastaloudis *et al.,* 2004).

Interestingly, even though post-exercise muscle soreness ratings were low compared to those following eccentric exercise, post-exercise milk consumption had a positive effect in attenuating increases in muscle soreness and tiredness over the 72h post-exercise period. This is somewhat unexpected given that there were no differences in muscle function or inflammation between the groups. Inflammation is a proposed mechanism for the manifestation of muscle soreness (MacIntyre *et al.,* 1995). Increased histamines and bradykinins sensitise nociceptors and increase the sensation of pain (Malm, 2001). Oedema may also contribute to the sensation of soreness, with swelling exerting increased osmotic pressure within the fibres and further sensitising nociceptors (Malm, 2001; Clarkson and Hubal, 2002). The disparity of results observed in this study may indicate different pathways for the recovery of muscle function and perception of soreness and tiredness which may or may not involve the inflammatory response. It is possible that hsCRP may not be reflective of the total cytokine activity in the post-exercise period and that measurement of other cytokines such as TNF-α or IL-6 may have provided greater insight. Nonetheless, because this was not a blind study it is plausible that information bias may have occurred through knowledge of the nature of the allocated intervention (Booth *et al*., 1992), where participants may have been aware of previously published research highlighting potential benefits of milk for recovery, thus leading to a perception of attenuated soreness and tiredness.

Surprisingly, the intermittent sprint protocol utilised in this study did not result in an increase in oxidative stress as indicated by PC and GSH:GSSG ratio, despite the considerable physical demand imposed by the exercise. This is in contrast with previous research that reported increases in post-exercise oxidative stress following sprint cycling (Jowko *et al.,* 2014), though in agreement with Bloomer *et al.,* (2007) and Farney *et al*., (2012). There are a number of possible reasons for this observation. Firstly, only selective biomarkers of oxidative stress were measured; the inclusion of additional markers or alternative assay techniques may have indicated an increase in oxidative stress. Both PC and GSH:GSSG have been identified as useful indicators of protein oxidation and redox disturbance, respectively (Powers *et al*., 2010). Protein Carbonyls are produced when ROS attack amino acids and thus concentration increases when oxidative stress increases. GSH is an antioxidant used in the reduction of lipid hydroperoxides generating oxidised glutathione (GSSG). When cells are exposed to oxidative stress, concentration of GSSG increases and

the ratio of GSSG to GSH increases. Nevertheless, the validity of many measures of oxidative stress has been questioned, with poor correlations between different markers of oxidative stress and between different methods for measuring individual markers (Finkler *et al*., 2014). Secondly, oxidative stress may have occurred in muscle tissue but was not detected in the serum samples as has been reported in animal research (You *et al*., 2005). Thirdly, a lack of observed oxidative stress may be because the participants were trained team-sport players, well-accustomed to the metabolic demands of repeat-sprinting albeit with a different exercise mode. This training may have resulted in adaptations that resulted in minimal change in PC and GSH:GSSG ratio over the duration of this study. Lending support to this idea Bloomer *et al*., (2012, 2006) reported no increase in oxidative stress in trained individuals following sprint cycling and Bogdanis *et al.,* (2013) reported attenuated oxidative stress responses following high intensity interval training. Finally, it is possible that a lack of oxidative stress may be because of the sex of the participants. Previous research has indicated that females experience lower oxidative stress than males (Ide *et al*., 2002) and that following exercise, markers of oxidative stress return to baseline quicker than males (Mastaloudis *et al*., 2004). This lower level of oxidative stress may be because of smaller muscle mass, leading to lower mitochondrial flux and lower ROS production (Ide *et al*., 2002). Additionally, Tiidus (1995, 2003) proposed that oestrogen may act as an antioxidant against lipid peroxidation of the cell membrane during exercise. Oestrogens possess an hydroxyl group on their A (phenolic) ring in a similar structure to tocopherol (Vitamin E) which is a known antioxidant (Persky *et al.,* 2000; Ayres *et al*., 1998). Acting in a similar way to tocopherol, oestrogen may donate hydrogen atoms leading to a termination of peroxidation chain reactions (Persky *et al.,* 2000; Ayres *et al*., 1998; Tiidus, 1995). Future investigations examining the effect of milk on oxidative stress should carefully consider exercise protocol design, training status and sex of the participants.

The majority of investigations examining the effects of nutritional interventions on recovery from metabolic stress employed a protocol that required participants to consume the nutritional substance over a prolonged period of time. For example, Samaras *et al.*, (2014) observed an increase in glutathione concentration when athletes were supplemented with a protein-carbohydrate bar for two months. The only investigation examining the effects of post-exercise consumption of a protein-carbohydrate supplement on oxidative stress markers was Kerasioti *et al.*, (2012).

While they observed a reduction in Thiobarbituric Acid Reactive Substances (TBARS) with the intake of an experimental cake post-2hours of cycling, there was no effect on subsequent time-trial performance. It is, therefore, likely that the timing of antioxidant supplementation is an important factor that could influence exercise-induced oxidative stress (Goldfarb *et al*., 2009) and that post exercise intake of antioxidants does not have a positive effect on oxidative stress markers and may even result in a pro-oxidant response (Nieman *et al.,* 2002). Thus, it is unlikely that one-off post-exercise consumption of nutritional products, as in the current study, will have any effect on recovery from oxidative stress. In this study, the beverage was consumed only after the exercise bout, as this is a regular practice of team sport athletes.

In conclusion, the consumption of 500ml of milk post non-eccentric exercise had minimal effect on the attenuation of losses in muscle function and no effect on increases in markers of muscle damage and inflammation following repeat-sprint cycling, though consumption of milk did reduce perceptions of soreness and tiredness. Speculatively, the benefit of milk for athletic recovery is likely to be greatest following activities that have an eccentric component. Further research is warranted in this area to investigate the effects of milk on recovery from exercise that induces high levels of oxidative stress. However, recently it has been suggested that minimising oxidative stress may inhibit adaptation to exercise (Buresh and Berg, 2015; Pingitore *et al.,* 2015; Paulsen *et al*., 2014). From this perspective, future research examining the effect of nutritional interventions on oxidative stress should carefully consider the practical application of outcomes. Consideration could be given to examining the intake of milk following exercise protocols that utilise activities typically used in sport participation and performace, aswell as the effect of milk on recovery from simulated or actual sport performance.

Chapter 6: The Effect of Milk On Recovery From Repeated Sprinting and Jumping

6.1 Introduction

Recent research has shown that the consumption of 500ml milk following muscle damaging exercise can attenuate decreases in muscle function (RSI and sprint performance) in males (Cockburn *et al*., 2010; 2008). These studies evoked muscle damage using an intense mechanical eccentric protocol on an isolated muscle group. Recently, Rankin *et al*., (2017, Chapter 5) reported that milk was of little benefit in recovery from repeat-sprint cycling (a predominantly concentric exercise) in female athletes, suggesting that the benefit of milk for athletic recovery is likely to be greatest following activities that have an eccentric component. Given that the mechanisms underpinning recovery following eccentrically biased and concentrically biased exercise may be different, it follows that choosing nutritional interventions that are specific to the mode of exercise and desired outcomes is required, and as recently discussed, a 'one size fits all' approach is inappropriate (Minett and Costello, 2015). Conceptualising a continuum of exercise from isolated mechanical eccentric exercise to isolated metabolic concentric exercise, the vast amount of exercise activities reside somewhere between inducing both mechanical and metabolic stress to varying degrees. Participation in many sports and exercise activities involves repeated high-intensity activity such as sprinting and jumping, change of direction and deceleration (Sirotic and Coutts, 2007; Spencer *et al*., 2004). Thus, for the purpose of exploring post-exercise recovery, opportunities exist for research utilising more sport-specific models which have greater ecological validity and practical significance. This has been addressed in investigations employing sprinting (Howatson and Milak, 2009), jumping (Marginson *et al*., 2005) and drop-jumping damage protocols (Howatson *et al*., 2012), which reported reductions in isometric strength, CMJ and squat jump performance, along with increased CK and muscle soreness. No investigations have been conducted utilising combined sprinting and jumping activities. Moving forward, the key strategy must be to match recovery strategies with the specific demands of exercise and thus, research exercise protocols must incorporate relevant activities.

Of significance, the aforementioned studies (Howatson *et al.,* 2012; Howatson and Milak, 2009; Marginson *et al*., 2005) were conducted with male participants. Our previous research has suggested sex differences in the response to eccentric exercise with females showing smaller increases in sprint time, passive soreness, active soreness and sTnI values (Rankin *et al*., 2015). Thus, this reinforces the

necessity of matching recovery interventions, not only to the nature of the exercise demands but also to the population group. There is undoubtedly a dearth of research focusing on reponses of female athletes to varied forms of exercise. Indeed, a recent study concluded that female participants are under-represented in sports and exercise research, comprising only 39% of participants across 1382 investigations (Costello *et al*., 2014). In an informative study Keane *et al.,* (Keane *et al*., 2015) investigated muscle damage in female athletes following a repeat-sprint protocol incorporating 15 x 30m sprints and reported reductions in maximal voluntary isometric contraction (MVIC), countermovement jump height and sprint performance, alongside increases in muscle soreness and serum creatine kinase over the 72h recovery period. Comparable results have been reported with female dancers following the same protocol (Brown *et al*., 2015). Similarly, Jakeman *et al.,* (2009) noted reduced squat jump height, CMJ height, isokinetic muscle strength alongside increased muscle soreness and CK in physically active females following the execution of 10 x 10 repetitions of drop jumps. While these investigations contribute to our understanding of responses to eccentrically loaded exercise in female athletes, there are few investigations examining nutritional interventions following exercise in females, and none exploring the effect of milk on recovery following this type of exercise.

Accordingly, specific strategies to ameliorate the deleterious effects of exercise and muscle damage and accelerate the recovery process are of importance to researchers, coaches and athletes. No studies have examined the potential of milk to enhance recovery from combined sprinting and jumping activities, yet this knowledge would be valuable for post-exercise nutritional prescription for athletes. Bovine milk, which comprises both whey and casein protein along with carbohydrate, lipids, vitamins and minerals, has become a popular post-exercise recovery drink. Its carbohydrate content (as lactose) is comparable to commercial sports drinks and may be beneficial in post-exercise glycogen synthesis (Ferguson-Stegall *et al*., 2011). Its whey and casein protein content enhances post-exercise muscle protein synthesis rates (Wilkinson *et al.,* 2007), while the high concentration of electrolytes facilitates fluid recovery following exercise (Desbrow *et al*., 2014). Moreover, establishing responses in female athletes can present new information on recovery strategies for this under-investigated population. Therefore, the aim of this study was

to examine the effect of milk on recovery from repeated sprinting and jumping in a female team-sport population.

6.2 Methods

6.2.1 Participants

Eighteen female team-sport (camogie and ladies Gaelic football) athletes (mean age 21.6 ± 3.4 y), training a minimum of twice per week, participated in this study. Mean (±SD) height and mass were 162.9 ± 9.3 cm and 64.3 ± 6.1 kg, respectively. Details of screening and exclusion criteria can be reviewed in Section 3.2. Ethics approval was provided by the London Sport Institute Ethics Sub-Committee at Middlesex University, and the Institute of Technology Carlow, where the data collection took place.

6.2.2 Study design

The study utilised an independent group design that required participants to visit the laboratory on seven occasions. The initial visit comprised familiarisation and the subsequent visit, no more than 3 days later, involved the determination of baseline (B) values for dependent variables. Participants were then randomly but equally divided into two groups: Milk (MILK) and carbohydrate (CHO). Completion of the exercise protocol took place no more than 5 days after familiarisation, and participants returned to the laboratory 2h (bloods only) 24h, 48h and 72h post-exercise for re-evaluation of the dependent variables (Figure 6.1). Visits took place at the same time of day preceded by an overnight fast and abstinence from alcohol, caffeine and exercise. Participants were requested to refrain from any strenuous activity for the duration of the study, and from treating symptoms of muscle soreness and tiredness with interventions such as massage, cryotherapy, supplements and non-steroidal anti-inflammatory drugs.

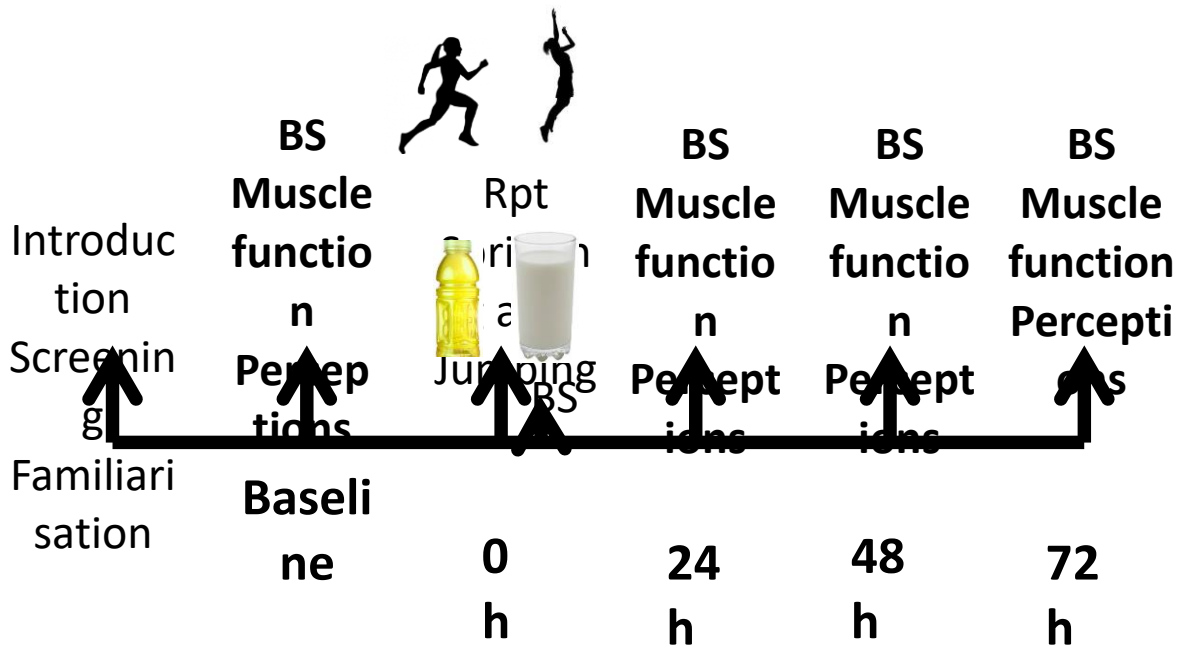

Figure 6. 1 Schematic of Study Design

6.2.3 Nutritional intervention and dietary control

Immediately upon completion of the protocol participants consumed either 500ml of milk or 500ml of an energy-matched carbohydrate solution as detailed in Section 3.4. Food diary details are also presented in Section 3.4. Analysis using Magnitude Based Inference indicated that there were no clear differences in energy, carbohydrate, protein or fat intake between groups.

6.2.4 Exercise protocol

The aim of the exercise protocol was to incorporate whole-body sport and exercise activities. Participants completed a 10min warm-up consisting of self-paced jogging, sprint drills and sprints at 60%, 80% and 100% of perceived maximum speed, followed by a series of dynamic stretches (Howatson and Milak, 2009). Participants then lined up at a marker 30cm from the start line and completed 15 x 20m maximal sprints stopping within a 10m deceleration zone marked out at the end of each sprint. Previous research has found that a similar protocol (15 x 30m) has resulted in EIMD in females (Keane *et al*., 2015). Following a 5min rest period, participants completed eight sets of 10 plyometric jumps, a protocol which has previously shown to induce muscle damage in males (Marginson *et al*., 2005). With feet shoulder width apart and hands on hips, participants were asked to flex their knees to 90 degrees, and to jump

as high as possible on each jump. Standardised verbal encouragement was provided to participants throughout the protocols.

6.2.5 Blood sampling

On each day, prior to other measures and following a 10min rest in the supine position, a venous blood sample was obtained via venepuncture from a forearm vein at the antecubital fossa. Details of analysis are provided in Section 3.8.1(CK) and Section 3.8.3 (hsCRP).

6.2.6 Peak torque

Peak torque was determined according to details in Section 3.9. Following a standardised warm-up, participants completed three maximal effort knee extension and flexion repetitions at 60°/s and 180°/s on the dominant leg.

6.2.7 Rate of force development

Rate of force development (RFD) over the first 200ms of an isometric contraction was determined as previously described (Aagaard et al., 2002). Refer to Section 3.10 for details.

6.2.8 Countermovement jump height

Countermovement jump height (cm) was determined according to the details in Section 3.11.

6.2.9 Reactive strength index

Reactive Strength Index (RSI) was determined from three maximal effort drop jumps from a height of 45cm onto an indoor sprint track. Refer to Section 3.12 for details.

6.2.10 Sprint performance

Five metre, 10m and 20m sprint performance, from a standing start 20cm behind the start line, were performed on an indoor sprint track and were recorded using timing gates. See Section 3.13 for detailed procedures.

6.2.11 Muscle soreness and muscle tiredness

Active muscle soreness was measured on a Visual Analogue Scale (VAS) during squatting to approximately 90° knee flexion and during isokinetic testing of knee extension at 60˚/s. A similar VAS was used to measure passive muscle tiredness. See details in Section 3.6.

6.2.12 Symptoms of stress

Symptoms of stress were monitored at the same time each day using the validated Daily Analysis of Life Demands (DALDA) questionnaire (Rushall, 1990). Section 3.7 provides details on the questionnaire.

6.2.13 Data analysis

Data was analysed by making probabilistic magnitude based inferences about the true values of the effect of intervention on outcomes. See Section 3.14 for details.

6.3 Results

6.3.1 Within-group effects

The exercise protocol employed resulted in Exercise Induced Muscle Damage (EIMD) for all participants. Analysis of within-group effects revealed post-exercise decreases in peak torque, CMJ, sprint performance and RFD, increases in serum CK and hsCRP and in muscle soreness and tiredness. Mean effects, ± 90%CI, with qualitative inferences, are presented in Table 6.1.

Table 6. 1 Within-group effects over time for dependent variables

Variable	Timeframe	Mean effect[a] ± or Factor effect x/÷ 90% CI[b]	Qualitative inference[c]
Peak Torque 60°/s Extension			
MILK	B-24	-8.2, ±2.7	Moderate decrease****
	B-48	-8.5, ±2.8	Moderate decrease****
	B-72	-4.1, ±4.8	Small decrease****
CHO	B-24	-13.4, ±6.0	Large decrease****
	B-48	-12.5, ±7.0	Large decrease***
	B-72	-10.6, ±7.8	Large decrease***
Peak Torque 60°/s Flexion			
MILK	B-24	-5.2, ±8.8	Small decrease*
	B-48	-10.4, ±9.1	Small decrease**
	B-72	-3.3, ±7.6	Unclear
CHO	B-24	-14.2, ±7.1	Moderate decrease***
	B-48	-13.3, ±5.4	Moderate decrease***
	B-72	-12.2, ±7.8	Moderate decrease***
Peak Torque 180°/s Extension			
MILK	B-24	-7.6, ±5.2	Small decrease**
	B-48	-5.8, ±7.6	Small decrease**
	B-72	-0.5, ±6.6	Unclear
CHO	B-24	-12.7, ±2.7	Large decrease****
	B-48	-14.5, ±7.4	Large decrease***
	B-72	-8.8, ±2.3	Large decrease****
Peak Torque 180°/s Flexion			
MILK	B-24	-2.8, ±8.0	Small decrease*
	B-48	-4.3, ±9.1	Small decrease*
	B-72	-1.6, ±9.3	Unclear
CHO	B-24	-14.0, ±7.7	Moderate decrease***
	B-48	-11.7, ±13.2	Moderate decrease**
	B-72	-10.8, ±6.1	Small decrease***
CMJ			
MILK	B-24	-1.2, ±3.4	Unclear
	B-48	-1.9, ±3.1	Small decrease*
	B-72	-0.6, ±1.4	Trivial***
CHO	B-24	-10.6, ±4.2	Moderate decrease****
	B-48	-9.9, ±5.0	Moderate decrease***
	B-72	-6.6, ±4.0	Small decrease***
RSI			
MILK	B-24	-7.0, ±5.2	Small decrease**
	B-48	-12.8, ±7.0	Small decrease***
	B-72	-5.3, ±8.0	Small decrease*
CHO	B-24	-10.8, ±9.0	Small decrease**
	B-48	-14.2, ±10.8	Small decrease**
	B-72	-10.8, ±7.8	Small decrease**
5m sprint			
MILK	B-24	-1.7, ±2.4	Small decrease*
	B-48	0.4, ±4.2	Unclear
	B-72	-0.6, ±3.3	Unclear
CHO	B-24	-4.8, ±3.2	Moderate decrease***
	B-48	-3.1, ±2.9	Small decrease**
	B-72	-1.4, ±2.0	Small decrease*

Variable	Timeframe	Mean effect[a] ± or Factor effect x/÷ 90% CI[b]	Qualitative inference[c]
10m sprint			
MILK	B-24	-1.2, ±2.1	Small decrease*
	B-48	-0.1, ±2.7	Unclear
	B-72	-0.3, ±3.2	Unclear
CHO	B-24	-2.4, ±3.0	Moderate decrease**
	B-48	-1.9, ±2.8	Unclear
	B-72	-0.5, ±2.8	Unclear
20m sprint			
MILK	B-24	-1.3, ±1.4	Small decrease*
	B-48	-0.6, ±1.3	Small decrease*
	B-72	-0.6, ±1.4	Small decrease*
CHO	B-24	-2.7, ±2.3	Moderate decrease**
	B-48	-2.2, ±2.8	Unclear
	B-72	-0.1, ±1.7	Unclear
RFD			
MILK	B-24	-11.1, ±11.9	Small decrease*
	B-48	-20.0, ±9.9	Small decrease***
	B-72	-9.9, ±14.5	Small decrease*
CHO	B-24	-23.0, ±9.3	Large decrease****
	B-48	-24.7, ±12.7	Large decrease***
	B-72	-18.5, ±16.4	Moderate decrease**
Creatine Kinase			
MILK	B-24	2.2 x/÷ 1.4	Moderate increase****
	B-48	1.8 x/÷ 1.5	Small increase***
	B-72	1.6 x/÷ 1.5	Small increase**
CHO	B-24	2.1 x/÷ 1.3	Large increase****
	B-48	1.4 x/÷ 1.5	Small increase**
	B-72	1.1 x/÷ 1.4	Unclear
hsCRP			
MILK	B-24	1.2 x/÷ 1.8	Unclear
	B-48	1.2 x/÷ 1.7	Unclear
	B-72	1.1 x/÷ 1.8	Unclear
CHO	B-24	1.0 x/÷ 1.2	Unclear
	B-48	0.9 x/÷ 1.2	Small decrease**
	B-72	0.8 x/÷ 1.3	Small decrease*
Soreness			
MILK	B-24	5.3, ±1.6	Large increase****
	B-48	4.3, ±1.5	Moderate increase****
	B-72	2.0, ±0.7	Small increase****
CHO	B-24	5.8, ±1.3	Large increase****
	B-48	4.8, ±1.1	Moderate increase****
	B-72	2.6, ±0.8	Small increase****
Tiredness			
MILK	B-24	4.3, ±1.1	Moderate increase****
	B-48	3.8, ±1.0	Moderate increase****
	B-72	1.8, ±0.8	Small increase***
CHO	B-24	5.1, ±0.9	Large increase****
	B-48	3.8, ±1.2	Moderate increase****
	B-72	2.3, ±0.8	Small increase****
DALDA			
MILK	B-24	3.4, ± 1.2	Small increase****
	B-48	3.9, ±1.7	Small increase****
	B-72	2.8, ±1.9	Small increase***

Variable	Timeframe	Mean effect[a] ± or Factor effect x/÷ 90% CI[b]	Qualitative inference[c]
CHO	B-24	4.7, ±1.7	Small increase****
	B-48	4.9, ±2.2	Small increase****
	B-72	3.2, ±1.6	Small increase***

*[a] Mean effect refers to Baseline minus 24h/48h/72h/96h; [b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude *Possible,**Likely, ***Very likely, ****Most likely*

6.3.2 Between-group effects

Peak torque

Baseline peak torque values for knee extension at 60°/s for MILK and CHO were 164.4 ± 17.9 Nm and 152.8 ± 10.0 Nm, respectively. Changes in peak torque between baseline and 24h for MILK and CHO were -8.1 ± 3.9% and -12.9 ± 9.3%, respectively, a likely small benefit (ES=0.38) for MILK. Unclear outcomes for MILK versus CHO were found at B-48h (-8.4 ± 4.2% v -11.9 ± 11.0%. A likely small benefit (ES=0.48) for MILK was found at B-72h (-3.9 ± 6.9% v -9.8 ± 11.6%).

At baseline, the peak torque values at 60°/s for knee flexion for MILK and CHO were 83.4 ± 16.5 Nm and 72.3 ± 11.2 Nm, respectively. Changes in peak torque between baseline and 24h for MILK and CHO were -4.4 ± 13.3%, and -13.6 ± 10.6%, respectively, a possible small benefit of milk (ES=0.22). There was an unclear outcome for MILK (-9.4 ± 14.2%) versus CHO (-12.9 ± 8.8%) at B-48h. A likely small benefit (ES=0.31) was observed for MILK (-2.7 ± 11.7%) versus CHO (-11.8 ± 11.6%) at B-72h.

Pre-exercise mean peak torque values for knee extension at 180°/s were 118.6 ± 14.5 Nm and 109.8 ± 7.8 Nm for the MILK and CHO conditions, respectively. A likely small benefit (ES=0.50) for MILK versus CHO was found at B-24h (-7.3 ± 7.6% v -12.6 ± 4.6%), a likely moderate benefit (0.84) B-48h (-5.3 ± 10.7% v -13.8 ± 10.8%) and a very likely moderate benefit (ES=0.94) for MILK from B-72h (0.0 ± 10.0% v -8.7 ± 3.7%). Changes in peak torque can be seen in Figure 6.2.

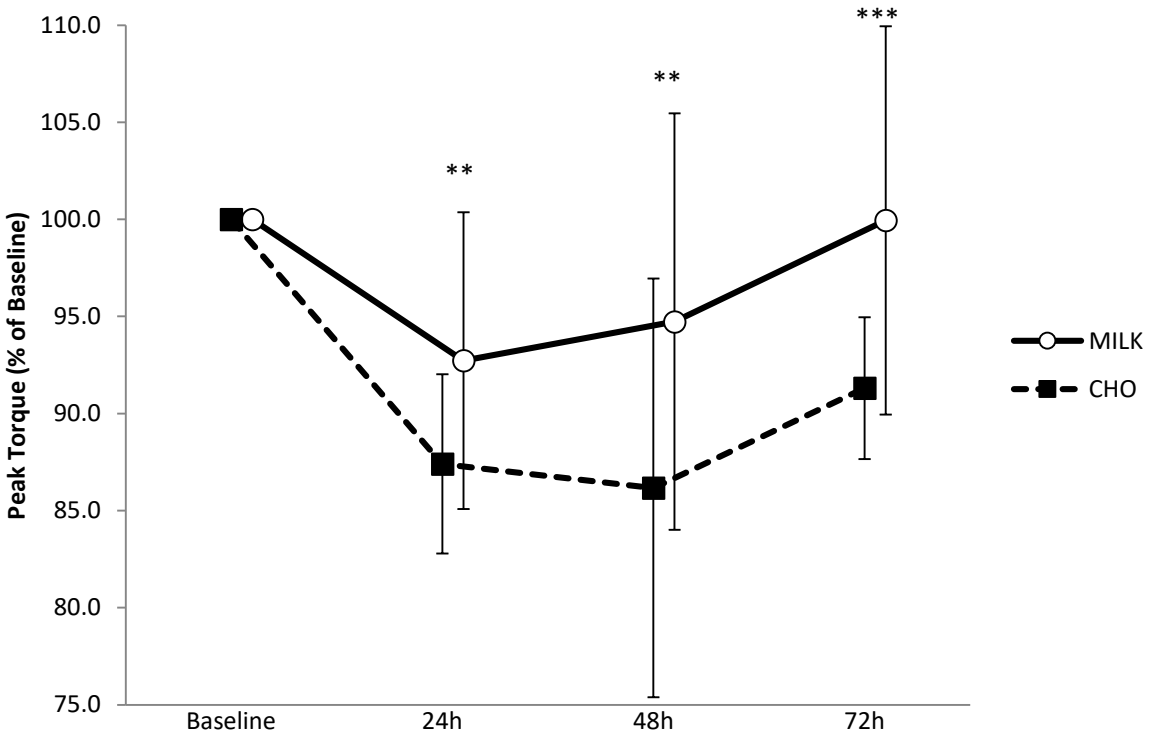

Figure 6. 2 Peak torque at 180°/s for dominant knee extension in response to repeated sprinting and jumping for MILK and CHO.

Values are presented as means ± SD. ** Likely benefit of MILK. ***Very likely benefit of MILK.

Baseline peak torque values for knee flexion at 180°/s for MILK and CHO were 64.4 ± 15.5 Nm and 62.2 ± 11.3 Nm. Changes in the peak torque between baseline and 24h for MILK and CHO were -2.2 ± 12.4% and -13.2 ± 11.2%, respectively, a likely small benefit (ES=0.51) of milk. There was an unclear outcome for MILK (-3.4 ± 13.8%) versus CHO (-10.0 ± 16.3%) at B-48h. A likely small beneficial effect (ES=0.41) was observed for MILK (-0.8 ± 14.2%) compared to CHO (-10.3 ± 9.4%) at B-72h.

Rate of force development

Baseline values for mean rate of force development were 555.1 ± 294.2 $Nm.s^{-1}$ and 634.3 ± 255.1 $Nm.s^{-1}$ for MILK and CHO, respectively. A likely trivial benefit (ES=0.03) for MILK versus CHO was found at B-24h (-8.5 ± 16.8% v -19.4 ± 15.0%), though unclear outcomes were observed at B-48h (-16.6 ± 14.3% v -20.2 ± 19.1%) and B-72h (-18.8 ± 36.6% v -13.8 ± 21.3%).

Countermovement jump performance

Baseline mean countermovement jump heights for MILK and CHO were 26.7 ± 2.7 cm and 26.7 ± 3.5 cm, respectively. A very likely moderate benefit (ES= 0.70) for MILK was seen at B-24h (-1.1 ± 5.2% v -9.7 ± 15.1%), a likely moderate benefit (ES=0.67) from B-48h (-1.8 ± 4.6% v -9.6 ± 8.1%) and a likely small benefit (0.47) from B-72h (-0.6 ± 2.1% v -10.0 ± 13.7%). Changes in CMJ performance can be seen in Figure 6.3.

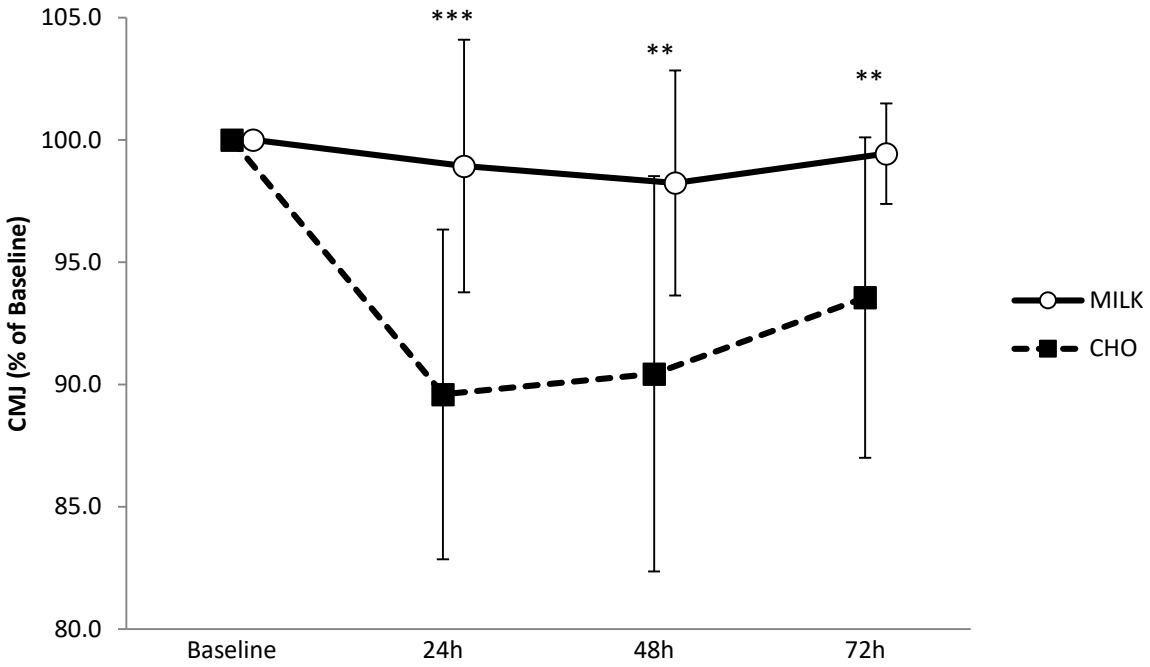

Figure 6. 3 Countermovement jump performance in response to repeated sprinting and jumping for MILK and CHO.

Values are presented as means ± SD. **Likely benefit of MILK. ***Very likely benefit of MILK.

Reactive strength index

Baseline RSI for the MILK group was 105.1 ± 21.9 cm.s^{-1} and for the CHO group was 104.0 ± 24.0 cm.s^{-1}. Comparisons of MILK and CHO at B-24h (-6.7 ± 7.7% v -9.7 ± 15.1%), B-48h (-12.3 ± 10.6% v -12.6 ± 18.7%) and B-72h (-4.7 ± 12.5% v -10.0 ± 13.7%) revealed unclear outcomes.

Sprint performance

At baseline, 5m sprint times were 1.23 ± 0.10 s and 1.25 ± 0.07 s for MILK and CHO, respectively. Between baseline and 24h there was a possible small benefit (ES=0.45) for MILK (-1.6 ± 3.4%) compared to CHO (-4.5 ± 4.5%). Comparisons of MILK and

CHO B-48h (0.5 ± 6.3% v -2.9 ± 4.4%) and B-72h (-0.5 ± 4.9% v -1.3 ± 3.2%) were unclear. Baseline 10m sprint times for MILK and CHO were 2.06 ± 0.12 s and 2.09 ± 0.08 s. All MILK v CHO comparisons gave an unclear outcome (B-24h -1.1 ± 3.0% v -2.3 ± 4.6%; B-48h 0.0 ± 4.0 v 1.8 ± 4.4%; B-72h -0.2 ± 4.8% v -0.4 ± 4.5%). Similarly, with baseline values of 3.57 ± 0.18 s (MILK) and 3.61 ± 0.11 s (CHO), unclear outcomes were observed for 20m sprint performance (B-24h -1.2 ± 2.0% v -2.6 ± 3.6%; B-48h -0.6 ± 1.9% v -2.0 ± 4.4%; B-72h -0.6 ± 2.1% v -0.1 ± 2.8%).

A summary of the statistical analysis for Peak torque, RFD, CMJ, RSI and sprint performance can be seen in Table 6.2.

Table 6. 2 Effects on muscle function following repeated sprinting and jumping

Variable	Time Frame	Mean effect[a], ±90% CI[b]	Qualitative Inference[c]
Peak Torque 60°/s Dominant leg extension	B – 24	6.0, ±7.7	Small benefit**
	B – 48	4.6, ±8.7	Unclear
	B – 72	7.2, ±10.3	Small benefit**
Peak Torque 60°/s Dominant leg flexion	B – 24	8.7, ±13.5	Small benefit*
	B – 48	3.3, ±13.4	Unclear
	B – 72	9.4, ±12.3	Small benefit*
Peak Torque 180°/s Dominant leg extension	B – 24	7.2, ±6.4	Small benefit**
	B – 48	10.8, ±9.6	Moderate benefit**
	B – 72	9.9, ±6.8	Moderate benefit***
Peak Torque 180°/s Dominant leg flexion	B – 24	12.4, ±13.8	Small benefit**
	B – 48	8.0, ±18.9	Unclear
	B – 72	10.2, ±12.1	Small benefit**
RFD (0-200ms)	B – 24	22.7, ±31.7	Trivial benefit**
	B – 48	21.6, ±43.4	Unclear
	B – 72	24.0, ±46.0	Unclear
CMJ	B – 24	9.9, ±6.2	Moderate benefit***
	B – 48	8.7, ±6.9	Moderate benefit**
	B – 72	6.1, ±4.7	Small benefit**
RSI	B – 24	2.6, ±11.3	Unclear
	B – 48	2.6, ±15.1	Unclear
	B – 72	5.6, ±12.2	Unclear
5m sprint	B – 24	-2.7, ±3.7	Small benefit*
	B – 48	-3.3, ±5.3	Unclear
	B – 72	-0.8, ±3.6	Unclear
10m sprint	B – 24	-1.4, ±3.5	Unclear
	B – 48	-1.8, ±3.9	Unclear
	B – 72	-0.5, ±3.9	Unclear
20m sprint	B – 24	-1.5, ±2.6	Unclear
	B – 48	-1.3, ±2.9	Unclear
	B – 72	0.3, ±2.2	Unclear

[a] Mean effect refers to MILK minus CHO; [b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; **[c]***Qualitative Inference represents the likelihood that the true value will have the observed magnitude *Possible,**Likely, ***Very likely, ****Most likely*

Muscle soreness and tiredness

Peak soreness and tiredness was observed 24h following exercise and values had not returned to baseline by 72h. A likely large benefit for MILK (27.1 ± 13.8%) versus CHO (31.7 ± 16.6%) was observed at 72h for soreness during isokinetic testing; all other comparisons were unclear.

DALDA

For DALDA B, a very likely very large benefit of MILK (13.5 ± 7.4%) compared to CHO (18.7 ± 11.0%) was observed at B-24h and a likely very large benefit was observed at B-48h (15.5 ± 10.1% v 19.6 ± 14.1%). From B-72h (11.0 ± 11.5% v 12.9 ± 10.5%) outcomes were unclear (See Figure 6.4).

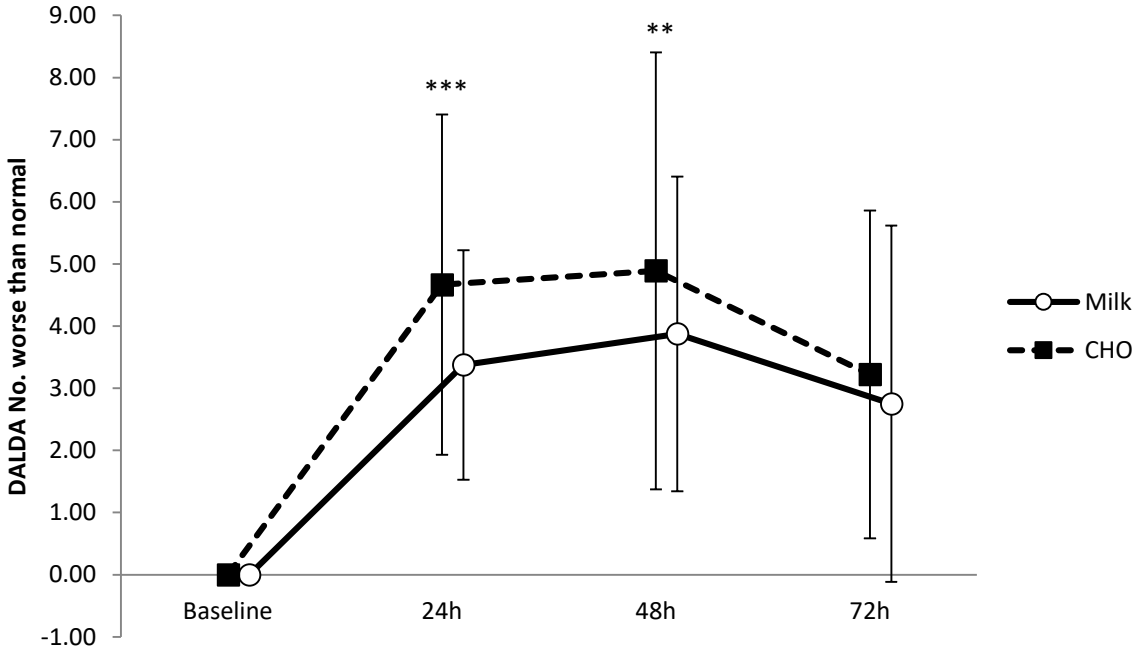

Figure 6. 4 DALDA B in response to repeated sprinting and jumping for MILK and CHO.

Values are presented as means ± SD. ***Very likely benefit of MILK. **Likely benefit of MILK.

Creatine kinase

Baseline serum CK values were 128.5 ± 118.4 U/l and 192.2 ± 103.6 U/l for MILK and CHO, respectively. Unclear outcomes for the comparison of MILK versus CHO were found at B-2h (1.8 x/÷ 1.3 v 1.7 x/÷ 1.3), B-24h (2.3 x/÷ 1.8 v 2.1 x/÷ 1.4), B-48h (1.8 x/÷ 1.9 v 1.4 x/÷ 1.8) and B-72h (1.4 x/÷ 1.9 and 1.1 x/÷ 1.8) (Figure 6.5).

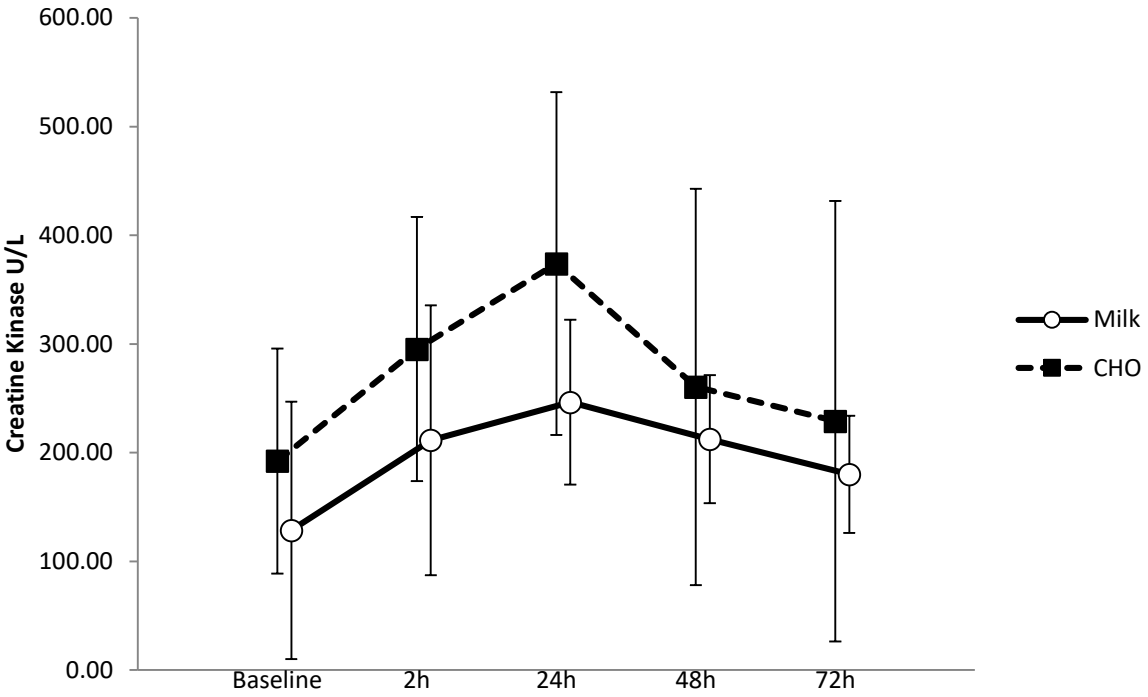

Figure 6. 5 Serum CK in response to repeated sprinting and jumping for MILK and CHO.

Values are presented as means ± SD.

hsCRP

Baseline hsCRP values were 1.0 ± 0.6 mg/l and 1.3 ± 0.6 mg/l for MILK and CHO. Unclear outcomes were observed for MILK versus CHO at B-2h (1.1 x/÷ 1.2 v 1.0 x/÷ 1.3), B-24h (1.3 x/÷ 2.0 v 1.0 x/÷ 1.3), B-48h (1.2 x/÷ 2.0 v 0.9 x/÷ 1.3) and B-72h (1.1 x/÷ 1.9 v 0.8 x/÷ 1.5). Mean effects for all measures of soreness and tiredness, symptoms of stress, CK and hsCRP can be seen in Table 6.3.

Table 6. 3 Effects on soreness, tiredness, symptoms of stress, CK and hsCRP following repeated sprinting and jumping

Variable	Time Frame	Mean effect[a] ± or Factor Effect x/÷ 90% CI[b]	Qualitative Inference[c]
Muscle soreness (Squat)	B – 24	-1.7, ±2.8	Unclear
	B – 48	-1.1, ±2.1	Unclear
	B – 72	-0.7, ±1.2	Unclear
Muscle soreness (Isokinetic knee extension/flexion)	B – 24	-1.4, ±2.2	Unclear
	B – 48	-1.1, ±2.3	Unclear
	B – 72	-1.0, ±2.6	Trivial benefit**
Muscle tiredness	B – 24	-2.0, ±3.0	Unclear
	B – 48	-1.6, ±3.2	Unclear
	B – 72	-1.4, ±2.3	Unclear
DALDA B	B – 24	-2.0, ±2.4	Small benefit***
	B – 48	-1.8, ±3.0	Trivial benefit**
	B – 72	-1.0, ±2.6	Unclear
CK	B – 2	1.1 x/÷1.3	Unclear
	B – 24	1.1 x/÷1.6	Unclear
	B – 48	1.3 x/÷ 1.8	Unclear
	B – 72	1.3 x/÷1.8	Unclear
hsCRP	B – 2	1.1 x/÷1.2	Unclear
	B – 24	1.2 x/÷1.8	Unclear
	B – 48	1.4 x/÷1.8	Unclear
	B – 72	1.3 x/÷1.7	Unclear

*[a] Mean effect refers to MILK minus CHO; [b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude **Likely, ***Very likely.*

6.4. Discussion

The key findings of this investigation indicate that consumption of 500ml of milk post-repeated sprinting and jumping attenuates losses in CMJ and peak torque at 60° and 180°/s for both extension and flexion, similar to previous research with female athletes following maximal eccentric contractions (Rankin *et al.,* 2015). Additionally, a benefit for MILK was seen for RFD and 5m sprint over the first 24h of recovery, but not for RSI, 10m or 20m sprint performance, muscle soreness and tiredness, CK and hsCRP.

There are a number of possible explanations for the observed benefits of milk on muscle function. The composition of milk, with ~80% casein and ~20% whey protein, provides both fast and slow delivery of amino acids for muscle protein turnover (Wilkinson *et al*., 2007). Previous research has indicated that the consumption of

amino acids following resistance training further stimulates protein synthesis, resulting in increased net protein synthesis (Tipton *et al*., 2004; Miller et al. 2003; Børsheim *et al*., 2002). The availability of amino acids, in particular, leucine, stimulates mammalian target of rapamycin (mTOR), the significant regulator of muscle protein synthesis (Norton and Layman, 2006). The consumption of protein early in the recovery period is likely to increase the phosphorylation of signalling proteins that regulate translation initiation and elongation, thus enhancing muscle protein synthesis (West *et al*., 2011). This effect has been observed with the consumption of protein in close temporal proximity to resistance training (Koopman *et al*., 2007), sprinting (Coffey *et al.,* 2011) and, more recently, concurrent training (Camera *et al*., 2015). Furthermore, it is possible that milk has a positive effect on the calpain degredative pathways that are initiated following eccentric exercise consequential to increased cellular concentrations of calcium (Croall *et al*., 1991). This would result in a preservation of myofibrillar and membrane protein integrity and force transmission ability, and an attenuation of losses in muscle function during the recovery period. Moreover, increased insulin has been observed following the consumption of milk (Gannon *et al*., 1986). Raised insulin inhibits muscle protein breakdown (Abdulla *et al.,* 2016), perhaps via the ubiquitin-proteasome pathway, further contributing to preservation of muscle function following EIMD.

The reasons for disparity in results between peak torque, RFD, 5m sprint and measures of RSI, 10m and 20m sprint are uncertain. The preferential damage of type 2 fibres with eccentric loading leads to reduced ability to generate power (Fridén *et al*., 1983) which is key in the explosive nature of the assessment of RFD and 5m sprint performance; the consumption of milk was beneficial in attenuating these losses. RSI is an assessment of the stretch shortening cycle (SSC). Twist and Eston (2005) proposed that a reduced reflex sensitivity during the SSC following EIMD could impair the ability to use ground impact forces, such as during drop jumping. It is conceivable that milk does not have an effect on this reduced neuromuscular function following EIMD, though this does not explain a lack of benefit of milk on 10m and 20m sprint performance. Further investigation may provide greater insight.

There was no effect of milk on serum CK concentration. Muscle membrane damage following eccentric loading occurs as a result of the mechanical stress during the primary phase of muscle damage with further disruption via the lysosomal pathway

during the secondary phase (Armstrong, 1990). The lack of an intervention effect on serum CK suggests that the consumption of milk does not affect this pathway. CK concentration, a marker of sarcolemma damage, was elevated during the recovery period, peaking 24h post-exercise and returning to baseline values by 72h. Mean peak CK values (246.4 ± 75.8 and 373.9± 157.82 U/l for MILK and CHO, respectively) were lower than observed for female athletes in an investigation employing isolated eccentric exercise (Rankin *et al.,* 2015). They were higher compared to those reported following repeat-sprint cycling with females (Rankin *et al*., 2017) but comparable to values reported following repeated sprinting in females (Keane *et al*., 2015). This is not surprising considering that the first investigation provoked muscle damage on an isolated muscle group utilising a maximal isokinetic eccentric loading while the second utilised a protocol lacking eccentric loading. The reported CK response is lower than that reported following similar exercise in males (Howatson and Milak, 2009), though not unexpected as it has been suggested that females experience reduced post-exercise muscle damage compared to males because of a protective effect of oestrogen (Stupka and Tiidus, 2001; Koumulainen *et al*., 1999). The observed beneficial effect of milk on muscle function and lack of effect on CK implies that serum CK is not a good indicator of muscle function following exercise, a conclusion alluded to most recently by Scott *et al*., (2016) following participation in a soccer match.

A comparison of conditions demonstrates little benefit for MILK compared to CHO in the perception of muscle soreness, though given that DOMS is a subjective measure, it is difficult to compare independent groups. Increases in soreness were lower than those reported in previous studies following repeated sprinting (Howatson and Milak, 2009) and repeated jumping (Marginson *et al*., 2005). However, these studies utilised male participants, and oestrogen may have an effect on the perception of pain and muscle soreness experienced following damaging exercise by playing a role in the binding of opioids to receptors in the brain and spinal cord, thereby modifying the transmission of pain (Kendall and Eston, 2002). Nonetheless, the observed soreness ratings were also lower than those reported by Keane *et al*., (2015) following a repeat-sprint protocol with females, despite the addition of repeated jumping in the current study. The reasons for this are unclear, though may reflect different degrees of prior exposure to repeated sprinting and jumping. Similar to the female participants in this study, those in Keane *et al*., were regular participants in team sport training

and competition, though in soccer, rugby union and netball. The participants in the current study were Gaelic football and camogie players and while the demands of these sports are somewhat similar to other team sports, the differences may contribute to the difference in the responses to the exercise protocol. In the current study, hsCRP did not increase following exercise despite an increase in soreness and inflammation being one proposed explanation for soreness. However, serum hsCRP may not be reflective of the total cytokine activity in the post-exercise period. The disparity of muscle function and soreness results observed indicate different pathways for the recovery of muscle function and perception of soreness, which may or may not involve the inflammatory response. Similar to CK, muscle soreness is a poor indicator of muscle function during the recovery period.

The measurement of symptoms of stress was completed using the DALDA questionnaire. Analysis indicated that those who consumed milk reported fewer 'worse than normal' symptoms 24h post-exercise. Physiological processes can be influenced by psychological status (Mehta and Agnew, 2012) and it is known that an individual's belief in the efficacy of an intervention can influence subsequent responses (Beedie and Foad, 2009). It is possible that this was the case in the current study as the participants were not blinded to the recovery drink received and may have been aware of potential benefits of milk for recovery through previously published research. However, given that there were no differences in perception of soreness or tiredness suggests genuine reduced symptoms of stress in the participants who consumed milk.

A limitation of the study is that there was no control for the phase of the menstrual cycle, and thus participants were likely to have had varying concentrations of circulating hormones, including oestrogen as mentioned above, at the time of testing. Nonetheless, it has been reported that oestrogen concentration may have limited effect on the symptoms of EIMD in exercising females (Markofski and Braun, 2014). It is possible that a larger intake of milk may have provided additional recovery benefits. While previous research has reported recovery benefits with consumption of 500ml milk (Rankin *et al*., 2015; Cockburn *et al*., 2010; 2008), this volume provides 17g of protein which is lower than the recommended 20g threshold for maximal stimulation of protein synthesis (Moore *et al.,* 2009), and substantially lower than the 40g recently found to be of greater benefit than 20g following whole-body exercise

(Macnaughton *et al*., 2016). Furthermore, given that post-exercise protein synthesis remains elevated for at least 24h post-resistance exercise (Burd *et al*., 2011), additional prescribed intake of milk during this time period may influence recovery. Research opportunities exist to extrapolate this further, particularly regarding recovery from team-sport specific exercise and in a female cohort.

In conclusion, the consumption of 500ml of milk post repeated sprinting and jumping had a positive effect on the attenuation of losses in muscle function, thus improving recovery, compared to an energy-matched carbohydrate drink. From a practical perspective, this intervention may serve to enhance the recovery of female team-sport athletes following activities that involve sprinting and jumping. This study reinforces the necessity for specific recovery interventions that are matched to the mode of exercise; in this case when the exercise includes eccentric loading. As is evident from this study, including milk in such a mode-specific approach is beneficial for recovery in female athletes.

Chapter 7: The Effect of Milk on Recovery From Repeated Simulated Team Sports in Females

7.1 Introduction

Minimising muscle damage and enhancing recovery between training sessions or games could be beneficial for maintaining performance in subsequent bouts. Recent research has shown that the consumption of milk following muscle damaging exercise can attenuate decreases in muscle functional capacity in males (Cockburn *et al.,* 2010; 2008) and females (Rankin *et al.,* 2015, 2018, Chapter 1 and Chapter 3) including peak torque, reactive strength index (RSI) and sprint performance. The mechanism for this effect is unknown though milk may increase post-exercise protein synthesis or reduce post-exercise degradation of muscle proteins (Phillips *et al.,* 2011). However, to date, it is unknown if the consumption of milk can enhance recovery from team sport competition and subsequent match performance.

Multi-sprint team sports are characterised by periods of high-intensity work interspersed with periods of low-intensity effort or rest. Participation in a single team sport match results in fatigue, muscle damage and oxidative stress (Silva *et al.,* 2013; Marin *et al.,* 2011). In reality, team-sport athletes engage in multiple training sessions or games within short periods of time resulting in insufficient recovery (Fatouros *et al.,* 2010; Ispirlidis *et al*., 2008). Repeated match play with limited recovery time has been found to result in fatigue, performance impairment, with strong correlations between muscle damage, muscle soreness, performance decrements and the number of sprints and changes of direction executed in subsequent games (Nedelec *et al*., 2014). Recently, Mohr *et al*., (2016) investigated muscle damage, inflammation and performance responses to three soccer games played within one week. Results indicated that the largest physiological stress, fatigue and decreased performance followed the second game which was preceded by only 3 days recovery. While the vast majority of investigations into the physiological demands of team sports come from the study of male athletes, Gravina *et al.,* (2011) explored the effects of soccer match play on markers of muscle damage and oxidative stress over an 18h recovery period in female soccer players. Similar to studies involving male players, they reported increased CK, LDH and Total Antixoxidant Status (TAS), though no measurements of muscle function were considered. In addition to increased CK (~152%), Andersson *et al.,* (2008) reported a decrease in sprint performance (-3%), CMJ (-4%), peak torque for knee extension (-7.1%) and knee flexion (-9.4%) following participation in a soccer match with female

athletes. Based on current literature, opportunities exist to explore the responses of female team sport athletes to repeated match play with short recovery duration.

Given the benefits of milk for enhanced recovery cited in the literature, and given the short recovery periods typically experienced by athletes between team-sport training sessions and games, it makes the expectation tenable that post-exercise consumption of milk may combat the negative effects of muscle damage and fatigue, thus accelerating the rate of recovery following simulated match-play. Accordingly, this study aims to examine the effect of milk on recovery from a simulated team game and a second simulated team game following a 48h recovery period, plus the effect of milk on 'in-game' measures in the second simulated game. Additionally, we aim to determine the impact of a second bout of exercise on the recovery profiles of athletes.

7.2 Methodology

7.2.1 Participants

Twenty female team sport athletes (mean age 20.6 ± 3.0 y; height 164.7 ± 5.0 cm; mass 65.4 ± 9.2 kg), drawn from the sports of camogie and ladies Gaelic football, participated in this investigation. Details on screening and inclusion/exclusion criteria are provided in Section 3.2. Ethics approval was gained from the Ethics Committees from both Middlesex University and Institute of Technology Carlow where the data collection took place.

7.2.2 Study design

Participants visited the laboratory on nine occasions. The first visit comprised study briefing and familiarisation with all variables. The purpose of the second visit was to determine baseline measures, following which participants were divided into two groups, MILK and CHO. Groups were matched according to player position and peak torque of the dominant leg knee extensors at 60°/s. All data collection took place at the same time of day following 24h abstinence from alcohol, caffeine and exercise.

Participants completed all measures following an overnight fast and a small standardised snack. No more than 5 days post-baseline measures, participants

completed an exercise protocol comprising a team sport circuit that simulates team sport games (STG1). Participants returned to the laboratory at 24 and 48h post exercise to repeat baseline measures. Upon completion of outcome measures 48h post-exercise, participants repeated the circuit (STG2) returning again to repeat baseline measures 72h and 96h post STG1 (Figure 7.1). Participants were requested to refrain from strenuous activity for the duration of the study, and from treating the symptoms of muscle damage and soreness with interventions such as nutritional supplements, massage, cryotherapy and non-steroidal anti-inflammatory drugs.

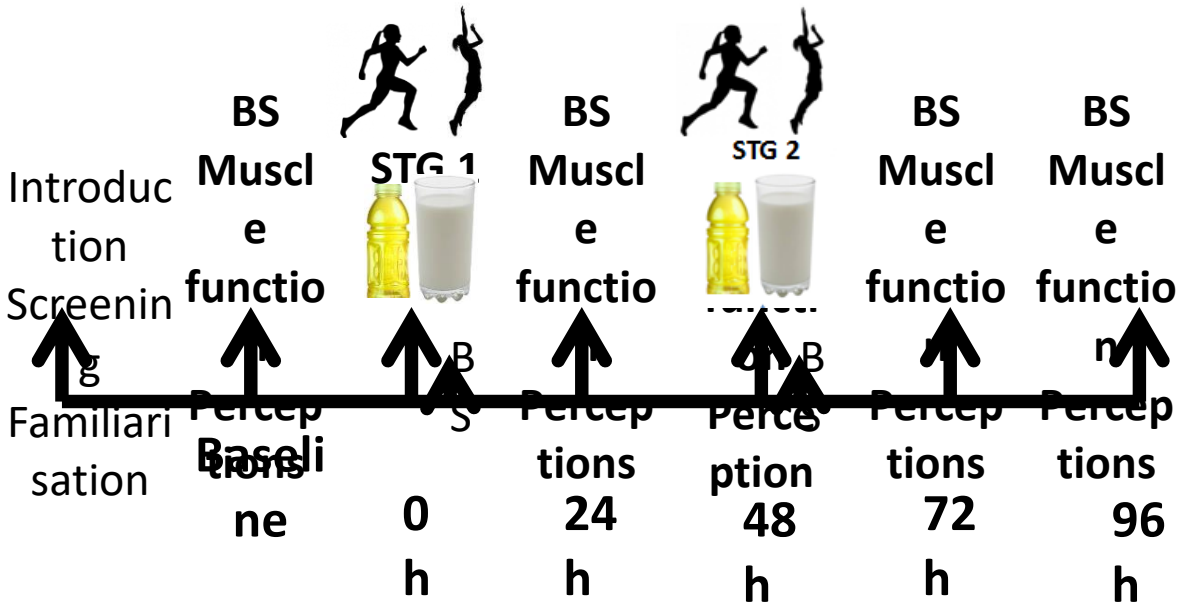

Figure 7. 1 Schematic of Study Design

7.2.3 Nutritional intervention and dietary control

Thirty minutes prior to commencing each STG each participant consumed a small snack (Oats and Honey cereal bar, Nature Valley, Middlesex, UK). The macronutrient composition of the bar was: energy 805kJ/192kcal, protein 3.4g, carbohydrate 27.1g, fat 7.2g. Immediately following the protocol, participants were provided with either 500ml of milk or 500ml of an energy-matched carbohydrate solution as detailed in Section 3.4. Each participant completed a food diary for 24h prior to baseline testing and for the subsequent 4 days. Food diary details are also presented in Section 3.4. Analysis using Magnitude Based Inference indicated that there were no clear differences in energy, carbohydrate, protein or fat intake between groups.

7.2.4 Exercise protocol

On two occasions, separated by 48h, participants completed a modified version of a validated exercise protocol described by Bishop *et al.,* (2001) which was designed to simulate team sport competition. Briefly, following a 10min warm-up, participants completed 2 x ~30min halves of intermittent running in a circuit layout, separated by a 10min half-time to simulate the format of camogie and ladies Gaelic football games. The STG consisted of a circuit that took ~51-53s to complete. Each lap commenced every 80s and 22 laps were completed in each half. The circuit was ~125m in length (total distance 5500m) and included three maximal sprints (2x10m and 1x20m), striding (27m), an agility exercise (12m) and countermovement jumps (2x), all separated by walking (20m) and jogging (26m) (Figure 7.1). Sprint performance (5m, 15m), CMJ, lap time, HR and RPE were recorded for each lap of the circuit. On the first day of the circuit, participants were allowed to drink water *ad libitum*; the same volume of water was provided to each participant for consumption during the second circuit. Upon completion of the STG, participants completed a standard cool-down, immediately after which they were provided with their assigned drink.

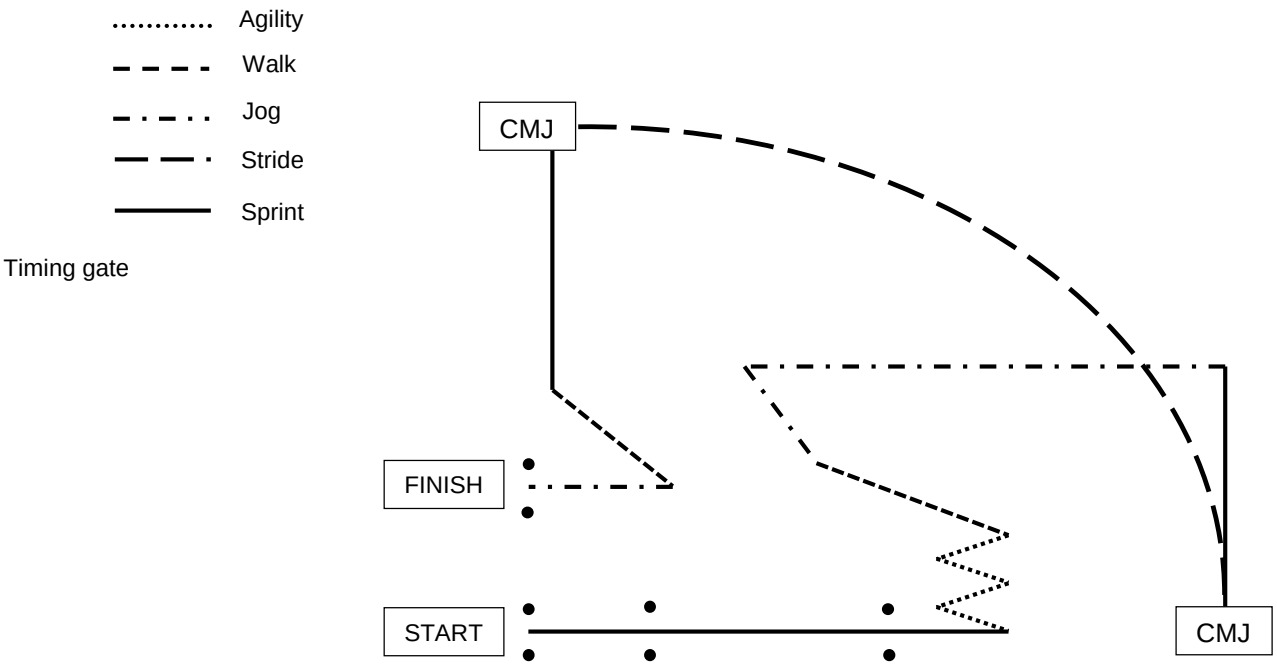

Figure 7. 2 Simulated team game circuit

7.2.5 Blood sampling

Following a 10min rest in the supine position, and prior to any other measurements, a venous blood sample was obtained via venepuncture from a forearm vein at the antecubital fossa. Details of analysis are provided in Section 3.8.1(CK), Section 3.8.3 (hsCRP), Section 3.8.4 (PC) and Section 3.8.6 (LOOH).

7.2.6 Peak torque

Peak isokinetic torque was determined on a Biodex System3 Isokinetic dynamometer according to details in Section 3.9.

7.2.7 Rate of force development

Rate of force development (RFD) was determined over 100-200ms of an isometric contraction (Peñailillo *et al.,* 2015), as detailed in Section 3.10.

7.2.8 Countermovement jump height

Countermovement jump height (cm) was determined according to the details in Section 3.11.

7.2.9 Reactive Strength Index

Reactive Strength Index (RSI) was measured from three maximal effort drop jumps from a height of 45cm onto an indoor sprint track. Refer to Section 3.12 for details.

7.2.10 Sprint performance

Five metre, 10m and 20m sprint performance, from a standing start 20cm behind the start line, were performed on an indoor sprint track. See Section 3.13 for detailed procedures.

7.2.11 Muscle soreness and muscle tiredness

Using a Visual Analogue Scale (VAS), active muscle soreness during squatting to approximately 90° knee flexion and during isokinetic testing of knee extension at

60°/s was determined. A similar VAS was used to measure passive muscle tiredness. See details in Section 3.6.

7.2.12 Symptoms of stress

The Daily Analysis of Life Demands (DALDA) questionnaire (Rushall, 1990) was used to monitor the symptoms of stress. The questionnaire was completed at the same time each day and Section 3.7 provides details on how the questionnaire was administered.

7.2.13 Data analysis

Data was analysed by making probabilistic magnitude based inferences about the true values of the effect of intervention on outcomes by expressing the uncertainty as 90% confidence limits (CL) as described by Batterham and Hopkins (2006). See Section 3.14 for details.

7.3 Results

7.3.1 Within-group effects

Post-STG1 analysis of within-group effects revealed post-exercise decreases in peak torque, CMJ, RSI, sprint performance and RFD, increases in serum CK and hsCRP and in muscle soreness and tiredness. Minimal effects were seen on markers of oxidative stress. The impact of STG2 on muscle function was minimal, with mostly trivial or unclear outcomes, though there was a trivial decrease in sprint performance following STG2. STG2 resulted in increased CK, hsCRP for CHO and LOOH for MILK. Mean effects, ± 90%CI, with qualitative inferences and Effect Size are presented in Table 7.1.

Table 7. 1 Within-group effects over time for dependent variables

Variable	Timeframe	Mean effect, ±90% CI or Factor effect x/÷ 90% CI[a]	Qualitative inference[b]
Peak Torque 60°/s Extension			
MILK	B-24	-6.1, ±3.6	Small decrease**
	B-48	-4.7, ±3.4	Small decrease*
	B-72	-4.5, ±4.7	Small decrease*
	B-96	-3.3, ±4.7	Trivial decrease*
	48-72	0.2, ±5.6	Trivial**

Variable	Timeframe	Mean effect, ±90% CI or Factor effect x/÷ 90% CI[a]	Qualitative inference[b]
	48-96	1.4, ±5.5	Trivial*
CHO	B-24	-10.2, ±2.9	Moderate decrease****
	B-48	-9.6, ±5.0	Moderate decrease***
	B-72	-6.8, ±4.2	Moderate decrease***
	B-96	-3.6, ±3.2	Small decrease**
	48-72	3.0, ±2.3	Small increase**
	48-96	6.7, ±4.9	Small increase***
Peak Torque 60°/s Flexion			
MILK	B-24	-5.0, ±6.1	Small decrease**
	B-48	-5.4, ±5.5	Small decrease**
	B-72	-3.0, ±5.1	Small decrease*
	B-96	-3.2, ±5.6	Unclear
	48-72	2.5, ±6.1	Unclear
	48-96	2.3, ±3.8	Unclear
CHO	B-24	-11.6, ±4.9	Moderate decrease***
	B-48	-15.3, ±9.5	Moderate decrease***
	B-72	-12.3, ±11.5	Moderate decrease**
	B-96	-5.4, ±9.7	Unclear
	48-72	3.6, ±3.4	Small increase*
	48-96	11.7, ±3.1	Moderate increase****
Peak Torque 180°/s Extension			
MILK	B-24	-4.0, ±4.6	Small decrease*
	B-48	-1.7, ±5.0	Trivial**
	B-72	-1.6, ±5.3	Trivial**
	B-96	-1.8, ±5.9	Trivial decrease*
	48-72	0.2, ±2.9	Trivial***
	48-96	-0.1, ±4.0	Trivial**
CHO	B-24	-3.2, ±2.9	Small decrease*
	B-48	-3.4, ±4.7	Small decrease*
	B-72	-4.6, ±4.4	Small decrease**
	B-96	-1.5, ±3.1	Trivial decrease*
	48-72	-1.2, ±3.7	Trivial decrease*
	48-96	2.0, ±3.5	Unclear
Peak Torque 180°/s Flexion			
MILK	B-24	-4.7, ±5.0	Small decrease*
	B-48	-3.1, ±1.8	Small decrease*
	B-72	-4.2, ±3.4	Small decrease*
	B-96	-4.5, ±5.9	Small decrease*
	48-72	-1.1, ±4.4	Trivial**
	48-96	-1.5, ±5.9	Unclear
CHO	B-24	-7.0, ±5.2	Small decrease**
	B-48	-9.1, ±3.7	Small decrease***
	B-72	-6.1, ±7.6	Small decrease*
	B-96	-5.3, ±7.3	Small decrease*
	48-72	3.3, ±6.1	Unclear
	48-96	4.2, ±6.5	Unclear
CMJ			
MILK	B-24	-6.1, ±2.1	Small decrease***
	B-48	-4.7, ±3.6	Small decrease**
	B-72	-4.7, ±2.8	Small decrease**
	B-96	-3.3, ±3.5	Trivial decrease*

Variable	Timeframe	Mean effect, ±90% CI or Factor effect x/÷ 90% CI[a]	Qualitative inference[b]
	48-72	0.1, ±3.7	Trivial**
	48-96	1.5, ±1.4	Unclear
CHO	B-24	-7.5, ±5.7	Moderate decrease**
	B-48	-8.6, ±7.3	Moderate decrease**
	B-72	-6.5, ±5.5	Small decrease**
	B-96	-5.3, ±7.0	Small decrease**
	48-72	2.4, ±4.0	Unclear
	48-96	3.6, ±3.7	Small increase**
RSI			
MILK	B-24	-8.5, ±6.1	Small decrease**
	B-48	-13.5, ±5.2	Moderate decrease****
	B-72	-17.8, ±5.4	Moderate decrease****
	B-96	-10.0, ±6.0	Small decrease***
	48-72	-5.0, ±6.4	Small decrease*
	48-96	4.1, ±6.7	Unclear
CHO	B-24	-15.0, ±6.4	Moderate decrease***
	B-48	-14.8, ±5.6	Moderate decrease****
	B-72	-10.8, ±10.7	Small decrease**
	B-96	-2.8, ±12.2	Trivial decrease*
	48-72	4.9, ±7.8	Unclear
	48-96	14.1, ±10.0	Moderate increase***
5m sprint			
MILK	B-24	1.3, ±1.5	Trivial decrease*
	B-48	1.5, ±2.3	Small decrease*
	B-72	2.2, ±2.6	Small decrease**
	B-96	-0.3, ±4.5	Unclear
	48-72	0.7, ±1.9	Trivial*
	48-96	-1.7, ±3.5	Unclear
CHO	B-24	3.1, ±2.1	Moderate decrease***
	B-48	3.8, ±2.7	Moderate decrease***
	B-72	4.8, ±3.2	Large decrease***
	B-96	2.8, ±3.0	Moderate decrease**
	48-72	1.0, ±1.5	Small decrease*
	48-96	-1.0, ±1.5	Unclear
10m sprint			
MILK	B-24	2.2, ±1.4	Small decrease**
	B-48	1.2, ±1.8	Small decrease*
	B-72	1.9, ±1.7	Small decrease**
	B-96	-0.8, ±0.8	Trivial increase*
	48-72	0.7, ±1.4	Trivial decrease*
	48-96	-1.9, ±1.5	Small increase**
CHO	B-24	2.2, ±1.9	Moderate decrease**
	B-48	2.8, ±2.0	Moderate decrease***
	B-72	2.5, ±2.1	Moderate decrease**
	B-96	1.1, ±1.4	Small decrease*
	48-72	-0.2, ±1.4	Unclear
	48-96	-1.6, ±1.9	Small increase**
20m sprint			
MILK	B-24	2.6, ±1.7	Small decrease**
	B-48	2.1, ±1.3	Small decrease**
	B-72	2.7, ±1.7	Small decrease**
	B-96	0.2, ±3.3	Unclear
	48-72	0.6, ±1.2	Trivial*
	48-96	-2.2, ±3.1	Unclear
CHO	B-24	2.0, ±1.1	Small decrease**

Variable	Timeframe	Mean effect, ±90% CI or Factor effect x/÷ 90% CI[a]	Qualitative inference[b]
	B-48	2.0, ±2.1	Small decrease**
	B-72	2.4, ±1.3	Small decrease***
	B-96	1.2, ±1.1	Small decrease*
	48-72	0.4, ±1.8	Trivial decrease*
	48-96	-0.8, 2.2	Unclear
RFD			
MILK	B-24	-12.1, ±17.4	Small decrease*
	B-48	-21.6, ±11.6	Moderate decrease***
	B-72	-14.6, ±13.7	Small decrease**
	B-96	-8.2, ±9.0	Trivial decrease*
	48-72	8.9, ±14.6	Unclear
	48-96	17.1, 20.4	Small increase*
CHO	B-24	-35.5, ±18.3	Moderate decrease***
	B-48	-30.4, ±14.0	Moderate decrease***
	B-72	-26.7, ±14.9	Moderate decrease***
	B-96	-33.2, ±22.3	Moderate decrease**
	48-72	-3.1, ±25.4	Trivial decrease*
	48-96	-4.0, ±23.6	Trivial decrease*
Creatine Kinase			
MILK	B-2	1.7 x/÷ 1.4	Moderate increase***
	B-24	1.7 x/÷ 1.3	Moderate increase***
	B-48	1.1 x/÷1.3	Trivial increase*
	B-52	2.3 x/÷1.2	Large increase****
	B-72	1.7 x/÷ 1.7	Moderate increase**
	B-96	1.2 x/÷1.3	Small increase*
	48-52	2.3 x/÷ 1.2	Large increase***
	48-72	1.6 x/÷ 1.7	Large increase**
	48-96	1.2 x/÷ 1.2	Small increase*
CHO	B-2	1.9 x/÷ 1.4	Large increase***
	B-24	1.7 x/÷ 1.7	Large increase**
	B-48	1.3 x/÷ 1.5	Small increase**
	B-52	2.3 x/÷ 1.2	Large increase****
	B-72	1.7 x/÷ 1.6	Moderate increase**
	B-96	1.2 x/÷ 1.4	Small increase*
	48-52	1.8 x/÷ 1.2	Large increase****
	48-72	1.3 x/÷ 1.4	Small increase **
	48-96	0.9 x/÷ 1.3	Unclear
hsCRP			
MILK	B-2	1.1 x/÷ 1.4	Unclear
	B-24	1.5 x/÷ 1.5	Small increase**
	B-48	1.2 x/÷ 1.6	Unclear
	B-52	1.1 x/÷ 1.3	Unclear
	B-72	1.4 x/÷1.6	Unclear
	B-96	1.1 x/÷ 1.8	Unclear
	48-52	1.0 x/÷ 1.1	Trivial**
	48-72	1.1 x/÷ 1.2	Trivial increase*
	48-96	0.9 x/÷ 1.4	Unclear
CHO	B-2	0.9 x/÷ 1.2	Small increase*
	B-24	0.9 x/÷ 1.4	Unclear
	B-48	0.7 x/÷ 1.4	Small increase**
	B-52	1.1 x/÷1.3	Unclear
	B-72	1.3 x/÷ 1.3	Unclear
	B-96	1.2 x/÷ 1.7	Unclear
	48-52	1.6 x/÷ 2.0	Very large increase**

Variable	Timeframe	Mean effect, ±90% CI or Factor effect x/÷ 90% CI[a]	Qualitative inference[b]
	48-72	1.8 x/÷ 2.1	Very large increase**
	48-96	1.6 x/÷ 2.0	Large increase**
Soreness (isokinetic)			
MILK	B-24	5.1, ±0.4	Large increase****
	B-48	6.0, ±0.6	Large increase****
	B-72	6.1, ±0.9	Large increase****
	B-96	4.4, ±1.3	Moderate increase****
	48-72	0.1, ±0.6	Unclear
	48-96	-1.6, ±1.2	Small decrease**
CHO	B-2	5.8, ±0.7	Large increase****
	B-48	6.1, ±0.9	Large increase****
	B-72	6.6, ±0.8	Large increase****
	B-96	5.2, ±1.0	Large increase****
	48-72	0.5, ±0.8	Trivial increase**
	48-96	-0.9, ±0.9	Trivial decrease**
Soreness (squat)			
MILK	B-24	3.3, ±1.4	Moderate increase***
	B-48	4.2, ±1.1	Moderate increase****
	B-72	4.4, ±1.4	Moderate increase****
	B-96	3.2, ±1.1	Moderate increase****
	48-72	0.2, ±1.1	Unclear
	48-06	-1.1, ±0.9	Small decrease**
CHO	B-24	3.4, ±1.2	Moderate increase****
	B-48	5.3, ±0.7	Large increase****
	B-72	5.0, ±1.0	Large increase****
	B-96	3.6, ±1.1	Moderate increase****
	48-72	-0.3, ±1.3	Unclear
	48-96	-1.7, ±1.2	Small decrease***
Tiredness			
MILK	B-24	4.2, ±1.2	Moderate increase****
	B-48	5.0, ±1.3	Large increase****
	B-72	4.9, ±1.0	Moderate increase****
	B-96	3.8, ±0.9	Moderate increase****
	48-72	-0.1, ±0.9	Unclear
	48-96	-1.2, ±1.1	Trivial decrease**
CHO	B-24	4.3, ±0.8	Moderate increase****
	B-48	6.0, ±1.0	Large increase****
	B-72	6.0, ±1.1	Large increase****
	B-96	4.3, ±1.1	Moderate increase****
	48-72	0.0, ±1.2	Unclear
	48-96	-1.2, ±1.1	Small decrease***
DALDA			
MILK	B-24	2.9, ±1.3	Small increase***
	B-48	4.3, ±2.1	Small increase***
	B-72	4.2, ±2.3	Small increase***
	B-96	3.8, ±2.9	Small increase***
	48-72	-0.1, ±1.2	Unclear
	48-96	-0.5, ±1.6	Unclear
CHO	B-24	5.3, ±1.6	Small increase****
	B-48	8.0, ±1.7	Moderate increase****
	B-72	7.4, ±2.1	Moderate increase****
	B-96	5.8, ±2.4	Small increase****
	48-72	-0.6, ±1.0	Trivial decrease**

Variable	Timeframe	Mean effect, ±90% CI or Factor effect x/÷ 90% CI[a]	Qualitative inference[b]
	48-96	-2.2, ±2.0	Trivial decrease**
LOOH			
MILK	B-2	1.0 x/÷ 1.4	Unclear
	B-24	1.0 x/÷ 1.1	Trivial**
	B-48	1.0 x/÷ 1.2	Trivial*
	B-52	0.9 x/÷ 1.2	Unclear
	B-72	1.0 x/÷ 1.3	Unclear
	B-96	1.0 x/÷ 1.3	Unclear
	48-52	1.0 x/÷ 1.1	Trivial**
	48-72	1.0 x/÷ 1.0	Trivial increase*
	48-96	1.0 x/÷ 1.2	Trivial increase*
CHO	B-2	1.1 x/÷ 1.1	Trivial increase*
	B-24	0.9 x/÷ 1.1	Small decrease*
	B-48	1.0 x/÷ 1.2	Unclear
	B-52	1.0 x/÷ 1.2	Unclear
	B-72	1.0 x/÷ 1.2	Trivial*
	B-96	0.8 x/÷1.3	Unclear
	48-52	1.0 x/÷ 1.3	Trivial*
	48-72	1.0 x/÷ 1.3	Unclear
	48-96	0.9 x/÷ 1.4	Trivial decrease*
PC			
MILK	B-2	1.0 x/÷ 1.8	Unclear
	B-24	1.1 x/÷ 1.5	Trivial*
	B-48	1.0 x/÷ 1.3	Unclear
	B-52	1.0 x/÷ 1.5	Unclear
	B-72	0.8 x/÷ 1.7	Unclear
	B-96	1.0 x/÷ 1.4	Unclear
	48-52	0.9 x/÷ 1.7	Unclear
	48-72	0.8 x/÷ 2.0	Unclear
	48-96	1.0 x/÷ 1.5	Unclear
CHO	B-2	0.9 x/÷ 1.5	Unclear
	B-24	1.0 x/÷ 1.2	Unclear
	B-48	1.0 x/÷ 1.4	Unclear
	B-52	0.9 x/÷ 1.2	Unclear
	B-72	0.9 x/÷ 1.2	Unclear
	B-96	1.0 x/÷ 1.4	Unclear
	48-52	0.9 x/÷ 1.5	Unclear
	48-72	0.9 x/÷ 1.5	Unclear
	48-96	1.0 x/÷ 1.4	Unclear

[a]± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [b]Qualitative Inference represents the likelihood that the true value will have the observed magnitude
**Possible, **Likely, ***Very Likely, ****Most likely*

7.3.2 Between-group effects

Peak torque

Baseline peak torque values for knee extension at 60°/s for MILK and CHO were 156.7 ± 27.3 Nm and 163.0 ± 14.9 Nm respectively, with both groups remaining below baseline values at 96h. Changes in peak torque between baseline and 24h for

MILK and CHO were -5.9 ± 6.8% and -10.0 ± 5.8% respectively, a likely small benefit (ES=0.26) for MILK. A possible small benefit (ES=0.28) for MILK versus CHO was found at B-48h (-4.5 ± 8.6% v -9.3 ± 7.8%); an unclear outcome at B-72h (-4.2 ± 8.6% v -6.6 ± 6.7%) and a trivial effect at B-96h (-2.9 ± 8.8% v -3.5 ± 4.9%, ES=0.04).

At baseline, the peak torque values at 60°/s for leg flexion for MILK and CHO were 81.4 ± 9.3 Nm and 81.7 ± 11.2 Nm, respectively. Changes in peak torque between baseline and 24h for MILK and CHO were -4.2 ± 13.7%, and -11.3 ± 7.8%, respectively, a likely small benefit (ES=0.45) of MILK. There was a likely moderate benefit (ES=0.61) for MILK versus CHO at B-48h (-4.7 ± 12.4% v -14.1 ± 13.5%) and a small benefit (ES=0.52) at B-72h (-2.2 ± 13.8% v -10.6 ± 15.8%) and an unclear outcome at B-96h (-2.6 ± 11.8% v -4.4 ± 13.1%). See Figure 7.3.

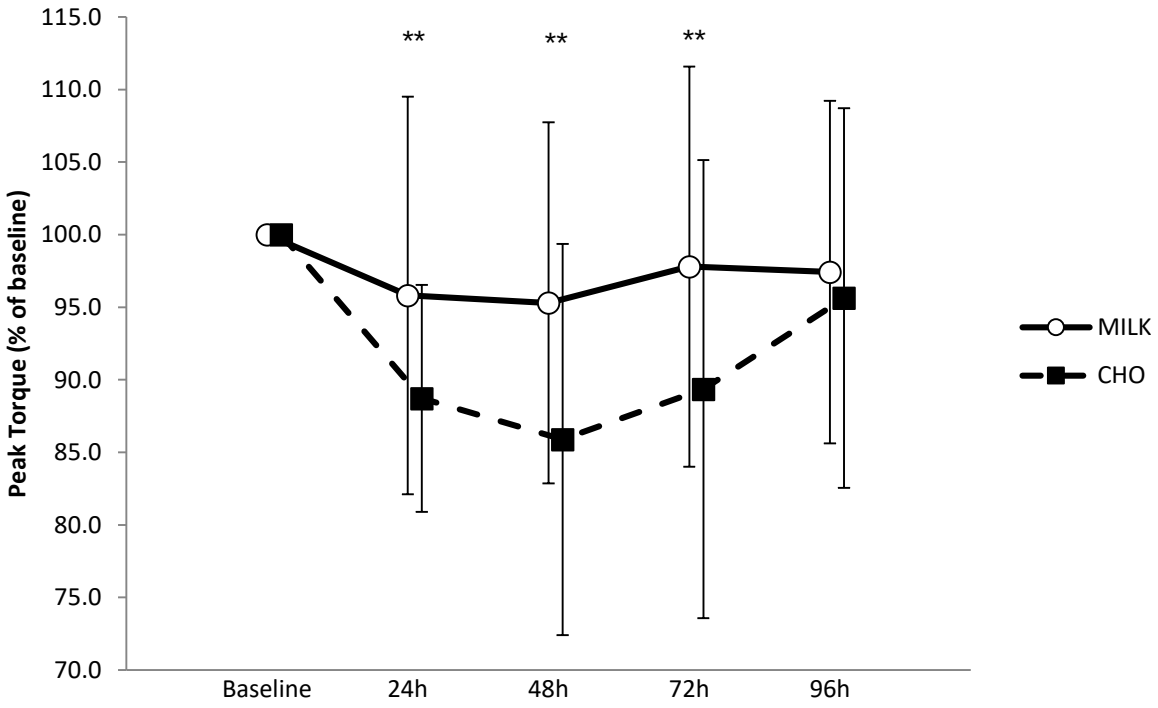

Figure 7. 3 Peak torque at 60°/s for dominant knee flexion in response to repeated simulated team sport games (following Baseline and 48h measures). Values are presented as means ± SD. **Likely benefit of MILK.

Pre-exercise mean peak torque values for knee extension at 180°/s were 106.5 ± 19.8 Nm and 113.0 ± 11.9 Nm for the MILK and CHO conditions, respectively. A possible trivial outcome (ES=0.13) was observed for MILK versus CHO at B-24h (-3.7 ± 8.6% v –3.1 ± 4.6%). There was an unclear outcome at B-48h (-1.5 ± 8.0% v -

3.2 ± 7.5%) and B-72h (-1.2 ± 8.3% v -4.4 ± 7.0%) and a trivial outcome (ES=0.07) from B-96h (-1.3 ± 10.6% v -1.4 ± 5.0%).

Baseline peak torque values for knee flexion at 180°/s for MILK and CHO were 67.8 ± 11.7 Nm and 66.9 ± 11.3 Nm. Changes in the peak torque between baseline and 24h for MILK and CHO were –4.4 ± 8.0%, and –6.6 ± 8.9%, respectively, an unclear outcome. A likely small benefit (ES=0.32) of milk was seen at B- 48h (-3.0 ± 2.8% v -8.9 ± 6.3%). Unclear outcomes were observed for B-72h (-4.0 ± 5.8% v -5.4 ± 12.1%) and B-96h (-4.1 ± 9.8% v -4.7 ± 11.1%).

Rate of force development

Baseline values for mean rate of force development (100-200ms) were 429.2 ± 125.2 $Nm.s^{-1}$ and 448.1 ± 151.9 $Nm.s^{-1}$ for MILK and CHO, respectively. An unclear outcome for MILK versus CHO was noted at B-24h (-7.2 ± 34.9% v -13.8 ± 57.0%) and a trivial outcome (ES=0.13) at B-48h (-19.3 ± 20.5% v -17.5 ± 36.2&). Unclear outcomes were observed at B-72h (-11.4 ± 25.9% v -17.0 ± 68.1%) and B-72h (-3.5 ± 30.9% v -16.9 ± 42.3%).

Countermovement jump performance

Baseline mean countermovement jump heights for MILK and CHO were 25.8 ± 4.5 cm and 27.7 ± 10.6 cm, respectively. All comparisons at all timepoints were unclear: B-24h (-6.0 ± 4.5% v -6.9 ± 10.6%), B-48h (-4.5 ± 6.3% v -7.8 ± 11.8%), B-72h (-4.5 ± 5.6% v -6.0 ± 9.1%) and B-96h (-3.1 ± 7.2% v -4.6 ± 11.8%).

Reactive strength index

Baseline RSI for the MILK group was 105.8 ± 17.2 $cm.s^{-1}$ and for the CHO group was 113.6 ± 18.3 $cm.s^{-1}$. Comparisons of MILK and CHO at B-24h (-7.9 ± 11.8% v -14.1 ± 12.0%), and B-48h (-13.1 ± 9.9% v -23.6 ± 29.5%) were unclear. Comparisons from B-72h (-17.3 ± 10.4% v -7.2 ± 24.9%, ES=0.47) and B-96h (-9.5 ± 10.8% v -0.3 ± 25.2%, ES=0.48) revealed a possible small harmful effect of milk.

Sprint performance

At baseline, 5m sprint times were 1.17 ± 0.10 s and 1.18 ± 0.04 s for MILK and CHO, respectively. Between baseline and 24h there was a possible small benefit (ES=0.40) of MILK (-1.4 ± 3.0%) compared to CHO (-3.1 ± 6.3%). A likely small benefit

(ES=0.51) of milk was observed at B-48h (-1.5 ± 4.1% v -3.9 ± 7.0%) and a likely small benefit (ES=0.58) at B-72h (-2.3 ± 4.4% v -5.0 ± 9.5%). Comparison of MILK and CHO B-96h (0.1 ± 6.9% v -2.8 ± 5.9%) was unclear. Changes can be see in Figure 7.4.

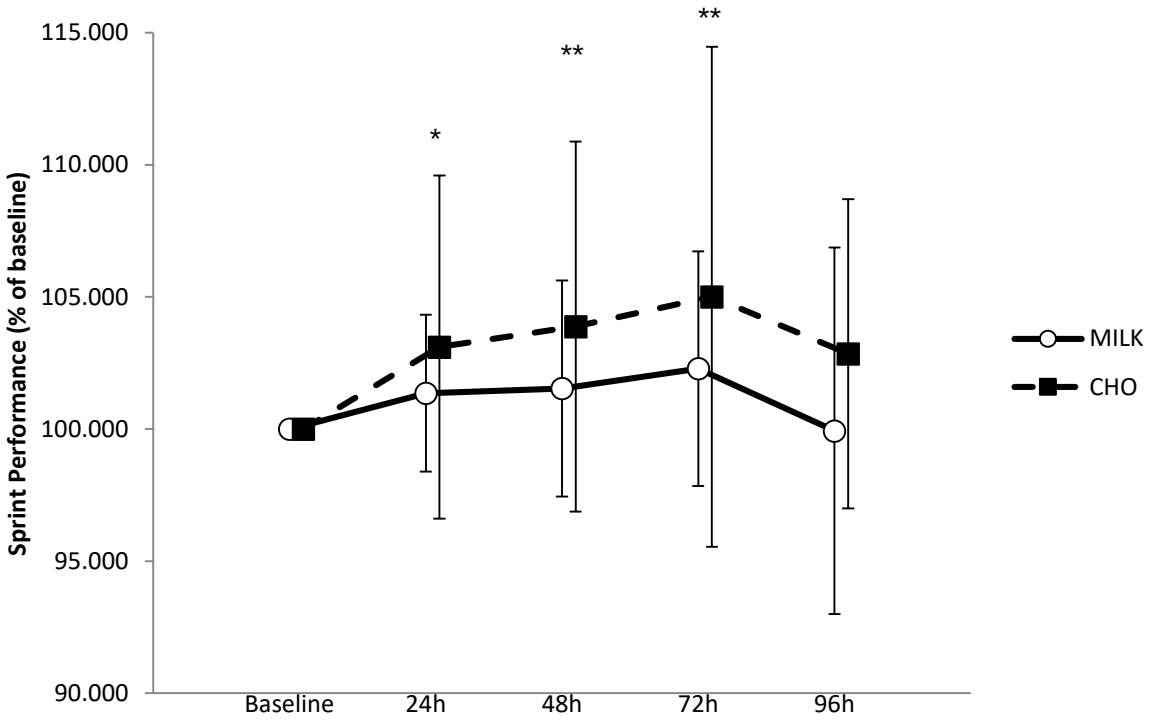

Figure 7. 4 5m sprint performance in response to repeated simulated team sport games (following Baseline and 48h measures).

Values are presented as means ± SD. *Possible benefit of MILK. **Likely benefit of MILK.

Baseline 10m sprint times for MILK and CHO were 2.00 ± 0.10 s and 2.04 ± 0.07 s. A trivial outcome was noted at B-24h (-2.3 ± 2.6% v -2.3 ± 3.8%). A likely small benefit of milk was seen from B-48h (-1.3 ± 3.1% v -2.8 ± 3.5%, ES=0.50) and from B-72h (-2.0 ± 2.9% v -2.6 ± 4.0%, ES=0.30). A possible small benefit (ES=0.53) of milk was seen at B-96h (0.7 ± 2.1% v -1.1 ± 2.4%).

With baseline values of 3.47 ± 0.20 s (MILK) and 3.55 ± 0.17 s (CHO), a possibly trivial outcome was observed for 20m sprint performance at B-24h (-2.6 ± 3.0% v -2.0 ± 2.7%, ES=0.02). An unclear outcome was seen at B-48h (-2.1 ± 2.2% v -2.1 v 3.7%). A possibly trivial effect was noted at B-72h (-2.7 ± 5.1% v -2.4 ± 2.4%, ES=0.02) with an unclear outcome at B-96h (0.1 ± 5.1% v -1.2 ± 1.8%).

A summary of the statistical analysis for Peak torque, RFD, CMJ, RSI and sprint performance can be seen in Table 7.2.

Table 7. 2 Effects on muscle function following repeated sprinting and jumping

Variable	Time Frame	Mean effect[a], ±90% CI[b]	Qualitative inference[c]
Peak Torque 60°/s Leg extension	B – 24h	5.1, ±5.2	Small benefit**
	B – 48h	5.2, ±6.7	Small benefit*
	B – 72h	2.5, ±6.5	Unclear
	B – 96h	0.3, ±5.6	Trivial*
Peak Torque 60°/s Leg flexion	B – 24h	7.4, ±8.6	Small benefit**
	B – 48h	11.4, ±13.6	Moderate benefit**
	B – 72h	10.5, ±15.2	Small benefit**
	B – 96h	2.3, ±11.5	Unclear
Peak Torque 180°/s Leg extension	B – 24h	-1.7, ±5.4	Trivial*
	B – 48h	1.5, ±6.9	Unclear
	B – 72h	3.0, ±7.1	Unclear
	B – 96h	-0.9, ±6.6	Trivial*
Peak Torque 180°/s Leg flexion	B – 24h	2.7, ±7.4	Unclear
	B – 48h	6.6, ±4.6	Small benefit**
	B – 72h	2.2, ±8.8	Unclear
	B – 96h	0.9, ±9.4	Unclear
RFD (0-200ms)	B – 24h	18.8, ±46.6	Unclear
	B – 48h	2.2, ±28.0	Trivial*
	B – 72h	1.0, ±35.8	Unclear
	B – 96h	23.9, ±45.6	Unclear
CMJ	B – 24h	0.4, ±6.8	Unclear
	B – 48h	2.9, ±9.2	Unclear
	B – 72h	1.5, ±6.8	Unclear
	B – 96h	1.5, ±8.4	Unclear
RSI	B – 24h	7.4, ±11.1	Unclear
	B – 48h	1.1, ±10.8	Unclear
	B – 72h	-8.4, ±15.9	Small harm*
	B – 96h	-7.8, ±14.3	Small harm*
5m sprint	B – 24h	-1.9, ±2.4	Small benefit*
	B – 48h	-2.5, ±3.2	Small benefit**
	B – 72h	-2.7, ±3.6	Moderate benefit**
	B – 96h	-3.2, ±5.0	Unclear
10m sprint	B – 24h	-0.8, ±2.3	Trivial**
	B – 48h	-2.1, ±2.6	Small benefit**
	B – 72h	-1.3, ±2.6	Small benefit**
	B – 96h	-2.3, ±1.6	Small benefit*
20m sprint	B – 24h	0.1, ±2.1	Trivial*
	B – 48h	-0.3, ±2.5	Unclear
	B – 72h	-0.1, ±2.0	Trivial*
	B – 96h	-0.1, ±3.5	Unclear

*[a] Mean effect refers to MILK minus CHO; [b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude, *Possible,**Likely*

Muscle soreness and tiredness

Peak soreness during a squat was observed at 48h for CHO and at 72h for MILK, and values had not returned to baseline by 96h. All comparisons between MILK and CHO were unclear. Peak soreness during isokinetic testing was seen at 24h post-circuit 2 for both MILK and CHO. A possible trivial benefit was seen for MILK versus B-24h (51.0 ± 7.4 v 65.5 ± 13.8%). All other comparisons were unclear. Tiredness followed a similar pattern. A likely trivial outcome was noted at B-24h (41.5 ± 20.6% v 43.0 ± 13.4%), with an unclear outcome at B-48h (50.0 ± 21.6% v 60.0 ± 19.4%). A likely small benefit of milk was seen at B-72h (49.0 ± 17.9 v 60.0 ±19.4%), with an unclear outcome at B-96h (38.0 ± 15.5% v 43.0 ± 19.5%).

DALDA

For DALDA B a likely small benefit of MILK (11.6 ± 8.9%) compared to CHO (21.2 ± 11.3%) was observed at B-24h and a very likely small benefit was observed at B-48h (17.2 ± 14.4% v 32.0 ± 11.8%). From B-72h (16.8 ± 15.5% v 29.6 ± 14.6%) a likely small benefit of MILK was seen, with an unclear outcome at B-96h (15.2 ± 19.8% v 23.2 ± 16.6%). See Figure 7.5.

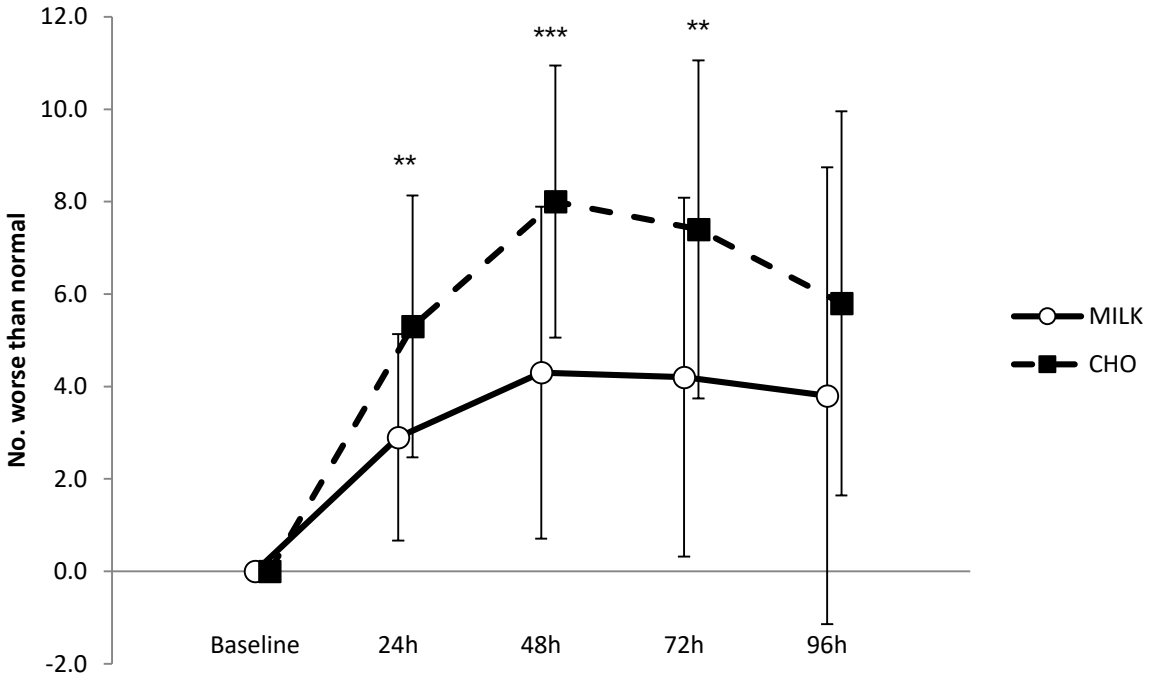

Figure 7. 5 Symptoms of stress measured by DALDA in response to repeated simulated team sport games (following Baseline and 48h measures).

Values are presented as means ± SD. **Likely benefit of MILK. ***Very likely benefit of MILK.

Creatine kinase

Baseline serum CK values were 196.2 ± 130.0 U/l and 188.6 ± 125.0 U/l for MILK and CHO respectively (Figure 7.6). Unclear outcomes for the comparison of MILK versus CHO were found at B-2h (1.7 x/÷ 1.6 v 1.9 x/÷ 1.7), B-24h (1.7 x/÷ 1.6 v 1.8 x/÷ 2.2), B-48h (1.1 x/÷ 1.5 v 1.3 x/÷ 1.9), B-52h (2.3 x/÷ 1.9 v 2.3 x/÷ 2.0), B-72h (1.7 x/÷ 2.5 and 1.7 x/÷ 2.1) and B-96h (1.2 x/÷ 1.6 v 1.2 x/÷ 1.8).

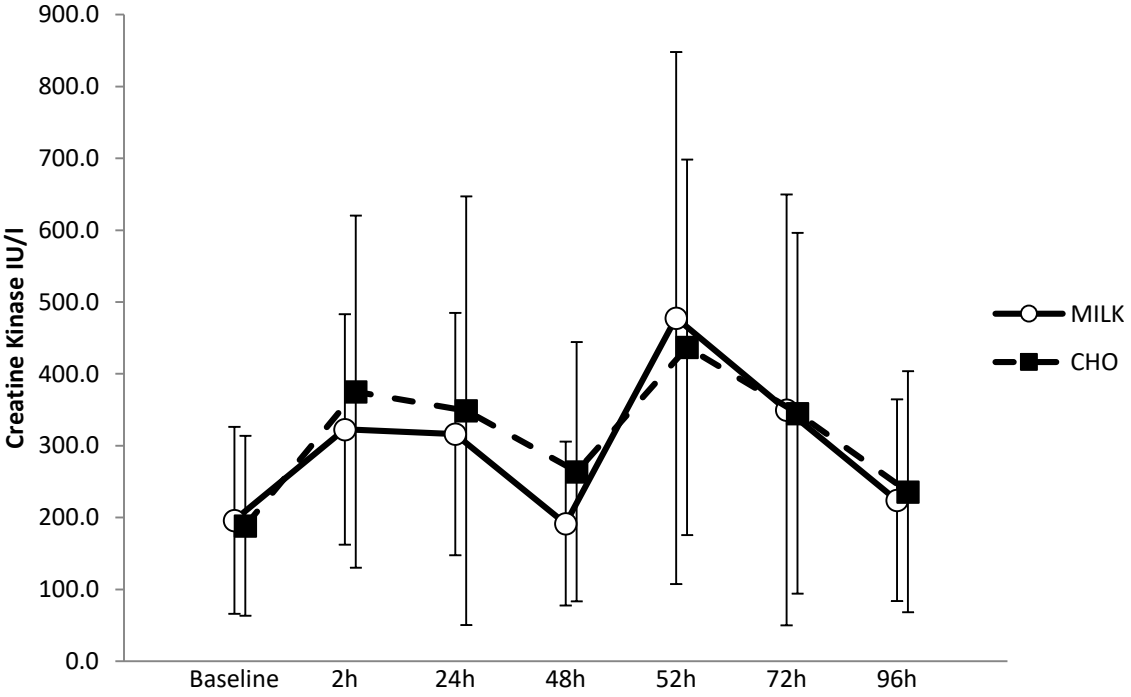

Figure 7. 6 Serum Creatine Kinase in response to repeated simulated team sport games (following Baseline and 48h measures) for MILK and CHO.

Values are presented as means ± SD.

hsCRP

Baseline hsCRP values were 1.6 ± 2.0 mg/l and 1.1 ± 0.6 mg/l for MILK and CHO. A likely small harmful effect was observed for MILK versus CHO at B-2h (1.1 x/÷ 1.6 v 0.9 x/÷ 1.6, ES=0.48), a moderate harmful effect B-24h (1.5 x/÷ 1.8 v 0.9 x/÷ 1.8, ES=0.76) and B-48h (1.2 x/÷ 2.0 v 0.7 x/÷ 1.9, ES=0.61). Unclear outcomes were seen at B-52h (1.1 x/÷ 2.0 v 1.1 x/÷ 3.0), B-72h (1.4 x/÷ 2.2 v 1.3 x/÷ 3.2) and B-96h (1.1 x/÷ 2.6 v 1.2 x/÷ 2.7).

Lipid hydroperoxides

Baseline LOOH values were 0.6 ± 0.2 µmol/l and 0.7 ± 0.2 µmol/l for MILK and CHO. An unclear outcome was observed for MILK v CHO at B-2h (0.9 x/÷ 1.7 v 1.1 x/÷ 1.2). A likely small harmful effect (ES=0.18) was observed for MILK versus CHO at B-24h

(1.0 x/÷ 1.2 v 0.9 x/÷ 1.2), with unclear outcomes seen at B-48h (0.9 x/÷ 1.3 v 1.0 x/÷ 1.3), B-52h (0.8 x/÷ 1.3 v 1.0 x/÷ 1.3). B-72h (0.9 x/÷ 1.5 v 1.0 x/÷ 1.3) and B-96h (0.9 x/÷ 1.5 v 0.8 x/÷ 1.4).

Baseline PC values were 0.34 ± 0.33 nmol/mg protein and 0.27 ± 0.06 nmol/mg protein for MILK and CHO. Unclear outcomes for MILK v CHO were found at all timepoints: B-2h (0.9 x/÷ 1.9 v 1.1 x/÷ 2.5). B-24h (1.0 x/÷ 1.3 v 1.1 x/÷ 1.8, ES=0.06), B-48h (0.9 x/÷ 1.8 v 1.0 x/÷ 1.6, ES=0.09, B-52h) (0.8 x/÷ 1.3 v 1.0 x/÷ 1.8), B-72h (0.8 x/÷ 1.3 v 0.8 x/÷ 1.4) and B-96h (0.7 x/÷ 1.7 v 1.0 x/÷ 1.6).

Mean effects for all measures of soreness and tiredness, symptoms of stress, CK, hsCRP, LOOH and PC can be seen in Table 7.3.

Table 7. 3 Effects on soreness, tiredness, symptoms of stress, CK, hsCRP, LOOH and PC following repeated sprinting and jumping

Variable	Time Frame	Mean effect[a], ±/ x/÷ 90% CI[b]	Qualitative Inference[c]
Muscle soreness (Squat)	B – 24h	-0.2, ±1.7	Unclear
	B – 48h	-1.1, ±1.3	Unclear
	B – 72h	-0.6, ±1.6	Unclear
	B – 96h	-0.5, ±1.5	Unclear
Muscle soreness (Isokinetic knee extension/ flexion)	B – 24h	-0.7, ±0.8	Trivial benefit*
	B – 48h	-0.1, ±1.1	Unclear
	B – 72h	-0.5, ±1.1	Unclear
	B – 96h	-0.8, ±1.5	Unclear
Muscle tiredness	B – 24h	-0.1, ±1.4	Unclear
	B – 48h	-1.0, ±1.5	Unclear
	B – 72h	-1.1, ±1.5	Small benefit**
	B – 96h	-0.5, ±1.4	Unclear
DALDA B	B – 24h	-2.4, ±2.0	Small benefit**
	B – 48h	-3.7, ±2.6	Small benefit***
	B – 72h	-3.2, ±2.9	Small benefit**
	B – 96h	-2.0, ±3.6	Unclear
CK	B – 2h	1.0, x/÷1.5	Unclear
	B – 24h	1.0, x/÷1.8	Unclear
	B – 48h	0.9, x/÷ 1.5	Unclear
	B – 52h	1.1, x/÷1.6	Unclear
	B – 72h	1.0, x/÷1.9	Unclear
	B – 96h	1.0, x/÷1.5	Unclear
hsCRP	B – 2h	1.3, x/÷1.5	Small harm**
	B – 24h	1.7, x/÷1.6	Moderate harm**
	B – 48h	1.7, x/÷1.7	Moderate harm**
	B – 52h	1.0, x/÷2.1	Unclear
	B – 72h	1.0, x/÷2.2	Unclear
	B – 96h	1.0, x/÷2.1	Unclear
LOOH	B – 2h	1.2, x/÷1.5	Unclear
	B – 24h	0.9, x/÷1.2	Small harm**
	B – 48h	1.1, x/÷1.3	Unclear
	B – 52h	1.4, x/÷1.7	Unclear
	B – 72h	1.1, x/÷1.4	Unclear
	B – 96h	0.9, x/÷1.4	Unclear
PC	B – 2h	0.9, x/÷2.0	Unclear
	B – 24h	0.9, x/÷1.5	Unclear
	B – 48h	0.8, x/÷1.6	Unclear
	B – 52h	0.8, x/÷1.5	Unclear
	B – 72h	0.9, x/÷1.3	Unclear
	B – 96h	0.7, x/÷1.6	Unclear

*[a] Mean effect refers to MILK minus CHO; [b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude, *Possible,**Likely, ***Very likely*

7.3.3 Within-group effects for STG variables

Changes in STG variables from STG1 to STG2 were observed. Analysis of within-group effects revealed a decrease in 5m sprint performance, but a likely increase in 15m sprint performance for both MILK and CHO. Trivial effects were seen for CMJ, laptime and HR for MILK and CHO. RPE was likely lower in STG2 for CHO, but the outcome was trivial for MILK. Changes can be seen in Table 7.4.

Table 7. 4 Within group effects from STG1 to STG2

Variable	Mean effect[a], ±90% CI[b]	Qualitative inference[c]
5m sprint performance		
MILK	3.0, ±1.7	Small decrease*
CHO	2.3, ±7.4	Small decrease***
15m sprint performance		
MILK	-3.3, ±2.5	Small increase**
CHO	-5.9, ±7.2	Moderate increase**
CMJ		
MILK	-1.0, ±4.0	Trivial**
CHO	-0.4, ±2.0	Trivial**
Lap time		
MILK	-0.5, ±3.5	Unclear
CHO	0.1, ±2.1	Trivial**
Heart rate		
MILK	0.9, ±1.0	Trivial**
CHO	0.6, ±0.9	Trivial**
RPE		
MILK	0.0, ±0.8	Trivial*
CHO	-0.8, ±0.7	Trivial decrease**

[a]± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [b]Qualitative Inference represents the likelihood that the true value will have the observed magnitude
**Possible, **Likely, ***Very Likely, ****Most likely*

7.3.4 Between-group effects for STG variables

STG 1 5m sprint times for MILK and CHO were 2.00 ± 0.10 s and 2.04 ± 0.07 s. A comparison of MILK v CHO revealed an unclear outcome from STG 1 to STG 2 (-3.0 ± 13.3% v -3.1 ± 2.0%). 15m sprint times in STG 1 were 3.24 ± 0.25 s and 3.29 ± 0.22 s for MILK and CHO, respectively. An unclear outcome was observed from STG 1–STG2 for MILK v CHO (3.21 ± 3.92% v 5.29 ± 10.32%). Mean CMJ heights in STG 1 were 21.4 ± 3.2 cm and 21.4 ± 2.8 cm for MILK and CHO. A possibly trivial outcome was observed for STG 1 v STG 2 (-0.8 ± 6.8% v -0.4 ± 3.3%, MILK v CHO). STG 1 Lap times for MILK and CHO were 51.1 ± 4.7 s and 52.2 ± 4.9 s. An unclear

outcome was seen for STG 1 v STG2 for MILK (-0.9 ± 6.4%) versus CHO (0.3 ± 4.2). Mean HR values from STG 1 were 180.5 ± 15.1 bpm and 182.0 ± 5.5 bpm for MILK and CHO, respectively. Comparison of MILK and CHO from STG 1 to STG 2 showed a likely trivial effect. Mean RPE for MILK and CHO for STG 1 were 14.0 ± 1.8 and 13.9 ± 1.7. A comparison of MILK v CHO from STG 1 to STG 2 was unclear. See Table 7.5 and Figure 7.7.

Table 7. 5 Effects on circuit variables; 5m sprint, 15m sprint, lap time, jump height, HR and RPE

Variable	Mean effect[a] ± 90% CI[b]	Qualitative Inference[c]
5m sprint	-1.5, ±7.4	Unclear
15m sprint	-0.8, ±3.2	Unclear
CMJ	-0.6, ±4.4	Trivial*
Lap time	-1.1, ±3.9	Unclear
HR	-0.3, ±1.3	Trivial**
RPE	-0.6, ±1.0	Unclear

*[a] Mean effect refers to MILK minus CHO; [b] ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence intervals for the true difference; [c]Qualitative Inference represents the likelihood that the true value will have the observed magnitude, *Possible,**Likely*

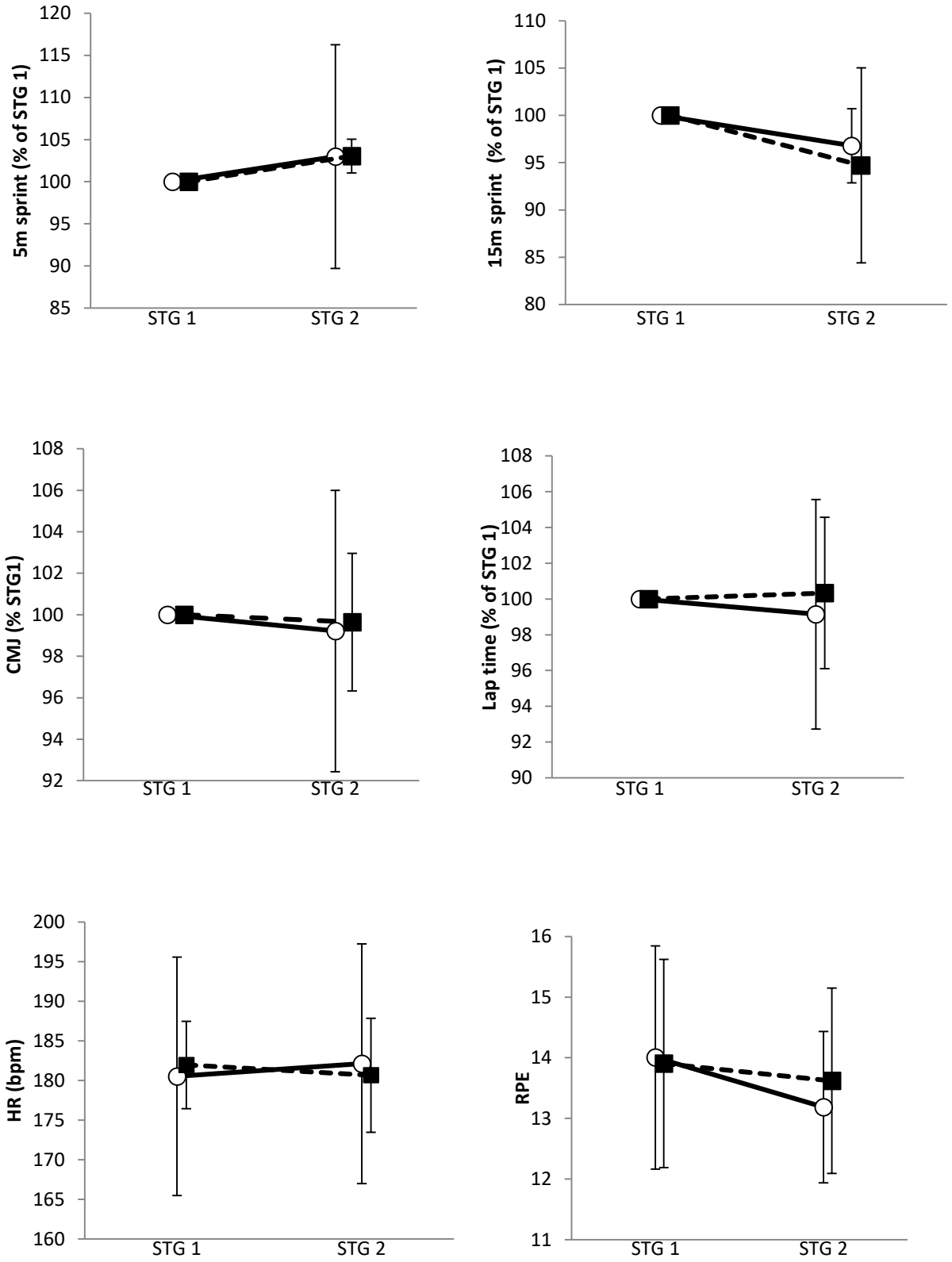

Figure 7. 7 Mean values for STG 1 v STG 2 a. 5m sprint performance. b. 15m sprint performance. c. Countermovement jump performance. d. Lap time. e Heart rate. f. Rate of Perceived Exertion

7.4 Discussion

The present study is the first to examine the effect of milk on recovery from repeated simulated team sport in females. The principal findings were that milk had a positive effect on attenuating losses in peak torque, sprint performance and symptoms of stress, and on increases in some measures of muscle soreness and tiredness following STG1. For the STG variables, there was no effect of milk on CMJ and HR from STG1 to STG2 with all other STG comparisons unclear. The second STG did not lead to a secondary decrease in muscle function (apart from sprint performance, though this was not consistent across both groups), soreness, tiredness or perceptions of stress. There was a post-STG2 increase in CK and in hsCRP (CHO) and LOOH (MILK).

The consumption of milk had a beneficial effect on muscle function, specifically peak torque and sprint performance (5m and 10m) over the recovery period. Similar benefits have been noted when milk was consumed following repeated sprinting and jumping in females (Rankin *et al*., 2018, Chapter 6) and the magnitude of loss observed was similar to that reported following match participation by female soccer players (Andersson *et al.,* 2008). The benefit of milk on recovery of muscle function in this study speculatively arises from a post-exercise enhancement of protein balance resulting in a stimulation of protein synthesis rates (Groen *et al.,* 2015). For RFD and RSI, comparisons of MILK v CHO revealed unclear outcomes with MILK being possibly harmful for RSI from B-72h and B-96h. There was considerable variability in both these variables perhaps due to the more technical nature of the measures. Unclear outcomes were also observed for CMJ. This is in contrast to previous research which reported a beneficial effect of milk on recovery of CMJ following repeated sprinting and jumping in females (Rankin *et al*., 2018, Chapter 6). The effect of milk on measures of soreness and tiredness were primarily unclear, though a likely benefit for MILK was noted for tiredness from baseline-72h. Given that soreness is a subjective measure and that this investigation employed an independent-groups design it is difficult to compare conditions. Regardless, the mechanism underpinning the manifestation of soreness is not fully elucidated. The soreness reported was comparable to those of female soccer players following match participation (Andersson *et al.,* 2008) though somewhat lower than male rugby players who completed a similar circuit (Duffield *et al.,* 2008). This was not unexpected as the perception of pain and post-exercise muscle soreness may be

influenced by oestrogen via a role in the binding of opioids to receptors in the brain and spinal cord modifying the transmission of pain (Kendall and Eston, 2002). Milk had beneficial effects on the perceptions of the symptoms of stress, as has been previously observed (Rankin *et al.,* 2018, Chapter 6), with participants who consumed milk reporting fewer 'worse than normal' symptoms over the recovery period. While it is plausible that bias existed because of a lack of binding to the drink consumed, an attenuation of negative aspects of psychological wellbeing is likely to be positive for athletes.

Comparison of hsCRP data indicates a likely harmful effect of MILK over the 48h post STG1, though an unclear outcome following STG2. This early benefit for CHO is a result of a decrease in hsCRP from baseline to 2h and baseline to 48h, while the MILK group showed possible increases during this time. It has been shown that carbohydrate consumption has an effect in attenuating increases in some inflammatory markers following intermittent exercise (Bishop *et al.,* 2002). This is probably due to the effect of increased blood glucose concentrations on cortisol and release of cytokines from monocytes and macrophages (Nieman *et al.,* 2001). However, this does not explain the lack of benefit for CHO following STG2, and importantly the 500ml of milk also contained carbohydrate (25.5g). Previous research with elite female soccer players reported an increase in both pro- and anti-inflammatory cytokine responses following a soccer match, with a dampened response following a second game 72h later (Andersson *et al.,* 2009). The findings of this study show an opposite effect for the CHO group, with an increase in hsCRP following STG2. Obviously hsCRP may not be reflective of the overall cytokine response and further research is warranted to investigate this further.

In accordance with previous research with female team sport athletes (Rankin *et al*., 2017; 2015, Chapters 4, 5), the effect of milk on the accumulation of serum CK was unclear. Peak CK was lower than that reported in previous studies that utilised variations of the STG (Singh *et al*., 2011; Ingram *et al.,* 2009; Duffield *et al.,* 2008). However, participants in the aforementioned studies were male and it has been reported that oestrogen has a protective effect on the myofibrillar membrane resulting in a smaller increase in serum CK following exercise (Stupka *et al*., 2001). Peak CK for female soccer players following 2 soccer games has been reported as 414 U/l (Andersson *et al.,* 2008), which is comparable to the peak in the current study. We

found no evidence that MILK attenuated oxidative stress as comparisons of MILK v CHO for LOOH and PC were typically unclear. However, this is consequential to the surprising observation that we did not see clear evidence of oxidative stress throughout the trial. This was unexpected as previous investigations have reported increases in oxidative stress following participation in both repeat-sprint trials (Deminice *et al*., 2013) and team sport matches (Silva *et al.,* 2013). The divergent findings in the oxidative stress response between this and previous studies could be explained by the use of different biochemical markers or analytical techniques. There is also the possibility that oxidative stress did occur but was confined to the muscle tissue and was not detected in the circulation, which is conceivable as the muscle is the prime producer of ROS following exercise (Jackson *et al*., 2016). Given that the markers of muscle damage increased but the markers of oxidative stress did not, it is reasonable to speculate that the participants' basal antioxidant status was more than sufficient to manage any increase in oxidative stress during and after exercise.

Interestingly, despite milk benefiting muscle function measures over the recovery period, a comparison of sprint performance and CMJ from STG1 to STG2 revealed no clear benefit of MILK. MILK also had no clear effect on lap time, HR or RPE. This is in contrast to an investigation by Poulios *et al.,* (2018) who reported that protein consumption following a game of soccer had a positive effect on aspects of locomoter activity in a second game performed 72h later. The protein intake in that study was greater than in this, with an intake of 80g during the 6h post-exercise which may explain the observed differences. A relevant observation by Mohr *et al.,* (2016) highlighted no difference in peak sprinting speed and sprinting distance when 3 simulated games were played within 7 days, despite increases in other markers of fatigue and muscle damage. Of note in the current study is that the average 5m sprint time in STG1 and STG2 (~1.37s) was considerably slower than the isolated sprint times measured during recovery (~1.20s), and CMJ height was approximately 80% of the isolated jump height indicating that participants did not sprint nor jump maximally during the STG. This is most likely related to the physiological demands of the STG but nonetheless, questions the validity of using isolated sprint and jump performance as a marker of recovery, or a predictor of performance for subsequent match situations. Despite the STG2 15m sprint times being likely faster for both groups, overall lap times from STG 1 to SGTG2 were unchanged. This demonstrates that athletes altered their activity profiles, a phenomenon that has been observed

during actual match-play where athletes alter their low intensity activity in order to preserve high intensity running capacity at a later stage (Smith *et al.,* 2015). Furthermore, based on the results of this study, it is conceivable that the positive effect of milk is greatest when participants are performing maximally. What we understand from this is that isolated measures of sprint performance and jump height are not indicators of the performance of these measures within a simulated team sport situation. Thus, crucially, future research should consider the validity of indicators of recovery.

Similar to previous research (Andersson *et al*., 2008; Ispirlidis *et al.,* 2008), the results of this study indicate that recovery of muscle function, soreness/tiredness and CK were not complete prior to the start of STG2, 48h following the first bout. Other investigations have reported decreases in CMJ performance following a soccer match, but there is variability in the recovery response of this measure. It has been reported that CMJ is still reduced 72h post-match (Andersson *et al*., 2008), while Silva *et al.,* (2013) noted that CMJ performance was restored 48h post-match. In these cases, variability may, in part, be due to the variability in the matches themselves, in particular with regard to higher speed activities (rather than distance covered which is quite stable from match to match, Gregson *et al*., 2010). In the current study, CMJ performance for all participants remained below baseline at 48h post STG1 and remained below baseline for the remainder of the recovery period. Surprisingly, STG2 had little impact on measures of muscle function, soreness, tiredness or perceptions of stress. Differences from 48-72h and 48-96h were typically trivial or unclear with some measures (peak torque, CMJ, RSI, soreness, tiredness, DALDA) continuing to return towards baseline values despite the demands of STG2. This could suggest a repeated bout effect whereby the exposure to the demands of STG1 may have provided a protective effect resulting in less muscle damage and fatigue following STG2 (McHugh, 2003). The exception to this was 5m sprint performance for MILK and CHO and 10m sprint performance for MILK which decreased following STG2. Nonetheless, performance of all muscle function variables remained below baseline at 96h, which has implications for coaches and athletes in terms of training programmes and competition fixtures. The two-fold increase in the pattern of serum CK was as expected, with an increase observed following STG1, returning towards baseline before a second increase following STG2. This is similar to the pattern observed following repeated match play (Mohr *et*

al., 2016; Andersson *et al.,* 2008), though this pattern is clearly not aligned to that of muscle function. The other serum markers were either not impacted by STG2 (PC) or not impacted across both groups (LOOH, hsCRP). This may reflect variability in the measures.

Despite the attempt to use an exercise protocol that simulates the physiological demands of team sports, there are some non-physiological factors not part of the protocol that can significantly influence physiological performance in real-game situations. These include match contextual factors such as match status (a score), lack of an opponent, an unrealistic match location and lack of physical contact. Importantly, the exercise protocol was conducted without a ball or hurl, and thus did not include kicking, catching or striking activities which would not only influence physiological factors during the protocol, but possibly post-exercise fatigue and muscle damage.

In conclusion, the present study clearly demonstrates that consumption of milk following two simulated team games separated by 48h can attenuate losses in peak torque, sprint performance and symptoms of stress compared to an energy-matched carbohydrate drink in female athletes, with benefits also for some measures of soreness and tiredness. A similar outcome was not observed for RSI, RFD and CMJ and serum biomarkers. From a practical perspective, this study indicates that milk may serve to enhance the recovery of female athletes following team-sport participation. No benefit of milk was observed for sprint performance, jump height or lap times within the second STG protocol which questions how transferable isolated measures of recovery are to real match performances**.** Further research is necessary to elucidate if milk can affect within-game performance variables when recovery periods are limited.

Chapter 8: Overall Discussion

8.1 Introduction

The overarching purpose of this thesis was to investigate the effect of milk on recovery from a variety of exercise protocols with a focus on the female athlete. The main finding was that postexercise consumption of 500ml of milk had a beneficial effect on recovery of muscle function following exercise, though a degree of variation was observed across a wide range of measures. However, milk offered little benefit when the exercise did not include any eccentric loading (Study 2). Overall dissociation between the effect of milk on muscle function and markers of muscle damage (soreness, tiredness, CK) was noted, suggesting that careful consideration needs to be given to the interpretation of markers of fatigue and recovery following exercise.

Previous research examining the effects of milk in an exercise context have focused on resistance training (Josse *et al*., 2010; Hartman *et al.,* 2007; Rankin *et al*., 2004) or recovery from eccentric exercise (Cockburn *et al.,* 2012; 2010; 2008). While providing valuable insight into the potential benefits of milk, the exercise protocols utilised in these studies do not accurately represent the nature of many sport and exercise activities. The investigations presented in this thesis aimed to expand on existing knowledge by examining the effects of milk on recovery from a variety of exercise protocols, in order to enhance the practical application of milk as a recovery beverage.

Furthermore, this thesis presents a series of investigations focusing on female athletes, a reasonably novel approach considering the underrepresentation of females in sport and exercise research (Costello et al, 2014; Brookshire, 2016). The dearth of research with female athletes has resulted in application to females of knowledge that has been generated solely from research involving male participants. It has been highlighted that there is a clear need to improve our understanding of female physiology (Bruinvels *et al*., 2016) and this thesis has attempted to address this deficit. Sex-comparison studies, as in Study 1, can contribute to our understanding of human physiology and how sex differences during exercise can impact recovery, but also inform nutritional intervention, identifying if customised nutrition recommendations on the basis of sex are necessary. Sex-specific investigations, as in Study 2, 3 and 4 allow for the further development of the understanding of the responses of this cohort to exercise and specific nutritional

plans. The results of this thesis indicate that the post-exercise consumption of milk is beneficial for recovery from varying forms of exercise in female athletes. This means that, for this population, the inclusion of milk as part of a recovery nutritional plan is a worthwhile consideration.

8.2 The Effect of Milk on Recovery From Exercise

The consumption of milk had positive effects on muscle function of female athletes across all investigations, though the probability of a beneficial effect varied across studies. The greatest positive effects were seen on the measurement of peak torque at both 60˚/s and 180˚/s. Sprint performance was benefited somewhat apart from Study 2 which may be due to the non-eccentric nature of the initial exercise protocol. The effects on CMJ, RSI and RFD were primarily unclear, apart from Study 3 for CMJ, and the sensitivity of CMJ, RSI and RFD as measures of recovery are discussed below. Overall, for muscle function, the greatest mean effect of milk and the largest effect size were observed for Study 1 and Study 3 when the eccentric loading was greatest. For sprint performance, the mean effect of milk was similar for Study 3 and Study 4. Notably, these results suggest that milk is probably of greatest benefit when the level of mechanical stress is high.

The positive effects of milk observed in previous investigations have been attributed to a positive protein balance following the consumption of milk, as a result of an increase in protein synthesis and a decrease in protein breakdown (Wilkinson *et al*., 2007; Elliott *et al*., 2006). Resistance exercise research has provided compelling evidence that post-exercise protein ingestion induces a synergistic combination of the two stimuli (Glynn *et al*., 2010; Biolo *et al.,* 1995), resulting in increased mRNA expression and changes in muscle protein content (McGlory *et al*., 2017). This is a credible explanation for the observed results in this thesis, particularly Study 1 which involved strenuous eccentric exercise. Speculatively, the ingestion of milk resulted in rapid aminoacidemia, providing a substrate for de novo protein synthesis (Miller *et al*., 2004). This increase in muscle protein synthesis could enhance the repair of damaged myofibres and cellular structures, having a positive effect on muscle function capacity in the recovery period. In addition to this, the consumption of milk may have reduced protein breakdown through its insulinogenic effect (Bird *et al.,* 2006). A reduction in protein breakdown would preserve myofibrillar proteins and

thus, attenuate losses in the muscle ability to produce force. The effect of milk on recovery from the cycling protocol utilised in Study 2 was not as clear. Exposure to endurance exercise also results in enhanced protein synthesis, but primarily resulting in enhanced mitochondrial content (Gibala *et al*., 2006) and may explain the lack of effect of milk on measures of muscle function that are not endurance based. The mechanisms underpinning the combined effect of repeated sprinting and protein ingestion, as examined in Study 3 and 4, on muscle protein synthesis has not been elucidated in the literature. Myofibrillar protein synthesis is elevated following high intensity interval training in older men (Bell *et al*., 2015) though not to the same extent following resistance training, and such exercise is likely to induce muscle fibre remodelling rather than accretion (McGlory *et al.,* 2017). Nitrogen balance, or rates of MPS and MPB were not measured in any of the studies in this thesis, which is a limitation of the investigations. Subsequently, it is not possible to identify exact mechanisms responsible for the effects observed. Future investigations that elucidate these processes promise to enhance our understanding of the exact mechanisms by which post-exercise milk consumption affects recovery.

Milk had no effect on the inflammatory marker hsCRP in Studies 2, 3 and 4. However, the exercise protocol in Study 2 was the only one that resulted in clear changes in CRP at all timepoints. It is possible that hsCRP is not reflective of the inflammatory state at all timepoints, explaining the unclear and varied results for the other exercise protocols. Interestingly, Study 4 found that CHO had a positive effect on the inflammatory marker in the early recovery period. This has been reported previously (Nieman *et al*., 2001), though considering that the volume of milk consumed delivered more than 25g of carbohydrate, further investigations are warranted in this area. An interesting and relevant insight into post-exercise inflammation has been provided by Singh *et al*., (2011) who compared the effects of intermittent running, either with or without body ‘contact’, on muscle damage and inflammatory response. They demonstrated that both ‘contact’ and ‘non-contact’ training resulted in elevated serum CK, while CRP only increased following training with body ‘contact’. Based on the present results and due to the fact that potential interferences with inflammation are not directly related to muscle damage (Singh *et al.,* 2011), it appears that CRP may not be a useful and specific enough marker for monitoring fatigue and recovery following exercise without body contact.

Milk had little effect on serum CK and sTnI following exercise. A benefit for milk was only observed for CK for males in Study 1 despite the 'extremely large' increases in CK for females, and increases in sTnI for both sex groups in this study. Moderate to large effect sizes were seen for both MILK and CHO increases in CK in Study 2, 3 and 4 but no clear benefit of milk was observed. Therefore, a benefit of milk when mechanical stress is highest (as observed for muscle function) was not observed for this variable in females. Furthermore, given the benefits noted for muscle function, the results highlight the disconnect between these commonly used markers of muscle damage. This lack of effect of milk may represent differing recovery processes and the use of CK as a valid marker of muscle damage and recovery is discussed below.

Markers of oxidative stress were not impacted by the exercise protocols completed (Study 2 and Study 4), and thus, no benefit of milk was noted during the recovery period. While milk has some anti-oxidant properties (Zulueta *et al.,* 2009; Haug *et al*., 2007; Elia *et al*., 2006), the post-exercise consumption of a moderate amount of milk was not expected to impact this stress. However, the lack of effect of the exercise protocol on these markers was surprising as increases in oxidative stress have been observed following many different types of exercise protocols (Kerasioti *et al.,* 2012; Child *et al.,* 2000; Marzatico *et al*., 1997). The conflicting results reported in this thesis may be as a result of the markers chosen or the analysis techniques used. It is also tenable that the antioxidant status of the participants was sufficient to manage any increase in oxidative stress in the post-exercise period. Of course, it is also possible that the sex of the participants played some role in the results observed as females typically have lower concentrations of these markers than their male counterparts (Ide *et al.,* 2002; Mastaloudis *et al*., 2004).

In Study 3 and Study 4, MILK had a positive effect on the perception of the symptoms of stress. The difficulties in using subjective measures as an indicator of recovery have been discussed in the previous chapters. It is possible that, because of an inability to blind participants to the drink received and available information regarding potential benefits of milk in recovery, there was a pre-existing perceptual bias that influenced these measures. However, if this was the case, positive outcomes would be expected in other variables that are also subjective in nature. However, across all four investigations, milk had an inconsistent effect on markers of

muscle soreness and tiredness, indicating an increased likelihood of a genuine reduction in the symptoms of stress. A thorough explanation for muscle soreness has yet to be elucidated, and different mechanisms are responsible for effects on other variables, with different magnitudes and timecourses. Nonetheless, as commented in Chapter 7, a positive effect on wellbeing may be beneficial to the athlete regardless of the clear identification of a mechanism.

8.3 Exercise Protocols

The exercise protocols utilised in this series of investigations resulted in reduced muscle function and an increase in markers of muscle damage. The effect, however, was influenced by the nature of the exercise protocol. The decreases in muscle function were greatest following the eccentrically biased exercise protocol in Study 1. The reduction in peak torque at 60˚/s and 180˚/s was greater than 25% for the CHO group, compared to ~4% in Study 2 and up to 14% in Study 3 and Study 4. This is not surprising considering that the protocol employed comprised maximal eccentric contractions on the isolated hamstring muscles. Accordingly, Study 1 also produced the highest muscle soreness and CK values with the mean peak value of ~12000 U/L compared to 225 U/L in Study 2 and 373 U/L and 477 U/L in Study 3 and Study 4, respectively. While isolated eccentric exercise provides a suitable model for the study of EIMD, it does not represent movements that are typically executed during 'real-life' sport or exercise. Similarly, the protocol utilised in Study 2 (concentric cycling) provided an appropriate model for enhancing our understanding of how milk might affect recovery (indicating that milk was of little benefit) but typically produced lower fatigue, performance decrements and muscle damage compared to the real world setting. The protocols selected for Study 3 and Study 4 have greater ecological validity and practical application for coaches and athletes. It is, thus, apparent that different exercise scenarios influence recovery differently, highlighting the necessity of ensuring that recovery interventions are closely matched to the original exercise demand and are not a 'one size fits all' (Minett and Costello, 2015). Thus, in the context of the overall aim of this thesis, it seems that milk is of greatest benefit when consumed following isolated eccentric exercise when performance decrements are greatest. However, from a practical perspective, milk can enhance recovery from valid exercise protocols that include eccentric loading.

The experimental design for Study 4 attempted to replicate the demands placed on many team sport athletes who train and compete regularly with short intervening periods. Fatigue and reduction in muscle function during match play continue post-exercise and usually take a number of days to resolve (Brownstein *et al*., 2017). Many players are participating in match schedules that include multiple matches with short intervening recovery periods. In Gaelic games, many players are playing and competing at numerous levels including minor (U-18), U-21 and adult grades, and at club and inter-county levels. Moreover, as is traditional in many areas, there are players simultaneously training and competing in both hurling/camogie and Gaelic football, so-called dual players. This level of participation leads to very short recovery periods between training sessions and matches, impacting both physiological and psychological ability to compete. In these situations, milk with it's multifactorial role may be a suitable recovery beverage, facilitating muscle repair, muscle remodelling, immune system restoration, glycogen replenishment and rehydration (James *et al.,* 2018).

For maximum practical application, it is imperative to develop exercise protocols that closely simulate the demands of different sports (Goodall *et al.,* 2017; Carling *et al*., 2015), thus allowing the assessment of recovery that will be valid and meaningful for athletes and coaches. There has been considerable progress in this area, including a treadmill-based soccer specific protocol (Page *et al.,* 2015) and a soccer protocol incorporating intermittent activity and ball dribbling tests (Russell *et al.,* 2011). Carling *et al*., (2015) recommended that simulations should include directional changes, accelerations and decelerations, and jumps. The protocol utilised in Study 4 attempted to include all of these variables. Nevertheless, there is a lack of valid and reliable protocols that are specific to different sports, including Gaelic games. While the physiological demands of these sports are not unlike other team sports, there are unique characteristics and skills that may significantly influence the recovery requirements. It is significant that while match simulations are necessary for the control of as many variables as possible while replicating the physiological demands of match-play, it is very difficult to develop a true simulation. Real match-play not only involves activities that are seldom included in simulations e.g. passing, tackling shooting, but also demands decision-making, reacting and anticipating which are perceptual demands that are often crucial in determining the performance of the player (Magalhaes *et al*., 2010). Yet, utilising real match play for investigations

means that there is a lack of control over the activities of participants, and there is high variability in the demands placed on players. Undoubtedly, what is needed are match simulations that incorporate as many match related activities as possible (passing, shooting, tackling) while placing the same physiological demands on each participant. These simulations should be specific for each sport so as to incorporate the activities that are specific to different sports. Furthermore the simulations should be adaptable to accurately simulate the demands for female players and those of different playing levels and ages. This would permit a greater understanding of the demands on these populations and would ultimately allow the development of more individualised recovery strategies. Ultimately, research on recovery can be enhanced by the development of valid exercise protocols which can then be utilised to examine potential benefits of milk or other interventions.

8.4 Measuring Recovery

There is considerable debate on how best to monitor recovery and what variables best represent the physiological state and preparedness of an athlete. This sequence of studies chose a number of variables that had the potential to give a realistic insight into the recovery of the participants while not influencing the recovery process or effect of the intervention.

Peak torque was measured in each of the investigations. The speeds chosen (60˚/s and 180˚/s) are commonly used, with low-velocity measures proving to be the more reliable (Greig *et al*., 2008). However, these velocities are still far removed from multijoint movement velocities with velocity as high as 970˚/s reported for knee flexion during sprinting (Baltzopoulos and Gleeson, 2001) and thus, may not be the most valid indicator. Interestingly, the post-exercise consumption of milk almost always had a beneficial effect on this variable, but not always on other variables, perhaps due to the level of variance in these measures. This included sprint performance. Using repeated sprint ability tests has greater validity than isolated maximal sprints and there is a strong correlation between RSA performance in a test and that in a match situation (Rampinini *et al.,* 2007). However, daily execution of an RSA test during the recovery period is likely to affect the recovery from the original exercise protocol and thus mask the effect of the original protocol and the

intervention applied. Perhaps the use of an RSA test with fewer sprints might be worth considering.

Countermovement jump height was also measured in each of the investigations as it is commonly used as a marker of neuromuscular fatigue. Nevertheless, there is considerable variability in the literature with regard to the effect of exercise participation on CMJ height, and discrepancy still exists in terms of exactly how sensitive the CMJ is as an indicator of neuromuscular fatigue (Cormack *et al.,* 2008). A number of investigations have reported no change in CMJ following high intensity sprint activity or competition (Gathercole *et al.,* 2015; Cormack *et al.,* 2008) but Thorpe *et al*., (2015) reported a positive association between high intensity distance covered and CMJ. The aforementioned authors suggest that CMJ may not be sensitive enough to measure fatigue during recovery. It is also suggested that individuals alter their jump technique in order to maintain their jump height (Gathercole *et al.,* 2015; Cormack *et al*., 2008), again questioning the usefulness of CMJ as a marker of both fatigue and recovery (de Hoyo *et al.,* 2016). These factors may explain the observed variance in the effect of milk on CMJ. It may be worth considering alternative CMJ measures for monitoring purposes (Gathercole *et al*., 2015), such as the ratio of flight time to contraction time (sum of the concentric and eccentric phase duration), the only CMJ variable which showed meaningful change post-match and across an AFL season (Cormack *et al*. 2008). Nevertheless, a CMJ test is easy and time efficient to administer and is a non-invasive, non-fatiguing test, making it a suitable choice for these investigations.

A number of investigations have used RSI as a measure of fatigue and as a monitoring tool and it is a reliable measure (Byrne *et al*., 2017), yet others consider that variability in the measure may negate its practical use (Beattie and Flanagan, 2015). Beattie and Flanagan (2015) noted that the level of variability (trial-to-trial and interday) may be greater than the smallest worthwhile change. The level of variability in the dynamic multi-joint measures of muscle function in this study may be greater than the effect of milk, thus masking benefits. Overcoming this difficulty by using participants who are very familiar with the test protocols is a possible solution.

In the context of real-world application, Study 4 provides an interesting insight in to the relevance of markers of fatigue and recovery. Study 4 showed that milk had a

positive effect on recovery of peak torque and sprint performance, yet had no effect when comparing sprint performance in STG 1 to sprint performance in STG 2. The reasons for this are unclear but it certainly questions the validity of utilising isolated measures of maximal sprint and jump performance as indicators of in-game sprint and jump performance.

The studies in this thesis have highlighted the necessity of carefully considering the variables used to measure recovery, particularly in relation to predicting subsequent performance. Study 3 and Study 4 highlighted the disconnect between measures of muscle function, soreness and serum markers of muscle damage, inflammation and oxidative stress. In particular, when the consumption of milk had a positive effect on recovery of a muscle function variable, the same benefit was not seen on muscle soreness. This would suggest that measures of muscle soreness are not always correlated with muscle function. A similar disconnect was observed between muscle function and CK, which has previously been reported (Johnston *et al*., 2016; Doeven *et al*., 2018), reinforcing the caution that must be exerted if using muscle soreness or CK as an indicator of muscle function during recovery. Marqués-Jiménez *et al.,* (2017) provide a basic but insightful review of objective and subjective measures as indicators of fatigue, recovery and EIMD in soccer players**,** concluding that there is a lack of consensus on the usefulness of some of them. Indeed a recent analysis has found poor association, in terms of time course and magnitude of change, between the variables used to monitor recovery (Damas *et al*., 2016). Combining performance, subjective and biochemical markers and the individualisation of the analysis, leading to individualised recovery strategies is a more constructive approach (Marqués-Jiménez *et al.,* 2017; Doeven *et al*., 2018).

Each study in this thesis utilised serum CK as an indicator of membrane damage. The variability in the CK response to exercise has been extensively reviewed (Baird *et al.,* 2012; Brancaccio *et al.,* 2007; Clarkson and Ebbling, 1988) and the appearance of CK in the blood is influenced not only by the nature of the exercise completed but the permeability of the muscle cell membrane and the percentage of fast twitch fibres, both of which are highly individual (Wiewelhove *et al.,* 2015). Indeed, peak CK values for individuals has been shown to vary ~358% in response to the same relative intensity exercise bout (Jackman *et al.,* 2010). This variability was noted in the current investigations. For example, in study 1 at 72h post-exercise,

one individual had a CK value of 103 U/l (105% of baseline) while another in the same group (MM) had a CK of 59157 U/l (>40000% of baseline). Such variability makes it difficult to have confidence in CK as a reliable measure of recovery in intervention studies.

Throughout the studies, milk had a positive effect on the objective measures of recovery including peak torque, sprint performance and CMJ, but typically did not have an effect on perception of soreness or tiredness. This disconnect between the objective and subjective measures is not novel in research. Saw *et al.,* (2015), in a systematic review, concluded that there was no consistent association between subjective and objective measures in the monitoring of athletes. While this review did not focus on short term recovery from exercise participation, it highlights the insinuations from the current sequence of investigations. From a practical perspective, athletes with similar soreness and tiredness do not display similar levels of reduced muscle function. Even when soreness was high (Study 1, 3 and 4) muscle function was returning to baseline values at a faster rate in the athletes who consumed milk compared to those who consumed carbohydrate. Although Saw and colleagues recommended that subjective measures are useful for athlete monitoring and using both subjective and objective measures logically provides a more comprehensive understanding of individual player recovery (Brownstein *et al*., 2017), the evidence from this thesis indicates that subjective measures are a poor indicator of muscle function at a given time and should not be exclusively relied on as a measure of how the athlete is recovering, or subsequent performance. This is of relevance for future research exploring recovery or the effects of recovery interventions.

8.5 Future Directions

When assimilating the findings reported in this thesis in the context of exercise recovery, the rationale for further investigation is apparent. This sequence of investigations has examined the effect of milk on recovery from different exercise protocols and importantly, is the first to explore the benefits of milk for female athletes.

While this thesis presents a series of investigations focusing solely on the intake of milk in the immediate post-exercise situation, it is likely that a planned periodised approach may be beneficial. This could include repeated ingestion of milk during the recovery period in order to maximise protein synthesis rates and enhance or accelerate recovery, as it is known that protein synthesis rates remain elevated for at least 24h post-exercise (Burd *et al.,* 2011) and an even distribution of protein during the day (rather than skewed towards an evening intake) results in greater protein synthesis (Mamerow *et al.,* 2014). It has been reported that augmented protein synthesis with the consumption of protein does not continue through the night (Beelan *et al*., 2008) but recent research has shown that the ingestion of protein before sleep may be beneficial in supporting muscle protein synthesis overnight (Res *et al*., 2012), possibly due to a maintenance of circulating amino acid concentrations. Future research could explore if the consumption of milk pre-bedtime is a practical means to maintain overnight MPS.

Previous research identified that 500ml was as effective as 1000ml when consumed following muscle-damaging exercise (Cockburn *et al.,* 2012) and thus, this intake volume formed the basis for the intervention in this sequence of studies. However, the exercise protocol utilised by Cockburn and colleagues and in Study 1 of this thesis was on an isolated muscle group quite different from the 'whole-body' activities of most sports and the protocols utilised in Study 3 and Study 4. It is credible that an increased intake may be more beneficial with such exercise. This was recently demonstrated by MacNaughton *et al*., (2016) when protein synthesis was enhanced with 40g of protein compared to 20g following whole-body resistance exercise and an opportunity exists to see if milk can produce a similar outcome.

In the context of ecological validity, it is not uncommon for athletes to combine more than one recovery intervention. Indeed, it is tenable that combining several strategies will be of greater benefit than one strategy in isolation (Beck *et al.,* 2015). In this series of investigations, milk had little effect on muscle soreness and tiredness. Other recovery strategies have been found to be beneficial in this regard (Machado *et al.,* 2016; Nelson, 2013; Bleakley *et al.,* 2012). Thus, opportunities exist to explore how milk might interact with other recovery strategies to provide optimal recovery for individual athletes.

Milk has been identified as a suitable post-exercise rehydration beverage, with its sodium and calorie content resulting in a slower gastric emptying and absorption time leading to greater fluid retention (Shireffs *et al.,* 2007; Watson *et al.,* 2008). Investigations examining post-exercise protein synthesis, hydration status and recovery markers would provide an insight into the potential for milk as an holistic recovery beverage, both in acute and chronic scenarios. It is also worth considering research of other dairy products. Yogurt, given its pleasant taste and ease of consumption along with its protein content, is considered an appropriate post-exercise dairy food (Josse and Phillips, 2013). Only 2 studies have investigated the effects of post-exercise consumption of yogurt and both were resistance training interventions (White *et al*., 2009; Thomas *et al*., 2011). Neither found yogurt consumption to benefit lean mass composition. However, the amount of yogurt consumed provided only 5 g protein which is a suboptimal stimulus for muscle protein synthesis (Moore *et al*., 2009). Further research is warranted to examine the effect of larger amounts of yogurt consumption, and possibly Greek or 'Greek-style' yogurts (typically 10 g protein per 100 g) on muscle protein synthesis and recovery from exercise.

It is also relevant that foods rich in high quality protein, such as milk, are typically rich in other nutrients (Phillips *et al.,* 2015). Dairy protein sources have high natural calcium content and therefore, can offer other potential benefits to the athlete, an area which could be explored further, especially in a female athlete cohort. A number of researchers have reported a positive effect of chronic resistance training plus dairy consumption on bone health outcomes in females (Josse *et al.,* 2012; Thomas *et al.,* 2010), though neither of the cohorts examined were an athletic population. Reid (2016) described how a structured milk intake with an elite sprint kayaker enhanced muscle mass, recovery and vitamin D status during intensive training periods and is an approach that warrants further investigation.

8.6 Other Limitations/Influencing Factors

All four investigations showed considerable inter-individual differences in the response to the exercise protocol employed. Despite large differences between the group means, many of the effect outcomes were unclear and larger participant numbers may have provided more certain results. Still, some of the variability may be

as a result of genetic variability and vulnerability to the exercise protocols (Baird *et al.,* 2012), leading to greater disruption of muscle fibres, alterations in calcium handling ability and varying inflammatory responses (Clarkson *et al*., 2005; Devaney *et al*., 2007; Yamin *et al*., 2008). Of relevance, is previously reported variability in the uptake and distribution of amino acids (Fouillet *et al.,* 2002; Bos *et al.,* 1999, 2003). Another source of this variability may be differences in the initial physical performance characteristics of the participants. While all participants fulfilled the inclusion criteria, differences in levels of conditioning may have impacted the response to the exercise protocol and thus, the intervention. Lower post-match muscle damage and decreases in CMJ have been reported in rugby league players with the highest high-intensity running ability and lower body strength (Johnston *et al*., 2015). It is also difficult to predict if the present results can be transferred to athletes exercising/competing at a higher or elite level.

A related influencing factor could be the time of the year when the data was collected. Data collection for this series of investigations was conducted during the period October- March. This is the competitive season for collegiate players. However, some of the participants may have completed their club season in the previous mid-summer while others were still participating in club fixtures at the start of the investigation. These players, therefore, were conceivably in better physical condition and more accustomed to the demands of the exercise protocols. This could have influenced the impact of the post-exercise nutritional intervention. Future research could explore the time course of recovery during different phases of the season (Thomas *et al.,* 2017) which could provide information to coaches and athletes regarding the necessity of specific recovery interventions at different periods during the year.

Finally, the overall, and day-to-day, dietary intakes may have impacted the results of the studies presented. To examine the effect of an intervention without interference from the rest of the diet is a challenge. Furthermore, the timing of nutrient intake, frequency of intake and source of nutrient intake may influence individual and group responses. In the four investigation of this thesis overall analysis of nutrient and energy intake over the experimental period revealed no clear differences between groups (apart from a likely higher carbohydrate intake in the MILK group in Study 2). Nonetheless, individual daily energy intake varied from 654 kcal to 3331 kcal across

the four investigations, and within-studies the difference in daily kcal intake ranged from 954 kcal (Study 3) to 2537 kcal (Study 1). Of relevance, there was a similar variation in protein intake with one individual reporting a protein intake of 24.3 g/day (Study 4) and another reporting 143.9 g/day (Study 3). This not only makes within-study analysis difficult, but also prevents conclusive between-study analysis. An obvious limitation in this regard is the use of Food Diaries to determine both energy and nutrient intake, with possibly up to 30% of participants under-reporting their food intake (Poslusna *et al.,* 2009). The gold standard for assessing energy intake is the observation method (Baker *et al.,* 2014) though this approach is very labour intensive and not compatible with most research environments. Providing pre-packaged food for all participants is a good alternative (Jeacocke and Burke, 2010) but this is a costly element, though some investigators have provided a standard pre-exercise meal to at least control pre-exercise energy and nutrient intake (Keane *et al.,* 2015). Otherwise, cross-referencing food diaries with a daily interview is a technique that has been recently used (Brown *et al.,* 2015) and allows the researchers to clarify any ambiguity and complete missing data. Adopting some of the above approaches to the monitoring of food intake in the studies of this thesis may have provided both greater accuracy, and greater insight into potential counfounding influences.

8.7 Conclusion

Collectively, the data from the four progressive studies presented suggest that post-exercise consumption of 500 ml of milk attenuates, at least partially, the short-term negative effects of exercise participation.

The study described in Chapter 4, which examined the effect of milk on recovery from eccentric exercise in males and females, demonstrated some sex differences in the responses. Milk:

- Attenuated losses in muscle function and increases in muscle soreness in females
- Attenuated increases in CK and muscle soreness in males.

The study described in Chapter 5 explored the effect of milk on recovery from a non-eccentric cycling protocol. In this case milk:

- Had minimal effect on the attenuation of losses in muscle function or increases in markers of muscle damage and oxidative stress.
- Reduced perceptions of soreness and tiredness.

Chapter 6 describes a study that investigated the effect of milk on recovery from repeated sprinting and jumping. In this study milk:

- Attenuated losses in peak torque, countermovement jump height, RFD and 5m sprint performance.
- Had no clear effect on RSI, 10/20m sprint performance, CK, hsCRP and muscle soreness.

Finally, the study dscribed in Chapter 7 examined the effect of milk on recovery from repeated simulated team sport performance. Milk

- Attenuated some losses in muscle function
- Reduced symptoms of stress
- Had no effect on 'in-game' measures, including sprinting and jumping

The positive effect on muscle function was not for all variables, nor all timepoints. While the effects of milk on muscle soreness and tiredness were mostly unclear, milk had a consistently positive effect on symptoms of stress as identified by DALDA. Practically, the results indicate, for female athletes at least, that milk is an effective recovery drink from varying types of exercise that include a degree of eccentric loading, with the greatest benefits observed following exercise with the greatest eccentric bias. This information is of value to athletes and coaches who aim to maximise the recovery process following exercise, and especially when the recovery period is short. There are a number of possible explanations for these results, though further investigation is warranted to elucidate these**.** In terms of methodological considerations, a key finding is that common isolated measures of recovery may not be related to in-game performance and there is certainly future research potential in this area. Importantly, the four progressive studies presented in this thesis contribute to the expansion of our understanding of female athletes, offering evidence for the formulation of effective recovery interventions for a cohort of athletes that has historically depended on strategies developed from their male counterparts. The

addition of knowledge to redress the sex imbalance in sports and exercise research can only serve to identify both sex similarities and sex differences, thus facilitating the enhancement of guidelines and sport specific education for optimising recovery and performance.

APPENDICES

APPENDIX A: Sample Participant Information Sheet and Informed Consent (Study 3)

MIDDLESEX UNIVERSITY
SCHOOL OF HEALTH AND EDUCATION

PARTICIPANT SHEET (PS)

Participant ID Code:……

1. Study title

The effects of milk consumption on recovery from repeated sprinting and jumping

2. Invitation paragraph

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.
Thank you for reading this.

3. What is the purpose of the study?

High-intensity repeated sprinting and jumping, core exercise activities in team sport performance, have been associated with increased muscle damage and oxidative stress, which reduces the ability of the muscle to produce force and causes increased muscle fatigue, leading to reduced performance. Nutritional intervention may be beneficial in limiting these changes. The aim of this study is to investigate the effect of milk consumption on muscle damage and oxidative stress following maximal repeated sprinting and jumping.

4. Why have I been chosen?

It is important that we assess as many participants as possible and you have indicated that you are interested in taking part in this study. You fulfil the following requirements for participants:

Sex: Female
Age: 18-30years
Sport played: Gaelic football or camogie; training/competing at least three times per week.

Also, you do not meet any of the exclusion criteria - lower limb or back injury in previous 3 months, surgery in previous 6 months, lactose intolerance, known coronary disease, uncontrolled metabolic disorder or respiratory disease, hepatitis, HIV, pregnancy, post-partum.

5. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you do decide to withdraw from the study then please inform the researcher as soon as possible and they will facilitate your withdrawal. If, for any reason, you wish to withdraw your data please contact the researcher within a month of your participation. After this, it may not be possible to withdraw your individual data as the results may have already been published. However, as all data are anonymised, your individual data will not be identifiable in any way. A decision to withdraw at any time, or a decision not to take part, will not affect your status as a student in any way.

6. What will I have to do?

You will be involved in this research project for 7-10days.

You will be asked to attend room C156 (Physiology Laboratory) at the Institute of Technology, Carlow on 6 separate occasions, with all testing taking place between 7am and 11am. The table at the end of this section summarises your involvement. On attending the first session (Familiarisation), you will be met by the investigator and allowed to ask any questions concerning what you will later be asked to do. You will be asked to complete a health screening questionnaire to find out about your current health status and your health history and sign a consent form. You will then perform a number of assessments (see below) to allow you to become familiar with the protocols. This visit will take approximately one hour.

The second visit will be for the conduction of baseline measurements (see below) and will take approximately 40min.

On visit 3, following a warm-up, you will be asked to perform 15 x 20m maximal sprints, with a 10m deceleration or ‘stopping’ zone after the finish line. You will have a rest after each sprint. You will then be asked to complete 8 sets of 10 maximal jumps, followed by a cool-down. Immediately after the cool down you will be provided with 500ml of milk or 500ml of a carbohydrate drink to consume immediately and you will be free to leave. Following the consumption of your allocated drink, you will be asked to fast for 2h, then return to the laboratory to provide a blood sample.

Visits 4, 5 and 6 will take place 24h, 48h and 72h after the exercise session, during which the baseline measurements will be repeats. These visits will take approximately 40min each.

Measurements you will be asked to complete during each visit:

(a) Provide a venous blood sample (BS) from a vein on your forearm.

(b) Peak torque test (PkTq) – this will measure the maximal amount of force you can generate in both your left leg and your right leg on a computer controlled strength testing device (isokinetic machine). You will be asked to flex (bend) and extend (straighten) your knee as hard as possible. You will complete three repetitions at two different speeds on both your left and right legs.

(c) Rate of force development (RFD) – this assessment will measure how quickly you can develop force in your leg muscles using a computer controlled strength testing device (isokinetic machine). You will be asked to straighten your leg against the immovable arm of the machine as hard as possible. You will complete three repetitions.

(d) Countermovement Jump test (CMJ) - from a standing position you will be asked to squat down to approximately 90 degrees and jump upwards as high as you can. Your jump height will be recorded. You will complete three jumps.

(e) Reactive Strength Index (RSI) – from a standing position on a step you will be asked to step off and upon contact with the ground, jump upwards as high as you can. Your time in contact with the ground and your jump height will be recorded. You will complete three jumps.

Twenty metre sprint test (20mSp) - you will be asked to run 20m as fast as you can. Your sprint time will be recorded. You will complete three sprints with a two minute rest between sprints.

(f) Soreness (S) and Tiredness (T) - you will be shown a scale and asked to indicate how sore you feel and how tired you feel when completing a squatting exercise.

(g) Perception of recovery- you will be asked to complete a short questionnaire asking you about how you feel you are recovering from the exercise trial with regard to the physical recovery, your energy levels and your enthusiasm and motivation.

For 24h prior to the exercise session and for the 72h after, you will be asked to record your food and fluid intake. You will be provided with a weighing scale, measuring jug and recording sheet for this purpose.

You will be asked to refrain from strenuous physical activity for the duration of the investigation, and from treating the symptoms of muscle fatigue or soreness with interventions such as massage, ice or medication, or consuming any nutritional supplements (e.g. anti-oxidants, HMB, casein/whey protein).
A summary of your involvement can be seen in the following table.

Visit	Duration	What I will do
Visit 1: Familiarisation	I hour	Health screening questionnaire; consent form; BS, PkTq, RFD, CMJ, RSI, Sp, S/T, PR
Visit 2: Baseline measures	40min	BS, PkTq, RFD, CMJ, RSI, Sp, S/T, PR
Visit 3: Exercise protocol	1 hour	10min warm up 15 x 20m maximal sprints 8 sets of 10 CMJ 10min cool down
Visits 4-6	30min	BS, PkTq, RFD, CMJ,RSI, Sp, S/T, PR

Table 1: Participant involvement

BS: blood sample; PkTq: peak torque; CMJ: countermovement jump; RSI: Reactive Strength Index; Sp: 20m sprint; S/T: soreness/tiredness; PR: perception of recovery.

Please note that in order to ensure quality assurance and equity, this project may be selected for audit by a designated member of the committee. This means that the designated member can request to see signed consent forms. However, if this is the case, your signed consent form will only be accessed by the designated auditor or member of the audit team.

7. Will I have to provide any bodily samples (i.e. blood/saliva/urine)?

Yes, you will be asked to provide a venous blood sample from a vein on your forearm. The researcher has been trained in venepuncture. You will be allowed to lie down for the procedure. Your skin will be cleaned, the needle inserted and stabilised with minimal discomfort to you. The correct volume of blood will be obtained in a blood collection tube and the needle will be removed. You will be asked to apply pressure with a cotton swab to the puncture site to minimise bruising and bleeding. Should the tests reveal an abnormality, the researcher will recommend to you that you seek further medical advice from your GP. Bear in mind though that a single test may not always provide an accurate reflection of your health status.

8. What are the possible disadvantages and risks of taking part?

Participation in this project will involve some physical discomfort.

The research project has been designed to induce muscle soreness and oxidative damage having you sprint and jump with maximal effort. You may experience feelings of exertion and local tiredness in your legs and it is likely you will experience delayed muscle soreness and stiffness afterwards. However, this discomfort is short-term and will dissipate after approximately five days.

When having your blood sample collected by a researcher trained in venepuncture, you will feel a needle prick in your arm. Every care will be taken to ensure that minimal discomfort is felt during the blood draw using standardised venepuncture techniques. In rare cases, bruising may be apparent but this should subside within days.

The sprint and jump tests require you to work at high intensities though they are of short duration. You may feel dizzy and tired. These feelings are temporary and are expected to subside within minutes of completing the exercise.

Appropriate risk assessments for all procedures have been conducted and will be followed throughout the duration of the study.

9. What are the possible benefits of taking part?

We hope that participating in the study will help you. However, this cannot be guaranteed. The information we get from this study may help to provide researchers, coaches and players with specific guidelines with regard to the benefits of nutritional supplementation for team sports athletes following high intensity training sessions or competition.

10. Will my taking part in this study be kept confidential?

The research team has put a number of procedures in place to protect the confidentiality of participants. You will be allocated a participant code that will always be used to identify any data you provide. Your name or other personal details will not be associated with your data, for example, the consent form that you sign will be kept separate from your data. All paper records will be stored in a locked filing cabinet, accessible only to the research team, and all electronic data will be stored on a password protected computer. All information you provide will be treated in accordance with the UK Data Protection Act.

11. What will happen to the results of the research study?

The results of the research study will be used as part of a PhD thesis. The results may also be presented at conferences or in journal articles. However, the data will only be used by members of the research team and at no point will your personal information or data be revealed.

12. Who has reviewed the study?

The study has received full ethical clearance from the Research Ethics Committee who reviewed the study. The committee is the Middlesex University, School of Science and Technology, London Sport Institute Ethics Sub-committee.

13. Contact for further information

If you require further information, have any questions or would like to withdraw your data, then please contact:

Paula Rankin, Department of Science and Health, 05991 75540,
paula.rankin@itcarlow.ie
Dr Emma Cockburn, London Sport Institute, Middlesex University,
e.cockburn@mdx.ac.uk

Thank you for taking part in this study. You should keep this participant information sheet as it contains your participant code, important information and the research teams contact details.

CONSENT FORM

Title of project: The effects of milk consumption on recovery from repeated sprinting and jumping

Name of Researcher: Paula Rankin

1. I confirm that I have read and understand the information sheet dated .....……..….for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.

3. I agree that this form that bears my name and signature may be seen by a designated auditor.

4. I agree that my non-identifiable research data may be stored in National Archives and be used anonymously by others for future research. I am assured that the confidentiality of my data will be upheld through the removal of any personal identifiers.

5. I agree to take part in the above study.

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of parent/guardian (if appropriate)	Date	Signature

1 copy for participant; 1 copy for researcher

APPENDIX B: Sample Health Screening Form

Health Questionnaire

STRICTLY CONFIDENTIAL

Participant Code:
Height:
Weight:
Please answer these questions truthfully and completely. The sole purpose of this questionnaire is to ensure that you are fit and healthy to follow the proposed research programme.

1. How would you describe your present level of activity?

Vigorous activity:

Less than once per month	Once a month
Once a week	Two/three times a week
Four/five times a week	More than five times a week

2. Are you currently taking any form of medication? If yes, please give brief details.

Yes ☐ No ☐

3. Have you suffered from a bacterial or viral infection in the last two weeks?

Yes ☐ *No* ☐

4. Have you had cause to suspend physical activity in the last two weeks for any reason?

Yes ☐ *No* ☐

5. If you currently suffer from or have previously suffered from any of the following conditions you will be unable to take part in the study. Please inform the researcher (without specifics) if as a result you will now be unable to take part in the study.
   - heart complaint/condition
   - asthma
   - diabetes (Type 1 or 2)

- high blood pressure
- blood borne disease or infection

6. Is there any reason why you should not embark on the proposed research programme?

Yes ☐ No ☐

7. Do you take any form of nutritional supplement (creatine, whey and casein protein, HMB etc)

Yes ☐ No ☐

9. Do you have a known allergy or intolerance to milk or other dairy products?

Yes ☐ No ☐

You are not required to provide specifics of any condition that precludes you from taking part in the study. However, if you are not sure if any such condition will affect your ability to participate, please feel free to discuss this with the research team, although you are not obliged to do so.

Signature: _____

Date: _____

Researcher: _____

APPENDIX C: Food Diary

Dietary Food Record Instructions

Name: _____

Please record dietary intake for the following days: day prior to exercise trial, day of exercise trial and the following two days of recovery.
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 (eg vitamins, minerals)
- Record the date and time of consuming each food, drink, 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 or toast

Include anything that was added to the food or drink

- Addition of sugar to tea
- Addition of butter to bread
- Addition of oil (what type) to cook steak

Use nutrient descriptors and brand names to describe foods

- Diet
- Low calorie
- Reduced fat
- Dairylea
- Cadbury

Write down how much you have consumed

- Use the scales provided to weigh ALL food
- Use the labels on food to help you determine amounts
- If you use ready prepared food return the label from the package
- Write down the number of sausages, fish fingers, slices of meat
- 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 a food diary

Day: _____ Date: _____

Time	Food/drink	Description	Amount eaten
8am	Cereal	Kellogg's Bran Flakes	50g
	Milk	Semi skimmed	100ml
	Orange juice	Smooth from concentrate	500ml
10.30am	Cereal bar	Brunch bar with hazelnut (see wrapper)	1 bar
12.30pm	Baked potato	Microwave	Large
	Butter	Dairygold	5g
	Cheese	Medium cheddar, grated	10g
	Beans	Heinz baked beans	100g
	Orange diluting juice	Tesco own with no added sugar	20ml with 100ml water
2.45pm	Water	Tap	500ml
7pm	Chicken	Diced and stir fried with extra virgin olive oil	200g; 10ml oil
	Carrots	2 medium stir fried, chopped	30g
	Rice	White boiled	75g
	Broccoli	3 florets stir fried	30g
	Mushrooms	Button, stir fried	30g
	Water	Tap	200ml
9pm	Toast	1 slice white bread	20g
	Jam	Strawberry with real fruit	10g
	Milk	Semi-skimmed	100ml

Name: _____ Day: _____ Date: _____

Time	Food/drink	Description	Amount

If more space is required, please write on the back.

APPENDIX D: Macronutrient and caloric content analysis

Study 1

Groups	Component	Mean Effect ± 90%CI	Qualitative Inference	Effect Size
FM v FC	Kcal	-19.1 ± 380.6	Unclear	Trivial
	Carbohydrate	19.1 ± 50.8	Unclear	Small
	Protein	-13.5 ± 16.9	Unclear	Moderate
	Fat	-5.8 ± 25.4	Unclear	Small
MM V MC	Kcal	-345.7 ± 471.8	Unclear	Small
	Carbohydrate	-46.6 ± 66.6	Unclear	Small
	Protein	-12.4 ± 40.4	Unclear	Small
	Fat	-17.0 ± 21.1	Unclear	Small

Study 2

Groups	Component	Mean Effect ± 90%CI	Qualitative Inference	Effect Size
MILK v CHO	Kcal	-53.2 ± 291.6	Unclear	Trivial
	Carbohydrate	33.5 ± 42.6	Likely Higher	Small
	Protein	-2.1 ± 24.9	Unclear	Trivial
	Fat	-2.3 ± 13.4	Unclear	Trivial

Study 3

Groups	Component	Mean Effect ± 90%CI	Qualitative Inference	Effect Size
MILK v CHO	Kcal	90.5 ± 253.9	Unclear	Small
	Carbohydrate	22.1 ± 34.8	Unclear	Small
	Protein	-29.6 ± 24.6	Unclear	Trivial
	Fat	2.6 ± 13.6	Unclear	Trivial

Study 4

Groups	Component	Mean Effect ± 90%CI	Qualitative Inference	Effect Size
MILK v CHO	Kcal	132.7 ± 368.6	Unclear	Small
	Carbohydrate	33.0 ± 50.3	Unclear	Trivial
	Protein	0.0 ± 25.9	Unclear	Trivial
	Fat	0.8 ± 16.6	Unclear	Trivial

Appendix D. DALDA Questionnaire

DALDA Questionnaire **Participant code**

Please tick the correct box for this moment

Date

Part A

	Worse than normal	**Normal**	**Better than normal**
Diet			
Home Life			
School/College/Work			
Friends			
Training and Exercise			
Climate			
Sleep			
Recreation			
Health			

Part B

	Worse than normal	**Normal**	**Better than normal**
Muscle Pains			
Techniques			
Tiredness			
Need for a Rest			
Supplementary Work			
Boredom			
Recovery Time			
Irritability			
Weight			

	Worse than normal	Normal	Better than normal
Throat			
Internal			
Unexplained Aches			
Technique Power			
Enough Sleep			
Between Sessions Recovery			
General Weakness			
Interest			
Arguments			
Skin Rashes			
Congestion			
Training Effort			
Temper			
Swellings			
Likeability			
Running Nose			

TABLE 2. DEFINITIONS OF PART B (SYMPTOMS OF STRESS) FOR THE DAILY ANALYSES OF LIFE DEMANDS FOR ATHLETES.

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1. Muscle pains. Do you have sore joints and/or pains in your muscles?
2. Techniques. How do your techniques seem/feel to you? Have your technical skills changed?
3. Tiredness. Your general state of tiredness is:
4. Need for a rest. Do you feel that you need a rest between training sessions?
5. Supplementary work. How strong do you feel when you do supplementary training (e.g., weights, resistance work, stretching)?
6. Boredom. How boring is training?
7. Recovery time. Do the recovery times between each training effort need to be longer?
8. Irritability. Are you irritable? Do things get on your nerves?
9. Weight. How is your weight?
10. Throat. Have you noticed your throat being sore or irritated?
11. Internal. How do you feel internally? Have you had constipation, upset stomachs, etc.?
12. Unexplained aches. Do you have any unexplained aches or pains?
13. Technique power. How do you rate the level of power you develop in your techniques?
14. Enough sleep. Are you getting enough sleep?
15. Between sessions recovery. Are you tired before you start your second training session of the day?
16. General weakness. Do you feel weak all over?
17. Interest. Do you feel that you are maintaining your interest in your sport?
18. Arguments. Are you having squabbles and arguments with people?
19. Skin rashes. Do you have any unexplained skin rashes or irritations?
20. Congestion. Are you experiencing congestion in the nose and/or sinuses?
21. Training effort. Do you feel that you can give your best effort at training?
22. Temper. Do you lose your temper?
23. Swellings. Do you have any lymph gland swellings under your arms, below your ears, in your groin, etc.?
24. Likability. Do people seem to like you?
25. Running nose. Do you have a running nose?

REFERENCES

Aagaard, P., Simonsen, E.B., Andersen, J.L., Magnusson, P., Dyhre-Poulsen, P. (2002). Increased rate of force development and neural drive of human skeletal muscle following resistance training. *Journal of Applied Physiology*, 93, 1318-1326.

Abdulla, H., Smith, K., Atherton, P. J. and Idri, I. (2016). Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown: a systematic review and meta-analysis. *Diabetologia*, 59, 44-55.

Aguilo, A., Tauler, P., Gimeno, I., Fuentespina, E. and Pons, A. (2000). Changes in erythrocyte antioxidant enzymes during prolonged exercise. *Biofactors*, 11, 27-30.

Alessio, H.M., Hagerman, A.E., Fulkerson, B.K., Ambrose, J., Rice, R.E. and Wiley, R.L. (1999). Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Medicine and Science in Sports and Exercise,* 32, 1576-1581.

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

Allen, D.G., Whitehead, N.P. and Yeung, E.W. (2005). Mechanisms of stretch-induced muscle damage in normal and dystrophic muscle: role of ionic changes. *Journal of Physiology*, 567, 723-725.

Amelink, G.J. and Bär, P.R. (1986). Exercise-induced muscle protein leakage in the rat: effects of hormonal manipulation. *Journal of the Neurological Sciences*, 76, 61-68.

Amelink, G.J., Koot, R.W., Erich, W.B., Van Gijin, J. and Bar, P.R. (1990). Sex-linked variation in creatine kinase release, and its dependence on oestradiol, can be demonstrated in an in vitro rat skeletal muscle preparation. *Acta Physiologica Scandanavica*, 138, 115-134.

Andersson, H., Raastad, T., Nilsson, J., Paulsen, G., Garthe, I. and Kadi, F. (2008). Neuromuscular fatigue and recovery in elite female: effects of active recovery. *Medicine and Science in Sports and Exercise*, 40, 372-380.

Andersson, H.Å., Randers, M.B., Heiner-Møller, A., Krustrup, P. and Mohr, M. (2010). Elite female soccer players perform more high-intensity running when playing in international games compared with domestic league games. *The Journal of Strength and Conditioning Research*, *24*, 912-919.

Andrade, F.H., Reid, M.B., Allen, D.G., Allen, D.G. and Westerblad, H. (1998). Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse. *Journal of Physiology*, 509, 565-575.

Anthony, J.C., Yoshizawa, F., Anthony, T.G., Vary, T.C., Jefferson, L.S. and Kimball, S.R. (2000). Leucine stimulates translation intitiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *Journal of Nutrition,* 130, 2413-2419.

Anzai, K., Ogawa, K., Ozawa, T. and Yamamoto, H. (2000). Oxidative modification of ion channel activity or ryanodine receptor. *Antioxidants and Redox Signalling,* 2, 35-40.

Appell, H.J., Soares, J.M.C. and Duarte, J.A.R. (1992). Exercise, muscle damage and fatigue. *Sports Medicine*, 13, 2, 108-115.

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

Armstrong, R.B., Ogilvie, R.W. and Schwane, J.A. (1983). Eccentric exericse-induced injury to rat skeletal muscle. *Journal of Applied Physiology*, 54, 90-93.

Armstrong, R.B., Warren, B.L. and Warren, J.A. (1991). Mechanisms of exercise-induced muscle fibre injury. *Sports Medicine*, 12, 3, 184-207.

Arnett, M.G., Hyslop, R., Dennehy, C.A. and Schneider, C.M. (2000). Age-related variations of serum CK and CKMB response in females. *Canadian Journal of Applied Physiology*, 25, 419-429.

Arthur, G.D., Booker, T.S. and Belcastro, A.N. (1999). Exercise promotes a subcellular redistribution of calcium-stimulated protease activity in striated muscle. Canadian Journal of Physiology and Pharmacology, 77, 42-47.

Ascensão, A., Rebelo, A., Oliveira, E., Marques, F., Pereira, L. and Magalhães, J. (2008). Biochemical impact of a soccer match—analysis of oxidative stress and muscle damage markers throughout recovery. *Clinical biochemistry*, *41*, 841-851.

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

Avery, N.G., Kaiser, J.L., Sharman, M.J., Scheett, T.E., Barnes, D.M., Gomez, A.L., Kraemer, W.J and Volek, J.S. (2003). Effects of vitamin E supplementation on recovery from repeated bouts of resistance exercise. *Journal of Strength and Conditioning Research*, 17, 801-809.

Ayres, S., Abplanalp, W., Liu, J.H. and Subbiah M.T.R. (1998). Mechanisms involved in the protective effect of estradiol -17β on lipid peroxidation and DNA damage. *American Journal of Physiology*, 274, E1002-1008.

Baba, S., Ebihara, S., Sakano, K. and Natsume, M. (2014). Whey protein-containing product reduces muscle damage induced by running in male adults. *Sport Science and Health*, 10, 85-95.

Baird, M.F., Graham, S.M., Baker, J.S. and Bickerstaff, G.F. (2012). Creatine kinase and exercise-related muscle damage implications for muscle performance and recovery. Journal of Nutrition and Metabolism, doi: 10.1155/2012/960363.

Baker, L.B., Heaton, L.E., Stein, K.W., Nuccio, R.P. and Jeukendrup, A.E. (2014). Validity and relative validity of a novel digital approach for 24-h dietary recall in athletes. *Nutrition Journal*, 13, 41.

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

Balnave, C.D., Davey, D.F. and Allen, D.G. (1997). Distribution of sarcomere length and $[Ca^{2+}]_i$ in single fibres from mouse skeletal muscle following stretch-induced injury. *Journal of Physiology*, 502, 649-659.

Baltzopoulos, V. and Gleeson, N.P. (2001). Skeletal muscle function. In: Eston, R.G., Reilly, T. editors. Tests, procedures and data. (Vol 2:Exercise Physiology). London, Routeledge, 7-35.

Bangsbo, J., Mohr, M. and Krustrup, P. (2006). Physical and metabolic demands of training and match-play in the elite football player. *Journal of Sports Sciences*, 24, 665-674.

Bar, P.R., Amelink, G.J., Oldenburg, B. and Blankenstein, M.A. (1988). Prevention of exercise-induced muscle membrane damage by oestradiol. *Life Sciences*, 42, 2677-2681.

Barash, I.A., Peters, D., Fridén, J., Lutz, G.J. and Lieber, R.L. (2002). Desmin cytoskeletal modifications after a bout of eccentric exercise in the rat. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*, 283, R958-R963.

Bar-Peled, L. and Sabatini, D.M. (2014). Regulation of mTORC1 by amino acids. *Trends in Cell Biology*, 24, 400-406.

Barroso, R., Roschel, H., Ugrinowitsch, C., Araújo, R., Nosaka, K. And Tricoli, V. (2010). Effect of eccentric contraction velocity on muscle damage in repeated bouts of elbow flexor exercise. *Applied Physiology Nutrition and Metabolism*, 35, 534-540.

Batterham, A.M. and Hopkins, W.G. (2006). Making meaningful inferences about magnitudes. *International Journal of Sports Physiology and Performance*, 1, 50-57.

Baty, J.J. Hwang, H., Ding, Z., Bernard, J.R., Wang, B., Kwon, B. and 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, 321-329.

Beaton, L.J., Allan, D.A., Tarnopolsky, M.A., Tiidus, P.M. and Phillips, S.M. (2002). Contraction-induced muscle damage is unaffected by vitamin E supplementation. *Medicine and Science in Sports and Exercise*, 34, 798-805.

Beaton, L.J., Tarnopolsky, M.A. and Phillips, S.M. (2002). Contraction-induced muscle damage in humans following calcium channel blocker administration. *Journal of Physiology,* 544.3, 849-859.

Beattie, K. and Flanagan, E.P. (2015). Establishing the reliability & meaningful change of the drop-jump reactive strength index. *Journal of Australian Strength and Conditioning Research*, *23*, 12-18.

Beck, K.L., Thomson, J.S., Swift, R.J. and von Hurst, P.R. (2015). Role of nutrition in performance enhancement and postexercise recovery. *Open Access Journal of Sports Medicine*, 6, 259.

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

Beelan, M., Tieland, M., Gijsen, A.P., Vandereyt, H., Kies, A.C., Kuipers, H, Saris, W.H., Koopman, R. and van Loon, L.J. (2008). Coingestion of carbohydrate and protein hydrolysate stimulates muscle protein synthesis during exercise in young

men, with no further increase during subsequent overnight recovery. *Journal of Nutrition*, 138, 2198-2204.

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

Belcastro, A.N., Rossiter, M., Low, M.P. and Sopper, M.M. (1981). Calcium activation of sarcoplasmic reticulum ATPase following strenuous activity. *Canadian Journal of Physiology and Pharmacology*, 59, 1214-1218.

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

Bell, J.A., Fujita, S., Volpi, D., Cadenas, J.G. and Rasmussen, B.B. (2005). Short-term insulin and nutritional energy provision do not stimulate muscle protein synthesis if blood amino acid availability decreases. *American Journal of Physiology. Endocrinology and Metabolism*, 289, E999-E1006.

Bell, K.E., Séguin, C., Parise, G., Baker, S.K. and Phillips, S.M. (2015). Day-to-day changes in muscle protein synthesis in recovery from resistance, aerobic, and high-intensity interval exercise in older men. *The Journals of Gerontology: Series A*, *70*, 1024-1029.

Bell, P.G., Walshe, I.H., Davison, G.W., Stevenson, E. and Howatson, G. (2014). Montmorency cherries reduce the oxidative stress and inflammatory responses to repeated days high-intensity stochastic cycling. *Nutrients*, 6, 829-843.

Benaroudj, N, Zwickl, Pl, Seemuller, E., Baumeister, W. and Goldberg, A.L. (2003). ATP hydrolysis by the proteasome regulatory complex PAN serves multiple functions in protein degradation. *Molecular Cell*, 11, 69-78.

Benziane, B., Burton, T.J., Scanlan, B., Galuska, D., Canny, B.J., Chibalin, A.V., Zierath, J.R. and Stepto, N.K. (2008). Divergent cell signaling after short-term

intensified endurance training in human skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism*, *295*, E1427-E1438.

Berg, A. and Keul, J. (1981). Physiological and metabolic responses of female athletes during laboratory and field exercise. *Medicine and Science in Sports and Exercise*, 14, 77-96.

Bernards, J.R., Sato, K., Haff, G.G. and Bazyler, C.D. (2017). Current research and statistical practices in sport science and a need for change. *Sports*, 5, 87.

Bessa, A.L, Oliveira, V.N., De Agostini, G.G., Oliveira, R.J.S., White, G.E., Wells, G.D., Teixeira, D.N.S., Espindola, F.S. (2016). Exercise intensity and recovery: biomarkers of injury, inflammation and oxidative stress. *Journal of Strength and Conditioning Research*, 30, 311-319.

Bijker, K., de Groot, G. and Hollander, A. (2002). Differences in leg muscle activity during running and cycling in humans. European Journal of Applied Physiology, 87, 556-561.

Bijur, P. E., Silver, W., and Gallagher, E. J. (2001). Reliability of the visual analog scale for measurement of acute pain. Academic Emergency Medicine, 8, 1153-1157.

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

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

Biolo, G., Williams, B.D., Fleming, R.Y., and Wolfe, R.R. (1999). Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes*, *48*, 949-957.

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

Bishop, D., Spencer, M., Duffield, R. and Lawrence, S. (2001). The validity of a repeated sprint ability test. *Journal of Science and Medicine in Sport*, 4, 19-29.

Bishop, N., Gleeson, M., Nicholas, C. and Ali, A. (2002). Influence of carbohydrate supplementation on plasma cytokine and neutrophil degranulation responses to high intensity intermittent exercise. *Interational Journal of Sport Nutrition and Exercise Metabolism,* 12, 145-156.

Bishop, P.A., Jones, E. and Woods, A.K. (2008). Recovery from training: a brief review. *Journal of Strength and Conditioning Research*, 22, 1015-1024.

Blazevich, A.J., Horne, S., Cannavan, D., Coleman, D.R. and Aagaard, P. (2009). Effect of contraction mode of slow-speed resistance training on the maximum rate of force development in the human quadriceps. *Muscle Nerve*, 38, 1133-1146.

Bleakely, C., McDonough, S., Gardner, E., Baxter, D.G., Hopkins, T.J. and Davison G.W. (2012). Cold-water immersion (cryotherapy) for preventing and treating muscle soreness after exercise. *Sao Paulo Medical Journal,* 130, 348.

Bloomer, R.J. (2007). The role of nutritional supplements in the prevention and treatment of resistance exercise-induced skeletal muscle injury. Sports Medicine, 37, 519-532.

Bloomer, R.J., Canale, R.E., McCarthy, C.G. and Farney, T.M., (2012). Impact of oral ubiquinol on blood oxidative stress and exercise performance. *Oxidative Medicine and Cellular Longevity*, doi:10.1155/2012/465020.

Bloomer, R.J., Falvo, M.J., Fry, A.C., Schilling, B.K., Smith, W.A. and Moore, C.A. (2006). Oxidative stress response in trained men following repeated squats or sprints. *Medicine and Science in Sports & Exercise*,38, 1436-1442.

Bobbert, M.F., Hollander, A.P. and Huijing, P.A. (1986). Factors in delayed onset muscle soreness of man. *Medicine and Science in Sports and Exercise*, 18, 75-81.

Bodganis, G.C., Nevill, ME., Boobis L.H. and Lakomy, H.K. (1985). Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *Journal of Applied Physiology*., 1996, 876-884.

Bogdanis, G. C., Stavrinou, P., Fatouros, I. G., Philippou, A., Chatzinikolaou, A., Draganidis, D.,Ermidis, G. and Maridaki, M. (2013). Short-term high-intensity interval exercise training attenuates oxidative stress responses and improves antioxidant status in healthy humans. *Food and Chemical Toxicology*, 61, 171-177.

Bombardier, E., Vigna, C., Iqbal, S. Tiidus, P.M. and Tupling, A.R. (2009). Effects of ovarian sex hormones and downhill running on fibre-type-specific HSP70 expression in rat soleus. *Journal of Applied Physiology*, 106, 2009-2015.

Booth, D.A., French, J.A., Wainwright, C.J., and Gatherer, A.J.H. (1992). Personal benefits from post-ingestional actions of dietary constituents. *Proceedings of the Nutrition Society*, 5, 335-341.

Borsa, P. A. and Sauers, E.L. (1999). The importance of sex on myokinetic deficits before and after microinjury. *Medicine and Science in Sports and Exercise*, 32, 891-896.

Børsheim, E., Aarsland, A. and Wolfe, R.R. (2004). Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise. *International Journal of Sports Nutrition and Exercise Metabolism*, 14, 255-271.

Børsheim, E., Cree, M.G., Tipton, K.D., Elliott, T.A., Aarsland, A. and Wolfe, R.R. (2004). Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. *Journal of Applied Physiology*, 96, 674-678.

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

Bos, C., Mahé, S., Gaudichon, C., Benamouzig, R., Gausserès, N. Luengo, C., Ferrière, Rautureau, J. and Tomé, D. (1999). Assesment of net postprandial protein utilization of ^{15}N-labelled milk nitrogen in human subjects. *British Journal of Nutrition*, 81, 21-226.

Bos, C., Metges, C.C., Gaudichon, C., Petzke, K.J., Pueyo, M.E., Morens, C., Everwand, J., Benamouzig, R. and Tomé, D. (2003). Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. *The Journal of Nutrition*, 133, 1308-1315.

Boveris, A., Oshino, N. and Chance, B. (1972). The cellular production of hydrogen peroxide. *Biochemical Journal*, 128, 617-630.

Bowtell, J.L., Sumners, D.P., Dyer, A., Fox, P. and Mileva, K.N. (2011). Montmorency cherry juice reduces muscle damage caused by intensive strength exercise. *Medicine and Science in Sports and Exercise*, 43, 1544, 1451.

Braakhuis, A.J., Hopkins, W.G. and Lowe, T.E. (2013). Effect of dietary antioxidants, training, and performance correlates on antioxidant status in competitive rowers. *International Journal of Sports Physiology and Performance*, 8, 565-572.

Branca, D., Gugliucci, A., Bano, D., Brini, M. and Carafoli, E. (1999). Expression, partial purification and functional properties of the muscle-specific calpain isoform p94. *European Journal of Biochemistry,* 265, 839-846.

Brancaccio, P., Maffulli, N. and Limongelli, F.M. (2007). Creatine kinase monitoring in sport medicine. *British Medical Bulletin*, 81 and 82, 209-230.

Breen, L., and Phillips, S.M. (2011). Skeletal muscle protein metabolism in the elderly: interventions to counteract the ‘anabolic resistance’ of ageing. *Nutrition and Metabolism*, 8, 68.

Bridgeman, L.A., Gill, N.D., Dulson, D.K. and McGuigan, M.R. (2017). The effect of exercise-induced muscle damage after a bout of accentuated eccentric load drop jumps and the repeated bout effect. *The Journal of Strength & Conditioning Research*, *31,* 386-394.

Brieger, K., Schiavone, S., Miller Jr, F.J. and Krause, K.H. (2012). Reactive oxygen species: from health to disease. *Swiss Medical Weekly*, 142, 13659.

Brookshire, B. (2016). Women in sports are often under-represented in science. *Science News, Magazine of the society for science and the public.* https://www.sciencenews.org/blog/scicurious/women-sports-are-often-underrepresented-science. Accessed 22/03/2018.

Brown, M.A., Howatson, G., Keane, K. and Stevenson, E.J. (2015). Exercise-induced muscle damage following dance and sprint specific exercise in females. *Journal of Sports Medicine and Physical Fitness,* 56, 1376-1383.

Brown, M.A., Howatson, G., Keane, K. and Stevenson, E.J. (2016). Exercise-induced muscle damage following dance and sprint specific exercise in females. *Journal of Sports Medicine and Physical Fitness,* 56, 1376-1383.

Brown, M.A., Stevenson, E.J. and Howatson, G. (2017). Whey protein hydrolysate supplementation accelerates recovery from exercise–induced muscle damage in females. Applied Physiology Nutrition and Metabolism. doi: 10.1139/apnm-2017-0412.

Brownstein, C.G., Dent, J.P., Parker, P., Hicks, K.M., Howatson, G., Goodall, S. and Thomas, K. (2017). Etiology and recovery of neuromuscular fatigue following competitive soccer match-play. *Frontiers in Physiology*, 8, 831. doi: 10.3389/fphys.2017.00831.

Bruinvels, G., Burden, R.J., McGregor, A.J., Ackerman, K.E., Dooley, M., Richards, T. and Pedlar, C. (2016). Sport exercise and the menstrual cycle: where is the research? *British Journal of Sports Medicine*, 51, 487-488.

Bruunsgaard, H.H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T., MacLean, D. and Pedersen, B. (1997). Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *Journal of Physiology*, 499-833-841.

Buckley, J.D., Thomson, R.L., Coates, A.M., Howe, P.R., Denichilo, M.O. and Rowney, M.K. (2010). Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. *Journal of Science and Medicine in Sport,* 13, 178-181.

Buckley-Bleiler, R., Maughan, R.J., Clarkson, P.M., Bleiler, T.L. and Whiting, P.H. (1989). Serum creatine kinase activity after isometric exercise in premenopausal and postmenopausal women. *Experimental Aging Research*, 15, 195-198.

Buford, T.W., Cooke, M.B., Shelmadine, B.D., Hudson, G.M., Redd, L. and Willoughby, D.S. (2009). Effects of eccentric treadmill exercise on inflammatory gene expression in human skeletal muscle. *Applied Physiology, Nutrition, and Metabolism*, 34, 745-753.

Bunt, J. (1990). Metabolic actions of estradiol: significance for actue and chronic exercise response. *Medicine and Science in Sports and Exercise*, 22, 286-290.

Burd, N.A., West, D.W., Moore, D.R., Atherton, P.J., Staples, A.W., Prior, T., Tang, J.E., Rennie, M.J., Baker, S.K. and Phillips, S.M. (2011). Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24h after resistance exercise in young men. Journal of Nutrition, 141, 568-573.

Buresh, R. and Berg, K. (2015). A tutorial on oxidative stress and redox signaling with application to exercise and sedentariness. *Sports medicine-open*, 1, 3.

Burgess, D.J., Naughton, G. and Norton, K.I. (2006). Profile of movement demands of national football players in Australia. Journal of Science and Medicine in Sport, 9, 334-341.

Burke, L. (2017). Practical issues in evidence-based use of performance supplements: supplement interactions, repeated use and individual responses. *Sports Medicine*, 47, 79-100.

Burke, L.M., Hawley, J.A., Wong, S.H. and Jeukendrup, A.E. (2011). Carbohdyrates for training and competition. *Journal of Sports Sciences*, 29, S17-27.

Burke, LM. (1997). Nutrition for post-exercise recovery. *Australian Journal of Science and Medicine in Sport*, 29:3-10.

Buse, M.G. and Reid, S.S. (1975). Leucine. A possible regulator of protein turnover in muscle. *Journal of Clinical Investigation*, 56, 1250-1261.

Byfield, M.P., Murray, J.T. and Backer, J.M. (2005). hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. *Journal of Biological Chemistry*, 280, 33076-33082.

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, 531-536.

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

Byrne, D.J., Browne, D.T., Byrne, P.J. and Richardson, N. (2017). Interday Reliability of the Reactive Strength Index and Optimal Drop Height. *Journal of Strength & Conditioning Research*, *31*, 721-726.

Camera, D.M., West, D.W.D., Phillips, S.M., Rerecich, T., Stellingwerff, T., Hawley, J.A. and Coffey, V.G. (2015). Protein ingestion increases myofibrillar protein

synthesis after concurrent training. *Medicine and Science in Sports and Exercise*, 47, 82-91.

Cantini, M., Massimino, M.L. and Rapizzi, E. (1995). Human satellite cell proliferation in vitro is regulated by autocrine secretion of IL-6 stimulated by a soluble factor(s) released by activated monocytes. *Biochemical and Biophysical Research Communication*, 16, 49-53.

Capetanaki, Y., Bloch, R.J., Kouloumenta, A., Mavroidis, M. and Psarras, S. (2007). Muscle intermediate filaments and their links to membranes and membranous organelles. *Experimental Research*, 313, 2063-2076.

Carafoli, E. (2004). Calcium-mediated cellular signals: a story of failures. *Trends in Biochemical Sciences,* 29, 371-379.

Carling, C. ,Gregson, W., McCall, A., Moreira, A., Wong, D.P. and Bradley, P. (2015). Match running performance during fixture congestion in elite soccer: research issues and future directions. *Sports Medicine,* 45, 605-613.

Carroll, S., Nicotera, P. and Pette, D. (1999). Calcium transients in single fibers of low-frequency stimulated fast-twitch muscle of rat. *American Journal of Physiology*, 277, C1122-C1129.

Carter, A., Dobridge, J. and Hackney, A.C. (2001). Influence of estrogen on markers of muscle tissue damage following eccentric exercise. *Human Physiology*, 27, 626-630.

Cermak, N.M., Noseworthy, M.D., Bourgeois, J.M., Tarnopolsky, M.A. and Gibala, M.J. (2012). Diffusion tension MRI to assess skeletal muscle disruption following eccentric exercise*. Muscle and Nerve*, 46, 42-50.

Chapman, D., Newton, M., Sacco, P. and Nosaka, K. (2006). Greater muscle damage induced by fast versus slow velocity eccentric exercise. *International Journal of Sports Medicine*, 27, 591-598.

Chapman, D.W., Newton, M., McGuigan, M. and Nosaka, K. (2008). Effect of lengthening contraction velocity on muscle damage of the elbow flexors. *Medicine and Science in Sports and Exercise*, 40, 5, 926-933.

Chapman, D.W., Simpson, J.A., Iscoe, S., Robins, T. And Nosaka, K. (2013). Changes in serum fast and slow skeletal troponin I concentration following maximal eccentric contractions. *Journal of Science and Medicine in Sport*, 16, 82-85.

Chatzinikolaou, A., Fatouros, I.G., Gourgoulis, V., Avloniti, A., Jamurtas, A.Z., Nikolaidis, M.B., Douroudos, I., Michailidis, Y., Beneka, A., Malliou, P., Tofas, T., Georgiadis, I., Mandalaidis, D. and Tazildaris, K. (2010). Time course of changes in performance and inflammatory responses after acute plyometric exercise. *Journal of Strength and Conditioning Research,* 24, 1389-1398.

Chen, Y.W., Hubal, M.J., Hoffman, E.P, Thompson, P.D. and Clarkson, P.M. (2003). Molecular responses of human muscle to eccentric exercise. *Journal of Applied Physiology*, 95, 2485-2494.

Cherednichenko, G., Zima, A.V., Feng, W., Schaefer, S., Blatter, L.A. and Pessah, I.N. (2004). NADH oxidase activity of rat cardiac sarcoplasmic reticulum regulates calcium-induced calcium release. *Circulation Research*, 94, 478-486.

Chesley, A., MacDougall, J.D., Tarnopolsky, M.A., Atkinson, S.A. and Smith, K. (1992). Changes in human muscle protein synthesis after resistance exercise. *Journal of Applied Physiology*, 73, 1383-1388.

Child, R.B., Saxton, J.. and 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 Science*, 16, 301-308.

Child, R.B., Wilkinson, D.M. and Fallowfield, J.L. (2000). Effects of a training taper on tissue damage indices, serum antioxidant capacity and half-marathon running performance. *International Journal of Sports Medicine*, 21, 325-331.

Choi, I.Y., Lee, P, Denney, D.R., Spaeth, K., Nast, O., Ptomey, L., Roth A.K., Lierman, J.A. and Sullivan, D.K. (2015). Dairy intake is associated with brain glutathione concentration in older adults. *American Journal of Clinical Nutrition*, 101, 287-293.

Choi, S.J. and Widrick, J.J. (2010). Calcium-activated force of human muscle fibres following a standardized eccentric contraction. *American Journal of Physiology-Cell Physiology*, 299, C1409-C1417.

Chow, L.S., Albright, R.C., Bigelow, M.L., Toffolo, G., Cobelli, C. and Nair, K.S. (2006). Mechanism of insulin's anabolic effect on muscle: measurements of muscle protein synthesis and breakdown using aminoacyl-tRNA and other surrogate measures. *American Journal of Physiology Endocrinology and Metabolism*, 291, E729-E736.

Churchward-Venne, A.A., Breen, L., Di Donato, D.M., Hector, A.J., MItchess, C.J., Moore, D.R., Stellingwerff, T., Breuille, D., Offord, E.A., Baker, S.K. and Phillips, S.J. (2014). Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomised trial. *The American Journal of Clinical Nutrition*, 99, 276-286.

Clarkson, P.M. and Ebbeling, C. (1988). Investigation of serum creatine kinase variability after muscle-damaging exercise. *Clinical Science*, 75, 257-261.

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

Clarkson, P.M. and Hubal, M.J. (2002). Exercise-induced muscle damage in humans. *American Journal of Physical Medicine and Rehabilitation*, 81, S52-S69.

Clarkson, P.M. and Sayers, S.P. (1999). Etiology of exercise-induced muscle damage. *Canadian Journal of Applied Physiology*, 24, 3, 234-248.

Clarkson, P.M., Hoffman, E.P., Zambraski, E., Gordish-Dressman, H., Kearns, A., Hubal, M., Harmon, B. and Devaney, J.M. (2005). ACTN3 and MLCK genotype associations with exertional muscle damage. *Journal of Applied Physiology*, 99, 564-569.

Clarkson, P.M., Kearns, A.K., Rouzier, P., Rubin, R. and Thompson, P.D. (2006). Serum creatine kinase levels and renal function measures in exertional muscle damage. *Medicine and Science in Sports and Exercise*, 38, 623-627.

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

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

Clifford, T., Allerton, D.M., Brown, M.A., Harper, L., Horsburgh, S., Keane, K.M. Stevenson, E.J. and Howatson, G. (2016). Minimal muscle damage after a marathon and no influence of beetroot juice on inflammation and recovery. *Applied Physiology Nutrition and Metabolism*, 42, 263-270.

Close, G.L., Hamilton, D.L., Philp, A., Burke, L.M. and Morton, J.P. (2016). New strategies in sport nutrition to increase exercise performance. *Free Radical Biology and Medicine*, *98*,144-158.

Cockburn, E., Bell, P. G., and Stevenson, E. (2013). Effect of milk on team sport performance after exercise-induced muscle damage. *Medicine and Science in Sports and Exercise*, *45*, 1585-1592.

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

Cockburn, E., Robson-Ansley, P., Hayes, P.R. and Stevenson, E. (2012). Effect of volume of milk consumed on the attenuation of exercise-induced muscle damage. *European Journal of Applied Physiology*, 112, 3187-3194.

Cockburn, E., Stevenson, E., Hayes, P. R., Robson-Ansley, P., and 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*, 270-277.

Coffey, V.G., Moore, D.R., Burd, N.A., Rerecich, T., Stellingwerff, T., Garnham, A.P., Phillips, S.M. and Hawley, J.A., (2011). Nutrient provision increases signalling and protein synthesis in human skeletal muscle after repeated sprints. *European Journal of Applied Physiology*, 111, 1473-1483.

Coirault, C., Guellich, A., Barbry, T., Samuel, J.L., Riou, B. and Lecarpentier, Y. (2007). Oxidative stress of myosin contributes to skeletal muscle dysfunction in rats with chronic heart failure. *American Journal of Physiology Heart and Circulatory Physiology*, 292, H1009-H1017.

Connolly, D.A.J., McHugh, M.P. and Padilla-Zakour, O.I., (2006). Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *British Journal of Sports Medicine,* 40, 679-683.

Consolino, C.M. and Brooks, S.V. (2004). Susceptibility to sarcomere injury induced by single stretches of maximally activated muscles of mdx mice. *Journal of Applied Physiology*, 96, 633-638.

Constable, J.K., Barclay, C.J. and Gibbs, C.L. (1997). Energetics of lengthening in mouse and toad skeletal muscles. *Journal of Physiology*, 505.1, 205-215.

Cooke, M.B., Rybalka, E., Stathis, C.G., Cribb, P.J. and Hayes, A. (2010). Whey protein isolate attenuates strength decline after eccentrically-induced muscle damage in healthy individuals. *Journal of the International Society of Sports Nutrition*, 7, 30.

Cormack, S.J., Newton, R.U. and McGuigan, M.R. (2008). Neuromuscular and endocrine responses of elite players to an Australian rules football match. *International Journal of Sports Physiology and Performance*, *3*, 359-374.

Cormack, S.J., Newton, R.U., McGuigan, M.R. and Cormie, P. (2008). Neuromuscular and endocrine responses of elite players during an Australian rules football season. *International Journal of Sports Physiology and Performance*, 3, 439-453.

Cosio-Lima, L.M., Desai, B., Stelzer, J.W. and Schuler, P.B. (2012). Effects of a 4:1 carbohydrate/protein solution versus a carbohydrate-alone solution on IL-6, TNF-a and cortisol during prolonged cycling in hot environmental conditions. *Open Access Journal of Sports Medicine*, 3, 21–26.

Costa, A., Orosz, Z., Apor, P., Cxaba, N., Siamilis, S., Csende, C., Racz, L. and Tihanyi, J. (2009). Impact of repeated bouts of eccentric exercise on sarcolemma disruption in human skeletal muscle. *Acta Physiologica Hungarica,* 96, 189-202.

Costa, M. L., Escaleira, R., Cataldo, A., Oliveira, F. And Mermelstein, C.S. (2004). Desmin: molecular interactions and putative functions of the muscle intermediate filament protein. *Brazilian Journal of Medical and Biological Research,* 37, 1819-1830.

Costello, J.T., Bieuzen, F. and Bleakely, C.M. (2014). Where are all the female participants in sports and exercise medicine research? *European Journal of Sport Sciences*, 14, 847-851.

Coutts, A.J. and Reaburn, P. (2008). Monitoring changes in rugby league players' perceived stress and recovery during intensified training. *Perceptual Motor Skills*, 106, 904-916.

Crameri, R.M., Aagaard, P., Qvortrup, K., Langberg, H., Olesen, J. and Kjær, M. (2007). Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. *Journal of Physiology*, 583, 365-380.

Crenshaw, A.G., Friden, J., Hargens, A.R., Lang, G.H. and Thornell, L.E. (1993). Increased technetium uptake is not equivalent to muscle necrosis: scintigraphic, morphological and intramuscular pressure analysis of sore muscles after exercise. *Acta Physiologica Scandanavica,* 148, 187-198.

Cribb, P., Williams, A.D., Carey, M.F. and Hayes, A. (2006). The effect of whey isolate and resistance training on strength, body composition and plasma glutamine. International *Journal of Sport Nutrition and Exercise Metabolism*, 16, 494-509.

Cribb, P.J. and Hayes, A. (2006). Effects of supplement-timing and resistance exercise on skeletal muscle hypertrophy. *Medicine and Science in Sports and Exercise*, 38, 1918-1925.

Croall, D.E. and Demartino, G.N. (1991). Calcium-activated neutral protease (calpain) system: structure, function, and regulation. *Physiological Reviews*, 71, 813-847.

Crozier, S.J., Kimball, S.R., Emmert, S.W., Anthony, J.C. and Jefferson, L.S. (2005). Oral leucine administration stimulated protein synthesis in rat skeletal muscle. *Journal of Nutrition,* 135, 376-382.

Cumming, G. (2014). The new statistics: why and how. *Psychological Science*, 25, 7-29.

Cunniffe, B., Hore, A.J., Whitcombe, D.M., Jones, K.P., Baker, J.S. and Davies, B. (2010). Time course of changes in immuneoendocrine markers following an international rugby game. *European Journal of Applied Physiology*, *108*, 113.

Cuthbertson, D.J., Babraj, J., Smith, K., Wilkes, E., Fedele, M.J., Esser, K. and Rennie, M. (2006). Anabolic signalling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise. *American Journal of Physiology Endocrinology and Metabolism*, 290, E731-E738.

D'Lugos, A.C., Luden, N.D., Faller, J.M., Akers, J.D., McKenzie, A.I. and Saunders, M.J. (2016). Supplemental protein during heavy cycling training and recovery impacts skeletal muscle and heart rate responses but not performance. *Nutrients*, 8, 550.

da Luz, C.R., Nicastro, H., Zanchi, N.E., Chaves, D.F.S. and Lancha, A.H. (2011). Potential therapeutic effects of branched-chain amino acids supplementation on resistance exercise-based muscle damage in humans. *Journal of the International Society of Sports Nutrition*, 8, 23.

Dahlstrom Burnley, E.C., Olson, A.N., Sharp, R.L., Baier, S.M. and Alekel, D.L. (2010). Impact of protein supplements on muscle recovery after exercise-induced muscle soreness. *Journal of Exercise Science and Fitness*, 8, 89-96.

Dairy Council (2018). The Nutritional Composition of Dairy Products. Dairy Council, London.

Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A. and Colombo, R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta*, 329, 23-38. damage. *Medicine and Science in Sports and Exercise, 43*, 491-500.

Damas, F., Nosaka, K., Libardi, C.A., Chen, T.C. and Ugrinowitsch, C. (2016). Susceptibility to exercise-induced muscle damage: a cluster analysis with a large sample. *International Journal of Sports Medicine*, *37*, 633-640.

Dannecker, E.A., Hausenblas, H.A., Kaminski, T.W. and Robinson, M.E. (2005). Sex differences in delayed onset muscle pain. *Clinical Journal* of *Pain*, 21, 120-126.

Dannecker, E.A., Knoll, V. And Robinson, M.E. (2008). Sex differences in muscle pain: self-care behaviours and effects on daily activities. *The Journal of Pain*, 9, 200-209.

Dannecker, E.A., Koltyn, K.F., Riley III, J.L. and Robinson, M.E. (2003). Sex differences in delayed onset muscle soreness. *Journal of Sports Medicine and Physical Fitness*, 43, 78-84.

Dannecker, E.A., Liu, Y., Rector, R.S., Thomas, T.R., Fillingim, R.B. and Robinson, M.E. (2012). Sex differences in exercise-induced muscle pain and muscle damage. *The Journal of Pain*, 13, 1242-1249

Dannecker, E.A., Liu, Y., Rector, R.S., Thomas, T.R., Sayers, S.P., Leeuwenburgh, C. and Ray, B.K. (2013). The effect of fasting on indicators of muscle damage. *Experimental gerontology*, 48, 1101-1106.

de Freitas, M.C., Gerosa-Neto, J., Zanchi, N.E., Lira, F.S. and Rossi, F.E. (2017). Role of metabolic stress for enhancing muscle adaptations: Practical applications. *World Journal of Methodology*, *7*, 46.

De Hoya, M., Cohen, D.D., Sanudo, B., Carrasco, L, Álvarez-Mesa, A., del Ojo, J.J., Dominguez-Cobo, S., Mañas, V. and Otero-Esquina, C. (2016). Influence of football match time-motion parameters on recovery time course of muscle damage and jump ability. *Journal of Sports Sciences*, 34, 14, 1363-1370.

Deldicque, L., Atherton, P., Patel, R., Theisen, D., Nielens, H., Rennie, M.J. and Francaux, M. (2008). Effects of resistance exercise with and without creatine supplementation on gene expression and cell signalling in human skeletal muscle. *Journal of Applied Physiology*, 104, 371-378.

Delecroix, B., Abaïdia, A.E., Leduc, C., Dawson, B. and Dupont, G. (2017). Curcumin and piperine supplementation and recovery following exercise induced muscle damage: a randomized control trial. *Journal of Sports Science and Medicine*, 16, 147.

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

Dernbach, A.R., Sherman, W.M., Simonsen, J.C., Flowers, K.M. and Lamb, D.R. (1993). No evidence of oxidant stress during high-intensity rowing training. *Journal of Applied Physiology,* 74, 2140-2145.

Desbrow, B., Jansen, S., Barrett, A., Leveritt, M.D. and Irwin, C. (2014). Comparing the rehydration potential of different milk-based drinks to a carbohydrate–electrolyte beverage. *Applied Physiology, Nutrition, and Metabolism*, *39*, 1366-1372.

Devaney, J.M., Hoffman, E.P., Gordish-Dressman, H., Kearns, A., Zambraski, E. And Clarkson, P. (2007). IGF-II gene region polymorphisms related to exertional muscle damage. *Journal of Applied Physiology*, 102, 1815-1823.

Di Donato, D.M., West, D.W., Churchward-Venne, T.A., Breen, L., Baker, S.K. and Phillips, S.M. (2014). Influence of aerobic exercise intensity on myofibrillar and mitochondrial protein synthesis in young men during early and late postexercise recovery. *American Journal of Physiology-Endocrinology and Metabolism*, *306*, E1025-E1032.

Dobridge, J.D. and Hackney, A.C. (2004). The effects of estrogen on indices of skeletal muscle tissue damage after eccentric exercise in postmenopausal women. *Human Physiology*, 30, 98-102.

Doeven, S.H., Brink, M.S., Kosse, S.J. and Lemmink, K.A.P.M. (2018). Postmatch recovery of physical performance and biochemical markers in team ball sports: a systematic review. *BMJ Open Sport and Exercise Medicine*, 4, e000264.

Dousset, E., Avela, J., Ishikawa, M., Kallio, J., Kuitunen, S., Kyro, H., Inen, L. Linnamo, V. and 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, 453-460.

Dreyer, H.C., Glynn, E.L., Jujan, H.L., Fry, C.S., Dicarlo, S.E. and Rasmussen, B.B. (2006). Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *Journal of Physiology*, 576, 613-624.

Drummond, M.J., Dreyer, H.C., Fry, C.S., Glynn, E.L and Rasmussen, B.B. (2009). Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC signalling. *Journal of Applied Physiology*, 106, 1374-1384.

Duan, C., Delp, M.D., Hayes, D.A., Delp, P.D. and Armstrong, R.B. (1990). Rat skeletal-muscle mitochondrial [Ca^{2+}]and injury from downhill walking. *Journal of Applied Physiology*, 68, 1241-1251.

Duchateau, J. and Baudry, S. (2013). Insights into the neural control of eccentric contractions. *Journal of Applied Physiology*, 116, 1418-1425.

Dufaux, B., Heine, O., Kothe, A., Prinz, U. and Rost, R. (1997). Blood glutathione status following distance running. *International Journal of Sports Medicine,* 18, 89-93.

Duffield, R., Edge, J., Merrells, R., Hawke, E., Barnes, M., Simcock, D. and Gill, N. (2008). The effects of compression garments on intermittent exercise performance and recovery on consecutive days. *International Journal of Sports Physiology and Performance*, 3, 454-468.

Dumke, C.L., Davis, J.M., Murphy, E.A., Nieman, D.C., Carmichael, M.D., Quindry, J.C., Triplett, N.T., Utter, A.C., Gowin, S.J.G., Henson, D.A. and McAnulty, S.R. (2009). Successive bouts of cycling stimulates genes associated with mitochondrial biogenesis. *European Journal of Applied Physiology*, 107, p.419.

Ebringer, L., Ferenčík, M. and Krajčovič, J. (2008). Beneficial health effects of milk and fermented dairy products. *Folia Microbiologica*, *53*, 378-394.

Eddens, L., Browne, S., Stevenson, E., Sanderson, B., van Someren, K. and Howatson, G. (2017). The efficacy of protein supplementation during recovery from muscle-damaging concurrent exercise. *Applied Physiology Nutrition Metabolism*, 999, 1-9.

Edward, T.H. (2005). A new perspective on the biology of C-reactive protein. *Circulation Research*, 97, 609-611.

Efeyan, A., Zoncu, R. and Sabatini, D.M. (2012). Amino acids and mTORC1: from lysosomes to disease. *Trends in Molecular Biology,* 18, 524-533.

Egan, B. and Zierath, J.R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metabolism Review*, 17, 162-184.

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

Eliasson, J., Elfegoun, T., Nilsson, J., Kohnke, R., Ekblom, B. and 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-Endocrinology and Metabolism*, 291, E1197-E1205.

Elliot, T.A., Cree, M.G., Sanford, A.P., Wolfe, R.R. and Tipton, K.D. (2006). Milk ingestion stimulates net muscle protein synthesis following resistance exercise. *Medicine and Science in Sports and Exercise*, 38, 667-674.

Elmer, S., McDaniel, J., Mattson, J. and Martin, J. (2012). Effect of a contusion injury on muscular force, power, work, and fatigue. *Scandinavian Journal of Medicine & Science in Sports*, *22*, 4, 488-494.

Enns, D.L. and Tiidus, P.M. (2008). Estrogen influences satellite cell activation and proliferation following downhill running in rats. *Journal of Applied Physiology*, 104, 347-353.

Enns, D.L. and Tiidus, P.M. (2010). The influence of estrogen on skeletal muscle: sex matters. *Sports Medicine*, 40, 41-58.

Enns, D.L., Iqbal, S. and Tiidus, P.M. (2008). Oestrogen receptors mediate oestrogen-induced increases in post-exercise rat skeletal muscle satellite cells. *Acta Physiologica*, 194, 81-83.

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

Esmarck, B., Andersen, J.L., Olsen, S., richter, E.A., Mizuno, M. and Kjaer, M. (2001). Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *Journal of Physiology*., 535, 301-311.

Esposito, F., Impellizzeri, F.M., Margonato, V., Vanni, R., Pizzini, G. and Veicsteinas, A., (2004). Validity of heart rate as an indicator of aerobic demand during soccer activities in amateur soccer players. *European Journal of Applied Physiology*, *93*, pp.167-172.

Farah, C.S. and Reinach, F.C. (1995). The troponin complex and regulation of muscle contraction. *The Journal of the Federation of American Societies for Experimental Biology*, 9, 755-767.

Farney, T.M., McCarthy, C.G., Canale, R., Schilling, B.K., Whitehead, P.N. and Bloomer, R.J. (2012). Absence of blood oxidative stress in trained men after strenuous exercise. *Medicine and Science in Sports and Exercise*, 44, 1855-1863.

Fatouros, I.G., Chatzinikolaou, A., Douroudos, I.I., Nikolaidis, M.G., Kyparos, A., Margonis, K., Michailidis, Y., Vantarakis, A., Taxildaris, K., Katrabasas, I. and Mandalidis, D. (2010). Time-course of changes in oxidative stress and antioxidant status responses following a soccer game. *The Journal of Strength and Conditioning Research*, *24*, 3278-3286.

Favero, T.G., Colter, D., Hooper, P.F. and Abramson, J.J. (1998). Hypochlorous acid inhibits Ca^{2+}-ATPase from skeletal muscle sarcoplasmic reticulum. *Journal of Applied Physiology*, 84, 425-430.

Feasson, L., Stockholm, D., Freyssenet, D., Richard, I., Duguez, S., Beckmann, J.S. and Denis, C. (2002). Molecular adaptations of neuromuscular disease-associated proteins in response to eccentric exercise in human skeletal muscle. *Journal of Physiology*, 543, 297-306.

Febbraio, M.A. and Pedersen, B.K. (2005). Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? *Exercise and Sport Science Reviews*, 33, 114-119.

Feng, X., Li, G.Z. and Wang, S. (2004). Effects of estrogen on gastrocnemius muscle strain injury and regeneration in female rats. *Acta Pharmacologica Sinica,* 25, 1489-1494.

Ferguson-Stegall, L., McCleave, E.L., Ding, Z., Doerner III, P.G., Wang, B., Liao, Y.H., Kammer, L., Liu, Y., Hwang, J., Dessard, B.M. and Ivy, J.L. (2011) Post-exercise carbohydrate-protein supplementation improves subsequent exercise performance and intracellular signalling for protein synthesis. *Journal of Strength and Conditioning Research*, 25, 1210-1224.

Fernandez-Gonzalo, R., Lundberg, T. R., Alvarez-Alvarez, L., and de Paz, J. A. (2014). Muscle damage responses and adaptations to eccentric-overload resistance exercise in men and women. *European Journal of Applied Physiology*,*114*, 1075-1084.

Fielding, R.A., Manfred, T.J., Ding, W., Fiatarone, M.A., Evans, W.J. and Cannon J.G. (1993). Acute phase response in exercise III. Neutrophil and IL-B accumulation in skeletal muscle. *American Journal of Physiology Regulatory Integrative and Comparative*, 265, R166-R172.

Figueiredo, V.C. and Cameron-Smith, D. (2013). Is carbohydrate needed to further stimulate muscle protein synthesis/hypertrophy following resistance exercise? *Journal of the International Society of Sports Nutrition*, 10, 42.

Finaud, J., Lac, G. and Filaire, E. (2006). Oxidative stress. Relationship with exercise and training. *Sports Medicine*, 36, 327-358.

Finkler, M. Lichtenberg, D. and Pinchuk, I. (2014). The relationship between oxidative stress and exercise. *Journal of Basic and Clinical Physiology and Pharmacology*, 25, 1-11.

Fischer, C.P. (2006). Interleukin-6 in acute exercise and training: what is the biological relevance? *Exercise Immunological Review*, 12, 6-33.

Fisher, A.J., Smith, C.A., Thoden, J., Smith, R., Sutoh, K., Holden, H.M. and Rayment, I. (1995). Structural studies of myosin: nucleotide complexes: a revised model for the molecular basis of muscle contraction. *Biophysical Journal*, 68, 19s-28s.

Fisher-Wellman, K. and Bloomer, R.J. (2009). Acute exercise and oxidative stress: a 30 year history. *Dynamic medicine*, 8, 1.

Flanagan, E.P. and Comyns, T.M. (2008). The use of contact time and the reactive strength index to optimize fast stretch-shortening cycle training. *Strength and Conditioning Journal*, *30,* 32-38.

Flores, D.F., Gentil, P., Brown, L.E., Pinto, R.S., Carregaro, R.L. and Bottaro, M. (2011). Dissociated time course of recovery between sexs after resistance exercise. *Journal of Strength and Conditioning Research,* 25, 3039-3044.

Fluckey, J.D., Knoz, M., Smith, L., Dupont-Vesteegden, E.E., Gaddy, D., Tesch, P.A. and Peterson, C.A. (2006). Insulin-facilitated increase of muscle protein synthesis after resistance exercise involves a MAP-kinase pathway. *American Journal of Physiology Endocrinology and Metabolism*, 290, E1205-E1211.

Foley, J.M., Jayaraman, R.C., Prior, B.M., Pivarnik, J.M. and Meyer, R.A. (1999). MR measurements of muscle damage after eccentric exercise. *Journal of Applied Physiology*, 87, 2311-2318.

Fouillet, H., Bos, C., Gaudichon, C., and Tomé, D. (2002). Approaches to quantifying protein metabolism in response to nutrient ingestion. *The Journal of Nutrition*, *132*, 3208S-3218S.

Fridén, J. and Lieber, R.L. (1992). Structural and mechanical basis of exercise-induced muscle damage. *Medicine and Science in Sport and Exercise*, 24, 5, 521-530.

Fridén, J. and Lieber, R.L. (1996). Ultrastructural evidence for loss of calcium homeostasis in exercised skeletal muscle. *Acta Physiologica Scandanavica*, 158, 381-382.

Fridén, J. and Lieber, R.L. (1998). Segmental muscle fibre lesions after repetitive eccentric contractions. *Cell Tissue Research*, 293, 165-171.

Fridén, J., Seger, J. and Ekblom, B. (1988). Sublethal muscle fibre injuries after high-tension anaerobic exercise. *European Journal of Applied Physiology*, 57, 360-368.

Fridén, J., Sjöström, M. and Ekblom, B. (1981). A morphological study of delayed muscle soreness*. Experientia*, 37, 506-507.

Fridén, J., Sjöström, M. and Ekblom, B. (1983). Myofibrillar damage following intense eccentric exercise in man. *International Journal of Sports Medicine*, 4, 170-176.

Fujita, S., Dreyer, H.C., Drummond, M.J, Glynn, E.L., Cadenas, J.G., Yoshizawa, F., Volpi, E. and Rasmussen, B.B. (2007). Nutrient signalling in the regulation of human muscle protein synthesis. *Journal of Physiology*, 582, 813-823.

Fujita, S., Rasmussen, B.B., Bell, J.A., Cadenas, J.G. and Volpi, E. (2006). Basal muscle intracellular aminoacid kinetics in women and men. *American Journal of Physiology*, 292: E77-E83.

Gabbett, T.J. (2010). GPS analysis of elite women's field hockey training and competition. *The Journal of Strength & Conditioning Research*, *24*, 1321-1324.

Gannon, M.C., Nuttall, F.Q., Krezowski, P.A., Billington, C.J. and 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, 784-791.

Gao, Y., Wineman, A.S. and Waas, A.M. (2008). Mechanics of muscle injury induced by lengthening contraction. *Annals of Biomedical Engineering*, 36, 1615-1623.

Garlick, P.J. and Grant, I. (1988). Amino Acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin. Effect of branched-chain amino acids. *Biochemistry Journal,* 254, 579-584.

Gathercole, R., Sporer, B., Stellingwerff, T. and Sleivert, G. (2015). Alternative countermovment jump analysis to quantify acute neuromuscular fatigue. *International Journal of Sports Physiology ad Human Performance.*, 10, 84-92.

Geeves, M.A. (1991). The dynamics of actin and myosin asociation and the crossbridge model of muscle contraction. *Biochemistry*, 274, 1-14.

Geeves, M.A. and Holmes, K.C. (1999). Structural mechanism of muscle contraction. *Annual Reviews in Biochemistry*, 68, 687-728.

Gelfand, R.A. and Barrett, E.J. (1987). Effect of physiological hyperinsulinemia on skeletal-muscle protein-synthesis and breakdown in man. *Journal of Clinical Investigation*, 80, 1-6.

Gentle, H.L., Love, T.D., Howe, A.S. and Black, K.E. (2014). A randomised trial of pre-exercise meal composition on performance and muscle damage in well-trained basketball players. *Journal of the International Society of Sports Nutrition*., 11, 33.

Gibala, M.J., Little, J.P., van Essen, M., Wilkin, G.P., Burgomaster, K.A., Safdar, A., Raha, S. and Tarnopolsky, M.A. (2006). Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *Journal of Physiology*, 575, 901-911.

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

Gilson, S.F., Saunders, M.J., Moran, C.W., Moore, R.W., Womack, C.J. and Todd, M.K. (2010). Effects of chocolate milk consumption on markers of muscle recovery following soccer trianing: a randomized cross-over study. *Journal of the International Society of Sports Nutrition*, 7, 19.

Gissel, H. (2000). Ca^{2+} accumulation and cell damage during muscle contraction. *European Journal of Applied Physiology*, 83, 175-180.

Gissel, H. and Clausen, T. (1999). Excitation-induced Ca^{2+} uptake in rat skeletal msucle. *American Journal of Physiology Regulative Integrative and Comparative Physiology*, 276, R331-R339.

Gissel, H. and Clausen, T. (2001). Excitation-induced Ca2+ influx and skeletal muscle cell damage. *Acta Physiologica Scandinavica*, 171, 327-334.

Glover, E.K., Phillips, S.M., Oates, B.R., Tang, J.E., Tarnopolsky, M.A., Selby, A., Smith, K. and Rennie, M.J. (2009). Immobilization induces anabolic resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion. *Journal of Physiology*, 586, 6049-6061.

Glynn, E.L., Fry, C.S., Drummond, M.J., Dreyer, H.C., Dhanani, S., Volpi, E. and Rasmussen, B.B. (2010). Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *American Journal of Physiology Regulatory Integrative and Comparative Physiology*, 299, R533-R540.

Glynn, E.L., Fry, C.S., Timmerman, K.L., Drummond, M.J., Volpi, E. And Rasmussen, B.B. (2013). Addition of carbohydrate or alanine to an essential amino acid mixture does not enhance human skeletal muscle protein anabolism. *The Journal of Nutrition*, 143, 307-314.

Gohil, K., Packer, L., and de Lumen, B. (1986). Vitamin E deficiency and vitamin C supplements: exercise and mitochondrial oxidation. *Journal of Applied Physiology,* 60, 1986-1991.

Goldfarb, A. H., Cho, C., Cho, H., Romano-Ely, B., and Kent Todd, M. (2009). Protein and antioxidants in an isocaloric carbohydrate drink: effect on plasma oxidative stress markers and IL-6. *International Journal of Sport Nutrition and Exercise Metabolism,* 19, 115-126.

Goldfarb, A.H., McIntosh, M.K., Boyer, B.T. and Fatouros, J. (1994). Vitamin E effects on indexes of lipid peroxidation in muscle from DHEA-treated and exercised rats. *Journal of Applied Physiology*, 76, 1630-1635.

Goldman, R.D., Grin, B., Mendez, M.G. and Kuczmarski, E.R. (2008). Intermediate filaments: versatile building blocks of cell structure. *Current Opinion in Cell Biology*, 20, 28-34.

Goll, D.E., Thompson, V.F., Li, H., Wei, W. and Cong., J. (2003). The calpain system. *Physiological Reviews*, 83, 731-801.

Gomez-Cabrera, M.C., Close, G.L., Kayani, A., McArdle, A., Vina, J. and Jackson, M.J. (2010). Effect of xanthine oxidse-generated extracellular superoxide on skeletal muscle force generation. *American Journal of Physiology Regulatory Integrative and Comparative Physiology*, 298, R2-R8.

Goodall, S., Thomas, K., Harper, L.D., Hunter, R., Parker, P. ,Stevenson, E., West, D., Russell, M. and HOwatson, G. (2017). The assessment of neuromuscular fatigue during 120min of simulated soccer exercise. *European Journal of Applied Physiology*, 117, 687-697.

Gorissen, S.H.M., Burd, N.A., Hamer, H.M., Gijsen, A.P., Groen, B.B. and van Loon, L.J.C. (2014) Carbohydrate co-ingestion delays dietary protein digestion and absorption but does not modulate postprandial muscle protein accretion. *The Journal of Clinical Endocrinology and Metabolism,* 99, 2250-2258.

Granata, C., Oliveira, R.S., Little, J.P., Renner, K. and Bishop, D.J. (2016). Mitochondrial adaptations to high-volume exercise training are rapidly reversed after

a reduction in training volume in human skeletal muscle. *The FASEB Journal*, 30, 3413-3423.

Gravina, L., Furiz, F., Lekue, J.A., Irazusta, J. and Gil, S.M. (2011). Metabolic impact of a soccer match on female players. *Journal of Sports Sciences,* 29, 1345-1352.

Green, M.S., Corono, B.T., Doyle, J.A. and 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, 1-18.

Greenhaff, P.L., Karagounis, L.G., Peirce, N. Simpson, E.J., Hazell, M., Layfield, R., Wackerhage, H., Smith, K., Atherton, P., Selby, A. And Rennie, M.J. (2008). Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *American Journal of Physiology Endocrinology and Metabolism*, 295, E595-E604.

Gregson, W., Drust, B., Atkinson, G. and Salvo, V.D. (2010). Match-to-match variability of high-speed activities in premier league soccer. *International Journal of Sports Medicine*, 31, 237-242.

Greig, M. (2008). The influence of soccer-specific fatigue on peak isokinetic torque production of the knee flexors and extensors. *American Journal of Sports Medicine*, 36, 1403-1409.

Griffiths, H. R. (2000). Antioxidants and protein oxidation. *Free radical research*, *33*(Supple), S47-S58.

Groen, B.B., Horstman, A.M., Hamer, H.M., De Hann, M., van Kranenburg, J., Bierau, J., Poeze, M., Wodzig, W.K., Rasmussen, B.B. and van Loon, L.J.C. (2015). Post-prandial protein handling: you are what you just ate. *PLoS One*, 10: e0141582.

Hall, A.H., Leveritt, M.D., Ahuja, K.D.K. and Shing, C.M. (2013). Coingestion of carbohydrate and protein during training reduces training stress and enhances

subsequent exercise performance. *Applied Physiology Nutrition and Metabolism*, 38, 597-604.

Halliwell, B. and Chirico, S. (1993). Lipid peroxidation: its mechanism, measurement, significance. *American Journal of Clinical Nutrition*, 57, 715S-725S

Halson, S.L., Bridge, M.W., Meusen, R., Busschaert, B., Gleeson, M., Jones, D.A. and Jeukendrup, A.D. (2002). Time course of performance changes and fatigue markers during intensified training in trained cyclists. *Journal of Applied Physiology*, 93, 947-956.

Ham, D.J., Caldow, M.K., Lynch, G.S. and Koopman, R. (2014). Leucine as a treatment for muscle wasting: a critical review. *Clinical Nutrition*, 33, 937-945.

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

Hamer, P.W., McGeachie, J.M., Davies, M.J. and 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, 69-79.

Hamilton, D. (2009). Drop jumps as an indicator of neuromuscular fatigue and recovery in elite youth soccer athletes following tournament match play. *Journal of Australian Strength and Conditioning*, 17(4).

Harber, M.P., Konopka, A.R., Jemiolo, B., Trappe, S.W., Trappe, T.A. and Reidy, P.T. (2010). Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *299*, R1254-R1262.

Harrison, A.J. and Gaffney, S.D. (2004). Effects of muscle damage on stretch-shortening cycle function and muscle stiffness control. *Journal of Strength and Conditioning Research*, 18, 771-776.

Hartman, J.W., Tang, J.E., Wilkinson, S.B., Tarnopolsky, M.A., Lawrence, R.L., Fullerton, A.V. and Phillips, S.M. (2007). Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. *American Journal of Clinical Nutrition*, 86, 373-381.

Hatchett, A., Berry, C., Oliva, C., Wiley, D., St Hilaire, J., LaRochelle, A. (2016). A comparison between chocolate milk and a raw milk honey solution's influence on delayed onset of muscle soreness. *Sports*, 4, 18.

Haug, A., Høstmark, A.T. and Harstad, O.M (2007). Bovine milk in human nutrition – a review. *Lipids in Health and Disease,* 6, 25.

Hayashi, K., Katanosaka, K., Yamanaka, A.M., Nosaka, K., Mizumura, K. and Taguchi, T. (2017). Muscular mechanical hyperalgesia after lengthening contractions in rats depends on stretch velocity and range of motion. *European Journal of Pain*, 21, 125-139.

Hayes, M., Smith, D., Castle, P.C., Watt, P.W., Ross, E.Z. and Maxwell, N.S. (2013). Peak power output provides the most reliable measure of performance in prolonged intermittent-sprint cycling. *Journal of Sports Sciences*, 31, 565-572.

Heaton, L.E., Davis, J.K., Rawson, E.S., Nuccio, R.P., Witard, O.C., Stein, K.W., Baar, K., Carter, J.M. and Baker, L.B. (2017). Selected in-season nutritional strategies to enhance recovery for team sport athletes: a practical overview. *Sports Medicine*, 47, 2201-2218.

Helfand, B.T., Chang, L. And Goldman, R.D. (2003). The dynamic and motile properties of intermediate filaments. *Annual Review of Cell and Developmental Biology*, 19, 445-467.

Herrmann, H., Bär, H., Kreplak, L., Strelkov, S.V. and Aebi, U. (2007). Intermediate filaments: from cell architecture to nanomechanics. *Nature Reviews Molecular Cell Biology*, 8, 562.

Herzog, W. (2013). Mechanisms of enhanced force produced in lengthening (eccentric) muscle contractions. *Journal of Applied Physiology*, 116, 1407-1417.

Herzog, W., Schappacher, G., DuVall, M., Leonard, T.R. and Herzog, J.A. (2016). Residual force enhancement following eccentric contractions: a new mechanism involving titin. *Physiology*, 31, 300-312.

Hessel, A.L., Lindstedt, S.L. and Nishikawa, K.C. (2017). Physiological mechanisms of eccentric contraction and its applications: a role fo the giant titin protein. *Frontiers in Physiology*, 8, 70.

Hicks, K.M., Onambele-Pearson, G., Winwood, K. and Morse, C.I. (2017). Oral contraceptive pill use and the susceptibility to markers of exercise-induced muscle damage. *European Journal of Applied Physiology*, 117, 7, 1393-1402.

Hicks, K.M., Onambélé, G.L., Winwood, K. and Morse, C.I. (2016). Muscle damage following maximal eccentric knee extensions in males and females. *PloS one*, 11, 3, p.e0150848.

Highton, J., Twist, C., Lamb, K. and Nicholas, C. (2013). Cabohydrate-protein coingestion improves multiple-sprint running performance. *Journal of Sports Sciences,* 31, 361-369.

Highton, J.M., Twist, C. and Eston, R.G. (2009). The effects of exercise-induced muscle damage on agility and sprint running performance. *Journal of Exercise and Science Fitness*, 7, 24-30.

Hill, D.R. and Newburg, D.S. (2015). Clinical applications of bioactive milk components. *Nutrition Reviews*, *73*, 463-476.

Hody, S., Rogister, B., Leprince, P., Wang, F., and Croisier, J. L. (2013). Muscle fatigue experienced during maximal eccentric exercise is predictive of the plasma creatine kinase (CK) response. *Scandinavian Journal of Medicine and Science in Sports*, *23*, 501-507.

Hoeger, B. M., Dicapo, J., Rasiarmos, R., and Hunter, S. K. (2008). Dose response of isometric contractions on pain perception in healthy adults. *Medicine and Science in Sports and Exercise*, *40*, 1880-1889.

Hoffman, J. and Falvo, M. (2004). Protein- which is best? *Journal of Sports Science and Medicine,* 3, 118-130.

Hoffman, J.R., Nusse, V., and Kang, J. (2003). The effect of an intercollegiate soccer game on maximal power performance. *Canadian Journal of Applied Physiology*, 28,807-817.

Holmes, K.C. (1997). The swinging lever-arm hypothesis of muscle contraction. *Current Biology,* 7, R112-R118.

Holmes, K.C., Schröder, R.R., Sweeney, H.L. and Houdusse, A. (2004). The structure of the rigor complex and its implications for the power stroke. *Philosophical Transactions of the Royal Society Biological Sciences*, 359, 1819-1828.

Hopkins, W., Batterham, A. (2016) Error Rates, Decisive Outcomes and Publication Bias with Several Inferential Methods. *Sports Medicine*, 46, 1563-1573.

Hopkins, W.G (2006b). Pre-post parallel groups trial (Excel spreadsheet). www.sportsci.org

Hopkins, W.G. (2002). Probabilities of clinical or practical significance. *Sportscience*, 6.

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

Hopkins, W.G. (2006a). Post-only crossover (Excel spreadsheet). www.sportsci.org

Hopkins, W.G. (2006c). Compare two groups means (Excel spreadsheet). www.sportsci.org

Hopkins, W.G. (2017). P values down but not yet out. Slideshow presented at the 8th International Scientific Conference on Kinesiology, May 2017, Croatia, www.sportsci.org

Hopkins, W.G. and Batterham, A.M. (2018). The vindication of Magnitude-Based Inference. *Sportscience*, *22*, 19-29.

Hopkins, W.G., Marshall, S.W., Batterham, A.M. and Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Medicine and Science in Sports and Exercise*, 41, 3-13.

Hortobágyi, T., Houmard, J., Fraser, D., Dudek, R., Lambert, J. and Tracy, J. (1998). Normal forces and myofibrillar disruption after eccentric exercise. *Journal of Applied Physiology*, 84, 492-498.

Howarth, K.R., Moreau, N.A., Phillips, S.M. and Gibala, M.J. (2009). Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *Journal of Applied Physiology*, 4, 1394-1402.

Howatson, G., Leeder, J. and van Someren, K. (2016). The BASES expert statement on athletic recovery strategies. www.bases.org.uk/The-BASES-Expert-Statement-on-Athletic-Recovery-Strategies. Accessed 12/02/2016

Howatson, G. and Milak, A. (2009). Exercise-induced muscle damage following a bout of sport specific repeated sprints. *Journal of Strength and Conditioning Research*, 23, 2419-2424.

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

Howatson, G., Hoad, M., Goodall, S., Tallent, J., Bell, P.G. and French, D.N. (2012). Exercise-induced muscle damage is reduced in resistance-trained males by

branched chain amino acids: a ranodmized, double-blind, placebo controlled study. *Journal of the International Society of Sports Nutrition,* 9, 20.

Howatson, G., McHugh, M. P., Hill, J. A., Brouner, J., Jewell, A. P., Van Someren, K. A., Shave, R.E. and Howatson, S. A. (2010). Influence of tart cherry juice on indices of recovery following marathon running. *Scandinavian Journal of Medicine and Science in Sports*, 20, 843-852.

Howatson, G., Van Someren, K. and Hortobagyi, T. (2007). Repeated bout effect after maximal eccentric exercise. *International Journal of Sports Medicine*, *28*, 557-563.

Hubal, M.J., Rubinstein, S.R. and Clarkson, P.M. (2008). Muscle function in men and women during maximal eccentric exercise. *Journal of Strength and Conditioning Research*, 22, 1332-1338.

Hubal, M.J., Rubinstein, S.R., Clarkson, P.M. (2008) Muscle function in men and women during maximal eccentric exercise. *Journal of Strength and Conditioning Research*, 22,1332-1338

Huxley, A.F. (1974). Muscular contraction. *Journal of Physiology*, 243, 1-43.

Huxley, A.F. and Niedergerke, R. (1954). Structural changes in muscle during contraction. Interference micropscopy of living msucle fibres. *Nature*, 173, 971-973.

Hyatt, J.P. and Clarkson, P.M. (1998). Creatine kinase release and clearance using MM variants following repeated bouts of eccentric exercise. *Medicine and Science in Sports and Exercise,* 30, 1059-1065.

Hyldahl, R.D., Chen, T.C. and Nosaka, K. (2017). Mechanisms and mediators of the skeletal muscle repeated bout effect. *Exercise and Sport Sciences Reviews*, *45*, 24-33.

Ide, B.N. (2012). Muscle damage and human skeletal muscle hypertrophy. *Biochemistry and Pharmacology*, 1, 1-2.

Ide, T., Tsutsui, H., Ohashi, N., Hayashidani, S., Suematsu, N., Tsuchihashi, M., Tamai, H. and Takeshita, A. (2002). Greater oxidative stress in healthy young men compared with premenopausal women. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *22*, 438-442.

Ingalls, C.P., Warren, G.L. and 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, 215-224.

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

Ingram, J., Dawson, B., Goodman, C., Wallman, K. and Beilby, J. (2009). Effect of water immersion methods on post-exercise recovery from simulated team sport exercise. *Journal of Science and Medicine in Sport*, 12, 417-421.

Ispirlidis, I., Fatouros, I.G., Jamurtas, A.Z., Nikolaidis, M.G., Michailidis, I., Douroudos, I., Margonis, K., Chatzinikolaou, A., Kalistratos, E., Katrabasas, I. and Alexiou, V. (2008). Time-course of changes in inflammatory and performance responses following a soccer game. *Clinical Journal of Sport Medicine*, *18*, 423-431.

Ives, S.J., Bloom, S., Matias, A., Morrow, N., Martins, N., Roh, Y., Ebenstein, D., O'Brien, G., Escudero, D., Brito, K., Glickman, L., Connelly, S. and Arciero, P.J. (2017). Effects of a combined protein and antioxidant supplement on recovery of muscle function and soreness following eccentric exercise. *Journal of the International Society of Sports Nutrition*, 14, 21.

Jackman, S.R., Witard, O.C., Jeukendrup, A.E. and Tipton, K.D. (2010). Branched-chain amino acid ingestion can ameliorate soreness from eccentric exercise. *Medicine and Science in Sports and Exercise,* 42, 962-970.

Jackson, M.J., Jones, D.A. and Edwards, R.H.T. (1984). Experimental skeletal muscle damage: the nature of the calcium-activated degenerative processes. *European Journal of Clinical Investigations,* 14, 369-374.

Jackson, M.J., Pye, D. and Palomero, J. (2007). The production of reactive oxygen and nitrogen species by skeletal muscle. *Journal of Applied Physiology,* 102, 1664-1670.

Jackson, M.J., Vasilaki, A. and McArdle, A. (2016). Cellular mechanisms underlying oxidative stress in human exercise. *Free Radical Biology and Medicie, 98*, 13–17.

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

Jakeman, J.R., Macrae, R. and Eston, R. (2009). A single 10-min bout of cold-water immersion therapy after strenuous plyometric exercise has no beneficial effect on recovery from the symptoms of exercise-induced muscle damage. *Ergonomics*, 52, 456-460.

Jamart, C., Francaux, M., Millet, G.Y., Deldicque, L., Frère, D. and Féasson, L. (2012). Modulation of autophagy and ubiquitin-proteasome pathways during ultra-endurance running. *Journal of Applied Physiology*, 112, 1529-1537.

James, L.J., Stevenson, E.J., Rumbold, P.L. and Hulston, C.J. (2018). Cow's milk as a post-exercise recovery drink: implications for performance and health. *European Journal of Sport Science*, 1-9.

Jayaraman, R.C., Reid, R.W., Foley, J.M., Prior, B.M., Dudley, G.A., Weingand, K.W. and Meyer, R.A. (2004). MRI evaluation of topical heat and static stretcing as therapeutic modalities for the treatment of eccentric exercise-induced muscle damage. *European Journal of Applied Physiology,* 93, 30-38.

Jeacocke, N.A. and Burke, L.M. (2010). Methods to standardize dietary intake before performance testing. *International Journal of Sport Nutrition and Exercise Metabolism*, *20*, 87-103.

Jeukendrup, A. (2017). Periodized nutrition for athletes. *Sports Medicine*, 47, 51-63.

Jewell, J.L., Russell, R.C. and Guan, K.L. (2013). Amino acid signalling upstream of mTOR. *Nature Reviews Molecular Cell Biology*, 14, 133-139.

Jimenez-Flores, R., Heick, J., Davis, S.C., Green Hall,. and Schaffner, A. (2012). A comparison of the effects of a high carbohydrate vs. a higher protein milk supplement following simulated mountain skirmishes. *Military Medicine*, 177, 723-731.

Johnston, M.J., Cook, C.J., Drake, D. Costley, L., Johnston, J.P. and Kilduff, L.P. (2016). The neuromuscular, biochemical and endocrine responses to a single-session vs. double-session training day in elite athletes. *The Journal of Strength and Conditioning Research*, 30, 3098-3106.

Johnston, R.D., Gabbett, T.J., Jenkins, D.G., and Hulin, B.T. (2015). Influence of physical qualities on post-match fatigue in rugby league players. *Journal of Science and Medicine in Sport,* 18, 209-213.

Josse, A.R. and Phillips, S.M. (2012). Impact of milk consumption and resistance training on body composition of female athletes. In *Acute Topics in Sport Nutrition* (Vol. 59, pp. 94-103). Karger Publishers.

Josse, A.R., Tang, J.E., Tarnopolsky, M.A. and Phillips, S.M. (2010). Body composition and strength changes in women with milk and resistance exercise. *Medicine and Science in Sports and Exercise*, 42, 1122-1130.

Jówko, E., Dlugolęcka, B., Makaruk, B. and Cieśliński, I. (2014). The effect of green tea extract supplementation on exercise-induced oxidative stress parameters in male sprinters. *European Journal of Nutrition*. 54, 783-791.

Joyce, S., Sabapathy, S., Bulmer, A. C., and Minahan, C. (2014). The effect of prior eccentric exercise on heavy-intensity cycling: the role of sex and oral contraceptives. *European Journal of Applied Physiology*, *114*, 995-1003.

Judge, A.R. and Dodd, S.L. (2004). Xanthine oxidase and activated neutrophils cause oxidative damage to skeletal muscle after contractile claudication. *American Journal of Physiology Heart and Circulatory Physiology*, 286, H252-H256.

Julian, R., Hecksteden, A., Fullagar, H.H. and Meyer, T. (2017). The effects of menstrual cycle phase on physical performance in female soccer players. *PloS one*, 12, 3, p.e0173951.

Kamandulis, S., Skurvydas, A., Brazaitis, M., Škikas, L. and Duchateau, J. (2010). The repeated bout effect of eccentric exercise is not associated with changes in voluntary activation. *European Journal of Applied Physiology*, *108*, 1065-1074.

Kanda, K., Sugama, K., Jayashida, H., Sakuma, J., Kawakami, Y, Miura, S., Yoshioka, H., Mori, U. And Suzuki, K. (2013). Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exercise Immunology Review*, 19, 72-85.

Kaneko, M., Suzuki, H. ,Masuda, H., Yan, G., Hayashi, H., Kobayashi, A. and Yamazaki, N. (1992). Effects of oxygen free radicals on Ca^{2+} binding to cardiac troponin. *Japanese Circulation Journal*, 56, 1288-1290.

Kano, Y., Masuda, K., Furukawa, Y., Sudo, M., Mito, K. and Sakamoto, K. (2008). Histological skeletal muscle damage and surface EMG relationships following eccentric contractions. *The Journal of Physiological Sciences*, 58, 349-355.

Kano, Y., Sonobe, T., Inagaki, T., Sudo, M. and Poole, D.C. (2012). Mechanisms of exercise-induced muscle damage and fatigue: intracellular calcium accumulation. *Journal of Physical Fitness and Sports Medicine*, 1, 505-512.

Kanter, M.M., Nolte, L.A. and Holloszy, J.O. (1993). Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. *Journal of Applied Physiology*, 74, 965-969.

Kanzaki, K., Kuratani, M., Matsunaga, S., Yanaka, N. and Wada, M. (2014). Three calpain isoforms are autolyzed in rat fast-twitch muscle after eccentric contractions. *Journal of Muscle Research and Cell Motility*, 35, 179-189.

Kanzaki, K., Kuratani, M., Mishima, T., Matsunaga, S., Yanaka, N., Usui, S. and Wada, M. (2010). The effects of eccentric contraction on myofibrillar proteins in rat skeletal muscle. *European Journal of Applied Physiology*, 120, 943-952.

Karfonta, K., Lunn, W., Colletto, M., Anderson, J.M. and Rodriguez, N.R. (2010). Chocolate milk and glycogen replenishment after endurance exercise in moderately trained males. *Medicine and Science in Sports and Exercise*, 42, 86.

Karolkiewisz, J., Szczesniak, L., Kasprzak, Z. and Ruchlewski, T. (2001). The influence of endurance training and carbohydrate and protein supplementation on concentration of reduced glutathione (GSH) in red blood cells and TBARS in blood plasma of athletes. *Medicine in Sports,* 17, 119-125.

Karp, J.R., Johnston, J.D., Tecklenburg, S., MIckleborough, T.D., Fly, A.D. and Stager, J.M. (2006). Chocolate milk as a post-exercise recovery aid. *International Journal of Sport Nutrition and Exercise Metabolism,* 16, 78-91.

Kasperek, G.J. and Snider, R.D. (1989). Total and myofibrillar protein degredation in isolated soleus muscles after exercise. *American Journal of Physiology*, 257, E1-E5.

Katsanos, C.S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A. and Wolfe, R.R. (2006). A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *American Journal of Physiology. Endocrinology and Metabolism*, 291, E381-E387.

Keane, K.M., Salicki, R., Goodall, S., Thomas, K. and Howatson, G. (2015). Muscle damage response in female collegiate athletes after repeated sprint activity. *The Journal of Strength and Conditioning Research*, 29, 2802-2807.

Kellmann, M., Bertollo, M., Bosquet, L., Brink, M., Coutts, A.J., Duffield, R., Erlacher, D., Halson, S.L., Hecksteden, A., Heidari, J. and Kallus, K.W. (2018). Recovery and performance in sport: consensus statement. *International Journal of Sports Physiology and Performance*, 13, 2, 240-245.

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

Kerasioti, E., Kiskini, A., Veskoukis, A., Jamurtas, A., Tsitsimpikou, C., Tsatsakis, A.M., Koutedakis, Y., Stagos, D., Kouretas, D. and Vaios, K. (2012). Effect of a special carbohydrate-protein cake on oxidative stress markers after exhaustive cycling in humans. *Food and Chemical Toxicology*, 50, 2805-2810.

Kerasioti, E., Stagos, D., Jamurtas, A., Kiskini, A., Koutedakis, Y., Goutzourelas, N., Pournaras, S., Tsatsakis, A.M. and Kouretas, D. (2013). Anti-inflammatory effects of a special carbohydrate-whey protein cake after exhaustive exercise in humans. *Food and Chemical Toxicology*, 61, 42-46.

Kerksick, C., Taylor IV, L., Harvey, A. and Willoughby, D. (2008). Sex-related differences in muscle injury, oxidative stress, and apoptosis. *Medicine and Science in Sports and Exercise,* 40, 1772-1780.

Kharraz, Y, Guerra, J., Mann, C.J., Serrano, A.L., and Munoz-Canoves, P. (2013). Macrophage plasticity and the role of inflammation in skeletal muscle repair. *Mediators of Inflammation*, 10, 491-497.

Kim, H.J., Jamart, C., Deldicque, L., Lee, Y.H., Kim, C.K., Raymackers, J.M. and Francaux, M. (2011). ER-stress markers and ubiquitin-proteasome pathway activity in response to 200-km run. *Medicine and Science in Sports and Exercise*, 43, 18-25.

Kirby, T.J., Triplett, N.T., Haines, T.L., Skinner, J.W., Fairbrother, K.R. and McBride, J.M. (2012). Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise. *Amino Acids*, 42, 1987-1996.

Kirk, B., Mitchell, J., Jackson, M., Amirabdollahian, F., Alizadehkhaiyat, O. and Clifford, T. (2017). A2 Milk Enhances Dynamic Muscle Function Following Repeated Sprint Exercise, a Possible Ergogenic Aid for A1-Protein Intolerant Athletes? *Nutrients*, 9, 94.

Koh, T.J. and Escobedo, J. (2004). Cytoskeletal disruptio and small heat shock protein translocation immediately after lengthening contractions. *American Journal of Physiology- Cell Physiology*, 286, C713-722.

Kokko, J. (2000). Rhabdomyolysis. In Goldman L, Ausielo D (Eds) Cecil's Textbook of Medicine. WB Saunders, Philadelphia, PA, 522-526

Komulainen, J., Koskinen, S.O., Kalliokoski, R., Takala, T. and Vihko, V. (1999). Sex differences in skeletal muscle fibre damage after eccentrically biased downhill running in rats. *Acta Physiologica Scandinavica*, 165, 57-63.

Kontrogianni-Konstantopoulos, A., Ackermann, M.A., Bowman, A.L., Yap, S.V. and Bloch, R.J. (2009). Muscle giants: molecular scaffolds in sarcomerogenesis. *Physiology Reviews*, 80, 1217-1267.

Koopman, R., Beelen, M., Stellingwerff, T., Pennings, B., Saris, W.H.M., Kies, A.K., Kuipers, H., van Loon, L.J.C. (2007). Coingestion of carbohydrate with protein does not further augment postexercsie muscle protein synthesis. *American Journal of Physiology Endocrinology and Metabolism,* 293, E833-E842.

Koppes, R.A., Herzog, W. And Corr, D.T. (2013). Force enhancement in lengthening contractions of cat soleus muscle in situ: transient and steady-state aspects. *Physiological Reports,* 1, e00017, doi: 10.1002/phy2.17.

Kourie, J.I. (1998). Interaction of reactive oxygen species with ion transport mechanisms. *American Journal of Physiology Cell Physiology*, 275, C1-C24.

Kubota, J., Ono, T., Araki, M., Torii, S., Okuwaki, T. And Fukubayashi, T. (2007). Non-uniform changes in magnetic resonance measurements of the semitendinosus muscle following intensive eccentric exercise. *European Journal of Applied Physiology*, 101, 713-720.

Labeit, S., Ottenheijm, C.A.C. and Granzier, H. (2011). Nebulin, a major player in muscle health and disease. *The Journal of the Federation of American Societies for Experimental Biology*, 25, 822-829.

Larsen, R.G., Ringgaard, S. And Overgaard, K. (2007). Localization and quantification of muscle damage by magnetic resonance imaging following step exercise in young women. *Scandanavian Journal of Medicine and Science in Sports*, 17, 76-83.

LaStayo, P.C., Woolf, J.M., Lewek, M.D., Snyder-Mackler, L., Reich, T. and Lindstedt, S.L. (2003). Eccentric muscle contractions: their contribution to injury, prevention, rehabilitation and sport. *The Journal of Orthopaedic and Sports Physical Therapy,* 33, 557-571.

Lauritzen, F., Paulsen, G., Raastad, T., Bergersen, L.H. and 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, 1923-1934.

Lavender, A.P. and Nosaka, K. (2008). Changes in markers of muscle damage of middle-aged and young men following eccentric exercise of the elbow flexors. *Journal of Science and Medicine in Sport*, 11, 124-131.

Lecker, S.H., Goldberg, A.L. and Mitch, W.E. (2006). Protein degredation by the Ubiquitin-proteasome pathway in normal and disease states. *Journal of the American Society Nephrology*, 17, 1807-1819.

Lee, J., Goldfarb, A.H., Rescino, M.H., Hegde, S., Partick, S. and 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, 443-448.

Leeder, J. D., van Someren, K. A., Gaze, D., Jewell, A., Deshmukh, N. I., Shah, I., and Howatson, G. (2014). Recovery and adaptation from repeated intermittent sprint exercise. *International Journal of Sports Physiology and Performance*, 9, 489-496.

Lek, A., Evesson, F.J., Lemckert, F.A., Redpath, G.M., Lueders, A.K., Turnbull, L., Whitchurch, C.B., North, K.N. and Cooper, S.T. (2013). Calpains, cleaved mini-dysferlinC72 and L-type channels underpin calcium-dependent muscle membrane repair. *The Journal of Neruoscience*, 33, 5085-5094.

LeResche, L. (2011). Defining sex disparities in pain management. *Clinical Orthopaedics and Related Research*, 469, 1871-1877.

Levenhagen, D.K., Carr, C., Carlson, M.G. (2002). Postexercise protein intake enhances whole-body and leg protein accretion in humans. *Medicine and Science in Sports and Exercise,* 34, 828-837.

Levenhagen, D.K., Gresham, J.D., Carlson, M.G. Maron, D.J., Borel, M.J. and 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, E982-E993.

Levine, R.L. and Statdman, E.R. (2001). Oxidative modification of proteins during aging. *Experimental Gerantology*, 36, 1495-1502.

Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S. and Stadtman, E.R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology*, 186, 465-478.

Lewis, P.B., Ruby, D. and Bush-Joseph, C.A. (2012). Muscle soreness and delayed-onset muscle soreness. *Clinical Sports Medicine*, 31, 255-262.

Lieber, R.L. (1992). Skeletal muscle structure and function. Williams and Wilkins, Maryland, USA.

Lieber, R.L. and Friden, J. (1993). Muscle damage is not a function of muscle force but active muscle strain. *Journal of Applied Physiology*, 72, 520-526.

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

Lieber, R.L., Shah, S. and Fridén, J. (2002). Cytoskeletal disruption after eccentric contraction-induced muscle injury. *Clinical Orthopaedics and Related Research*, 403S, S90-S99.

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

Lindena, J., Küpper, W., Friedel, R. And Trautschold, I. (1979). Lymphatic transport of cellular enzymes from muscle into the intravascular compartment. *Enzyme*, 24, 120-131.

Lindstedt, S.L., LaStayo, P.C. and Reich, T.E. (2001). When active muscles lengthen: properties and consequences of eccentric contractions. *News in Physiological Science*, 16, 256-261.

Little, J.P., Safdar, A., Wilkin, G.P., Tarnopolsky, M.A. and Gibala, M.J. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *The Journal of Physiology*, 588, 1011-1022.

Little, J.P., Safdar, A., Wilkin, G.P., Tarnopolsky, M.A. and Gibala, M.J. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *The Journal of Physiology*, *588*, 1011-1022.

Lockie, R.G., Murphy, A.J., Knight, T.J. and de Jonge, X.A.J. (2011). Factors that differentiate acceleration ability in field sport athletes. *The Journal of Strength and Conditioning Research*, 25, 2704-2714.

Lovering, R.M. and De Deyne, P.G. (2004). Contractile function, sarcolemma integrits, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *American Journal of Physiology Cell Physiology*, 286, C230-C238.

Lovering, R.M., McMicllan, A.B. and Gullapalli, R.P. (2009). Location of myofiber damage in skeletal muscle after lengthening contractions. *Muscle and Nerve*, 40, 589-594.

Lunn, W.R., Pasiakos, S., Colletto, M.R., Karfonta, K.E., Carbone, J.W., Anderson, J.M. and Rodriguez, N.R. (2012). Chocolate milk and endurance exercise recovery: protein balance, glycogen and performance. *Medicine and Science in Sports and Exercise*, 44, 682-691.

Lymn, R.W. and Taylor, E.W. (1971). Mechanism of adenosine triphosphate hydrolysis of actomyosin. *Biochemistry*, 10, 4617-4624.

Lynch, S. (2013). The differential effects of a complex protein drink versus isocaloric carbohydrate drink on performance indices following high-intensity resistance training: a two arm crossover design. *Journal of the International Society of Sports Nutrition,* 10, 31.

MacDougall, J.D., Gibala, M.J., Tarnopolsky, M.A., MacDonald, J.R., Interisano, S.A. and Yarasheski, K.E. (1995). The time course for elevated muscle protein synthesis following heavy resistance exercise. *Canadian Journal of Applied Physiology*, 20, 480-486.

Machado, A.F., Ferreira, P.H., Micheletti, J.K., de Almeida, A.C., Lemes, Í.R., Vanderlei, F.M., Junior, J.N. and Pastre, C.M. (2016). Can water temperature and immersion time influence the effect of cold water immersion on muscle soreness? A systematic review and meta-analysis. *Sports Medicine*, 46, 503-514.

MacInnis, M.J., Zacharewicz, E., Martin, B.J., Haikalis, M.E., Skelly, L.E., Tarnopolsky, M.A., Murphy, R.M. and Gibala, M.J. (2017). Superior mitochondrial adaptations in human skeletal muscle after interval compared to continuous single-leg cycling matched for total work. *Journal of Physiology*, *595*, 2955-2968.

MacIntyre, D.L., Reid, W.D. and McKenzie, D.C. (1995). Delayed muscle soreness. *Sports Medicine*, 20, 24-40.

MacIntyre, D.L., Reid, W.D., Lyster, D.M. and McKenzie, D.C. (2000). Difference effects of strenuous eccentric exercise on the accumulation of neutrophils in muscle in women and men. *European Journal of Applied Physiology*, 81, 47-53.

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

MacLean, D.A., Graham, T.E. and Saltin, B. (1994). Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. *American Journal of Physiology*, 267, E1010-E1022.

Macnaughton, L.S., Wardle, S.L., Witard, O.C., McGlory, C., Hamilton, D.L., Jeromson, S., Lawrence, C.E., Wallis, G.A. and Tipton, K.D. (2016). The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein. *Physiological Reports*, 4, e12893.

Macpherson, P.C., Dennis, R.G. and Faulkner, J.A. (1997). Sarcomere dynamics and contraction-induced injury to maximally activated single muscle fibres from soleus muscles of rats. *The Journal of Physiology*, 500, 523-533.

Madureira, A.R., Pereira, C.I., Gomes, A.M.P., Pintado, M.E. and Malcata, F.X. (2007). Bovine whey proteins – overview of their main biological properties. *Food Research International*, 40, 197-1211.

Maeo, S., Ando, Y., Kanehisa, H., and Kawakami, Y. (2017a). Localization of damage in the human leg muscles induced by downhill running. *Scientific Reports*, *7*.

Maeo, S., Saito, A., Otsuka, S., Shan, X., Kanehisa, H. and Kawakami, Y. (2017). Localization of muscle damage within the quadriceps femoris induced by different types of eccentric exercises. *Scandanavian Journal of Medicine and Science Sports*, 28, 95-106.

Maffiuletti, N.A., Aagaard, P., Blazevich, A.J., Folland, J., Tillin, N. and Duchateau J. (2016). Rate of force development: physiological and methodological considerations. *European Journal of Applied Physiology,* 110, 223-234.

Magalahães, J. , Rebelo, A., Oliveira, E., Silva, J.R., Marques, F. and Ascenão, A. (2010). Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters. *European Journal of Applied Physiology*, 108, 39-48.

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

Malm, C. and Yu, J.G. (2012). Exercise-induced muscle damage and inflammation: re-evaluation by proteomics. *Histochemical Cell Biology*, 138, 89-99.

Malm, C., Nyberg, P., Engstrom, M., Sjodin, B., Lenkei, R., Ekblom, B. and Lundberg, I. (2000). Immunological changes in human skeletal muscle and blood after eccentric exercise and muscle biopsies. *Journal of Physiology*, 529, 243-262.

Malm, C., Sjödin, B., Sjöberg, B., Lenkei, R., Renström, P., Lundberg, I.E. and Ekblom, B. (2004). Leukocytes, cytokines, growth factors and hormones in human

skeletal muscle and blood after uphill or downhill running. *Journal of Physiology*, 556.3, 983-1000.

Malone, S, Solan, B., Collins, K.D. and Doran, D.A. (2016a). Positional match running performance in elite Gaelic football. *Journal of Strength and Conditioning Research*, 30, 2292-2298.

Malone, S., Solan, B. and Collins, K. (2017b). The running performance profile of elite Gaelic football match-play. *Journal of Strength and Conditioning Research*, 31, 30-36.

Malone, S., Solan, B., Collins, K. and Doran, D. (2016b). The metabolic power and energetic demands of elite Gaelic football match play. *The Journal of Sports Medicine and Physical Fitness*, 57, 543-549.

Malone, S., Solan, B., Hughes, B. and Collins, K. (2017a). Duration specific running performance in elite Gaelic football. *Journal of Strength and Conditioning Research*, doi: 10.1519/JSC.0000000000001972.

Mamerow, M.M., Mettler, J.A., English, K.L., Casperson, S.L., Arentson-Lantz, E., Sheffield-Moore, M., Layman, D.K. and Paddon-Jones, D. (2014). Dietary Protein Distribution Positively Influences 24-h Muscle Protein Synthesis in Healthy Adults–3. *The Journal of Nutrition*, 144, 876-880.

Mara, J.K., Thompson, K.G, Pumpa, K.L. and Morgan, S. (2016). The acceleration and deceleration profiles of elite female soccer players during competitive matches. *Journal of Sciences and Medicine in Sport,* 20, 867-872.

Marginson, V., Rowlands, A.V., Gleeson, N.P. and 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 obys. *Journal of Applied Physiology*, 99, 1174-1181.

Maridakis, V., O'Connor, P.J., Dudley, G.A. and McCully, K.K. (2007). Caffeine attenuates delayed-onset muscle pain and force loss following eccentric exercise. *The Journal of Pain*, 8, 237-243.

Marin, D.P., dos Santos, M., de Cassia, R., Bolin, A.P., Guerra, B.A., Hatanaka, D. and Otton, R. (2011). Cytokines and oxidative stress status following a handball game in elite male players. *Oxidative Medicine and Cellular Longevity*, doi: 10.1155/2011/804873.

Markofski, M.M. and Braun, W.A. (2014). Influence of menstrual cycle on indices of contraction-induced muscle damage. *Journal of Strength and Conditioning Research*, 9, 2649-2656.

Marqués-Jiménez, D., Calleja-González, J., Arraitibel, I, Delextrat, A and Terrados, N. (2017). Fatigue and Recovery in Soccer: Evidence and Challenges. *The Open Sports Sciences Journal,* 10, 52-70.

Marshall, K. (2004). Therapeutic applications of whey protein. *Alternative Medicine Review*, 9, 136-156.

Maruyama, K. (1997). Connectin/titin, giant elastic protein of muscle. *The Journal of the Federation of American Societies for Experimental Biology,* 11, 341-345.

Marzatico, F., Pansarasa, O., Bertorelli, L., Somenzini, L. and Della Valle, G. (1997). Blood free radical entioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. *Journal of Sports Medicine and Physical Fitness,* 37, 235-239.

Mascher, H., Andersson, H., Nilsson, P.A., Ekblom, B. and Blomstrand, E. (2007). Changes in signalling pathways regulating protein synthesis in human muscle in the recovery period after endurance exercise. *Acta Physiologica*, *191*, 67-75.

Mastaloudis, A., Morrow, J.D., Hopkins, D.W., Devaraj, S. and Traber, M.G. (2004). Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not

inflammation, in ultramarathon runners. *Free Radical Biology and Medicine*, 36, 1329-1341.

Mastaloudis, A., Traber, M. G., Carstensen, K., and Widrick, J. J. (2006). Antioxidants did not prevent muscle damage in response to an ultramarathon run. *Medicine and Science in Sports and Exercise*,*38*, 72-80.

Matsumoto, K., Koba, T., Hamada, K., Sakurai, M., Higuchi, T. and Miyata, H. (2009). Branched-chain amino acid supplementation attenuates muscle soreness, muscle damage and inflammation during an intensive training program. *Journal of Sports Medicine and Physical Fitness*, *49,* 424.

McAnulty, S.R., McAnulty, L.S., Morrow, J.D., Nieman, D.C., Owens, J.T. and Carper, C.M. (2007). Influence of carbohydrate, intense exercise, and rest intervals on hormonal and oxidative changes. *International Journal of Sport Nutrition and Exercise Metabolism*, 17, 478-490.

McAnulty, S.R., McAnulty, L.S., Nieman, D.C., Morrow, J.D., Utter, A.C., Henson, D.A., Dumke, C.L. and Vinci, D.M. (2003). Influence of carbohydrate ingestion on oxidative stress and plasma antioxidant potential following a 3 h run. *Free radical research*, 37, 835-840.

McComas, A.J. (1996). Skeletal muscle. Form and function. Human Kinetics, Illinois, USA.

McCully , K.K. and Faulkner, J.A. (1986). Characteristics of lengthening contractions associated with injury to skeletal muscle fibers. *Journal of Applied Physiology*, 61, 1, 293-299.

McCully, K.K. and Faulkner, J.A. (1985). Injury to skeletal msucle fibers of mice following lengthening contractions. *Journal of Applied Physiology*, 59, 1, 119-126.

McErlean, C., Murphy, M. and O'Donoghue, P. (1998). Time-motion analysis of work rate within various positional roles of elite ladies' Gaelic football. Journal of Sport Sciences, 16, 21-22.

McGlory, C., Devries, M.C. and Phillips, S.M. (2017). Skeletal muscle and resistance exercise training: the role of protein synthesis in recovery and remodelling. *Journal of Applied Physiology*, 122, 541-548.

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. *Scandanavian Journal of Medicine and Science in Sports*, 13, 88-97.

McKinnon, N.B., Graham, M.T. and Tiidus, P.M. (2012). Effect of creatine supplementation on muscle damage and repair following eccentrically-induced damage to the elbow flexor muscles. *Journal of Sports Science and Medicine*, 11, 653-659.

McLeay, Y., Barnes, M.J., Mundel, T., Hurst, S.M., Hurst, R.D. and Stannard, S.R. (2012). Effect of New Zealand blueberry consumption on recovery from eccentric exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition,* 9, 19.

McLellan, C., Lovell, D.I. and Gass, G.C. (2011). Markers of postmatch fatigue in professional rugby league players. *Journal of Strength and Conditioning Research*, 25, 1030-1039.

McLellan, C.P., Lovell, D.I. and Gass, G.C. (2010). Creatine kinase and endocrine responses of elite players pre, during, and post rugby league match play. *The Journal of Strength and Conditioning Research*, *24*, 2908-2919.

McLellan, T.M., Pasiakos, S.M. and Lieberman, H.R. (2014). Effects of protein in combination with carbohydrate supplements on acute or repeat endurance exercise performance: A systematic review. *Sports Medicine*, 44, 535-550.

McNeil, P.L. and Khakee, R. (1992). Disruptions of muscle fiber plasma membranes. Role in exercise-induced damage. *American Journal of Pathology*, 140, 1097-1109.

McPherson, R.A. and Hardy, G. (2011). Clinical and nutritional benefits of cysteine-enriched protein supplements. *Current Opinion in Clinical Nutrition and Metabolic Care*, 14, 562-568.

Mehta, R. K. and Agnew, M. J. (2012). Influence of mental workload on muscle endurance, fatigue, and recovery during intermittent static work. *European Journal of Applied Physiology*, 112, 2891-2902.

Mellgren, R.L., Zhang, W., Miyake, K. And McNeil, P.L. (2007). Calpain is required for the rapid, calcium-dependent repair of wounded plasma membrane. *The Journal of Biological Chemistry,* 282, 2567-2575.

Meltzer, H.T. (1971). Factors affecting serum creatine phosphokinase levels in the general population: the role of race, activity and sex. *Clinica Chimica Acta*, 33, 165-172.

Melzer, W., Herrmann-Frank, A. and Lüttgau, H.Ch. (1995). The role of Ca^{2+} ions in excitation-contraction coupling of skeletal muscle fibres. *Biochimica et Biophysica Atca*, 1241, 59-116.

Meyer, F.A. and Lieber, R.L. (2012). Skeletal muscle fibrosis develops in response to desmin deletion. *American Journal of Physiology Cell Physiology*, 302, C1609-C1620.

Mickleborough, T.D., Sinex, J.A., Platt, D., Chapman, R.F. and Hirt, M. (2015). The effects PCSO-524®, a patented marine oil lipid and omega-3 PUFA blend derived from the New Zealand green lipped mussel (Perna canaliculus), on indirect markers of muscle damage and inflammation after muscle damaging exercise in untrained men: a randomized, placebo controlled trial. *Journal of the International Society of Sports Nutrition*, *12*,10.

Mikkelsen, U.R., Fredsted, A., Gissel, H. and Clausen, T. (2004). Excitation-induce Ca2+ influx and msucle damage in the rat: loss of membrane integrity and impaired force recovery. *The Journal of Physiology*, 559, 271-285.

Milanez, V.F., Ramos, S.P.., Okuno, N.M., Boullosa, D.A. and Nakamura, F. (2014). Evidence of a non-linear dose-response relationship between training load and stress markers in elite female futsal players. *Journal of Sports Science and Medicine*, 13, 22-29.

Miles, M.P., Andring, J.M., Pearson, S.D., Gordon, L.K., Kasper, C., Depner, C.M. and Kidd, J.R. (2008). Diurnal variation, response to eccentric exercise, and association of inflammatory mediators with muscle damage variables. *Journal of Applied Physiology*, 104, 451-458.

Miles, M.P., Clarkson, PM., Smith, L.L., Howell, J.N. and McCammon, M.R. (1994). Serum creatine kinase activity in males and females following two bouts of eccentric exercise. *Medicine and Science in Sports and Exercise*, 26, S168.

Miller, B.F., Hansen, M., Olesen, J.L., Flyvbjerg, A., Schwarz, P., Babraj, J.A., Smith, K., Rennie, M.J. and Kjaer, M. (2006). No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in contracting skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism*, 290, E163-E168.

Miller, S.L., Tipton, K.D., Chinkes, D.L., Wolf, S.E. and Wolfe, R.R. (2003). Independent and combined effects of amino acids and glucose after resistance exercise. *Medicine and Science in Sports and Exercise*, 35, 449-455.

Milne, K.J. and Noble, E.G. (2008). Response of the myocardium to exercise: sex specific regualtion of hsp70. *Medicine and Science in Sports and Exercise*, 40, 655-663.

Milner, D., Weitzer, G., Tran, D., Bradley, A. and Capetanaki, Y. (1996). Disruption of muscle architecture and myocardial degeneration in mice lacking desmin. *The Journal of Cell Biology*, 134, 1255-1270.

Minett, G.M. and Costello, J.T. (2015). Specificity and context in post-exercise recovery: it is not a one-size-fits-all approach. *Frontiers in Physiology*, *6*.

Moens, P., Baatsen, P.H.W.W. and Maréchal, G. (1993). Increased susceptibility of EDL muscles from mdx mice to damage induced by contractions with stretch. *Journal of Muscle Research & Cell Motility*, 14, 446-451.

Mohr, M., Draganidis, D., Chatzinikolaou, A., Barbero-Álavarez, J.C., Castagna, C, Douroudos, I, Avloniti, A., Margeli, A., Papassotiriou, I, Flouris, A.D., Jamurtas, A.Z., Krustrup, P. and Fatouros, I.G. (2016). Muscle damage, inflammatory, immune and performance responses to three football games in 1 week in competitive male players. *European Journal of Applied Physiology,* 116, 179-193.

Molina, R. and Denadai, B.S. (2012). Dissociated time course recovery between rate of force development and peak torque after eccentric exercise. *Clinical Physiology and Functional Imaging*, 32, 179-184.

Montgomery, P.G., Pyne, D.B., Cox, A.J., Hopkins, W.G., Minahan, C.L. and Hunt, P.H. (2008). Muscle damage, inflammation, and recovery interventions during a 3-day basketball tournament. *European Journal of Sport Science*, *8*, 241-250.

Moore, D., Phillips, S., Babraj, J., Smith, K. and Rennie, M. (2005). Myofibrillar and collagen protein synthesis in human skeletal muscle in young mend after maximal shortening and lengthening contractions. *American Journal of Physiology Endocrinology and Metabolism*, 288, E1153-E1159.

Moore, D.R. (2015). Nutrition to support recovery from endurance exercise: optimal carbohydrate and protein replacement. *Current Sports Medicine Reports*, *14*, 294-300.

Moore, D.R., Robinson, M.J., Fry, J.L., Tang, J.E., Glover, E.I., Wilkinson, S.B., Prior, T., Tarnopolsky, M.A. and Phillips, S.M. (2009). Ingested protein dose-response of muscle and albumin protein synthesis after resistance exercise in young men. *American Journal of Clinical Nutrition,* 89, 161-168.

Morgan, D.L. (1990). New insights into the behavior of muscle during active lengthening. *Biophysical Journal*, 57, 209-221.

Morgan, D.L. and Allen, D.G. (1999). Early events in stretch-induced muscle damage. *Journal of Applied Physiology*, 87, 6, 2007-2015.

Morgan, D.L. and Proske, U. (2004). Popping sarcomere hypothesis explains stretch induced muscle damage. *Proceedings of the Australian Physiological and Pharmacological Society,* 34, 19-23.

Morifuji, M., Ishizaka, M., Baba, S., Fukuda, K., Matsumoto, H., Koga, J., Kanegae, M. and Higuchi, M. (2010). Comparison of different sources and degrees of hydrolysis of dietary protein: effect on plasma amino acids, dipeptides, and insulin responses in human subjects. *Journal of agricultural and food chemistry*, 58, 8788-8797.

Morton, R.W., McGlory, C. and Phillips, S. (2015). Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. *Frontiers in Physiology*, 6, 245.

Mougios, V. (2007). Reference intervals for serum creatine kinase in athletes. *British Journal of Sports Medicine, 41*, 674-678.

Mujika, I. and Padilla, S. (2001) Physiological and performance characteristics of male professional road cyclists. *Sports Medicine*, 31, 479-487.

Muller, F.L., Liu, Y., and van Remmen, H. (2004). Complex III releases superoxide to both sides of the mitochondrial membrane. *Journal of Biological Chemistry*, 279, 49064-49073.

Murase, S., Terazawa, E., Queme, F., Ota, H., Matsuda, T., Hirate, K., Kozaki, Y., Katanosaka, K., Taguchi, T., Urai, H. And Mizumura, K. (2010). Bradykinin and nerve growth factor play pivotal roles in muscular mechanical hyperalgesia after exercise (delayd-onset muscle soreness). *The Journal of Neuroscience*, 30, 3752-3761.

Murphy, E., and Steenbergen, C. (2007). Sex-based differences in mechanisms of protection in myocardial ischemia–reperfusion injury. *Cardiovascular Research*, *75*(3), 478-486.

Murphy, R. (2009). Calpains, skeletal muscle function and exercise. *Proceedings of the Australian Physiological Society*, 40, 95-102.

Murphy, R.M. and Lamb, G.D. (2009). Endogenous calpain-3 activation is primarily governed by small increases in resting cytoplasmic Ca^{2+} and is not dependent on stretch. *Journal of Biological Chemistry*, 284, 7811-7819.

Murphy, R.M., Goodman, C.A., McKenna, M.J., Bennie, J., Leikis, M. And Lamb, G.D. (2007). Calpain-3 is autolyzed and hence activated in human skeletal muscle 24h following a single bout of eccentric exericse. *Journal of Applied Physiology*, 103, 926-931.

Murton, A.J., Constantin, D. And Greenhaff, P.L. (2008). The involvement of hte ubiquitin proteasome system in human skeletal muscle remodelling and atrophy. *Biochimica et Biophysica Acta*, 1782, 730-743.

Naclerio, F., Larumbe-Zabala, E., Cooper, R., Allgrove, J. and Earnest, C.P. (2015). A multi-ingredient containing carbohydrate, proteins L-glutamine and L-carnitine attenuates fatigue perception with no effect on performance, muscle damage or immunity in soccer players. *PloS one*, *10,* e0125188.

Naclerio, F., Larumbe-Zabala, E., Cooper, R., Jimenez, A. and Goss-Sampson, M. (2014). Effect of a carbohydrate-protein multi-ingredient supplement on intermittent sprint performance and muscle damage in recreational athletes. *Applied Physiology, Nutrition, and Metabolism*, 39, 1151-1158.

Nader, G. and Esser, K. (2001). Intracellular signalling specificity in skeletal muscle in response to different modes of exercise. *Journal of Applied Physiology*, 90, 1936-1942.

Nair, K.S., Schartz, R.G. and Welle, S. (1992). Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *American Journal of Physiology Endocrinology Metabolism*, 263. E928-E934.

Nakhostin-Roohi, B., Bohlooli, S., Barmaki, S. and Khoshkhahesh, F. (2012). Influence of spinach supplementation on exercise-induced muscle damage. *Annals of Biological Research*, 3, 4551-4554.

Nedelec, M., McAll A., Carling, C. Legall, F., Berthoin, S. and Dupont, G. (2014). The influence of soccer playing actions on the recovery kinetics after a soccer match. *Journal of Strength and Conditioning Research*, 28, 1517-1523.

Nedelec, M., McCall, Carling, C., Legall, F., Berthoin, S. and Dupont, G. (2012). Recovery in soccer: part 1 – post-match fatigue and time course of recovery. *Sports Medicine*, 42, 997-1015.

Nelson, N. (2013). Delayed onset of muscle soreness: is massage effective? *Journal of Bodywork and Movement Therapies*, 17, 475-482.

Nevill, A. and Lane, A. (2007). Why self-report 'Liekert' scale data should not be log-transformed. *Journal of Sports Sciences*, 25, 1-2.

Newham, D.J., McPhail, G., Mills, K.R. and Edwards, R.H.T. (1983). Ultrastructural changes after concentric and eccentric contractions of human muscle. *Journal of the Neurological Sciences*, 61, 109-122.

Nie, H., Kawczynski, A., Madeleine, P. and Arendt-Nielsen, L. (2005). Delayed onset muscle soreness in neck/shoulder muscles. *European Journal of Pain,* 9, 653-660.

Nieman, D.C., Henson, D.A., McAnulty, S.R., McAnulty, L., Swick, N.S., Utter, A.C., Vinci, D.M., Opiela, S.J. and Morrow, J.D. (2002). Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *Journal of Applied Physiology,* 92, 59-66.

Nieman, D.C., Henson, D.A., Smith, L.L., Utter, A.C., Vinci, D.M., Davis, J.M., Kaminsky, D.E. and Shute, M. (2001). Cytokine changes after a marathon race. *Journal of Applied Physiology,* 92, 109-114.

Nieman, D.C., Konrad, M., Henson, D.A., Kennerly, K., Shanely, R.A. and Wallner-Liebmann, S.J. (2012). Variance in the acute inflammatory response to prolonged cycling is linked to exercise intensity. *Journal of Interferon and Cytokine research*, 32, 12-17.

Nieman, D.C., Luo, B., Dréau, D., Henson, D.A., Shanely, R.A., Dew, D. And M.P. Meany (2014). Immune and inflammation responses to a 3-day period of intensified running versus cycling. *Brain, Behavior, and Immunity*, 39, 180-185.

Norton, L.E. and Layman, D.K. (2006). Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. *Journal of Nutrition,* 136, S533-537.

Nosaka, K. and Sakamotor, K. (2001). Effect of elbow joint angle on the magnitude of muscle damage to the elbow flexors: *Medicine and Science in Sports and Exercise*, 33, 1, 22-29.

Nosaka, K., and Clarkson, P.M. (1996). Variability in serum creatine kinase response after eccentric exercise of the elbow flexors. *International Journal of Sports Medicine*, *17*, 120-127.

Nosaka, K., Chapman, D., Newton, M. and 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, 313-319.

Nurenberg, P., Giddings, C.J., Stray-Gundersen, J., Fleckenstein, J.L., Gonyea, W.J. and 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*, 865-869.

Ogilvie, R.W., Armstrong, R.B., Baird, K.E. and Bottoms, C.L. (1985). Lesions in the rat soleus muscle following eccentrically biased exercise. *American Journal of Anatomy*, 182, 335-346.

Oliver, J., Armstrong, N. and Williams, C. (2008). Changes in jump performance and muscle activity following soccer-specific exercise. *Journal of Sports Sciences*, 26, 141-148.

Ormsbee, M.J., Gorman, K.A., Miller, E.A., Baur, D.A., Eckel, L.A., Contreras, R.J., Panton, L.B. and Spicer, M.T. (2016). Nighttime feeding likely alters morning metabolism but not exercise performance in female athletes. *Applied Physiology Nutrition and Metabolism*, 41, 1-9.

Ormsbee, M.J., Ward, E.G., Bach, C.W., Arciero, P.J., McKune, A.J. and Panton, L.B. (2015). The impact of a pre-loaded multi-ingredient performance supplement on muscle soreness and performance following downhill running. *Journal of the International Society of Sports Nutrition*, *12*, 2.

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P. and Klarlund Pedersen, B. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *Journal of Physiology*, 515.1, 287-291.

Overgaard, K., Fredsted, A., Hyldal, A., Ingemann-Hansen, T., Gissel, H. and Clausen, T. (2004). Effects of running distance and training on Ca^{2}+ content and damage in human muscle. *Medicine and Science in Sports and Exercise*, 36, 821-829.

Overgaard, K., Lindstrøm, Ingemann-Hansen, T. and Clausen, T. (2002). Membrane leakage and increased content of Na^{+}-K^{+} pumps and Ca^{2+} in human muscle after a 100km run. *Journal of Applied Physiology*, 92, 1891-1898.

Paddon-Jones, D., Keech, A., Lonergan, A. And Abernethy, P. (2005). Differential expression of muscle damage in humans following acute fast and slow velocity eccentric exercise. *Journal of Science and Medicine in Sport,* 8, 3, 255-263.

Page, R.M., Marrin, K., Brogden, C.M. and Greig, M. (2015). Biomechanical and physiological response to a contemporary soccer match-play simulation. *The Journal of Strength and Conditioning Research*, 29, 2860-2866.

Panchangam, A. and Herzog, W. (2011). Sarcomere overextension reduces stretch-induced tension loss in myofibrils of rabbit psoas. *Journal of Biomechanics*, 44, 2144-2149.

Papacosta, E., Nassis, G.P. and Gleeson, M. (2015). Effects of acute postexercise chocolate milk consumption during intensive judo training on the recovery of salivary hormones, salivary SIgA, mood state, muscle soreness, and judo-related performance. *Applied Physiology Nutrition and Metabolism*, 40, 1-7.

Park, Y.W. and Nam, M.S. (2015). Bioactive peptides in milk and dairy products: a review. *Korean Journal for Food Science of Animal Resources*, *35*, 831-840.

Paschalis, V., Koutedakis, Y., Jamurtas, A.Z., Mougios, V. and Baltzopoulos, V. (2005). Equal volumes of high and low intensity of eccentric exercise in relation to damage and performance. *Journal of Strength and Conditioning Research*, 19, 1, 184-188.

Pascoe, G.A. and Reed, D.J. (1989). Cell calcium, vitamin E, and the thiol redox system in cytotoxicity. *Free Radical Biology and Medicine*., 6, 209-224.

Pasiakos, S.M., Lieberman, H.R. and McLellan, T.M. (2014). Effects of protein supplements on muscle damage, soreness and recovery of muscle function and physical performance: A systematic review. *Sports Medicine*, 44, 655-670.

Patel, T.J. and Lieber, R.L. (1997). Force transmission in skeletal muscle: from actomyosin to external tendons. *Exercise and Sport Sciences Reviews*, 25, 321-363.

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

Paulsen, G., Cumming, K.T., Holden, G., Hallén, J., Rønnestad, B.R., Sveen, O., Skaug, A., Paur, I., Bastani, N.E., Østgaard, H.N. and Buer, C. (2014). Vitamin C and E supplementation hampers cellular adaptation to endurance training in humans: a double-blind, randomised, controlled trial. *The Journal of Physiology*, 592, 1887-1901.

Paulsen, G.,P, Ramer Mikkelsen, U., Raastad, T. and Peake, J.M. (2012). Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise. *Exercise Immunology Review*, 18, 42-97.

Peake, J., Nosaka, K. and Suzuki, K. (2005a). Characterization of inflammatory responses to eccentric exercise in humans. *Exercise Immunology Review*, 11, 64-85.

Peake, J.M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K. and Coombes, J.S. (2005b). Plasma cytokine changes in relation to exercise intensity and muscle damage. *European Journal of Applied Physiology,* 95, 514-521.

Peake, J.M., Suzuki, K., Wilson, G., Hordern, M., Nosaka, K., Mackinnon, L. and Coombes, J.S. (2005c). Exercise- induced muscle damage, plasma cytokines, and markers of neutrophil activation. *Medicine and Science in Sports and Exercise*, 37, 737-745.

Peñailillo, L., Blazevich, A., Numazawa, H. and Nosaka, K. (2015). Rate of force development as a measure of muscle damage. *Scandinavian Journal of Medicine and Science in sports*, *25*(3), pp.417-4.

Persky, A.M., Green, P.S., Stubley, L., Howell, C.O., Zaulyanov, L., Braxeau, GA. and Simpkins, J.W. (2000). Protective effect of estrogen against oxidative damage to heart and skeletal muscle in vivo and in vitro. *Proceedings of the Society for Experimental Biology and Medicine*, 223, 59-66.

Phillippou, A., Bogdanis, G., Maridaki, M., Halapas, A., Sourla, A. and Koutsilieris, M. (2009). Systemic cytokine response following exercise-induced muscle damage in humans. *Clinical Chemistry and Laboratory Medicine,* 47, 777-782.

Phillips, A., Hamilton, D.L. and Baar, K. (2011). Signals mediating skeletal muscle remodelling by resistance exercise: P13-kinase-independent activation of mTORC1. *Journal of Applied Physiology*, 110, 561-568.

Phillips, S. (2017). Current concepts and unresolved questions in dietary protein requirements and supplements in adults. *Frontiers in Nutrition*, 4, 13.

Phillips, S.M. (2014). A brief review of critical processes in exercise-induced muscular hypertrophy. *Sports Medicine*, 44, 71-77.

Phillips, S.M. and Van Loon, L.J. (2011). Dietary protein for athletes: from requirements to optimum adaptation. *Journal of Sports Sciences*, 29, S29-38.

Phillips, S.M., Fulgoni, V.L., Heaney, R.P., Nicklas, T.A., Slavin, J.L. and Weaver, C.M. (2015). Commonly consumed protein foods contribute to nutrient intake, diet quality, and nutrient adequacy. *American Journal of Clinical Nutrition*, 101, 1356S-1352S.

Phillips, S.M., Tipton, K.D., Aarsland, A., Wolf, S.E., and Wolfe, R.R. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *American Journal of Physiology Endocrinology and Metabolism*, 273, E99-E107.

Pingitore, A., Lima, G.P.P., Mastorci, F., Quinones, A., Iervasi, G. and Vassalle, C. (2015). Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. *Nutrition*, *31*(7), pp.916-922.

Pizza, F.X., Peterson, J.M., Baas, J.H. and Koh, T.J. (2005). Neutrophils contribute to muscle injury and impair its resolution after lengthening contractions in mice. *Journal of Physiology*, 562.3, 899-913.

Pliauga, V., Kamandulis, S., Dargeviciute, G., Jaszczanin, J., Kliziene, I., Stanislovaitiene, J. and Stanislovaitis, A. (2015). The effect of a simulated basketball game on players' sprint and jump performance, temperature and muscle damage. *Journal of Human Kinetics*, 46, 167-175.

Poslusna, K., Ruprich, J., de Vries, J.H., Jakubikova, M. and van't Veer, P. (2009). Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. *British Journal of Nutrition*, 101, S73-S85.

Posterino, G.S., Cellini, M.A. and Lamb, G.D. (2003). Effects of oxidation and cytosolic redox conditions on excitation-contraction coupleing in rat skeletal muscle. *Journal of Physiology,* 547, 807-823.

Potter, J.D., Robertson, S.P. and Johnson, J.D. (1981). Magnesium and the regulation of muscle contraction. *Federation Proceedings*, 40, 2653-2656).

Poudevigne, M.S., O'Connor, P.J. and Pasley, J.D. (2002). Lack of both sex differences and influence of resting blood pressure on muscle pain intensity. *Clinical Journal of Pain*, 18, 386-393.

Poulios, A., Fatouros, I.G., Mohr, M., Draganidis, D.K., Deli, C., Papanikolaou, K., Sovatzidis, A., Nakopoulou, T., Ermidis, G., Tzatzakis, T. and Laschou, V.C. (2018). Post-Game High Protein Intake May Improve Recovery of Football-Specific Performance during a Congested Game Fixture: Results from the PRO-FOOTBALL Study. *Nutrients*, 10, 494.

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

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

Powers, S.K. and Jackson, M.J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiology Reviews,* 88, 1243-1276.

Powers, S.K., Nelson, W.B. and Hudson, M.B. (2011). Exercise-induced oxidative stress in humans: cause and consequences. *Free Radical Biology and Medicine,* 51, 942-950.

Powers, S.K., Smuder, A.J., Kavazis, A.N. and Hudson, M.B. (2010). Experimental guidelines for studies designed to investigate the impact of antioxidant supplementation on exercise performance. *International Journal of Sport Nutrition and Exercise Metabolism*, 20, 2-14.

Pritchett, K. and Pritchett, R. (2012). Chocolate milk: a post-exercise recovery beverage for endurance sports. *Acute Topics in Sport Nutrition*, 59, 127-134.

Pritchett, K., Bishop, P., Pritchett, R., Green, M. and Katica, C. (2009). Acute effects of chocolate milk and a commercial recovery beverage on post-exercise recovery indices and endurance cycling performance. *Applied Physiology Nutrition and Metabolism*, 34, 1017-1022.

Proske, U. and Allen, T.J. (2005). Damage to skeletal muscle from eccentric exercise. *Exercise and Sport Sciences Reviews*, 33, 2, 98-104.

Proske, U. and Morgan, D.L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *Journal of Physiology*, 537.2, 333-345.

Pyne, D. (1994). Exericse-induced muscle damage and inflammation: a review. *The Australian Journal of Science and Medicine in Sport*, 26, 49-58.

Raastad, T., Ow, S.G., Paulsen, G., Enns, D., Overgaard, K., Crameri, R.M., Kiil, S., Belcastro, A.N., Bergersen, L. and Hallen, J. (2010). Changes in calpain activity,

muscle structure, and function after eccentric exercise. *Medicine and Science in Sports and Exercise*, 42, 86-95.

Racine, M., Tousignant-Laflamme, Y., Kloda, L.A., Dion, D., Dupuis, G. And Choinière, M. (2012). A systematic literature review of 10 years of research on sex/sex and experimental pain perception – Part 1 : Are there really differences between women and men? *Pain*, 153, 602-618.

Raj, D. A., Booker, T.S. and Belcastro, A.N. (1998). Striated muscle calcium-stimulated cysteine protease (calpain-like) activity promotes myeloperoxidase activity with exercise. *Pflügers Arch*, 435, 804-809.

Rampinini, E., Bishop, D., Marcora, S.M. et al (2007). Validity of simple field tests as indicators of match-related physical performance in top-level professional soccer players. *International Journal of Sports Medicine*, 28, 228-235.

Rankin, J.W., Goldman, L.P., Puglisi, M.J., Nickols-Richardson, S.M., Earthman, C.P. and Gwazdauskas, F. C. (2004). Effect of post-exercise supplement consumption on adaptations to resistance training. *Journal of the American College of Nutrition*, 23, 322-330.

Rankin, P., Landy, A., Stevenson, E. and Cockburn, E. (2018). Milk: an effective recovery drink for female athletes. *Nutrients*, 10, 228.

Rankin, P., Lawlor, M.J., Hills, F.A., Bell, P.G., Stevenson, E.J. and Cockburn, E. (2017). The effect of milk on recovery from repeat-sprint cycling in female team-sport athletes. *Applied Physiology Nutrition and Metab*olism**,** 999, 1-10.

Rankin, P., Stevenson, E. and Cockburn, E. (2015). The effect of milk on the attenuation of exercise-induced muscle damage in males and females. *European Journal of Applied Physiology,* 115, 1245-1261.

Rasmussen, B.B., Tipton, K., Miller, S., Wolf, S. and Wolfe, R. (2000). An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *Journal of Applied Physiology,* 88, 386-392.

Rawson, E.S., Miles, M.P. and Larson-Meyer, D.E. (2018). Dietary Supplements for Health, Adaptation, and Recovery in Athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, 1-33.

Rayment, I., Holden, H.M., Whittaker, M., Yohn, C.B., Lorenz, M., Holmes, K.C. and Milligan, R.A. (1993). Structure of the actin-myosin complex and its implications for muscle contraction. *Science*, 261, 58-65.

Rebalka, I.A. and Hawke, T.J. (2014). Potential biomarkers of skeletal muscle damage. *Biomarkers in Medicine*, 8, 375-378.

Reid, K. (2016). Case study: the role of milk in a dietary strategy to increase muscle mass and improve recovery in an elite sprint kayaker. *International Journal of Sport Nutrition and Exercise Metabolism,* 26, 179-184.

Reid, M.B. (2005). Response of the ubiquitin-proteasome pathway to changes in muscle activity. *American Journal of Physiology Regulatory Integrative Comparative Physiology*, 288, R1423-R1431.

Reid, M.B., Khawli, F.A. and Moody, M.R. (1993). Reactive oxygen in skeletal muscle III. Contractility of unfatigued muscle. *Journal of Applied Physiology*, 75, 1081-1087.

Reilly, T, and Keane, S. (2002). The effect of carbohydrate supplementation on the work-rate of Gaelic football players. In W. Spinks, T. Reilly, and A. Murphy (Eds.), *Science and Football IV* (pp234-238). London, Routledge.

Reilly, T. (2000). Endurance aspects of soccer and other field games. In R.J. Shephard, & P.O. Astrand (Eds*.), Endurance in sport* (pp 900-903). Oxford, Blackwell Science.

Rennie, M.J., Edwards, R.H., Davies, C.T., Krywawych, S., Halliday, D., Waterlow, J.C. and Millward, D.J. (1980). Protein and amino acid turnover during and after exercise. *Biochemical Society Transactions*, 8,499-501.

Res, P.T., Groen, B., Pennings et al., (2012). Protein ingestion before sleep improves postexercise overnight recovery. *Medicine and Science in Sports and Exercise*, 44, 1560-1569.

Rieu, I., Balage, M., Sornet, C., Giraudet, C., Pujos, E., Grizard, I., Mosoni, L. and Dardevet, D. (2006). Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidemia. *Journal of Physiology*, 575, 305-315.

Riley, J.L., Robinson, M.D., Wise, E.A. and Price, D. (1999). A meta-analytic review of pain perception across the menstrual cycle. *Pain*, 81, 225-235.

Rinard, J., Clarkson, P.M., Smith,L.L. and Grossman, M. (2000). Response of males and females to high-force eccentric exercise. *Journal of Sports Sciences,* 18, 229-236.

Rival, S.G., Fornaroli, S. Boeriu, C.G. and Wichers, H.J. (2001). Caseins and casein hydrolysates. Lipoxygenase inhibitory properties. *Journal of Agriculture and Food Chemistry*, 49, 287-294.

Roberts, S.P., Stokes, K.A., Trewartha, G., Hogben, P., Doyle, J. and Thompson, D. (2011). Effect of combined carbohydrate-protein ingestion on markers of recovery after simulated rugby union match-play. *Journal of Sports Sciences*, *29,* 1253-1262.

Rochford, D (2004). Data presented by Young, D (2008). An analysis of hurling. Does training meet the demands of the game? Paper presented at the Gaelic Athletic Association Annual Conference, Croke Park. www.gaa.ie/content/documents/publications/coaching_and_games/conference_2008/damien_young_demands_of_the_game_100123150634.pdf

Rode, C., Siebert, T. And Blickhan, R. (2009). Titin-induced force enhancement and force depression: A ‘sticky-spring’ mechanism in muscle contractions? *Journal of Theoretical Biology,* 259, 350-360.

Rodrigues, B.M., Dantas, E., de Salles, B.F., Miranda, H., Koch, A.J., Willardson, J.M. and Simão, R. (2010). Creatine Kinase and Lactate Dehydrogenase responses after upper-body resistance exercise with different rest intervals. *Journal of Strength and Conditioning Research*, 24, 1657-1662.

Roengrit, T., Wananon, R., Prasertsri, P., Kanpetta, Y., Sripanidkulchai, B. and Leelayuwat, N. (2014). Antioxidant and anti-nociceptive effects of Phyllanthus amarus on improving exercise recover in sedentary men: a randomized crossover (double-blind) design. *Journal of the International Society of Sports Nutrition*. 11, 9.

Rogers, M.A., Stull, G.A. and Apple, F.S. (1985). Creatine kinase isoenzyme activities in men and women following a marathon race. *Medicine and Science in Sports and Exercise*, 17, 679-682.

Rokitzki, L., Logemann, E., Sagredos, A.N., Murphy, M., Wetzel-Roth, W. and Keul, J. (1994). Lipid peroxidation and antioxidant vitamins under extreme endurance stress. *Acta Physiologica Scandanavica,* 151, 149-158.

Rowlands, D.S., Rössler, K., Thorp, R.M., Graham, D.F., Timmons, B.W., STannard, S.R. and Tarnopolsky, 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 Physiology*, 33, 39-51.

Rowsell, G.J., Coutts, A.J., Reaburn, P. and Hill-Haas, S. (2009). Effects of cold-water immersion on physical performance between successive matches in high-performance junior male soccer players. *Journal of Sports Sciences*, *27*, 565-573.

Rushall, B.S. (1990). A tool for measuring stress tolerance in elite athletes. *Journal of Applied Sport Psychology*, 2, 51-66.

Russell, M., Northeast, J., Atkinson, G., Shearer, D.A., Sparkes, W., Cook, C and Kilduff, L. (2015). Between-match variability of peak power output and creatine kinase responses to soccer match-play. *Journal of Strength and Conditioning Research*, 29, 2079-2085.

Russell, M., Rees, G., Benton, D. and Kingsley, M. (2011). An exercise protocol that replicates soccer match-play. *International Journal of Sports Medicine*, 32, 511-518.

Russell, M., Sparkes, W., Northeast, J., Cook, C., Love, T.D., Bracken, R.M. and Kilduff, L. (2016). Changes in acceleration and deceleration capacity throughout professional soccer match-play. *Journal of Strength and Conditioning Research*, 30, 2839-2844.

Ryan, L. and Petit, S. (2010). Addition of whole, semiskimmed, and skimmed bovine milk reduces the total antioxidant capacity of black tea. *Nutrition Research*, 30, 14-20.

Ryan, M., Malone, S. and Collins, K. (2017). An acceleration profile of elite Gaelic football match play. *Journal of Strength and Conditioning Research,* 32, 812-820.

Sachdev, S. and Davies, K.J. (2008). Production, detection, and adaptive responses to free radicals in exercise. *Free Radical Biology and Medicine,* 44, 215-233

Sainani, K.L. (2018). The Problem with" Magnitude-based Inference". *Medicine and Science in Sports and Exercise*, *50*, 10, 2166-2176.

Salazar, J.J., Michele, D.E. and Brooks, S.V. (2010). Inhibition of calpain prevents muscle weakness and disruption of sarcomere structure during hindlimb suspension. *Journal of Applied Physiology*, 108, 120-127.

Salminen, A. and Vihko, V. (1981). Effects of age and prolonged runing on proteolytic capacity in mouse cardiac and skeletal muscles. *Acta Physiologica Scandanavica*, 112, 89-95.

Samadi, A., Gaeini, A.A., Kordi, M.R., Rahimi, M., Rahnama, N. and Bambaeichi, E. (2012). Effect of various ratios of carbohydrate-protein supplementation on resistance exercise-induced muscle damage. Journal *of Sports Medicine and Physical Fitness*, 52, 151-157.

Samaras, A., Tsarouhas, K., Paschalidis, E., Giamouzis, G., Triposkiadis, F., Tsitsimpikou, C., Becker, A.T., Goutzourelas, N. and Kouretas, D. (2014). Effect of a special carbohydrate-protein bar and tomato juice supplementation on oxidative stress markers and vascular endothelial dynamics in ultra-marathon runners. *Food and Chemical Toxicology*. 69, 231-236.

Sandri, M., Carraro, U., Podhorska-Okolov, M., Rizzi, C., Arslan, P., Monti, D. and Franceschi, C. (1995). Apoptosis, DNA damage and ubiquitin expression in normal and mdx muscle fibers after exercise. *FEBS Letters*, 373, 291-295.

Sandri, M., Podhorska-Okolov, M., Geromel, V., Rizzi, C., Arslan, Pl, Franceschi, C. and Carraro, U. (1997). Exercise induces myonuclear ubiquitination and apoptosis in dystrophin-deficient muscle of mice. *Journal of Neuropathology and Experimental Neurology,* 56, 45-57.

Saunders, M.J. (2011). Carbohydrate-protein intake and recovery from endurance exercise: is chocolate milk the answer? *Current sports medicine reports*, 10, 203-210.

Saunders, M.J., Kane, M.D. and Todd, M.K. (2004). Effects of a carbohydrate-protein beverage on cycling endurance and muscle damage. *Medicine and Science in Sports and Exercise*, 36, 1233-1238.

Saunders, M.J., Luden, N.D. and 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, 678-684.

Saw, A.E., Main, L.C. and Gastin, P.,B. (2015). Monitoring the athlete training response: subjective self-reported measures trump commonly used objective measures: a systematic review. *British Journal of Sports Medicine*, 50, 281-291.

Saxton, J.M., Clarkson, P.M., James, R., Miles, M., Westerfer, M., Clark, S. and Donnelly, A.E. (1995). Neuromuscular dysfunction following eccentric exercise. *Medicine and Science in Sports and Exercise*, 27, 1185-1193.

Sayers, S.P. and Clarkson, P.M. (2001). Force recovery after eccentric exercise in males and females. *European Journal of Applied Physiology*, 84, 122-126.

Scalzo, R.L., Peltonen, G.L., Binns, S.E., Shankaran, M., Giordano, G.R., Hartley, D.A., Klochak, A.L., Lonac, M.C., Paris, H.L., Szallar, S.E. and Wood, L.M. (2014). Greater muscle protein synthesis and mitochondrial biogenesis in males compared with females during sprint interval training. *The FASEB Journal*, *28*, 2705-2714.

Schiaffino, S., Dyar, K.A., Ciciliot, S., Blaauw, B. and Sandri, M. (2013). Mechanisms regulating skeletal muscle growth and atrophy. *The FEBS journal*, *280*, 4294-4314.
Schilaty, N.D., Bates, N.A. and Hewett, T.E. (2017). Relative dearth of 'sex differences' research in sports medicine. *Journal of Science and Medicine in Sport*, 21, 440-441.

Schoenfeld, B.J. (2013). Potential mechanisms for a role of metabolic stress in hypertrophic adaptations to resistance training. *Sports Medicine*, 43,179–194.

Sciberras, J.N., Galloway, S.D.R., Fenech, A., Grech, G., Farrugia, C., Duca, D and Mifsud, J. (2015). The effect of turmeric (Curcumin) supplementation on cytokine and inflammatory marder responses following 2 hours of endurance cycling. *Journal of the International Society of Sports Nutrition,* 12, 5.

Scott, A., Malone, J. J., Morgans, R., Burgess, D., Gregson, W., Morton, J. P. and Drust, B. (2016). The relationship between physical match performance and 48h post-game creatine kinase concentrations in English Premier League soccer players. *International Journal of Sports Science and Coaching*, 11, 846-852.

Seifert, J.G., Kipp, R.W., Amann, M. And Gazal, O. (2005). Muscle damage, fluid ingestion, and energy supplementation during recreational alpine skiing. *International Journal of Sport Nutrition and Exercise Metabolism,* 15, 528-536.

Semark, A., Noakes, T.D., Gibson, A.S.C. and 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*, 197-203.

Sen, C.K. (2001). Antioxidants in exercise nutrition. *Medicine and Science in Sports and Exercise*, 33, 368-370.

Sesto, M.E., Radwin, R.G., Block, W.F. and Best, T.M. (2005). Anatomical and mechanical changes following repetitive eccentric exertions. *Clinical Biomechanics*, 20, 41-49.

Sewright, K.A., Hubal, M.J., Kearns, A., Holbrook, M.T. and Clarkson, P.M. (2008). Sex differences in response to maximal eccentric exercise. *Medicine and Science in Sports and Exercise*, 40, 242-251.

Sharma, R. and Shah, N. (2010). Health benefits of whey proteins. *Nutrafoods*, 9, 39-45.

Sharp, C.P. and Pearson, D.R. (2010). Amino acid supplements and recovery from high-intensity resistance training. *Journal of Strength and Conditioning Research*, 24, 1125-1130.

Sheel, A.W. (2016). Sex differences in the physiology of exercise: an integrative perspective. *Experimental Physiology*, 101.2, 211-212.

Shepstone, T.N., Tang, J.E., Dallaire, S.E., Schuenke, M.D., Staron, R.S. and Phillips, S.M. (2005). Short-term high-vs low-velocity isokinetic lengthening training results in greater hypertrophy of the elbow flexors in young men. *Scandinavian Journal of Medicine and Science in Sports*, 15, 135-136.

Shimomura, Y., Inaguma, A., Watanabe, S., Uamamoto, Ul, Muramatsu, Y., Bajotto, G., Sato, J., Shimomura, N., Kobayashi, H. and Mawatari, K. (2010). Branched-chain amino acid supplementation before squat exercise and delayed-onset muscle

soreness. *International Journal of Sports Nutrition and Exercise Metabolism,* 20, 236-244.

Shimomura, Y., Kitaura, U. and Shimomura, N. (2012). Effects of protein and amino acid supplementation on muscle protein metabolism in relation to exercise. *Journal of Physical Fitness and Sports Medicine,* 1, 219-225.

Shimomura, Y., Yamamoto, Y., Bajotto, G., Sato, J., Murakami, T., Shimomura, N., Kobayashi, H. and Mawatari, K. (2006). Nutraceutical effects of branched-chain amino acids on skeletal muscle. *Journal of Nutrition*, 136, 5295-5325.

Shirreffs, S.M., Watson, P. and Maughan, R.J. (2007). Milk as an effective post-exercise rehydration drink. *British Journal of Nutrition,* 98, 173–180.

Short, K.R., Vittone, J.L., Bigelow, M.L., Proctor, D.N. and Nair, K.S. (2004). Age and aerobic exercise training effects on whole body and muscle protein metabolism. *American Journal of Physiology Endocrinology and Metabolism*, 286, E92-E101.

Shumate, J.B., Brooke, M.H., Carroll, J.E. and Davis, J.E. (1979). Increased serum creatine kinase afer exercise: a sex-linked phenomenon. *Neurology*, 29, 902-904.

Sies, H. and Jones, D.P. (2007). Oxidative Stress. Elsevier, Amsterdam.

Silva, J.R., Ascensão, A., Marques, F., Seabra, A., Rebelo, A. and Magalhães, J. (2013). Neuromuscular function, hormonal and redox status and muscle damage of professional soccer players after a high level competitive match. European Journal of Applied Physiology, 113, 2193-2201.

Simonson, S.R. and Jackson, C.R. (2004). Leukocytosis occurs in response to resistance exercise in men. *Journal of Strength and Conditioning Research*, 18, 266-271.

Singh, T.K., Guelfi, K.J., Landers G. Dawson, B. and Bishop, D. (2011). A comparison of muscle damages, soreness and performance following a simulated

contact and non-contact team sport activity circuit. *Journal of Science and Medicine in Sport*, 14, 441-446.

Sipavičienė, S, Daniusevičiutė, L., Klizienė, I., Kamandulis, S. and Skurvydas, A. (2013). Effects of estrogen fluctuation during the menstrual cycle on the response to stretch-shortening exercise in females. *BioMed Research International*, doi: 10.1155/2013/243572.

Sirotic, A.C. and Coutts, A.J. (2007). Physiological and performance test correlates of prolonged, high-intensity intermittent running performance in moderately trained women team sport athletes. *Journal of Strength and Conditioning Research*, 21, 138-44.

Small, J.V., Fürst, D.O. and Thornell, E-J. (1992). The cytoskeletal lattice of muscle cells. *European Journal of Biochemistry*, 208, 559-572.

Smart, D.J., Gill, N.D., Beaven, C.M., Cook, C.J. and Blazevich, A.J. (2008). The relationship between changes in interstitial creatine kinase and game-related impacts in rugby union. *British Journal of Sports Medicine*, *42*, 198-201.

Smith, G.I., Atherton, P.J., Reeds, D.N., Mohammed, B.S., Jaffrey, H., Rankin, D., Rennie, M.J. and Mittendorfer, B. (2009). No major sex differences in muscle protein synthesis rates in the postabsorptive state and during hyperinsulinemia-hyperaminoacidemia in middle-aged adults. *Journal of Applied Physiology*, 107, 1308-1315.

Smith, L.L. (1991). Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Medicine and Science in Sports and Exercise*, 23, 542-551.

Smith, M.R., Marcora, S.M. and Coutts, A.J. (2015). Mental fatigue impairs intermittent running performance. *Medicine and Science in Sports and Exercise,* 47, 1682-1690.

Smithers, G.W. (2008). Whey and whey proteins – from ‘gutter-to-gold’. International Dairy Journal, 18, 695-704.

Sonobe, T., Inagaki, T., Poole, D.C. and Kano, Y. (2008). Intracellular calcium accumulation following eccentric contractions in rat skeletal muscle in vivo: role of stretch-activated channels. *American Journal of Physiology Regulative Integrative and Comparative Physiology*, 294, R1329-1337.

Sorichter ,S., Mari, J., Koller, A., Calzlari, C., Hounker, M., Pau, B. and Puschendorf, B. (2001). Release of muscle proteins after downhill running in male and female subjects. *Scandanavian Journal of Medicine and Science in Sport*, 11, 28-32.

Sorichter, S., Mair, J., Killer, A., Müller, E., Kremser, C., Judmaier, W., Haid, C., Rama, D., Calzolari, C. and Puschendorf, B. (1997). Skeletal muscle troponin I release and magnetic resonance imaging signal intensity changes after eccentric exercise-induced skeletal muscle injury. *Clinica Chimica Acta*, 262, 139-146.

Sorichter, S., Mair, J., Koller, A., Gebert, W., Rama, D., Calxolari, C., Artner-Dworzak, E. and Puschendorf, B. (1997). Skeletal troponin 1 as a marker of exercise-inducd muscle damage. *Journal of Physiology*, 83, 1076-1082.

Sorimachi, H., Ishiura, S. and Suzuki, K. (1997). Structure and physiological function of calpains. *Biochemical Journal*, 328, 721-732.

Sorimachi, H., Ohmi, S., Emori, Y., Kawasaki, H., Siado, T.C., Ohno, S., Minami, Y., and Suzuki, K. (1990). A novel member of the calcium-dependent cysteine protease family. *Biological Chemistry Hoppe-Seyler*, 371, 171-176.

Sousa, M., Teixeira, V.H. and Soares, J. (2014). Dietary strategies to recover from exercise-induced muscle damage. *International Journal of Food Sciences and Nutrition*, 65, 15-163.

Spanidis, Y., Stagos, D., Orfanou, M., Goutzourelas, N., Bar-Or, D., Spandidos, D. and Kouretas, D. (2017). Variations in Oxidative Stress Levels in 3 Days Follow-up in

Ultramarathon Mountain Race Athletes. *The Journal of Strength and Conditioning Research*, 31, 582-594.

Spencer, M., Lawrence, S., Rechichi, C., Bishop, D., Dawson, B. and Goodman, C. (2004). Time–motion analysis of elite field hockey, with special reference to repeated-sprint activity. *Journal of Sports Sciences*, *22*, 843-850.

Spencer, M.J. and Tidball, J.G. (1992). Calpain concentration is elevated although net calcium-dependent proteolysis is suppressed in dystrophin-deficient muscle. *Experimental Cell Research*, 203, 107-114.

Spencer, M.J., Croall, D.E. and Tidball, J.G. (1995). Calpains are activated in necrotic fibers from mdx dystrophic mice. *Journal of Biological Chemistry*, 270, 10909-10914.

St. Pierre Schneider, B. , Correia, L.A. and Cannon, J.G. (1999). Sex differences in leukocyte invasion in injured murine skeletal muscle. *Research in Nursing and Health*, 22, 243-250.

Staples, A.W., Burd, N.A., West, D.W. Currie, K.D., Aterton, P.J., Moore, D.R., Rennie, M.J., MacDonald, M.J., Baker, S.K. and Phillips, S.M. (2011). Carbohydrate does not augment exercise-induced protein accretion versus protein alone. *Medicine and Science in Sports and Exercise*, 43, 1154-1161.

Staron, R.S., Hikida, R.S., Murray, T.F., Nelson, M.M., Johnson, P. And Hagerman, F. (1992). Assessment of skeletal muscle damage in successive muscle biopsies from strength-trained and untrained men and women. *European Journal of Applied Physiology*, 65, 258-264.

Steinbacher, P. and Eckl, P. (2015). Impact of oxidative stress on exercising skeletal muscle. *Biomolecules*, 5, 356-377.

St-Pierre, J., Buckingham, J.A., Roebuck, S.J. and Brand, M.D. (2002). Topology of superoxide production from different sites in the mitochondrial electron transport chain. *Journal of Biological Chemistry,* 277, 44784-44790.

Stupka, N., and Tiidus, P.M. (2001). Effects of ovariectomy and estrogen on ischemia-reperfusion injury in hindlimbs of female rats. *Journal of Applied Physiology*, 91, 1828-1835.

Stupka, N., Lowther, S., Chorneyko, K., Bourgeois, J.M., Hogben, C. and Tarnopolsky, M.A. (2000). Sex differences in muscle inflammation after eccentric exercise. *Journal of Applied Physiology*, 89, 2325-2332.

Stupka, N., Tarnopolsky, M. A., Yardley, N.J. and Phillips, S.M. (2001). Cellular adaptation to repeated eccentric exercise-induced muscle damage. *Journal of Applied Physiology*, 91, 1669-1678.

Sugama, K., Suzuki, K., Yoshitani K., Shiraishi, K. and Kometani, T. (2012). IL-17, neutrophil activation and muscle damage following endurance exercise. *Exercise Immunology Review*, 18. 116-127.

Sugioka, K., Shimosegawa, Y. And Nakano, M. (1987). Estrogens as natural antioxidants of membrane phospholipid peroxidation. *FEBS Letters*, 210, 37-39.

Sunderland, C.D. and Edwards, P.L. (2017). Activity profile and between-match variation in elite male field hockey. *Journal of Strength and Conditioning Research*, 31, 758-764.

Taillandier , D., Combaret, L., Purch, M.N., Samuels, S.E., Béchet, D. and Attaix, D. (2004). The role of ubiquitin-proteasome-dependent proteolysis in the remodelling of skeletal muscle. *Proceedings of the Nutrition Society*, 63, 357-361.

Takahashi, H., Kuno, S., Miyamoto, T., Yoshioka, H., Inaki, M, Akima, H., Katusta, S. Anno, I. And Itai, Y. (1994). Changes in magnetic resonance images in human

skeletal muscle after eccentric exercise. *European Journal of Applied Physiology,* 60, 408-413.

Takarada, Y. (2003). Evaluation of muscle damage after a rugby match with special reference to tackle plays. *British Journal of Sports Medicine*, *37*, 416-419.

Takekura, H., Fujinami, N., Nishizawa, T., Ogasawara, H. And 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, 571-583.

Talbot, J.A. and Morgan, D.L. (1996). Quantitative analysis of sarcomere non-uniformities in active muscle following a stretch. *Journal of Muscle Research and Cell Motility*, 17, 261-268.

Talbot, J.A. and 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. 237-245.

Tang, J.E., Manolakos, J.J., Kujbida, G.W., Lysecki, P.J., Moore, D.R. and 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, 1132-1138.

Tang, J.E., Moore, D.R., Kujbida, G.W., Tarnopolsky, M.A. and 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. *Journal of Applied Physiology*, 107, 987-992.

Tanner, A. and Day, S. (2017). The effects of a 4-week, intensified training, and competition period on salivary hormones, immunoglobulin A, illness symptoms, and mood state in elite synchornosied swimmers. *Sports*, 5, 64.

Terzis, G., Georgiadis, G., Stratakos, G., Vogiatzis, I., Kavouras, S., Manta, P., Mascher, H. and Blomstrand, E. (2008). Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects. *European Journal of Applied Physiology*, 102, 145-152.

Thomas, D.T., Wideman, L. and Lovelady, C.A. (2010). Effects of calcium and resistance exercise on body composition in overweight premenopausal women. *Journal of the American College of Nutrition*, 29, 604-611.

Thomas, D.T., Wideman, L., Lovelady, C.A., (2011). Effects of a dairy supplement and resistance training on lean mass and insulin-like growth factor in women. *International Journal of Sports Nutrition and Exercise Metabolism*, 21, 181-188.

Thomas, K., Dent, J., Howatson, G. and Goodall, S. (2017). Etiology and Recovery of Neuromuscular Fatigue after Simulated Soccer Match Play. *Medicine and Science in Sports and Exercise*, *49*, 955-964.

Thomas, K., Morris, P. and Stevenson, E. (2009). Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sports drinks. *Applied Physiology Nutrition and Metabolism,* 34, 78-82.

Thompson, D., Nicholas, C.W., Wiliams, C. (1999). Muscular soreness following prolonged intermittent high-intensity shuttle running. *Journal of Sports Sciences*, 17, 387-395.

Thompson, H. and Scordilis, S. (1994). Ubiquitin changes in human biceps muscle following exercise induced muscle damage. *Biochemical and Biophysical Research Communications*, 204, 1193-1198.

Thomson, J. S., Ali, A. and Rowlands, S. (2011). Leucine-protein supplemented recover feeding enhances subsequent cycling performance in well-trained men. *Applied Physiology Nutrition and Metabolism*, 36, 242-253.

Thorlund, M.B., Aagard, P., Madsen, K., (2009). Rapid muscle force capacity changes after soccer match play. *International Journal of Sports Medicine*, 30, 273-278.

Thorpe, R. and Sunderland, C. (2012). Muscle damage, endocrine, and immune marker response to a soccer match. *Journal of Strength and Conditioning Research*, 26, 2788-2790.

Thorpe, R.T., Strudwick, A.J., Buchheit, M., Atkinson, G., Drust, B. and Gregson, W. (2015). Monitoring fatigue during the in-season competitive phase in elite soccer players. *International Journal of Sports Physiology and Performance*, 10, 958-964.

Tidball, J.G. (2005). Inflammatory processes in muscle injury and repair. *American Journal of Physiology, Regulatory Integrative and Comparative Physiology,* 288, R345-353.

Tiidus, P.M. (1995). Can estrogens diminish exercise induced muscle damage? *Canadian Journal of Applied Physiology*, 20, 26-38.

Tiidus, P.M. (2001). Oestrogen and sex influence on muscle damage and inflammation: evidence from animal models. *Current Opinion in Clinical Nutrition and Metabolic Care*, 4, 509-513.

Tiidus, P.M. (2003). Influence of estrogen on skeletal muscle damage, inflammation, and repair. *Exercise and Sport Sciences Reviews*, 31, 40-44.

Tiidus, P.M. (2011). Influence of estrogen on muscle plasticity. *Brazillian Journal of Biomotricity,* 5, 143-155.

Tiidus, P.M., Holden, D., Bobmardier, E., Zajchowski, S., Enns, D. and Belcastro, A. (2001). Estrogen effect on post-exercise skeletal muscle neutrophil infiltration and calpain activity. *Canadian Journal of Physiology and Pharmacology*, 79, 400-406.

Tipton, K.D., Elliott, T.A., Cree, M.G., Aarsland, A.A., Sanford, A.P. and Wolfe, R.R. (2007). Stimulation of net protein synthesis by whey protein ingestion before and after exercise. *American Journal of Physiology Endocrinology and Metabolism*, 292, E71-E76.

Tipton, K.D., Elliott, T.A., Cree, M.G., Wolf, S.E., Sanford, A.P. and 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: 2073-2081

Tipton, K.D., Ferrando, A.A., Philips, S.M., Doyle, D. Jr., Wolfe, R.R. (1999). Postexercise net protein synthesis in human muscle from orally administered amino acids. *American Journal of Physiology Endocrinology and Metabolism,* 276, E628-E634.

Tipton, K.D., Hamilton, D.L. and Gallagher, I.J. (2018). Assessing the role of muscle protein breakdown in response to nutrition and exercise in humans. *Sports Medicine*, 48, 53-64.

Tipton, K.D., Rasmussen, B.B., Miller, S.L., Wolf, S.E., Owens-Stovall, S.K., Petrini, B.E and Wolfe, R.R. (2001). Timing of amino acid-carbohdyrate ingestion alters anabolic response of muscle to resistance exercise. *American Journal of Physiology*, 281, E197-E206.

Toivola, D.M., Tao, G-Z., Habtezion, A., Liao, J. and Bishr Omary, M. (2005). Cellular integrity plus: organelle-related and protein-targeting functions of intermediate filaments. *Trends in Cell Biology,* 15, 608-617.

Tomiya, A., Aizawa, T., Nagatomi, R., Sensui, H. and Kokubun, S. (2004). Myfibers express IL-6 after eccentric exercise. *The Amercian Journal of Sports Medicine*, 32, 503-508.

Trappe, T.A., Carrithers, J.A., White, F., Lambert, C.P., Evans, W.J. and Dennis, R.A. (2002). Titin and nebulin content in human skeletal muscle following eccentric resistance exercise. *Muscle and Nerve*, 25, 569-579.

Trost, Z., France, C.R. and Thomas, J.S. (2011). Pain-related fear and avoidance of physcial exertion following delayed-onset muscle soreness. *Pain,* 152, 1540-1547.

Tsatalas, T., Giakas, G., Spyropoulos, G., Sideris, V., Lazaridis, S., Kotzamanidis, C. and Koutedakis, Y. (2013). The effects of eccentric exercise-induced muscle damage on running kinematics at different speeds. *Journal of Sports Sciences,* 31, 288-298.

Tvede, N., Pedersen, B.K., Hansen, F.R., Bendix, T., Christensen, L.D., Galbo, H. and Halkjaer-Kristensen, J. (1989). Effect of physical exercise on blood mononuclear cell subpopulations and in vitro proliferative responses. *Scandanavian Journal of Immunology*, 29, 383-389.

Twist, C. and Eston, R. (2005). The effects of exercise-induced muscle damage on maximal intensity intermittent exercise performance. *European Journal of Applied Physiology*, 94, 652-658.

Urso, M.L. and Clarkson, P.M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, 189, 41-54.

Valentine, R.J., Saunders, M.J., Todd, M.K. and 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, 363-378.

Van der Meulen, J.H. Kuipers, H. and Drukker, J. (1991). Relationship between exercise-induced muscle damage and enzyme release in rats. *Journal of Applied Physiology*, 71, 999-1004.

van Loon, J.C. (2014). Is there a need for protein ingestion during exercise? *Sports Medicine,* 44, S105-111.

van Loon, L.J., Greenhaff, P.L., Constantin-Teodosiu, D., Saris, W.H. and Wagenmakers, A.J. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *Journal of Physiology*, 536, 295-304.

Verburg, E., Murphy, R.M., Stephenson, D.G and Lamb, G.D. (2005). Disruption of excitation-contraction coupling and titin by endogenous Ca^{2+}-activated proteases in toad muscle fibres. *The Journal of Physiology*, 564, 775-790.

Vescovi, J.D. (2016). Locomotor, heart-rate, and metabolic power characteristics of youth women's field hockey: Female Athletes in Motion (FAiM) study. *Research Quarterly for Exercise and Sport*, 87, 68-77.

Vijayan, K., Thompson, J.L., Norenberg, K.M., Fitts, R.H. and Riley, D.A. (2001). Fiber-type susceptibility to eccentric contraction-induced damage of hindlimb-unloaded rat AL muscles. *Journal of Applied Physiology*, 90, 770-776.

Vissing, K., Overgaard, K., Nedergaard, A., Fredsted, A. and 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, 323-332.

Voges, D., Zwickl, P. And Baumeister, W. (2000). The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annual Review of Biochemistry,* 68, 1015-1068.

Volek, J.S., Volk, B.M., Gomez, A.L., Kunces, L.J., Kupchak, B.R., Freidenreich, D.J., Aristizabal, J.C., Saenz, C., Dunn-Lewis, C., Ballard, K.D., Quann, E.E., Kawiecki, D.L., Flanagan, S.D., Comstock, B.A., Fragala, M.S., Earp, J.E., Fernandez, M.L., Bruno, R.S., Ptolemy, A.S., Kelogg, M.D., Maresh, C.M. and Kraemer, W.J. (2013). Whey protein supplementation during resistance training augments lean body mass. *Journal of the American College of Nutrition*, 32, 122-135.

Vollaard, N.B., Shearman, J.P. and Cooper, C.E. (2005). Exercise-induced oxidative stress. *Sports medicine*, 35, 1045-1062.

Volpi, E., Lucidi, P., Bolli, G.B., Santeusanio, F. And De Feo, P. (1998). Sex differences in basal protein kinetics in young adults. *Journal of Clinical Endocrinology and Metabolism*, 83, 4363-4367.

Wadley, A.J., Killer, S.C., Svendsen, I.S. and Gleeson, M. (2015). The impact of intensified training with a high or moderate carbohydrate feeding strategy on resting and exercise-induced oxidative stress. *European Journal of Applied Physiology,* 115, 1757-1767.

Warren, G.L., Ingalls, C.P., Lowe, D.A. and Armstrong, R.B. (2001). Excitation-countraction uncoupling: major role in contraction-induced muscle injury. *Exercise and Sport Sciences Reviews*, 29, 82-87.

Warren, G.L., Lowe, D.A. and Armstrong, R.B. (1999). Measurement tools used in the study of eccentric contraction-induced injury. *Sports Medicine,* 27, 43-59.

Warren, G.L., Lowe, D.A., Hayes, D.A., Karwoski, C.J., Prior, B.M. and Armstrong, R.B. (1993). Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *The Journal of Physiology*, 468,.487-499.

Warren, G.L., O'Farrell, L., Rogers, K.R., Billings, K.M., Sayers, S.P. and Clarkson, P.M. (2006). CK-MM auto-antibodies: prevalance, immune complexes, and effects on CK clearance. *Muscle and Nerve*, 34, 335-346.

Watson. P., Love, T.D., Maughan, R.J. and Shirreffs, S.M. (2008) A comparison of the effects of milk and a carbohydrate-electrolyte drink on the restoration of fluid balance and exercise capacity in a hot, humid environment. *European Journal of Applied Physiology,* 104, 633–642

Webber, L.M., Byrnes, W.C., Rowland, T.W. and Foster, V.L. (1989). Serum creatine kinase activity and delayed onset of muscle soreness in prepubescent children: a preliminary study. *Pediatric Exercise Science*, 1, 351-359.

Welle, S., Thornton, C. Statt, M., McHenry B. (1994). Postprandial myofibrillar and whole body protein synthesis in young and old human subjects. *American Journal of Physiology Endocrinology and Metabolism*, 267, E599-604.

Werstein, K.M. and Lund, R.J. (2012). The effects of two stretching protocols on the reactive strength index in female soccer and rugby players. *The Journal of Strength and Conditioning Research*, 26, 1564-1567.

West, D.W., Burd, N.A., Churchward-Venne, T.A., Camera, D.M., Mitchell, C.J., Baker, S.K., Hawley, J.A., Coffey, V.G. and Phillips, S.M. (2012). Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state. *Journal of Applied Physiology* 112, 1805-1813.

West, D.W., Burd, N.A., Coffey, V.G., Baker, S.K., Burke, L.M., Hawley, J.A., Moore, D.R., Stellingwerff, T. and Phillips, S.M. (2011). Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *The American Journal of Clinical Nutrition*, 94, 795-803.

White, J.P., Wilson, J.M., Austin, K.G., Greer, B.K., St. John, N. and Panton, L.B. (2008). Effect of carbohydrate-protein supplement timing on acute exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition*, 5, 1-7.

White, K.M, Bauer, S.J., Hartz, K.K. and Baldridge, M. (2009). Changes in body composition with yogurt consumption during resistance training in women. *Journal of the International Society of Sports Nutrition*, 19, 18-33.

Whiting, K.P., Restall, C.J. and Brain, P.F. (2000). Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms. *Life Sciences*, 67, 743-757.

Wiewelhove, T., Raeder, C., Meyer, T., Kellmann, M., Pfeiffer, M. and Ferrauti, A. (2015). Markers for routine assessment of fatigue and recovery in male and female team sport athletes during high-intensity interval training. *PloS one*, 10, .e0139801.

Wilborn, C.D., Taylor, L.W., Outlaw, J., Williams, L., Campbell, B., Foster, C.A., Smith-Ryan, A., Urbina, S. and Hayward, S. (2013). The effects of a pre- and post-exercise whey vs casein protein consumption on body composition and performance

measures in collegiate female athletes. *Journal of Sports Science and Medicine*, 12, 74-79.

Wilkes, E.A., Selby, A.L., Atherton, P.J., Patel, R., Rankin, D., Smith, K. and Rennie, M.J.(2009). Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. *American Journal of Clinical Nutrition*, 90, 1343-1350.

Wilkinson, D.J., Hossain, T., Hill, D.S., Phillips, B.E., Crossland, H., Williams, J., Loughna, P., Churchward-Venne, T.A., Breen, L., Phillips, S.M. and Etheridge, T. (2013). Effects of leucine and its metabolite beta-hydroxy-beta-methylbutyrate on human skeletal muscle protein metabolism. *Journal of Physiology*, 591, 2911-2923.

Wilkinson, M. (2014). Distinguishing between statistical significance and practical/clinical meaningfulness using statistical inference. *Sports Medicine,* 44, 295-301.

Wilkinson, S.B., Phillips, S.M., Atherton, P.J., Patel, R., Yarasheski, K.E., Tarnopolsky, M.A. and Rennie, M.J. (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *The Journal of Physiology*, *586*, 3701-3717.

Wilkinson, S.B., Tarnopolsky, M.A., MacDonald, M.J., MacDonald, J.R., Armstrong, D. And Phillips, S.M. (2007). Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. *The American Journal of Clinical Nutrition*, 85, 1031-1040.

Willoughby, D., Rosene, J. and Myers, J. (2003b). HSP-72 and ubiquitin expression and caspas-activity after a single bout of eccentric exercise. *Journal of Exercise Physiology*, 6, 96-104.

Willoughby, D., Taylor, M. and Taylor, L. (2003a). Glucocorticoid receptro and ubiquitin expression after repeated eccentric exercise. *Medicine and Science in Sports and Exercise*, 35, 2023-2031.

Willoughby, D.S., VanEnk, C. and Taylor, L. (2003c). Effects of concentric and eccentric contractions on exerciseinduced muscle injury, inflammation, and serum il-6. *Journal of Exercise Physiology online*, *6*.

Wiseman, H. And Quinn, P. (1994). The antioxidant action of synthetic oestrogens involves devreased membrane fluidity: relevance to their potential use as an anticancer and cardioprotective agents compared to tamoxifen. *Free Radical Research*, 21, 187-194.

Witard, O.C., Jackman, S.R., Breen, L., Smith, K., Selby, A. and Tipton, K.D. (2014). Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *American Journal of Clinical Nutrition,* 99, 86-95.

Witard, O.C., Tieland, M., Beelen, M., Tipton, K., van Loon, L. and Koopman, R. (2009). Resistance exercise increases postprandial muscle protein synthesis in humans. *Medicine and Science in Sports and Exercise,* 41, 144-154.

Witard, O.C., Wardle, S.L., Macnaughton, L.S., Hodgson, A.B. and Tipton, K.D. (2016). Protein considerations for optimising skeletal muscle mass in healthy young and older adults*. Nutrients,* 8, 181.

Wojcik, J.R., Walberg-Rankin, J., Smith, L.L. and Gwazdauskas, F.C. (2001). Comparison of carbohdyrate and milk-based beverages on muscle damage and glycogen following exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 11, 406-419.

Wolff, S.P. (1994). Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hypoperoxides. *Methods in Enzymology*, 233:183–189.

Yadavalli, V.K., Forbes, J.G. and Wang, K. (2009). Nanomechanics of full-length nebulin: an elastic strain gauge in the skeletal muscle sarcomere. *Langmuir*, *25*, 7496-7505.

Yamin, C., Duarte, J.A.R., Oliveira, J.M.F., Amir, O., Sagiv, M., Eynon, N., Sagiv, M., Amir, R.E. (2008). IL6 (-174) and TNFA (-308) promoter polymorphisms are associated with systemic creatine kinase response to eccentric exercise. *European Journal of Applied Physiology*, 104, 579-586.

Yanagida, T., Arata, T. and Oosawa, F. (1985). Sliding distance of actin filament induced by a myosin crossbridge during one ATP hydrolysis cycle. *Nature*, 316, 366-369.

Yasuda, T., Sakamoto, K., Nosaka, K., Wada, M. and Katsuta, S. (1997). Loss of sarcoplasmic reticulum membrane integrity after eccentric contractions. *Acta Physiologica Scandanavica*, 161, 581-582.

Yeung, E.W. and Allen, D.G. (2004). Stretch-activated channels in stretch-induced muscle damage: role in muscular dystrophy. *Clinical and Experimental Pharmacology and Physiology*, 31, 551-556.

Yeung, E.W., Head, S.I. and Allen, D.G. (2003). Gadolinium reduces short term stretch-induced muscle damage in isolated mdx mouse muscle fibres. *Journal of Physiology*, 552, 449-458.

You, T., Goldfarb, A.H., Bloomer, R.J., Nguyen, L., Sha, X. And McKenzie, M.J. (2005). Oxidative stress response in normal and antioxidant supplemented rats to a downhill run: changes in blood and skeletal muscles. *Canadian Journal of Applied Physiology*, 30, 677-689.

Young, D. (2003). Data presented by Young, D (2008). An analysis of hurling. Does training meet the demands of the game? Paper presented at the Gaelic Athletic Association Annual Conference, Croke Park.

www.gaa.ie/content/documents/publications/coaching_and_games/conference_2008/damien_young_demands_of_the_game_100123150634.pdf

Young, W.B., Hepner, J. and Robbins, D.W. (2012). Movement demands in Australian rules football as indicators of muscle damage. *The Journal of Strength and Conditioning Research,* 26, 492-496.

Young, W.B., Miller, I.R. and Talpey, S.W., (2015). Physical Qualities Predict Change-of-Direction Speed but Not Defensive Agility in Australian Rules Football. *Journal of Strength & Conditioning Research*, 29, 206-212.

Yu, J., Malm, C. and 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, 29-34.

Yu, J.G. and Thornell, L.E. (2002). Desmin and actin alterations in human muscles affected by delayed onset muscle soreness: a high resolution immunocytochemical study. *Histochemisty and Cell Biology*, 118, 171-179.

Yu, J.G., Carlsson, L. and Thornell, L.E. (2004). Evidence for myofibril remodeling as opposed to myofibril damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study. *Histochemistry and Cell Biology*, 121, 219-227.

Yu, J.G., Liu, J., Carlsson, L., Thornell, L.E. and Stål, P.S. (2013). Re-evaluation of sarcolemma injury and muscle swelling in human skeletal muscles after ecentric exercise. *Plos One*, 8, e62056.

Yu, L.C., Dowben, R.M. and Kornacker, K. (1970). The molecular mechanism of force generation in striated muscle. *Proceedings of the National Academy of Science*, 66, 1199-1205.

Zavorsky, G.S., Kubow, S., Grey, V., Riverin, V. and Lands, L.C. (2007). An open-label dose-response study of lymphocyte glutathione levels in healthy men and

women receiving pressurized whey protein isolate supplements. *International Journal of Food Science and Nutrition,* 58, 429-436.

Zhang, B.T., Yeung, S.S., Allen, D.G., Qin, L. And Yeung, E.W. (2008). Role of the calcium-calpain pathway in cytoskeletal damage after eccentric contractions. *Journal of Applied Physiology*, 105, 352-357.

Zulueta, A., Maurizi, A. Frígola, A., Esteve, M.J., Coli, R. and Burini, G. (2009). Antioxidant capacity of cow milk, whey and deproteinized milk. *International Dairy Journal,* 19, 380-385.