



**EFFECT OF A MILK-BASED PROTEIN CARBOHYDRATE BEVERAGE ON ATTENUATING
DELAYED ONSET MUSCLE SORENESS, MAKERS OF MUSCLE DAMAGE AND INFLAMMATION,
AND SPRINT PERFORMANCE FOLLOWING GAELIC FOOTBALL MATCH PLAY**

Kevin. J. Reilly, BSc.

June 28, 2019

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Kevin J. Reilly, BSc.

Submitted for the award of MSc.

Dublin City University

School of Health and Human Performance

Supervisor: Prof. Niall M. Moyna

Submitted: Jun 28, 2019

Volume 1 of 1

Declaration

I hereby certify that this material, which I now submit for assessment of the program of study leading to the award of MSc. is entirely my own work, that I have exercised reasonable care to ensure that the work is original and, to the best of my knowledge, does not breach any law of copyright and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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ABSTRACT

Purpose

To compare the effect of milk based protein-carbohydrate beverage (MP-CHO) and a carbohydrate only beverage (CHO) immediately following Gaelic football match play on markers of muscle damage and inflammation, muscle soreness and sprint performance.

Methods

Male Gaelic football players with a mean age of 17.4 years played a competitive Gaelic football game after which they consumed a 500 mL of either a MP-CHO beverage or a CHO only beverage. Blood samples were taken and subjective muscle soreness was measured before, immediately post-game and at 12 h, 36 h and 60 h post-game. Sprint performance was measured pre-game and at 12 h, 36 h and 60 h post-game. Heart rate and movement patterns were continuously measured using telemetry and GPS tracking, respectively.

Results

Measured activity characteristics and impact zones were similar in MP-CHO and CHO during match-play. No significant main effect for group or interaction effects between time and group was observed for CK, circulating leukocytes, muscle soreness or 5 m and 20 m sprint times. Creatine kinase (CK) levels were elevated ($p < 0.001$) in MP-CHO and CHO immediately after, and 12 h post-game. Muscle soreness was elevated at 12 h ($p < 0.001$) and 36 h ($p < 0.01$) in MP-CHO and at 12 h ($p < 0.001$) in CHO compared to pre-game. Compared to pre-game values, circulating leukocyte increased significantly in both MP-CHO and CHO immediately post-game, and decreased significantly in both experimental conditions at 12 h, 36 h and 60

h post-game. There was a decrease ($p<0.001$) in 5 m and 20 m sprint times at 12 h, 36 h and 60 h in MP-CHO and CHO.

Conclusion:

Consuming MP-CHO or an isocaloric CHO beverage immediately following Gaelic football match play has no effect on attenuating DOMS, decrements in 5 m and 20 m sprint times or circulating levels of CK and leukocytes.

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Table of Contents

	Page Number
Title Page	i
Declaration	iii
Abstract	iv
Acknowledgements	vi
Table of Contents	vii
List of Figures	ix
List of Tables	x
Definition of Terms	xi
CHAPTER I INTRODUCTION	
Introduction	1
Study Aims	4
Study Hypotheses	4
CHAPTER II REVIEW OF LITERATURE	
Muscle structure	5
Extracellular matrix	7
Exercise induced muscle damage	9
Muscle soreness	13
Creatine kinases	14
Recovery nutrition	18
Bovine based supplementation and EIMD	25
CHAPTER III METHODS	
Participants	30
Study overview	30
Nutritional supplement	31
Height and weight	32
Running velocity	32
Movement patterns and gravitational forces	33
Movements classification system	34
Impacts	35
Heart rate	36
Muscle soreness	36
Maximal aerobic capacity	36
Open circuit spirometry	37
Blood sampling and analysis	37
Statistical Analysis	38
CHAPTER IV RESULTS	
Physical and physiological characteristics	39
Activity characteristics	39

Impact zones	40
Creatine kinase	41
Muscle soreness	41
Circulating leukocytes	42
Sprint speed	43
CHAPTER V DISCUSSION	
Discussion	44
Study limitations	51
Practical applications	51
BIBLIOGRAPHY	52
APPENDIX I	
Ethics submission	73
APPENDIX II	
Summary of studies examining the effect of milk supplementation and EIMD	91

List of Figures

Figure 2.1: Basic structure of skeletal muscle	5
Figure 2.2: Structure of a muscle fibre	6
Figure 2.3: Sarcomere structure	7
Figure 2.4: Extracellular matrix	8
Figure 2.5: Costamere structure	9
Figure 2.6: Satellite cell location	12
Figure 3.1: Study protocol	31
Figure 3.5: Muscle soreness visual analogue scale	36

List of Tables

Table 2.1	Studies examining the effect of milk based supplementation on EIMD	35
Table 3.1	Speed zone classifications	35
Table 3.2	Impact zones	35
Table 4.1	Physical and physiological characteristics	39
Table 4.2	Activity characteristics during Gaelic football match play	40
Table 4.3	Average number of impact zone entries during Gaelic football match play	40
Table 4.4	Serum levels of creatine kinase pre-game and at 12 h, 36 h and 60 h post-game	41
Table 4.5	Muscle soreness pre-game and at 12 h, 36 h and 60 h post-game	42
Table 4.6	Absolute number of circulating leukocytes pre-game and at 12 h, 36 h and 60 h post-game	42
Table 4.7	Sprint performance pre-game and at 12 h, 36 h and 60 h post game	43

Definition of Terms

AFL	Australian football league
BCAA	Branch chain amino acids
CHO	Carbohydrate
CK	Creatine kinase
DOMS	Delayed onset muscle soreness
ECM	Extracellular matrix
EIMD	Exercise induced muscle damage
FSR	Fractional synthesis rate
GAA	Gaelic Athletic Association
GPS	Global positioning system
h	Hour
MVC	Maximal voluntary contraction
MP-CHO	Milk based protein carbohydrate supplementation
MU	Motor unit
LDL	Lactate dehydrogenase
PAX7	Transcription factor paired box 7
ROS	Reactive oxygen species
RPE	Rating of perceived exertion
BMI	Body mass index
VAS	Visual analogue scale
$\dot{V}O_2\text{max}$	Maximal aerobic capacity

NMF Neuromuscular fatigue

CHAPTER 1

INTRODUCTION

Exercise-induced muscle damage (EIMD) caused by unaccustomed, eccentric exercise (Clarkson et al, 2002, Pereira Panza et al., 2015) or blunt force trauma (McLellan CP et al., 2010) can result in loss of both contractile protein and cell membrane integrity, altered neuromuscular recruitment, myofibrillar and cytoskeleton disruption, altered excitation-contraction coupling and inflammatory cell infiltration (Hyldahl et al., 2014; Tidball, 2011). Symptoms including prolonged decrements in the force generating capacity of skeletal muscle and delayed onset muscle soreness (DOMS) may present for 7-14 days following the initial exercise bout (Doma et al., 2018; Owens et al., 2019).

A number of therapeutic (Dupuy et al., 2018) pharmaceutical (Ranchordas et al., 2017; Lundberg et al., 2018) and nutritional interventions (Owens et al., 2019) have been used to limit ultra-structural damage and attenuate the reductions in skeletal muscle force producing capability. In particular, protein supplementation or a combination of protein and carbohydrates (CHO) by triggering a net positive protein balance may help to limit ultra-structural damage and hasten the recovery of muscle function (Sousa et al., 2014; Pasiakos et al., 2015; Tipton, 2015).

Bovine milk contains a mixture of protein, CHO and fat and has a number of characteristics that theoretically makes it a convenient, accessible, low-cost beverage to blunt contractile protein loss and hasten the recovery of muscle function (Tipton et al.,

2004; Roy, 2008). Casein and whey protein are present in bovine milk in a ratio of 3:1. Whey protein is rapidly digested and leads to a large, transient rise in aminoacidemia, particularly branched chain amino acids (BCAA) that stimulates the synthesis of muscle proteins (Brown et al., 2018; Ra et al., 2018; VanDusseldorp et al., 2018). In contrast, casein protein results in a slower and more sustained elevation of blood amino acid concentrations, particularly leucine, and suppresses proteolysis. The high concentration of electrolytes found in milk also makes it ideal to promote fluid recovery and electrolyte balance following exercise.

Previous research has indicated that consumption of 500 mL of milk following a laboratory based muscle damage protocol is beneficial for adult male athletes (Cockburn et al., 2012). The addition of CHO to the ingestion of a whole protein supplement or amino acids has an additive effect to promoting physiological adaptations following exercise due in part to the synergistic effect on plasma insulin responses (Rasmussen et al., 2000; Elliot et al., 2006).

The majority of studies that have examined the effect of bovine milk-based protein-CHO supplementation (MP-CHO) on limiting myofibrillar and cytoskeleton disruption and muscle performance decrements in response to EIMD have used laboratory-based protocols to induce muscle damage. Furthermore, studies demonstrating a benefit of MP-CHO supplements on limiting decrements in muscle function have primarily measured concentric peak torque using isokinetic dynamometry (Cockburn et al., 2008; Cockburn et al., 2013; Rankin et al., 2015). The findings from these

laboratory-based studies may have limited external validity when extrapolated to athletes involved in dynamic invasion team sports such as rugby, soccer and Australian football. The putative benefits of a MP-CHO beverage on markers of muscle damage, subjective muscle soreness, leukocyte trafficking, and indices of muscle performance needs to be examined in a dynamic sports setting.

Gaelic football is an invasion field-based team sport that can be described as a hybrid of soccer, rugby, Australian football and basketball. It is characterized by repeated bouts of high-intensity short-duration activities involving accelerations, decelerations and directional changes superimposed on the technical activities. Many of the short-duration high intensity activities involve muscle damaging eccentric muscle loading. Gaelic football also involves frequent high-impact body collisions that may contribute to skeletal muscle damage (Murphy et al., 2012).

The direct assessment of EIMD involves the morphological evaluation of the skeletal muscle that requires serial muscle biopsies. Given the invasive nature of muscle biopsies, indirect markers have been used extensively as surrogate measures in both laboratory and field-based studies of EIMD. These include the myocellular protein, myoglobin (Mb) and muscle-specific derived enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH) (Brancaccio et al., 2008). In addition, active and passive muscle soreness (Vickers, 2001) and decrements in skeletal muscle function (West et al., 2014) have also been used as surrogate measures of EIMD.

The purpose of this study is to compare the effects of a MP-CHO beverage and an energy-matched carbohydrate only beverage (CHO) immediately following Gaelic football match play on markers of muscle damage and inflammation, muscle soreness and sprint performance.

Study Aims

1. To compare the effects of MP-CHO and an energy-matched CHO beverage on circulating levels of CK immediately before and after GF match play
2. To compare the effects of MP-CHO and an energy-matched CHO beverage on leukocyte trafficking before and after GF match play
3. To compare the effects of MP-CHO and an energy-matched CHO beverage on muscle soreness before and after GF match play
4. To compare the effects of MP-CHO and an energy-matched CHO beverage on 10-m and 20-m sprint speed before and after GF match play

Study Hypothesis

1. Circulating levels of CK will be significantly lower in MP-CHO than CHO following Gaelic football match play
2. Circulating levels of leukocytes will be significantly lower in MP-CHO than CHO following Gaelic football match play
3. Muscle soreness levels will be significantly greater in CHO than MP-CHO following Gaelic football match play

4. Decrements in running velocity will decrease to a significantly greater extent in CHO than MP-CHO following Gaelic football match play

CHAPTER III

REVIEW OF LITERATURE

Muscle Structure

Skeletal muscle comprises approximately 40% of the total body mass and has a turnover rate of 1-2% per day (Mc Comas et al., 2006; Francaux et al., 2019). The primary role of skeletal muscle is to generate force to produce movement, provide structural support, maintain posture and adapt to external stimuli. In addition to representing the largest protein/amino acid reservoir in the human body, skeletal muscle is also the largest metabolically active tissue, glucose disposal site and fuel reservoir for other organs in fasting and pathological conditions. Skeletal muscle also possesses marked adaptive and regenerative capacity in response to exercise and injury (Lieber et al., 2017).

Structurally, skeletal muscle consists of multinucleated contractile myofibrils, and elastic components, supported by extracellular matrix. Each muscle is divided into compartments with each compartment containing a bundle of muscle fibres (fasciculus) surrounded by a layer of connective tissue, the perimysium (Figure 2.1.)

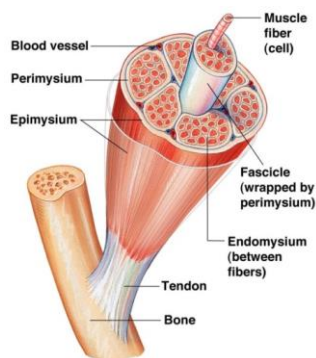


Figure 2.1 Basic structure of skeletal muscle

Individual muscle fibers comprised of multinucleated myofibrils, lie parallel to one another (Figure 2.2). Each individual muscle fibre is enclosed in a tubular cellular membrane, the sarcolemma, and surrounded by a sheath of collagenous endomysium (Schneck, 1992). Myofibrils contain sarcomeres, the contractile unit of a muscle fiber. Sarcomeres extend between two successive Z discs (Figure 2.3) and contain actin and myosin protein myofilaments that interact to produce muscle shortening or lengthening (Schneck, 1992). The Z-discs provide structural integrity by anchoring the sarcomeres. Desmin is a muscle-specific, type III intermediate filament that maintains the longitudinal and transverse passive mechanical properties of the fibre by integrating the sarcolemma, Z disk and nuclear membrane. Interestingly, the widest Z lines are found in slow twitch muscle fibers whereas the thinnest Z lines are present in fast twitch fibers which generate the highest shortening velocities (1).

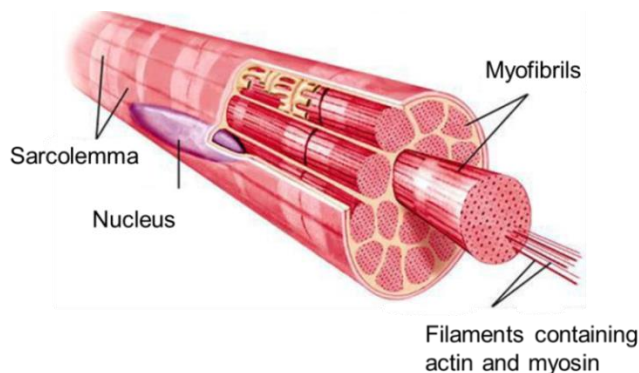


Figure 2.2. Structure of a muscle fibre

Sarcomeres are comprised of actin and myosin filaments, aligned in a particular sequence (Figure 2.3). The I band contains actin filaments whereas the A band contains both actin and myosin filaments. The H zone is the central portion of the A band and

contains only myosin filaments. The M line which is located in the centre of the A band helps to stabilize the sarcomere.

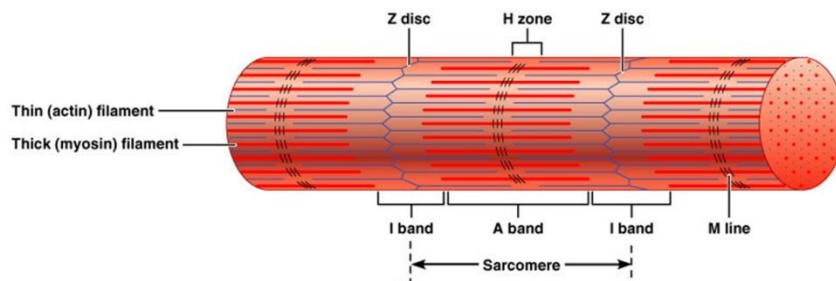


Figure 2.3. Sarcomere structure

Extracellular Matrix

The ECM is the non-cellular component present within skeletal muscle cells that connects reticular lamina, basal lamina, sarcolemma, and the cytoskeletal structure and is critical for force transmission and for the passive elastic response of skeletal muscle (Wang et al., 2016). It consists of the epimysium surrounding the entire muscle, the perimysium dividing the fascicles within the muscle, and the endomysium surrounding the individual muscle fibers. Through a series of networks containing collagens, laminins, fibronectin, and proteoglycans, the ECM provides mechanical support and protection to individual myofibers during contractions (Wang et al., 2016). The transmission of contractile force is facilitated by the mechanical link between membrane spanning integrin, dystroglycan and proteoglycan complexes and the muscle fibre cytoskeleton (Figure 2.4). In addition to providing mechanical support and protection to individual myofibers during contractions, the ECM provides muscle tissue with elastic properties, participates in the transmission of force from the myofiber to the tendon, serves as a

basic mechanical support for nerves and vessels present in skeletal muscle tissue, and determines the spatial barrier between endothelium and muscle cell surface. A dynamic balance exists among deposition, remodelling, and degradation of the ECM, particularly in response to mechanical loading (Wang et al., 2016).

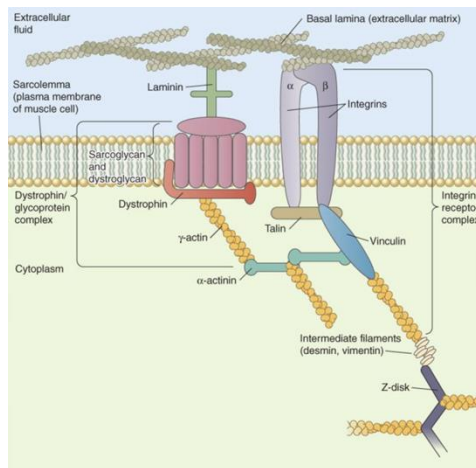


Figure 2.4. Extracellular matrix

The contractile machinery of the skeletal muscle cells is connected to the ECM by costameres, transverse structures organized in a rib-like pattern, which lie at the sarcolemma over the Z and M lines of nearby myofibrils (Figure 2.4). Dystroglycan complex integrins connect the contractile apparatus to the costameres at the sarcolemma. Costameres along with ECM provide integrity to the skeletal muscle and play an important role in stabilizing myofilaments (Mathes, et al., 2019)

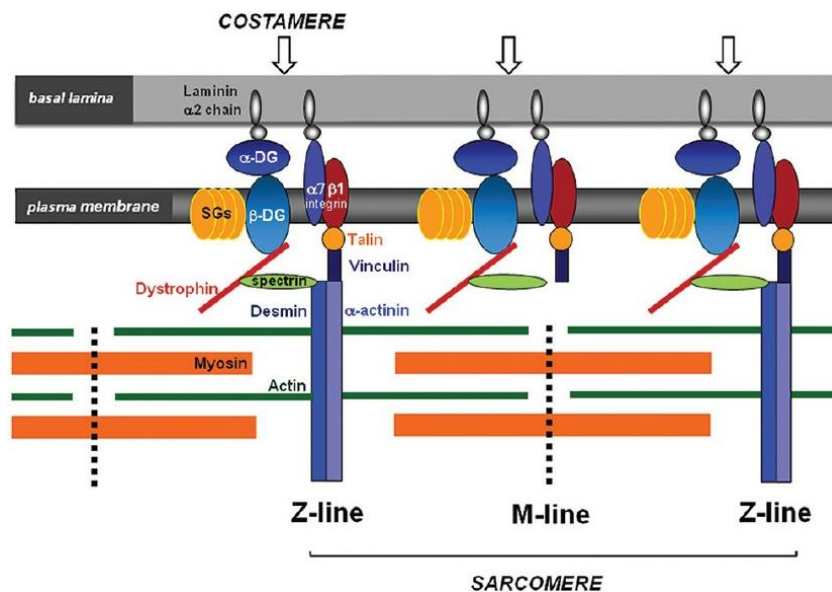


Figure. 2.4 Costamere structure

Exercise Induced Muscle Damage

Invasion team sports such as soccer, rugby league, rugby union, Australian football, ice hockey and Gaelic football involve eccentric loading of musculature through considerable acceleration, deceleration and directional changes. Activities involving eccentric muscle action during which the muscle is lengthened while it is active can result in physical damage to the macro-and microstructure of muscle fibres, disruption to cytoskeletal elements, a loss of membrane integrity, swollen mitochondria, displaced organelles, disrupted calcium signalling, impaired excitation-contraction coupling and activation of several muscle protein degradation pathways (Hyldahl et al., 2014; Douglas et al., 2017, Naughton et al., 2018). Furthermore, frequent high-impact body collisions associated with participation in many of these sports also results in exposure to impact related muscular damage.

Structural disruption of sarcomeres may be related to the heterogeneity in their abilities to resist eccentric actions (Morgan, 1990; Friden et al., 2001; Hyldahl et al., 2014). When stretched beyond the optimal overlap, weaker sarcomeres pop, resulting in Z disk streaming with slight myofibrillar disarray and mild muscle injury. In contrast, more severe injury is associated with Z-disc disruption, thickening, focal losses and displacement into adjacent sarcomeres (Sorichter et al., 1999). Fast twitch fibers are more sensitive than slow twitch fibres to Z-disk streaming. This is due in part to the fact that fast twitch fibres have thinner Z-lines and generate the very high shortening velocities (Raastad et al., 2010).

It has been hypothesized that lateral force transmission between adjacent muscle fibres and subsequent loss in muscle force following muscle damage may be due to disruption to the cytoskeletal and extracellular matrix proteins (Hyldahl et al., 2014). Inflammation and increased reactive oxygen species (ROS) production may further degrade the muscle architecture in the days following muscle-damaging exercise (Qaisar et al., 2016). Such changes may also reduce the ability to generate power, potentiate nerve endings and perceptions of pain, and increase the release of intramuscular proteins.

Skeletal muscle has a remarkable ability to remodel in response to injury (Baumert et al., 2016). This involves a tightly regulated inflammatory response that is initiated to clear the injured area in-preparation for repair and regeneration (Peak et al., 2005; Tidball, 2011). Typically, inflammatory cells, cytokines and growth factors accumulate at

the damaged site to initiate the repair process. In addition to releasing proteolytic enzymes to remove damaged tissue, inflammatory mononuclear phagocytic cells release ROS that further degrade muscle proteins that may contribute to delayed muscle injury (Pereira Panza et al., 2015).

The time-course of the inflammatory response varies and is dependent on a number of factors including the type, duration and intensity of exercise, the muscle group damaged and the method of detection. Neutrophils are considered to be the most effective phagocyte and are among the first cells to be recruited to the injury site. Along with macrophages, they play a major role in proteolysis and the phagocytosis of necrotic tissue (Cannon et al., 1990; MacIntyre et al., 2000). Leukocyte invasion begins within 1 h of increased muscle use (Peak et al., 2005; Tidball et al., 2011) and the leucocytosis can last for up to 5 d. The initial influx of neutrophils stimulates the production and release of monocytes and lymphocyte derived cytokines to assist in the regulation of the inflammatory response.

Satellite cells are a heterogeneous population of stem and progenitor cells that are required for growth, maintenance and repair of skeletal muscle (Wang et al., 2011; Feige et al., 2018). These cells are located beneath the basal lamina but outside the plasma membrane (sarcolemma). In the unperturbed state, satellite cells remain in a non-proliferative, quiescent state. In response to stimuli such as molecular triggers from exercise or myotrauma, they become activated, proliferate, and express myogenic markers. Satellite cells expressing myogenic markers (myoblasts) fuse together or to

existing muscle fibers to form new myofibers during regeneration of damaged skeletal muscle. In response to gross muscle damage, satellite cells migrate as myoblasts to areas that need to undergo regeneration (Bazgir et al, 2017).

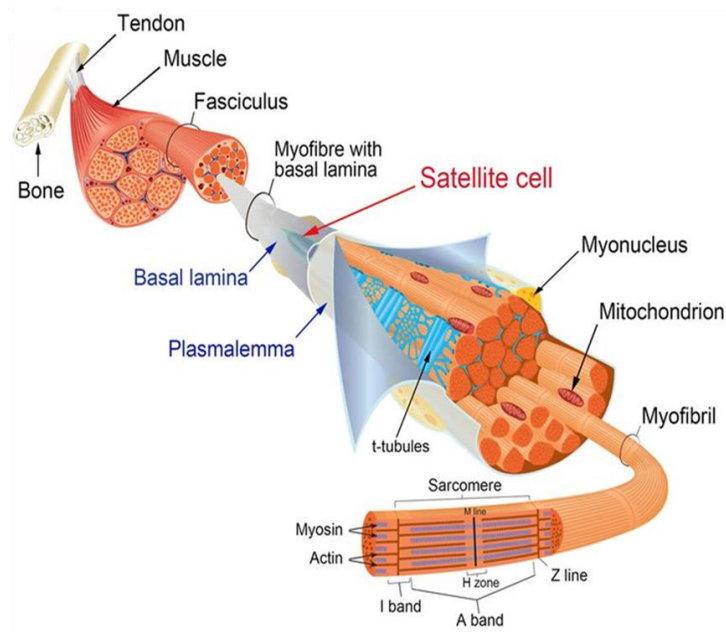


Figure 2.5. Satellite cell location

The transcription factor paired box 7 (PAX7) is expressed by adult muscle satellite cells (Buckingham, 2007). Pharmacologic and genetic ablation studies indicate that muscle does not regenerate in the absence of PAX7. Instead of the formation of myofibres, adipocyte infiltrates replace muscle in the absence of PAX7. Other cells types are recruited during muscle regeneration and can modulate the behaviour of satellite cells by secreting cytokines. For example, the secretion of pro-inflammatory cytokines by macrophages can induce myoblast entry into terminal differentiation during the early phase of muscle regeneration.

Muscle Soreness

Delayed-onset muscle soreness (DOMS) refers to the sensation of pain, soreness, or stiffness of the activated musculature after intense or unfamiliar exercise (Clarkson et al., 1992, Cheng et al., 2003), and is one of the most common symptoms of EIMD. Symptomology of muscle soreness is more pronounced following unaccustomed exercise with an eccentric component (Cheung et al., 2003). The pain associated with DOMS is normally due to activation of pain receptors in response to mechanical disruption of muscle fibers, disturbance of calcium homeostasis, and firing of nerve fibers in response to the synthesis and release of bradykinin, prostaglandins, histamine, and nerve growth factor by pro-inflammatory cells (Armstrong et al., 1984). Stimulation of nociceptors of C-fibers in response to nitric oxide produced by muscle nitric oxide synthase (Radak et al., 2012) and the presence of generation of free radicals in the injured tissue (Paulsen et al., 2012) have also been implicated in the onset of DOMS.

The delay in soreness may vary greatly, but related symptoms generally peaks 24 to 48 h post-exercise and slowly resolves within 4 to 7 d after the inciting activity (Evans et al., 1998). Symptoms can range from muscle tenderness to severe debilitating pain (Armstrong et al., 1984). DOMS is associated with a reduced range of motion, prolonged strength loss, decrements in dynamic sports movements and elevated levels of intramuscular proteins in the blood. In contrast to muscle strength loss, there is a weak relation between DOMS and histological evidence of EIMD, probably due to large inter-individual variability (Lewis et al., 2012). The perception of soreness after eccentric

exercise is often out of phase with serum markers and restoration of muscle strength from the causative exercise may take up to 2 weeks to occur. Edema and swelling in response to inflammatory cell infiltration and accumulation are coincident with the time of DOMS, and may be evident long after the presentation of soreness.

Histological evidence of myofibrils is required to directly assess muscle damage. However, collecting tissue samples to assess muscle damage is an invasive procedure requiring multiple biopsies from the same muscle. A number of surrogate markers including muscle function, sprint and endurance performance, delayed onset muscle soreness (DOMS), inflammatory markers and activity of intramuscular enzymes in the blood are often used to assess EIMD (Clarkson et al., 1992; Clarkson et al., 2001; Nie et al., 2011). None of the surrogate markers are considered “gold standard”, but each has its own advantages and disadvantages

Creatine Kinase

Alterations in membrane integrity in response to mechanical damage of muscle fibers, is followed by the release of intramuscular proteins into the general circulation. Muscle enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase, carbonic anhydrase isoenzyme II, creatine kinase (CK) and other proteins including myoglobin (Mb), heart fatty acid binding protein, troponin, and myosin heavy chain have all been used as surrogate markers of muscle damage (Sorichter et al., 1999, Poprzecki et al., 2004).

CK is the most popular marker of muscle damage because of the magnitude of elevation post exercise and the relative and low cost of analysis (Wu, 1998). It is easily assessed in the serum and is a sensitive, but not specific marker of muscle tissue damage. Elevated levels of CK in the circulation may also occur in response to intramuscular injection, myocardial infarction, muscular dystrophy, polymyositis, hypothyroidism and cerebral infarction (Nigro et al., 1983; Pfeiffer et al., 1983; Stadhouders et al., 1994; Borrayo-Sanchez et al., 2008).

CK is a dimeric globular protein that catalyzes the reversible transfer a high-energy phosphate from ATP to creatine, producing creatine phosphate. It consists of a pair of two different monomers B (brain) and M (muscle) each with a MW of approximately 40,000 Da (Wu, 1998). The M and B subunits are antigenically distinct proteins encoded by different genes. Three isoenzymes of CK exist, CK-BB, CK-MB and CK-MM. A unique dimeric mitochondrial form not composed of either M or B subunits also exists. CK-BB is found primarily in the brain, smooth muscle, prostate, thyroid, gut and lungs. The myocardium expresses primarily CK-MM (70-80%) and 20-30% CK-MB whereas skeletal muscle expresses 99% MM and only 1% MB.

Circulating levels of CK depend on a number of factors including age, gender, race, muscle mass and physical activity levels (Brancaccio et al., 2008; Baird et al., 2012). Normal circulating CK levels are approximately 80-200 U/L in men and 60-140 U/L in women (Wu, 1998). Levels are higher in athletes than non-athletes (Mougios, 2007, Evans et al., 1986) and considerable differences exist between Black, Caucasian and

Hispanic men (Black et al., 1986). A study examining serum levels of CK in 483 male athletes and 245 female athletes across a number of sports found that values ranged from 82 to 1083 U/L in male athletes and 47 to 513 U/L in female athletes (Mougios, 2007).

Weight-bearing exercises that involve eccentric muscle contractions induce the greatest increases in serum levels of CK (Malm et al., 2004). Activation patterns during eccentric muscle actions are different to those controlling static or concentric muscle actions (Bigland et al., 1954; Nardone et al., 1988; Enoka et al., 1996;). Among collegiate women, perceived muscle soreness was found to be significantly higher following eccentric than either concentric or isometric activity (Clarkson et al., 1986). However, post-exercise CK serum levels did not differ significantly among the three activities. Using the forearm flexor muscles Clarkson et al., (1992) examined the time course of changes in muscle function and other correlates of muscle damage following maximal effort eccentric actions. Peak soreness was experienced 2-3 d post-exercise while peak swelling occurred 5 d post-exercise. Plasma CK levels peaked 2 d after exercise, which is also the time when spontaneous muscle shortening was most pronounced. A large number of studies have found increases in serum CK levels for 2 to 7 d following eccentric exercise (Serrão et al., 2003; StÄubli, et al., 1985).

Among female soccer players, serum CK levels peaked at 152% above pre-game values and did not return to baseline for 69 h (Anderson et al., 2008). Pre-match CK levels were found to be 485% greater than baseline levels among Australian football players suggesting that the recovery time between games may be inadequate. In contrast,

Kraemer et al., (2013) found little change in CK levels among collegiate football players and suggested that optimal strength and conditioning and sports medicine programs may have contributed to the findings. Among collegiate level American Football players, circulating CK levels remained elevated for at least 7 d following two practice sessions per day (Soares et al., 2012).

Among elite rugby union players the average change in CK level (Δ CK) during an average of 3.4 regular season games was 927 ± 204 IU with the values being significantly higher in forwards (1439 ± 677 IU) than backs (545 ± 341 IU) (Cunniffe et al., 2009). The large positional difference in Δ CK could be explained by the fact that compared to backs, the forward players performed a higher number of tasks involving collisions. The addition of physical contact to small sided 6 v 6 rugby league games was also found to result in marked and longer lasting increases in CK compared to no physical contact (Johnston et al., 2014).

EIMD and accompanying increase in circulating CK levels in response to eccentric muscle actions is further accentuated by repeated blunt force trauma related collisions that occur frequently in invasion team-based sports such as rugby, Australian football, and American football. Compared to pre-game values, circulating levels of CK were found to be significantly elevated at 14 h and 38 h after a senior international rugby union game (Cunniffe et al., 2009). There was a significant relation between serum CK activity at both 14 h and 38 h post-game and the number of tackles and games contacts undertaken by each player. Similarly, compared to 24 h pre-match values, CK levels were significantly

higher for up to 120 h following rugby league match play (McLellan et al., 2010). Plasma CK peaked at 267% above the pre-match value 24 h after the game. The extent of muscle damage as measured by plasma CK levels was related to the impacts associated with repeated collisions > 7.1 G during match play. Furthermore, the combined total number of hit-ups and tackles was significantly related to plasma CK levels at 30 min, 24 h, 48 h and 72 h post-match. Takarada et al., (2003) also found a significant positive relation between the number of tackles performed during collegiate level rugby union match play and peak CK level measured 24 h post-match.

Skeletal muscle may adapt to repeated bouts of eccentric exercise in order to attenuate muscle damage in subsequent exercise bouts of the same or similar exercise. Jamurtas et al., (2000) found no significant difference in circulating CK levels in response to three separate bouts of plyometric, eccentric and concentric exercises. DOMS decreased significantly after the second exercise session independent of treatment. CK also decreased significantly after the second session and the decrease was independent of the treatment. Nosaka et al., (1994) also found a diminished CK response after a second bout of eccentric exercise in 26 participants with elevated CK levels following the first bout of eccentric exercise.

Recovery Nutrition

The regular scheduling of competitive fixtures in team sports such as soccer, Australian football, rugby and basketball often involves short recovery times that may negatively impact on the inability to recover adequately and impair subsequent

performances. A variety of therapeutic, nutritional and pharmacological interventions are commonly used to attenuate the negative effects of EIMD on muscle soreness and function and promote recovery following exercise (Beelan et al., 2010; Owens et al., 2019). These include massage, (Zainuddin et al, 2005), compression garments, (Hill et al., 2014) contrast water therapy, (Bieuzen et al., 2013; Higgins et al., 2017), active recovery (Suzuki et al., 2004), myofascial release through foam rolling, (MacDonald et al., 2013; Schroeder et al., 2015), stretching, (Torres et al., 2013), pharmaceutical (Cheng et al., 2003; Lundberg et al., 2018) and nutritional strategies, (Sousa et al., 2014;Owens et al., 2019).

Nutrition is an integral component of any sport training and performance program and nutritional strategies to enhance performance are quite different, and at times are not always compatible with those required to optimize training adaptations. For example, recent evidence indicates that CHO restriction may enhance mitochondrial biogenesis and potentially long-term adaptations, although it may impair performance in a given training session (Beelen et al., 2010). Competition nutrition is focused on sufficient nutrient intake to promote energy availability to delay the biochemical determinants of fatigue and to provide fluids to offset fluid losses due to sweating. In contrast, post-training nutrition is focused on promoting muscle recovery and maximizing training adaptations (Beelen et al., 2010).

Protein supplementation, by stimulating muscle protein synthesis and attenuating muscle protein breakdown represents an important putative recovery strategy to

facilitate repair and growth in response to EIMD (Pasiakos et al., 2015); VanDusseldorp et al., 2018). The anabolic effects of dietary protein intake are driven by the transfer and incorporation of amino acids captured from dietary protein sources into skeletal muscle proteins (Gorissen et al., 2016; Mitchell et al., 2016). The anabolic effects are both dose dependent and transient in nature. Maximal increases in muscle protein synthesis are achieved with provision of 10 g essential amino acids, equivalent to approximately 20 g protein (Moore et al., 2015). After a lag of approximately 30-60 min there is a 3-fold rise with muscle protein synthesis peaking around 1.5 to 2.0 h after oral intake. Thereafter, muscle protein synthesis declines back to baseline, despite the maintenance of substrate availability and anabolic signalling.

Protein supplementation aimed at mitigating the deleterious effects of muscle damage are targeted on activating both anabolic and anti-proteolytic signalling pathways. The mammalian target of rapamycin (mTOR) signalling pathway serves as a central regulator of exercise/nutrient-induced alterations in muscle protein synthesis (Atherton et al., 2012). mTORC1 is a multiprotein complex that integrates signals from nutrients, skeletal muscle contraction and growth factors. Leucine is the most effective essential amino acid in increasing the activity of mTORC1 (Drummond et al., 2009) and its downstream effectors involved in mRNA translation initiation (p70S6K, 4E-BP1) and elongation (eEF2).

Proteolysis in skeletal muscle is controlled by multiple systems including the lysosomal, calcium activated, caspase system and the ubiquitin-proteasome-dependent

pathway (Pasiakos., 2014). The ATP dependent ubiquitin-proteasome pathway (UPP) is the primary degradation system for skeletal muscle. It consists of several hundred proteins and is responsible for conjugating damaged proteins into poly-ubiquinated proteins through activity of a ubiquitin conjugating enzyme (E2). Polyubiquinated proteins are subsequently channelled into the catalytic 26S proteasome where proteolysis occurs. Total ubiquitin content can be used as an indicator of protein degradation and an increase in total ubiquitination occurs in conditions of high proteolysis (Greenhaff et al., 2008).

Co-ingestion of CHO with protein has an additive effect on protein synthesis due to the synergistic effect on the plasma insulin response beyond that seen with whole protein or amino acids supplements alone (Ivy et al., 2002). Insulin positively effects net protein balance by supressing muscle protein breakdown and increasing blood flow, and therefore amino acid delivery. The insulin required for optimal stimulation of muscle protein synthesis is only two to three times basal resting levels for most, healthy individuals, and can be achieved at protein doses ≥ 20 g protein per kg body weight. Indeed, recent studies have not found a synergistic effect of consuming amino acids and CHO together than either nutrient alone on the rate of protein synthesis (Greenhaff et al., 2008; Glynn et al., 2013), especially if protein intake is sufficient to stimulate muscle protein synthesis maximally (Staples et al, 2011).

The majority of studies that have examined whether protein supplementation attenuates the extent of EIMD and impairment in muscle function and physical

performance have used branch chain amino acids or a combination of protein and CHO (Pasiakos et al., 2015). Study findings are inconsistent due in large part to large variations in the duration, frequency and daily dose of the supplement and the extent of muscle damage. In a recent systematic review, Fouré et al., (2017) found little evidence that BCAA alleviates structural alterations or decrements in muscle function following EIMD.

Milk is an emulsion of butterfat globules within a water-based fluid that contains dissolved CHO and protein aggregates along with minerals (Uluko et al., 2016). Protein content in milk is dependent on breed, stage of lactation, health, and feeding of the animal. Milk proteins are subdivided into caseins which represent approximately 80% of the total protein and the remaining 20% is whey protein (Fox et al., 2011). Both casein and whey are complete proteins and contains all the essential amino acids required to facilitate an increase in positive muscle protein net balance (Uluko et al., 2016).

Whey protein is isolated from whey, the liquid portion of milk that separates from the curds as a by-product of cheese production. It is a mixture of 50% β -lactoglobulin (50%), 20% α -lactalbumin (20%), bovine serum albumin, immunoglobulins, lactoferrin, transferrin, and other minor proteins and enzymes. Native, concentrated, isolated and hydrolysed forms of whey protein are found in food.

Whey protein concentrate has a protein concentration that ranges from 30-90% and contains low levels of fat and lactose. Whey protein isolates are further processed to remove all the fat and lactose and contains at least 90% protein. Whey protein hydrolysate is the product of partial hydrolysis and is more rapidly-digested than the other two forms of whey protein. Per gram of protein, whey provides greater amounts

of essential amino acids, particularly leucine, than does casein (Pennings et al., 2011) and is emptied from the stomach quickly, resulting in a rapid rise in aminoacidemia. The digestibility of whey protein coupled with the high levels of BCAA, particularly leucine, translates into a rapid but transient increase in protein synthesis, while protein breakdown is not affected.

The casein family of proteins consists of alpha-s1 (45%), alpha-s2 (12%), beta (35%) and kappa (8%) caseins and each has its own amino acid composition, genetic variations, and functional properties (Phelan et al., 2011). In the present review, the term casein will be in singular form, but will encompass all 4 fractions of casein proteins. Casein is relatively insoluble and tends to form colloidal particles called micelles that increase the proteins solubility in water. It is an excellent anti-catabolic nutrient because it provides a slow, sustained release of plasma amino acids into the circulation (Phelan et al., 2011). The superior long-lasting effect of casein is attributed to a delayed gastric emptying and slower absorption rate from the gastrointestinal tract to the blood. The delay in gastric emptying results from the clotting of casein in the stomach. Casein can also be enzymatically hydrolysed (casein hydrolysate) during processing to improve its rate of absorption (Phelan et al., 2011; Uluko et al., 2016). The increase in digestion/absorption rate and amino acid delivery to skeletal muscle tissue arises from the fact that casein hydrolysate is pre-digested into smaller peptide fractions from its intact, original structure.

Slow and fast proteins differently modulate postprandial protein accretion Phillips et al., 2009; Pennings et al., 2011). Boirie et al., (1997) measured the anabolic and catabolic effects for 7 h following the ingestion 30 g of either ^{14}C -leucine-labelled whey or casein. Whey protein resulted in a rapid but transient increase in blood amino acids and protein synthesis. In contrast, there was a prolonged increase in blood amino acids following casein ingestion that resulted in a 34% reduction in protein breakdown. The net protein balance remained more positive after intake of casein protein over a 7 h period. The superior long-lasting effect of casein was attributed to a delayed gastric emptying and slower absorption rate from the gastrointestinal tract to the blood.

Bovine Based Supplementation and EIMD

Results of studies that examined the effects of milk-based protein supplementation on attenuating indices of EIMD have been equivocal with some research suggesting efficacy and others finding no beneficial effect (Table 2.1). Wojcik et al., (2001), compared the effects of a carbohydrate only beverage (CHO), ($1.3 \text{ g}\cdot\text{kg}^{-1}\cdot\text{CHO}$), a milk-based carbohydrate-protein (CHO-P) ($0.9 \text{ g/kg/CHO} + 0.4 \text{ g}\cdot\text{kg}^{-1} \text{ protein}$) beverage and a flavoured placebo on inflammatory markers and muscle function following eccentric quadriceps contractions with the dominant leg. Based on an average weight of 75.6 kg, the average protein intake in the CHO-P group was 25 g. Participants consumed a control diet for 4 d prior to and throughout the 5 d of the experiment. Experimental beverages were consumed immediately and 2 h following the bout of eccentric exercise. Fasting CK levels increased significantly for 5 d following the acute bout of lower limb

eccentric exercise with peak CK occurring on days 4 and 5. The treatment beverages had no significant effect on blood levels of CK on any of the 5 days following exercise. Serum CK did however, tended to be lowest ($p < 0.08$) for CHO-P 6 h after the acute bout of lower limb eccentric exercise. None of the post-exercise nutritional interventions significantly affected muscle soreness, inflammatory markers (IL-1, IL-6, and TNF- α) or isokinetic strength. The fact that participants commenced the study in a glycogen depleted state may have impacted on the external validity of the findings.

The timing of MP-CHO ingestion may influence protein metabolism following EIMD. Cockburn et al., (2010) compared the effect of consuming 1 L of a semi-skinned MP-CHO supplement containing 33.4 g of protein or water before, immediately after or 24 h following muscle-damaging eccentric-concentric knee flexions. Supplementation immediately after or 24 h following exercise blunted the increases in DOMS and decreases in muscle performance over 48 h, compared with pre-exercise supplementation. Consuming MP-CHO at any of the three time points was beneficial in attenuating increases in CK activity, compared with water.

Another study compared the effects of consuming milk, MP-CHO, CHO sports drink or water on attenuating EIMD in 32 healthy young men who regularly competed in individual and team sports (Cockburn et al., 2008). Participants completed a unilateral isokinetic eccentric–concentric knee flexion exercise after which they consumed 500 mL of their allocated nutritional supplement and again 2 h later. The total 1000 mL serving equated to 34 g protein, 118 g CHO, 16.4 g fat and 707 kcal for the MP-CHO supplement

group, 34 g protein, 49 g CHO, 19 g fat and 480 Kcal for the milk supplement group and 64 g CHO, trace amounts of protein and fat and 280 kcal for the CHO supplement group. Isokinetic muscle performance, DOMS and biomarkers of muscle damage (CK, and myoglobin) were assessed immediately before, and 24 h and 48 h after the bout of exercise. Milk and MP-CHO supplementation had no effect on passive muscle soreness but attenuated decreases in peak torque and increases in CK and MB. Using a similar study design, Cockburn et al., (2010) compared the effect of consuming 500 mL of milk, 1000 mL of milk or 1000 mL of water 24 h, 48 h and 72 h following EIMD caused by unilateral isokinetic dynamometry eccentric- concentric knee flexions. Blood levels of CK, Mb and interleukin-6 (IL-6), active and passive DOMS and peak torque were measured at each time point. Both 500 mL and 1000 mL of milk limited the decrements in peak torque and increases in CK and IL-6

Studies demonstrating a benefit of MP-CHO on attenuating reductions in muscle force producing capability after EIMD have measured concentric peak torque using isokinetic dynamometry. The relevance of these laboratory based findings to athletes is questionable as such static movements rarely occur during many sports due to the lack of high velocities and the absence of the stretch shortening cycle (Cockburn et al., 2013, Rankin et al., 2015). In a study involving semi-professional soccer players, Cockburn et al., (2013) examined the effect of 500 mL of semi-skimmed milk or water consumed after muscle-damaging exercise on muscle soreness, muscle proteins and a number of performance tests specific to field-based team sports - countermovement jump height, reactive strength index, 15-m sprint, and agility time and the Loughborough Intermittent

Shuttle Test (LIST). Participants completed 60 repetitions of unilateral isokinetic eccentric–concentric knee flexion, at a speed of $1.05 \text{ rad}\cdot\text{s}^{-1}$ to induce hamstring muscle damage after which they consumed their allocated supplement. The milk provided whey and casein protein, CHO in the form of lactose and 1.7% fat. Passive and active muscle soreness, CK, Mb, countermovement jump height, reactive strength index, 15-m sprint, and agility time were assessed before and 24 h, 48 h, and 72 h after EIMD. Blood samples were taken, perception of muscle soreness was evaluated and performance tests were performed at 24 h, 48 h, and 72 h. Participants completed the LIST 48 h after EIMD.

Milk consumption limited the decrements in agility, 10 m and 15 m sprint performance and the ability to perform repeated 15 m sprints during the LIST. There was no benefit of milk consumption on active or passive muscle soreness, increases in the circulation levels of CK and Mb, decrements in counter movement jump performance, reactive strength index as a measure of the ability to use the stretch-shortening cycle, and heart rate and RPE measured during the LIST.

More recently Rankin et al., (2015) found that 500 mL of a MP-CHO supplement immediately following bilateral isokinetic dynamometry attenuated increases in skeletal troponin I and CK and both passive and active muscle soreness in male sport athletes, compared to the consumption of a volume-and energy-matched CHO drink. However, MP-CHO consumption had minimal effect on limiting the decrease in peak torque and 20 m sprint performance.

Using a randomized cross-over design Pritchett et al., (2009) compared the effectiveness of post-exercise chocolate flavoured milk (CHOC) and a carbohydrate beverage (CHO) on exercise performance, muscle damage, rating of perceived exertion and DOMS. Ten trained cyclists and triathletes completed a high-intensity intermittent exercise protocol on a cycle ergometer followed 15-18 h later by a performance trial to exhaustion at 85% VO_2max . Participants were randomly assigned to isocaloric CHOC or CHO recovery drinks immediately and 2 h following the bout of high intensity exercise. The same protocol was repeated 7 d later with the other beverage. A blood sample was taken before and 15-18 h after the high-intensity intermittent exercise session.

The change in creatine kinase (CK) was significantly greater in the CHO than the CHOC trial. Muscle soreness levels were decreased significantly after 15-18 h of recovery with both CHOC and CHO equally effective in attenuating muscle soreness. Cycling time to exhaustion was not significantly different between trials. Similarly, Ferguson-Stegall et al., (2011) found that consuming low-fat chocolate milk had no effect on CK levels following a metabolically demanding protocol consisting of a 100-min glycogen depleting exercise followed by a 40-km time trial.

CHAPTER III

METHODS

PARTICIPANTS

Male Gaelic Football players (value are mean $\pm$ SD; age 17.41 ± 0.78 years, height 176.42 ± 7.13 m, and mass 72.03 ± 6.49 kg) from two separate GAA clubs volunteered to participate in the study. Each player had a minimum of 5 years playing experience. The teams trained on average 2 d per week and played at least one game per week during the competitive season. Written informed consent and assent were obtained from the parents/guardians and children, respectively. The study was approved by the Research Ethics Committee at Dublin City University.

STUDY OVERVIEW

A two group pre-post-match repeated-measures design was used in this study. The testing protocol is outlined in figure 3.1. Participants played a single competitive Gaelic Football game after which they consumed a MP-CHO beverage (500 mL) or an energy-matched CHO solution (500 mL). Blood samples were taken before the game (Pre), immediately post-game (Post) and 12 h, 36 h and 60 h after the game. Subjective muscle soreness and sprint performance were measured before the game and (Pre) and at 12 h, 36 h and 60 h. Heart rate and movement patterns were continuously measured throughout the game using telemetry and GPS tracking, respectively. The GPS device and a heart rate monitor were fitted 30 min prior to start the game. Body composition and

aerobic fitness were measured prior to the study. With the exception of post-game blood draws, all other measurements were taken in the Human Performance Laboratory at Dublin City University. Participants were asked to refrain from strenuous physical activity during the 24 h period before the game and for 60 h after the game.

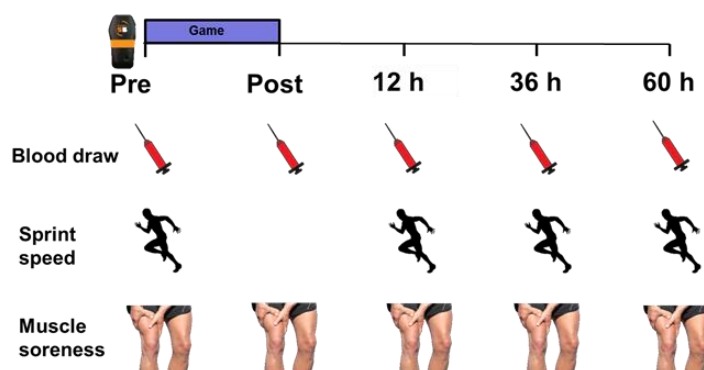


Figure 3.1 Study protocol

NUTRITIONAL SUPPLEMENT

Immediately after the post-match blood draw, participants in the MP-CHO group were provided with 500 mL of milk (Avonmore 1% Light, Glanbia, Kilkenny) containing 215 kcal, 17.0 g of protein, 25.5 g of CHO and 5 g of fat. At the same time participants in the CHO group consumed 500 mL of an energy-matched CHO solution (CHO) (Nutricia Polycal, 100 mL, Energy; 247 Kcal, 0.0 g protein, 61.9 g CHO 0.0 g fat).

PROCEDURES

Height and Body Mass

Height and body mass were measured to the nearest 0.1 cm using a portable scale (Seca 707 Balance Scales, GmbH, Hamburg, Germany). Participants were instructed to

wear a light top and shorts, and to remove their shoes prior to the measurement. Participants stood with their feet together on the base plate with their arms loosely by their side. Each participant was asked to take a deep breath and to stand with their back as straight as possible against the vertical measuring rods and to look straight ahead. Body mass was measured to the nearest 0.1 kg.

Running Velocity

A 10 min warm-up including jogging, striding and dynamic movement patterns was completed prior to the sprint test. Wireless electronic timing gates (Fusion Sport International) were positioned on the starting line and at a distance 5 m and 20 m from the start line. Participants placed their front foot on a marked line that was located 50 cm behind the first timing gate. They were instructed to start sprinting in their own time from a stationary start and to sprint as fast as possible through the last gate. Three trials were performed and the 5 m and 20 m times were recorded to the nearest millisecond (ms). Each trial was separated by a 3 min recovery period. Following each sprint, the time attained was provided to the participant for motivational purposes.

Movement Patterns and Gravitational Forces

A 10-Hz global positioning system (GPS) (SPI-Pro, GPSports, Canberra, ACT, Australia) with integrated triaxial accelerometry was used to measure movement patterns and gravitation forces (G), during match play. GPS technology provides information on time, speed, distance, position, altitude and direction. The intensity,

number, and distribution of gravitational forces (G) experienced by players during collision is recorded simultaneously via satellite communication with the GPS receiver and integrated accelerometer.

GPS typically uses a network of satellites in orbit around the Earth. Each satellite is equipped with an atomic clock that emits, at the speed of light, the exact time and position of the satellite. The GPS receiver compares the time emitted by each satellite signal with the lag time, measured by each receiver, translated into distance by trigonometry. By calculating the distance to at least 4 satellites, the exact position and altitude of the receiver on the Earth's surface can be determined. Speed of displacement is determined via the Doppler shift method, by measuring the rate of change of the satellites' signal frequency attributable to movement of the receiver (Townshend et al., 2008). The integrated accelerometer within the GPS unit measures accelerations and decelerations ($\text{m}\cdot\text{s}^{-2}$) for each plane, with known gravity of $9.8 \text{ m}\cdot\text{s}^{-2}$ equal to 1 G. The integrated accelerometer measures the rate of acceleration and deceleration on each plane and divides the value by $9.8 \text{ m}\cdot\text{s}^{-2}$, to determine the combined G-force as the sum of the G-force measured on each directional axis. The accuracy and reliability of the SPI-Pro have been reported previously (Larson, 2003; Coutts et al., 2010).

The GPS device which weighted 76 g was supported in a purpose designed harness fitted in the upper thoracic region underneath their playing kit. None of the participants complained of discomfort or impediment to their normal range of movement or performance from wearing the GPS equipment during match play. The playing number

of each participant and unit number of each device were manually recorded for identification purposes. The GPS system was synced with an electronic mobile device (iPhone 4S, Apple Inc. San Francisco, USA) that was used to manually record the exact start and end time of each playing half. The exact times of all tactical or injury enforced substitutions of monitored players were also manually recorded. Data were later downloaded from each GPS unit to a personal laptop computer where further analysis was carried out using specifically designed computer software analysis programme.

Movement Classification System

Data provided from the GPS integrated triaxial accelerometer for examination in this study included impact (G Force) data (intensity, number, and distribution) and total distance travelled characteristics. Six different speed zones are classified by the Statsports software (Table 3.1) and the frequency and duration of each entry was recorded.

Table 3.1. Speed zone classifications

Movement classification	Zone	m·sec⁻¹	km·h⁻¹
Standing	1	<0.19	<0.7
Walking	2	0.19 – 2.0	0.7-7.2
Jogging	3	2.0 – 4.0	7.2-14.0
Running	4	4.0 – 5.5	14.0-20.0
High speed running	5	5.5 – 7.0	20.0-25.0

Maximal speed running	6	> 7.0	> 25
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Impacts

The impact classification system was based on methods presented previously in rugby league (McLellan et al., 2011) and rugby union (Cunniffe et al., 2009) and manufacturer guidelines (GPSports). Each impact was coded to 1 of 6 zones based on acceleration G-force characteristics recorded via portable accelerometry. The impact zone characteristics used in this study are listed in Table 3.2

Table 3.2. Impact zones

Zone	Gravitational force (G force)	Collision classification
1	<5.0-6.0	Very light impact
2	6.1-6.5	Light to moderate impact
3	6.5-7.0	Moderate to heavy impact
4	7.1-8.0	Heavy impact
5	8.1-10.0	Very heavy impact
6	<10.	Severe impact

Heart Rate

Heart rate was continuously measured with a sampling frequency of 5 sec using a wireless team system (Polar Team Pro, Polar Precision Performance SW 3.0, Kempele, Finland). Heart rate data was stored on a receiver that was attached to an elastic strap and placed around the participant's chest. The data was transferred to a personal laptop computer for analysis. Peak HR was identified for each participants in order to categorise HR data obtained during match play in to one of four zones: 0-60%, 61-70%, 71-80%, 81-90% and 91-100% maximal heart rate (HRmax). Similar zones have previously been used to evaluate the heart rate response in elite youth soccer players (Wong et al., 2009).

Muscle Soreness

Subjective rating of active muscle soreness was measured using a 100 mm visual analogue scale (VAS) (Figure 3.2). The scale ranged from 0-100, with 0 being no soreness whatsoever and 100 being the worst pain imaginable. Soreness was indicated on the VAS after the participant performed a squat to a knee angle of approximately 90° with hands on hips and feet shoulder width apart and then returning to the standing position. The VAS was anchored to written cues from left (no pain) to right (worst pain imaginable).

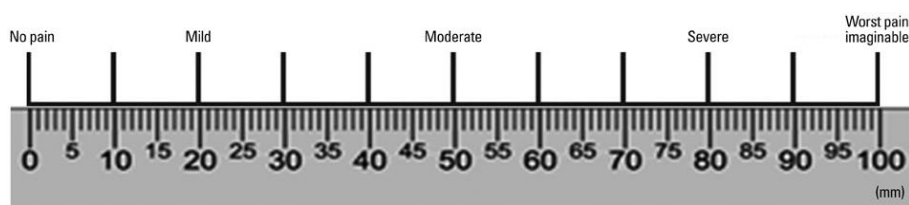


Figure 3.2. Muscle soreness visual analogue scale

Maximal Aerobic Capacity ($\dot{V}O_2\text{max}$)

Maximal aerobic capacity ($\dot{V}O_2\text{max}$) was determined on a treadmill (Woodway ELG 55, Waukesha, WI) using a ramp protocol. The treadmill velocity was set at a constant velocity of 10 km·h⁻¹ and the gradient was increased by 1% every min until volitional exhaustion. $\dot{V}O_2\text{max}$ was determined by averaging the two highest consecutive 30 sec values. Heart rate, ratings of perceived exertion (RPE), expired O₂, CO₂, and ventilatory volume were continuously recorded. RPE was obtained using the 15-point Borg category RPE scale. Heart rate was measured using telemetry (Polar Vantage NV™ Polar, Port Washington, NY).

Open Circuit Spirometry

Respiratory metabolic responses were determined using standard open-circuit spirometry techniques (Sensormedics Vmax 229, SensorMedics Corp., CA). Prior to testing, the gas analysers were calibrated with standard gases of known concentration. The calibration gas was sampled at a rate of 125 Hz. A mass flow sensor (Sensormedics, Loma Linda, CA, USA) was used to collect breath-by-breath measurement of ventilation. A 3 L volume syringe (Sensormedics, Loma Linda, CA, USA) was used to calibrate the mass flow sensor prior to each test.

Blood Sampling and Analysis

Approximately 6.0 mL of venous blood was drawn at each time point from a forearm vein using standard venepuncture. Whole blood samples were centrifuged (Beckman Coulter, UK) at 3000 rpm for 15 min at 4°C. Plasma was transferred to micro centrifuge tubes and stored at -80°C for subsequent analysis.

Routine complete blood counts (CBC) were performed prior to centrifugation on whole blood collected in EDTA vacutainers using an automated cell counter (Coulter Ac.Tdiff2, Beckman Coulter). Plasma CK was measured using a Randox Datona™ clinical chemistry analyser using a CK-NAC assay. The enzyme used for the CK assay was hexokinase and the substrate was creatine phosphate $2\text{Na}_4\text{H}_2\text{O}$. The absorbance level for the reaction was 340 nm. Normal reference values for CK range from 25-140 IU-L according to manufacturer's guidelines.

Statistical Analysis

Goalkeepers were excluded from the analysis. Prior to statistical analysis the data was checked for normality using the Shapiro-Wilks test. Mean differences in physical and physiological characteristics, activity patterns and impact zone entries were determined using an independent t-test. A group x time repeated measures ANOVA was used to compare the mean differences in serum levels of CK, muscle soreness, number of circulation leukocytes and sprint performance within and between group. Significant main effects were probed using a Bonferroni post hoc test. SPSS for Windows statistical software (ver 22.0) was used to perform the statistical analysis. The criterion for significance (alpha) was set at 0.05.

CHAPTER IV

RESULTS

Physical and physiological characteristics

Participant's physical and physiological characteristics are presented in table 4.1.

There was no significant difference in any of the measured physical or physiological parameters between MP-CHO and CHO

Table 4.1 Physical and physiological characteristics

	Group	
	MP-CHO	CHO
Age (y)	17.36 ± 0.84	17.47 ± 0.74
Height (cm)	173.84 ± 8.29	178.81 ± 5.03
Weight (kg)	70.89 ± 7.91	73.10 ± 4.90
BMI (kg.m ⁻²)	22.12 ± 6.00	22.86 ± 1.32
VO ₂ max (mL.kg ⁻¹ .min ⁻¹)	58.58 ± 7.83	57.37 ± 3.40
Maximal heart rate (b.min ⁻¹)	191.33 ± 10.60	191.58 ± 9.28
Total playing time (min)	61.34 ± 1.97	60.52 ± 3.87

Values are mean ± SD

Activity characteristics

There were no significant differences between groups for any of the measured activity characteristics during match-play. (Table 4.2)

Table 4.2 Activity characteristics during Gaelic football match play

	Group	
	MP-CHO	CHO
Total distance (m)	6059.58 ± 1271.46	6203.90 ± 913.36
Distance per min (m)	98.85 ± 20.74	101.21 ± 14.90
Maximal velocity (m·sec ⁻¹)	8.14 ± 0.22	8.18 ± 0.50
Walking (m)	2379.66 ± 258.10	2517.51 ± 252.43
Jogging (m)	1916.94 ± 650.78	1998.01 ± 485.00
Running (m)	1140.04 ± 412.86	1066.26 ± 328.60
High speed running (m)	528.97 ± 193.05	523.70 ± 220.51
Max speed running (m)	93.67 ± 57.44	98.42 ± 72.51

Values are mean ± SD; Standing <0.19 m·sec⁻¹; Walking 0.19 - 2.0 m·sec⁻¹; Jogging 2.0 - 4.0 m·sec⁻¹; Running 4.0 - 5.5 m·sec⁻¹; High speed running 5.5 - 7.0 m·sec⁻¹; Max speed running > 7.0 m·sec⁻¹

Impact zones

There was no significant difference within or between groups for the number of impact zones. (Table 4.3) No significant interaction effects between time and group were observed for the number of impact zones. (Table 4.3)

Table 4.3 Average number of impact zone entries during Gaelic football match play

Gravitational forces	Group	
	MP-CHO	CHO
1 (<5.0-6.0)	2475.60 ± 749.45	2542.07 ± 730.62
2 (6.1-6.5)	90.27 ± 34.09	110.47 ± 49.47
3 (6.5-7.0)	56.73 ± 23.05	77.47 ± 39.20
4 (7.1-8.0)	69.40 ± 27.86	86.73 ± 42.37
5 (8.1-10.0)	46.60 ± 24.70	64.33 ± 39.45
6 <10.	19.87 ± 12.62	25.20 ± 21.06

Values are mean ± SD

Creatine kinase

Serum levels of CK are summarized in table 4.4. There was a significant time main effect for circulating levels of CK with levels significantly elevated in MP-CHO and CHO post-game ($p < 0.001$) and 12 h ($p < 0.001$) compared to pre-game. No significant main effect for group or interaction effects between time and group were observed for circulating levels of CK.

Table 4.4 Serum levels of creatine kinase (IU/L) pre-game, post-game and at 12 h, 36 h and 60 h post-game

Time	Group	
	MP-CHO	CHO
Pre-game	514.40 $\pm$ 437.32	510.79 $\pm$ 369.05
Post-game	812.47 $\pm$ 587.02‡	793.43 $\pm$ 504.13‡
12 h	1011.40 $\pm$ 443.00‡	925.93 $\pm$ 375.16‡
36 h	706.27 $\pm$ 554.23	534.07 $\pm$ 318.69
60 h	459.67 $\pm$ 284.02	494.271 $\pm$ 355.46

Values are mean $\pm$ SD; ‡ $p < 0.001$ vs. pre-game

Muscle soreness

There was a significant time effect for subjective muscle soreness (Table 4.5). Muscle soreness was significantly elevated from baseline at 12 h ($p < 0.001$) and 36 h ($p < 0.01$) in MP-CHO and at 12 h ($p < 0.001$) in CHO. Peak muscle soreness occurred at 12 h in both MP-CHO and CHO. No significant main effect for group or interaction effects between time and group were observed for muscle soreness. (Table 4.5)

Table 4.5 Muscle soreness pre-game and at 12 h, 36 h and 60 h post game

Time	Group	
	MP-CHO	CHO
Pre-game	19.73 ± 14.43	23.60 ± 11.85
12 h	55.13 ± 13.64‡	50.00 ± 15.00‡
36 h	36.53 ± 19.99*	30.00 ± 11.02
60 h	14.33 ± 7.53	15.33 ± 7.67

Values are mean ± SD, *p<0.01 vs. pre; ‡p<0.001 vs. pre-game

Circulating leukocytes

Circulating leukocyte levels before and after Gaelic football match-play is summarized in table 4.6. There was a significant time effect for the number of circulating leukocytes. Blood levels of leukocytes were significantly elevated immediately postgame in both MP-CHO (p<0.001) and CHO (p<0.01). Compared to pre-game levels, the circulating number of leukocytes was significantly decreased in MP-CHO at 12 h (p<0.001), 36 h (p<0.001) and 60 h (p<0.001). Compared to pre-game levels, the circulating number of leukocytes was significantly decreased in CHO at 12 h (p<0.05), 36 h (p<0.01) and 60 h (p<0.001). No significant main effect for group or interaction effects between time and group were observed for circulating leukocytes.

Table 4.6 Absolute number of circulating leukocytes pre-game and at 12 h, 36 h and 60 h post game

Time	Group	
	MP-CHO	Milk
Pre-game	9.21 ± 1.92	7.98 ± 1.82
Post-game	11.58 ± 2.46†	11.17 ± 1.97‡
12 h	7.56 ± 1.69‡	6.89 ± 1.12*
36 h	7.19 ± 1.50‡	6.48 ± 1.38†
60 h	7.23 ± 1.65‡	6.33 ± 1.07‡

Values are mean ± SD, *p<0.05 vs. pre; †p<0.01 vs. pre;

‡p<0.001 vs. pre

Sprint speed

Table 4.9 summarizes the 5 m and 20 m sprint times before and after Gaelic football match play. There was a significant time main effect for 5 m and 20 m sprints. There was a significant decrease in 5 m and 20 m sprint times at 12 h ($p<0.001$), 36 h ($p<0.001$) and 60 h ($p<0.001$) in both MP-CHO and CHO. There was no significant effect for group or group x time interaction.

Table 4.7 Sprint performance pre-game and at 12h, 36h and 60h post game

	Group	
	Milk	CHO
5m (s)		
Pre-game	0.97 ± 0.04	0.95 ± 0.04
12 h	1.06 ± 0.07‡	1.07 ± 0.06‡
36 h	1.06 ± 0.07‡	1.08 ± 0.05‡
60 h	1.07 ± 0.07‡	1.07 ± 0.07‡
20m (s)		
Pre-game	2.97 ± 0.10	2.91 ± 0.11
12 h	3.11 ± 0.13‡	3.12 ± 0.11‡
36 h+	3.10 ± 0.13‡	3.11 ± 0.10‡
60 h+	3.11 ± 0.13‡	3.13 ± 0.14‡

Values are mean ± SD, ‡ $p<0.001$ vs. pre-game

CHAPTER V

DISCUSSION

Mechanical and metabolic stresses along with the physical impacts associated with participation in many invasion team sports elicits muscle damage that may temporarily limit subsequent training and match performance (Clarkson et al., 2002). This investigation sought to examine whether acute ingestion of a MP-CHO beverage as opposed to a CHO only beverage immediately following Gaelic football match play would have an impact on biomarkers of muscle damage, DOMS, inflammatory responses and sprint speed. The results do not support the hypothesis that MP-CHO would attenuate increases in CK, leukocyte trafficking, and DOMS and decrements in speed in response to EIMD following participation in competitive Gaelic football match-play.

Pre-match CK levels were higher than reported in previous studies involving prepubescent boys (Deli et al., 2017 Gorianovas et al., 2013) and adolescent males (Pal et al., 2018, Pullinen et al., 2011), but were similar to elite 19-year-old rugby league players (Johnston et al., 2015). Many of the study participants participated regularly in other team sports, and the elevated baseline values may be attributed to residual muscle damage from previous training and/or game activities, or the cumulative effect of muscle damage associated with the rigors of training and game participation prior to participation in the present investigation.

Blood levels of CK peaked at 97% and 82% above pre-game values at 12 h in MP-CHO and CHO respectively, and returned to pre-game values in both groups at 36 h. A

less than 20% decline of the force-generating capacity of muscles along with blood CK levels $<1,000$ U/L, limited inflammation and recovery within 12-48 h is classified as mild EIMD and is usually not accompanied by histological evidence of EIMD (Paulsen et al., 2012). The peak CK values were 1011 and 926 IU·L⁻¹ in MP-CHO and CHO, respectively. The modest elevation in blood levels of CK in the present study would therefore indicate the presence of mild muscle damage.

It is possible that the true peak CK value may have been missed as no blood sample was taken between 12 h and 24 h post-game. The relative increase in CK immediately post-game was similar in both groups and was almost identical to post-game values reported following competitive match-play in elite adolescent rugby league players (Cunniffe et al., 2009). The similar increase in CK in MP-CHO and CHO immediately post-exercise is not surprising considering that the mean total distance and the distance covered per minute during match play was similar in both groups.

In addition to repeated high speed eccentric muscle actions, repetitive blunt force trauma associated with high-impact collisions (Cunniffe et al., 2009, McLelland et al., 2010) may also cause muscle damage and increases in CK. There was no difference in the average number of impact zone entries between MP-CHO and CHO. The fact that the vast majority of the impacts recorded in both groups were in the very light (zone 1) and light to moderate (zone 2), zones indicates that blunt force trauma was not a major contributing factor to the elevated CK levels in the present study.

Alternatively, the modest increase in CK may be due to the fact that players through regular training and competitive match play had become accustomed to high force eccentric contractions and impact tackles, and were therefore less susceptible to muscle damage (Hyatt et al., 1998, Gorianovas et al., 2013). There is evidence that the degree of muscle damage and associated symptoms is greatly reduced by conditioning of the muscle through prior activity of a similar nature, a well-established phenomenon referred to as the repeated bout effect (Nosaka et al., 2001). The repeated bout effect has been found to facilitate a faster recovery of muscle function following a superseding bout of damaging exercise for up to 9 months. Alterations in pro and anti-inflammatory gene expression resulting in a reduced inflammatory response, cellular, muscle and extracellular matrix remodelling and neural changes have been proposed to explain the repeated bout effect (Baumert et al., 2016).

Relative to CHO, ingestion of MP-CHO did not reduce markers of muscle damage at any time following the game. The evidence supporting the role of MP-CHO beverage to attenuate indices of EIMD is equivocal, with research suggesting both efficacy and (Hoffman et al., 2009; Cockburn et al., 2010;) and a lack of benefits (Wojcik et al., 2001, White et al., 2008). Considering that muscle remodelling as measured by fractional synthesis rates (FSR) and fractional net balance can occur for at least 48 h, it is possible that providing additional protein across this timeframe may increase bioavailability compared to single bolus supplementation. (Phillips et al., 1997). The lack of a response to MP-CHO may also be indicative of differences in the effects of administering whole

proteins as compared to individual amino acids, particularly enriched leucine.(White et al., 2008)

Discrepancies could also be attributed to the lack of a controlled diet throughout the investigation. Outside of a single bolus of MP-CHO or CHO, participants were free to continue with their regular dietary habits which may have impacted the results. The amount of protein provided by the 500 mL of milk was 17 g. Previous research has shown that at least 20 g of intact protein is required to maximally stimulate muscle protein synthesis after resistance exercise (Moore et al., 2015). The lower relative intake of protein may not have been sufficient to maximally stimulate protein synthesis and attenuate muscle function losses, primarily due to the lower leucine content. Perhaps a greater intake of milk would have resulted in more positive effects.

Increases in muscle soreness normally peaks 24r to 48 h after muscle damaging exercise (Clarkson et al., 1992; Saxton et al., 1996; Lewis et al., 2012,118). In the present study, perceived active muscle soreness was significantly elevated from baseline at 12 h and 36 h in CHO-P and at 12 h in CHO. The peak muscle soreness values were approximately 50% of maximal attainable values and occurred at 12 h in both CHO-P and CHO. Previous studies examining the effect of milk consumption on active soreness following EIMD have yielded conflicting results. Some studies found no difference in muscle soreness in response to MP-CHO ingestion compared to CHO alone (Coutts et al., 2007; Wojcik et al., 2001; Cockburn et al., 2013) while others reported a benefit of MP-CHO in blunting increases in active soreness (Cockburn et al., 2013; Rankin et al., 2015).

The conflicting findings may be due in part to the considerable inter-individual variability in the response to muscle damaging exercise (Baumert et al., 2013). In particular, large inter-individual variability in CK levels makes it more difficult to detect group differences. Specific gene polymorphisms that influence muscle disruption, calcium handling and inflammatory responses may help to explain the considerable inter-individual variability in the response to muscle damaging exercise (Yamin et al., 2008; Pescatello et al., 2013) and warrant further investigation.

Disruption of force generating capacity is believed to be one of the primary mechanisms responsible for the decrements in muscle performance in the hours and days following EIMD. Compared to pre-game values, there was a significant decrement in 5 m and 20 m sprint performance in both MP-CHO and CHO at 12 h. Performance decrements in 5 m and 20 m sprint performance was still evident in both groups at 36 h and 60 h. Rankin et al., (2015) also found that 500 mL of a MP-CHO supplement immediately following EIMD had minimal effect on limiting the decrease in peak torque and 20 m sprint performance.

Peripheral and/or central mechanisms are thought to be responsible for the prolonged decrements in the force generating capacity of the muscle following EIMD (McLellan et al., 2011; Strojnik et al., 1998). EIMD increases the degradation of protein and membrane structures, leading to myofibrillar and cytoskeletal disruption. The subsequent loss of both contractile protein and cell membrane integrity may lead to impaired action potential propagation over the sarcolemma or excitation-contraction

coupling mechanisms. Compared to peripheral mechanisms of EIMD, alterations in neural control strategies such as motor unit recruitment threshold, discharge rate, conduction velocity and synchronization remains comparatively poorly understood. It is plausible that altered afferent signalling to the CNS may modify the motor unit recruitment strategy following EIMD and delay the recovery of force production during muscle contraction. Since high force producing type II muscle fibres are more susceptible to EIMD than type I fibers, it is likely that there will be preferential impairments of fast twitch motor units.

In a recent study MacGregor et al., (2018) examined how motor unit behaviour controlled force production during EIMD in knee extensor muscle and over a 7 d recovery period. The unique aspect of this study was the fact that fast twitch motor units were investigated in isolation from the entire active motor unit pool. Motor unit action potential trains (MUAP) were extracted from surface electromyographical signals using precision decomposition EMG (dEMG). dEMG investigates the behaviour of a sample of motor units, representative of the active motor unit pool and allows assessments of MU firing properties during contraction up to levels close to MVC, and facilitates evaluation of different MU within the recruited pool up to close to MVC.

The eccentric exercise protocol resulted in a 31% decrease in MVC 48 h post exercise and was accompanied by a diminished rate of torque development and elevated muscle soreness up to 72 h post exercise. The mean firing rate of high-threshold (high force producing type II) motor units declined by 22% at 48 h post exercise and along with

MVC recovered 7 d post EIMD. The mid-range recruited motor units demonstrated a tendency towards lower mean firing rates whereas early recruited motor units remained unaltered. An increased common drive of motor units probably due to impaired proprioception from muscle spindles secondary to EIMD was found at 48 h and suggest that changes in motor unit activity following exercise-induced muscle damage may mediate recovery.

EIMD results in both local and systemic inflammatory responses. Granulocytes and macrophages infiltrate the injured tissue within the first 24 h and remove cellular debris by synthesizing and releasing pro-inflammatory cytokines and chemo-attractants, increasing the expression of leukocyte receptors and releasing proteolytic enzymes and reactive oxygen species. The infiltration of immune cells is directly related to the intensity, duration, and type of exercise, muscle mass involved and the degree of muscle damage (Peake et al., 2005). In the present study, circulating leukocytes were significantly higher in both the MP-CHO and CHO immediately after the game. Leucocytosis is a well-established phenomenon following exercise and is related to demargination of leukocytes and their release from the spleen (Gleeson et al., 2007). Leukocyte numbers dropped below baseline levels at 12 h and had not returned to baseline by 60 h. This is in line with several studies which report that circulating leukocyte and neutrophil count increase within several hours after eccentric exercise, but that is dependent on the intensity, duration, and type of exercise and muscle mass. (Peake et al., 2005).

Study limitations

- The diet of the participants was not monitored before or after the Gaelic football match-play.
- Although MP-CHO and CHO were matched for carbohydrate content, the total caloric content was higher in the MP-CHO group
- Many of the study participants participated regularly in other team sports. Participants were instructed not to engage in any other activity for a period before and during the present study. This however, could not be confirmed.
- It is possible that the true peak CK value may have been measured as no blood sample was taken between 12 h and 24 h post-game.
- The games played were out of competition which may have had an effect of motivation levels.
- There was no direct measure of muscle damage.

Practical applications

The present study examined whether acute ingestion of a MP-CHO beverage as opposed to a CHO only beverage immediately following Gaelic football match play would have an impact on biomarkers of muscle damage, DOMS, inflammatory responses and sprint speed. MP-CHO or CHO supplementation immediately following Gaelic football match play did not attenuate DOMS, decrements in 5m and 20 sprint speed or circulating levels of CK and leukocytes. There was no adverse effect of consuming either MP-CHO or CHO immediately following Gaelic football match play.

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Appendix 1



Dublin City University
RESEARCH ETHICS
COMMITTEE

APPLICATION FOR
APPROVAL OF A
PROJECT INVOLVING
HUMAN PARTICIPANTS

Application No. (office use
only)

DCUREC/2015/180

Please read the following information carefully before completing your application. Failure to adhere to these guidelines will make your submission ineligible for review.

- ☐ **Applications must be e-mailed to the DCU Research Ethics Committee at rec@dcu.ie –no hardcopy required.**
- ☐ **Student applicants must cc their supervisor on that e-mail – this applies to all masters and postgraduate students.**
- ☐ **The application should consist of one electronic file only, with an electronic signature from the PI. The completed application must incorporate all supplementary documentation, especially that being given to the proposed participants. It must be proofread and spellchecked before submission to the REC.**
- ☐ **All sections of the application form must be answered as instructed and within the word limits given.**

Applications which do not adhere to all of these requirements will not be accepted for review and will be returned directly to the applicant.

Applications must be completed on the form; answers in the form of attachments will not be accepted, except where indicated. No hardcopy applications will be accepted. **Research must not commence until written approval has been received from the Research Ethics Committee.**

Note: If your research requires approval from the Biosafety Committee (BSC), or review by the Nursing Ethics Advisory Committee (NEAC), this must be in place prior to REC submission. Please attach the responses from these committees to this submission as directed below.

PROJECT TITLE	COMPARISON OF TWO NUTRITIONAL INTERVENTIONS ON MUSCLE DAMAGE, RUNNING SPEED AND MUSCLE POWER FOLLOWING A GAELIC FOOTBALL GAME
PRINCIPAL INVESTIGATOR(S)	Niall Moyna
START AND END DATE	July 2015 - September 2015
LEVEL OF RISK	Full committee

Please confirm that **all** supplementary information is included in your application (in electronic copy). If questionnaire or interview questions are submitted in draft form, please indicate this by putting (draft) after YES. A copy of the final documentation must be submitted for final approval when available.

My application has been collated as one electronic file which includes the following documentation:	INCLUDED (mark as YES)	NOT APPLICABLE (mark as N/A)
Bibliography		N/A
Recruitment advertisement		N/A
Plain language statement/Information Statement	YES	
Informed Consent form	YES	
Evidence of external approvals related to the research		N/A
Questionnaire/Survey	YES	
Interview/Focus Group Questions		N/A
Debriefing material		N/A
Other (e.g. BSC approval, NEAC review letter)		N/A

Please note:

1. Any amendments to the original approved proposal must receive prior REC approval.
2. As a condition of approval investigators are required to document and report immediately to the Secretary of the Research Ethics Committee any adverse events, any issues which might negatively impact on the conduct of the research and/or any complaint from a participant relating to their participation in the study

1. ADMINISTRATIVE DETAILS

PROJECT TYPE: (mark Y to as many as apply)	Research Project	Y...	Funded Consultancy	Y
			Clinical Trial	...
	Student Research Project (please indicate level, e.g. PhD/MSc Research/MSc Taught)	Y	Other - Please Describe:	...
	PhD	...		
	MSc Research	Y		
	MSc Taught	...		
	Final Year Project	...		

1.1 INVESTIGATOR CONTACT DETAILS

PRINCIPAL INVESTIGATOR(S): *Doctoral researchers and Research Masters or their supervisors may be listed as Principal Investigators, depending on the conventions of the discipline and on the individual case. It should be made clear, in subsequent sections of this application, who is carrying out the research procedures.*

NAME	SCHOOL/UNIT	EMAIL
Niall Moyna	Health and Human Performance	Niall.moyna@dcu.ie

OTHER INVESTIGATORS: *(including Taught Masters students)*

NAME	SCHOOL/UNIT	EMAIL
Kevin Reilly	Health and Human Performance	KReilly.CNI@lmetb.ie

1.2 WILL THE RESEARCH BE UNDERTAKEN ON-SITE AT DUBLIN CITY UNIVERSITY?

YES

(If NO, state details of the off-campus location – provide details of the approval to gain access to that location in section 2.7.)

1.3 IS THIS PROTOCOL BEING SUBMITTED TO ANOTHER ETHICS COMMITTEE, OR HAS IT BEEN PREVIOUSLY SUBMITTED TO AN ETHICS COMMITTEE?

YES

(If YES, please provide details and attach copies of approval(s) received etc.)

A similar protocol was approved last year (SHHP/REC/2013/009).
Effect of three competitive Gaelic football games in 8 days on peak power and markers of muscle damage and immune function

DECLARATION BY PRINCIPAL INVESTIGATOR(S)

The information contained herein is, to the best of my knowledge and belief, accurate. I have read the University's current research ethics guidelines, and accept responsibility for the conduct of the procedures set out in the attached application in accordance with the form guidelines, the REC guidelines (https://www4.dcu.ie/researchsupport/research_ethics/guidelines.shtml), the University's policy on Conflict of Interest, Code of Good Research Practice and any other condition laid down by the Dublin City University Research Ethics Committee. I have attempted to identify all risks related to the research that may arise in conducting this research and acknowledge my obligations and the rights of the participants.

If there exists any affiliation or financial interest for researcher(s) in this research or its outcomes or any other circumstances which might represent a perceived, potential or actual conflict of interest this should be declared in accordance with Dublin City University policy on Conflicts of Interest.

I and my co-investigators or supporting staff have the appropriate qualifications, experience and facilities to conduct the research set out in the attached application and to deal with any emergencies and contingencies related to the research that may arise.

Electronic Signature(s):

Principal investigator(s): 

Print Name(s): Niall Moyna

Date: 08 July 2015

2. PROJECT OUTLINE

2.1 LAY DESCRIPTION (APPROX. 300 WORDS)

PLEASE OUTLINE, IN TERMS THAT ANY NON-EXPERT WOULD UNDERSTAND, WHAT YOUR RESEARCH PROJECT IS ABOUT, INCLUDING WHAT PARTICIPANTS WILL BE REQUIRED TO DO. PLEASE EXPLAIN ANY TECHNICAL TERMS OR DISCIPLINE-SPECIFIC PHRASES.

During a game of Gaelic football muscles may fatigue and temporarily weaken. Sometimes the muscles may become damaged and it may take a number of days for them to recover. This type of muscle damage has been named 'exercise-induced muscle damage'. It results in proteins leaking from damaged muscle cells into the blood followed by an inflammatory response to assist with healing. In addition, many individuals can experience soreness and/or pain and a decreased ability to sprint and develop muscle power. Drinking 500 ml of milk has been found to limit increases in muscle soreness. Milk contains a lot of important nutrients and some studies have shown that it is a convenient, accessible, low-cost alternative to commercial powdered recovery supplements that are being promoted to children. The purpose of this study is to compare the effect of consuming 500 mL of milk on muscle damage, running speed and muscle power following a minor level (under-18) Gaelic football game.

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH (Approx. 400 words)

State the aims and significance of the project. Where relevant, state the specific hypothesis to be tested. Please provide a brief description of background research, a justification as to why this research project should proceed in that context and an explanation of any expected benefits to the community. NB – all references cited should be listed in an attached bibliography.

Exercise-induced muscle damage (EIMD) is commonly associated with participation in invasion team sports. It involves the mechanical disruption of the muscle fibres and release of cellular components such as creatine kinase (CK) into the blood and delayed-onset muscle soreness (DOMS). Inflammation occurs to clear debris from the injured area in preparation for regeneration. The inflammatory response results in a rapid and sequential invasion of muscle fibers by inflammatory cell populations and signalling molecules. From a performance perspective, EIMD results in decrements in muscle force production, agility, speed, repeated sprint ability and proprioception which can limit subsequent training and competitive performance

A number of pharmaceutical, therapeutic and nutritional interventions have been used to limit ultrastructural damage and attenuate the reductions in force producing capability following EIMD. Milk contains electrolytes, and both carbohydrates and proteins, and has been proposed as a convenient, accessible, low-cost alternative to commercial powdered recovery supplements. Ingestion of milk immediately post exercise has been shown to diminish decrements in speed, reactive strength and increases in CK³. It is possible that milk consumption improves muscle protein balance by replacing amino acids lost by myofibrillar protein degradation. This in turn may limit ultrastructural damage as measured by CK and other intramuscular proteins and may attenuate reductions in force producing capability. Studies to date that have examined the effect of milk on indices of muscle damage and performance have primarily used laboratory based interventions involving a single muscle group which has limited external validity when extrapolated to dynamic sports settings. The purpose of this study is to investigate the effects of milk consumption immediately following Gaelic football on markers of muscle damage and indices of muscle performance.

2.3 DESCRIBE THE METHODOLOGY BEING USED TO ACHIEVE YOUR STATED AIMS

Members of two local minor football teams will be recruited to participate in the research study. Participants will play a competitive Gaelic Football match after which they will consume milk (500 mL) or an energy-matched carbohydrate solution (500 mL). The game will be recorded and participants will wear a heart rate monitor and 10 Hz GPS tracking device during the game. Blood samples will be taken before the game (PrG), immediately post-game (PoG), 24 h post-game (+24 h), 48 h after the game (+48h) and 72 h after the game (+72 h). Subjective muscle soreness, sprint performance and muscle power will be measured PrG, +24 h, +48h and +72 h. Body composition and aerobic fitness will be measured prior to the study. With the exception of the post-game blood draw, all other measurements will be undertaken in the Human Performance Laboratory at DCU (figure)

³ Cockburn E et al., Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. Appl Physiol Nutr Metab. 2008, 33(4):775-83.

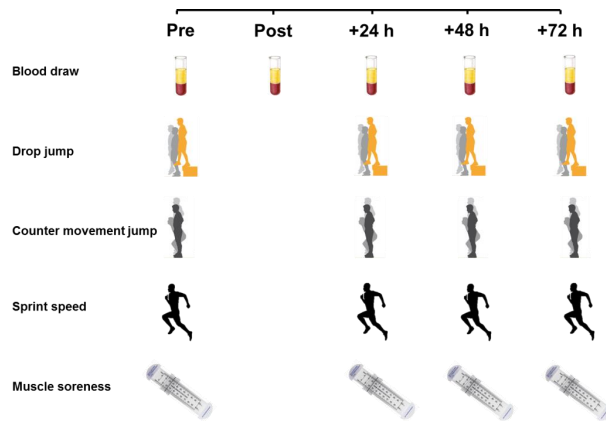


Figure. Study measurements and time points

Nutritional Supplement: Immediately after the post-match blood draw, participants in the milk group will be provided with 500 mL of milk (Avonmore 1% Light, Glanbia, Kilkenny) containing 215 kcal, 17.0 g of protein, 25.5 g of carbohydrates and 5 g of fat. At the same time participants in control group (C) will be provided with 500 ml of an energy-matched carbohydrate solution.

Blood Sampling: An individual trained in phlebotomy will draw the blood samples into vacutainers

Complete Blood Count: Routine complete blood counts (CBC) will be performed on whole blood collected in EDTA vacutainers using an automated cell counter (Coulter Ac.Tdiff2 Analyzer by Beckman Coulter).

Creatine Kinase: CK will be measured with a Randox Datona™ clinical chemistry analyser using a CK-NAC assay.

Plasma Cytokines: Plasma concentrations of IL-6, TNF- α , IL1- β and IFN- γ will be quantified simultaneously using a multiplex immunoassay system that employs electrochemiluminescent detection. Plates will be read using the Mesoscale 2400 Sector Imager.

Countermovement Jump Height: Countermovement jump height will be assessed using a light timing system (Smartjump, Fusion Sport, Coopers Plains, Queensland, Australia). Participants will squat down and jump vertically, with their hands on their hips throughout. Three trials with a 60 s rest will be performed and the best score will be used for analysis.

Drop jump: Participants will maintain an upright posture with their hands on their hips throughout the exercise. They will step off a 30 cm height onto a JumpMat (Smartjump, Fusion Sport, Coopers Plains, Queensland, Australia) and then immediately propel upwards and finish when they land on the JumpMat a second time. Three trials with a 60 s rest will be performed and the best score will be used for analysis.

Maximal Oxygen Uptake (VO_2max): Participants will undertake a maximal exercise test on a treadmill using a ramp protocol. Treadmill velocity will remain constant and the gradient will be increased at a rate of 0.2% every 12 sec until the participant reaches volitional exhaustion. Heart rate, rating of perceived exhaustion (RPE) and expired gases will be monitored throughout the test using open circuit spirometry.

Muscle Soreness: Subjective muscle soreness (DOMS) will be measured using a 100 mm visual analogue scale (VAS). Soreness will be indicated on the VAS after the participant performs a squat to a knee angle of approximately 90° with hands on hips and feet shoulder width apart and then returning to the standing position. The VAS is anchored to written cues from left (no muscle soreness) to right (muscles too sore to move), which corresponded to a number (0–10 unseen by the participant) on the reverse side. Participants will indicate their level of perceived soreness based upon the rating scale. This scale has been established as a valid and reliable measurement tool of soreness (Price et al., 1983)

GPS Tracking: A 10Hz GPS tracking device using earth orbiting satellites will be used to triangulate location, yielding time, distance, and velocity data. The GPS tracking device will be worn in a pouch attached to a harness type vest between the shoulder blades in the upper thoracic spine region. The tri-axial accelerometer in the unit will be used to calculate running velocities. The recorded location and running velocities will be stored within the tracking unit.

Heart Rate Determination: Heart rate will be continuously measured using a wireless Polar team system (Polar Precision Performance SW 3.0, Kempele, Finland). A transmitter will be attached to an elastic strap and placed around the chest. The strap length will be adjusted to fit comfortably and the buckle locked. The grooved electrode area on the back of the transmitter will be moistened.

Heart rate will be recorded and stored on the GPS device. The data will be subsequently transferred from the receiver to a PC for analysis.

Body Composition: Participants will be instructed to wear a light top and shorts and to remove their shoes prior to the measurement. Height will be measured to the nearest 0.1 cm using a portable scale (Seca 707 Balance Scales, GmbH, Hamburg, Germany). Body mass will be measured to the nearest 0.1 kg (Seca model 220 GmbH, Hamburg, Germany). Lang skinfold callipers (Cambridge Scientific Industries, MD) will be used to measure double thickness subcutaneous triceps and subscapular adipose tissue. Measurements will be taken on the right side of the body. Percent body fat will be calculated using an equation developed for children and youth⁴

Video Analysis: The electronic recording of the game will be downloaded and stored on a dedicated hard drive. Footage will be analyzed using a special software program (Dartfish) that combines video and statistical analysis.

2.4 PARTICIPANT PROFILE

Provide the number, age range and source of participants. Please provide a justification of your proposed sample size. Provide a justification for selecting a specific gender, age, or any other group if this is done in your project.

Inclusion Criteria:

- Current member of a minor football panel
- ≥ 16 years
- Clinically stable and in good health

Exclusion Criteria:

- Clinical conditions that may preclude them from exercise or injury, which would affect their performance in the games or tests, or which may be aggravated by participating in the games or performing the tests.

2.4(a) PARTICIPANT VULNERABILITY

Are some or all of participants vulnerable in any way? (e.g., by virtue of the group they belong to, people who have undergone traumatic or adverse emotional events, people with diminished cognitive ability, power relations between researchers and participants etc.)? If they are, state what this vulnerability (or vulnerabilities) is and justify why this research is being done with such participants.

No

2.4(b) CHILD PARTICIPANTS

If your participants include children, please confirm that you are in compliance with the following research specific guidelines as detailed in the DCU Child Protection framework. Further information on the framework is available online at <http://www.dcu.ie/equality/crc.shtml>

Yes

Please indicate your compliance with the following guidelines:

1. Have you made contact with the DCU Child Protection Officer? **Yes**
2. Informed consent is being obtained from the parents/guardians of children under 18 **Yes**
3. Informed assent must also be obtained from the children themselves **Yes**
4. The effect of the research on the child must be monitored to ensure that they feel comfortable with continuing with the research **Yes**
5. In addition to the child one other person should be present during the research. There may be rare occasions when a confidential interview or a one-to-one meeting is necessary and in such circumstances, the interview should be conducted in a room with an open door or visual access **Yes**

YES or NO

2.5 EXPLAIN HOW PARTICIPANTS ARE TO BE RECRUITED

Please provide specific details as to how you will be recruiting participants. How will people be informed that you are doing this research? How will they be approached and asked if they are willing to participate? If you are mailing or phoning people, please explain how you have obtained their names and contact details. If a recruitment advertisement is to be used, please ensure you attach a copy to this application.

A letter (Appendix 1) providing a brief summary of the research project will be sent to the manager of North Dublin GAA clubs with a minor boys football team completing in the Dublin football league/championship. Professor Niall Moyna (PI) will make a follow up phone call to each manager

⁴ Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, et al. Skinfold equations for estimation of body fatness in children and youth. Human Biology 1988; 60(5):709-72

and will arrange to give a presentation to teams interested in participating. The purpose of the study will be outlined and a brief summary of what is involved will be explained to all potential participants. The nature, benefits and risks of the study will be explained. In addition, they will be provided with a plain language statement. They will be encouraged to ask questions. They will be provided with an informed consent to be signed by a parent/guardian. In addition, they will sign an informed assent form and complete a Physical Activity Readiness Questionnaire (Appendix 2).

2.6 PLEASE EXPLAIN WHEN, HOW, WHERE, AND TO WHOM RESULTS WILL BE DISSEMINATED, INCLUDING WHETHER PARTICIPANTS WILL BE PROVIDED WITH ANY INFORMATION AS TO THE FINDINGS OR OUTCOMES OF THE PROJECT?

The results will form the basis for an MSc in exercise physiology and may be presented at scientific meetings and published in scientific journals. The identity of individual participants will not be divulged. Group information will only be presented. Participants will be provided with a copy of their baseline laboratory results and a summary of the overall study findings.

2.7 ARE OTHER APPROVALS REQUIRED TO GAIN ACCESS TO ANOTHER LOCATION, ORGANISATION ETC.?

No

2.8 HAS A SIMILAR PROPOSAL BEEN PREVIOUSLY APPROVED BY THE DCU REC?

YES -

(If YES, please state both the REC Application Number and Project Title)

SHHPREC/2013/009 - Effect of three competitive Gaelic football games in 8 days on peak power and markers of muscle damage and immune function

3. RISK AND RISK MANAGEMENT

3.1 JUSTIFICATION OF STATED LEVEL OF RISK TO RESEARCH PARTICIPANTS

You must provide a justification for the stated level of risk, as indicated on the cover page of your application. Note that the level of risk may be influenced by the vulnerability of the research group, the methods employed and the nature of the research itself. For further information on risk levels, please refer to the Types of Submission information on the website: https://www4.dcu.ie/researchsupport/research_ethics/guidelines/types.shtml

The study will collect standard laboratory based measures of human performance and blood collection. An almost identical submission underwent expedited review last year.

3.2 DOES THE RESEARCH INVOLVE:

	YES or NO
* use of a questionnaire? (attach copy**)?	Yes
* interviews (attach interview questions)?	No
* observation of participants without their knowledge?	No
* participant observation (provide details in section 2)?	No
* audio- or video-taping interviewees or events?	Yes
* access to personal and/or confidential data (including student, patient or client data) without the participant's specific consent?	No
* administration of any stimuli, tasks, investigations or procedures which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process?	Yes
* performance of any acts which might diminish the self-esteem of participants or cause them to experience embarrassment, regret or depression?	No
* investigation of participants involved in illegal activities?	No
* procedures that involve deception of participants?	No
* administration of any substance or agent?	Yes
* use of non-treatment of placebo control conditions?	No
* collection of body tissues or fluid samples?	Yes
* collection and/or testing of DNA samples?	No
* participation in a clinical trial?	No
* administration of ionising radiation to participants?	

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES

Identify, as far as possible, all potential risks to participants (physical, psychological, social, legal, economic, etc.), associated with the proposed research. Please explain what risk management procedures will be put in place to minimise these risks.

Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks. The volume of blood drawn is not harmful.

3.4 ARE THERE LIKELY TO BE ANY BENEFITS (DIRECT OR INDIRECT) TO PARTICIPANTS FROM THIS RESEARCH?

YES

(If YES, provide details.)

Participants will be provided with a summary of their baseline measurements

3.5 ARE THERE ANY SPECIFIC RISKS TO RESEARCHERS?

Examples include use of dangerous materials, asking certain types of questions, research being undertaken in certain locations, researchers working alone in isolated areas, etc.

YES

(If YES, please describe and explain what risk management procedures will be put in place to minimise these risks.)

Working with blood and needles carries risks. However, the exposure to blood and needles is minimal and the School of Health and Human Performance has standard operating procedures for the handling of biological products.

3.6 DEALING WITH ADVERSE/UNEXPECTED OUTCOMES

Please describe what measures/protocols you have put in place in the event that there are any unexpected outcomes or adverse effects to participants arising from involvement in the project.

The School of Health and Human Performance has the facilities to implement all aspects of this study and has an emergency plan for adverse events. In the unlikely event of a major adverse outcome, an ambulance will be called and the participant will immediately be sent to Beaumont Hospital, Dublin. In the unlikely event of a minor adverse outcome during the game, the situation will be dealt with by an Athletic Trainer and an adverse outcome that occurs in the Human Performance Laboratory will be dealt with by Prof. Moyna.

3.7 HOW WILL THE CONDUCT OF THE PROJECT BE MONITORED?

Please explain how the principal investigator will monitor the conduct of the project (especially where several people are involved in recruiting or interviewing, administering procedures, etc.) to ensure that it conforms with the procedures set out in this application. In the case of student projects please give details of how the supervisor(s) will monitor the conduct of the project.

The principal investigator will be involved in all aspects of the research, including participant recruitment and data collection. The research team will have daily meetings during the data collection phase to update on all aspects of the study. The School of Health and Human Performance has a detailed list of Standard Operating Procedures for each of the protocols in this study. All researchers, including students, will be familiar with the procedures and the Safety Statement before beginning data collection.

3.8 SUPPORT FOR PARTICIPANTS

Depending on risks to participants you may need to consider having additional support for participants during/after the study. Consider whether your project would require additional support, e.g., external counselling available to participants. Please advise what support will be available.

This project does not require additional support for participants

3.9 DO YOU PROPOSE TO OFFER PAYMENTS OR INCENTIVES TO PARTICIPANTS?

YES

(If YES, please provide further details.)

Each participant will receive a pair of football boots for their participation in the study

3.10 DO ANY OF THE RESEARCHERS ON THIS PROJECT HAVE A PERSONAL, PHILOSOPHICAL, FINANCIAL OR COMMERCIAL INTEREST IN ITS OUTCOME THAT MIGHT INFLUENCE THE INTEGRITY OF THE RESEARCH, OR BIAS THE CONDUCT OR REPORTING OF THE RESEARCH, OR UNDULY DELAY OR OTHERWISE AFFECT THEIR PUBLICATION?

NO

(If YES, please specify how this conflict of interest will be addressed.)

4. INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS (Approx. 200 words)

List the academic qualifications and outline the experience and skills relevant to this project that the PI, other researchers and any supporting staff have in carrying out the research and in dealing with any emergencies, unexpected outcomes, or contingencies that may arise.

Prof. Moyna is an exercise physiologist and has extensive experience
Kevin Reilly completed an BSc Physical Education/Biology in DCU and is familiar with all of the laboratory based procedures to be used in the study

5. CONFIDENTIALITY/ANONYMITY

5.1 WILL THE IDENTITY OF THE PARTICIPANTS BE PROTECTED?

YES

(If NO, please explain why.)

IF YOU ANSWERED YES TO 5.1, PLEASE ANSWER THE FOLLOWING QUESTIONS:

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED?

Please bear in mind that where the sample size is very small, it may be impossible to guarantee anonymity/confidentiality of participant identity. Participants involved in such projects need to be advised of this limitation in the Plain Language Statement/Information Sheet.

Confidentiality is an important issue during data collection. Participant's identity and other personal information will not be revealed, published or used in further studies. Participants will be assigned an ID number under which all personal information will be stored. The principal investigator and graduate student listed on this ethics application will have access to the data.

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY

Participants need to be made aware that confidentiality of information provided cannot always be guaranteed by researchers and can only be protected within the limitations of the law - i.e., it is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions. This information should be included in your Plain Language Statement and Informed Consent Form. Depending on the research proposal and academic discipline, you may need to state additional specific limitations.

State how and where participants will be informed of these limitations, or give a justification if this will not be done.

Appropriate information relating to legal limitations to data confidentiality is provided in information in the plain language statement and consent form.

6. DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL

For the purpose of this section, "Data" includes that in a raw or processed state (e.g. interview audiotape, transcript or analysis). "Samples" include body fluids or tissue samples.

6.1 HOW AND WHERE WILL THE DATA/SAMPLES BE STORED?

Note that the REC recommends that all data be stored on campus – please justify any off-site storage.

Data collection sheets will be stored in a secure locked cabinet in the Vascular Health Research Unit, DCU. Electronic data will be saved in a password-protected computer in the Vascular Health Research Unit, DCU. Blood samples will be stored in a -80° freezer in the School of Health and Human Performance, DCU. The principal investigator will be responsible for the security of the data and blood samples.

6.2 WHO WILL HAVE ACCESS TO DATA/SAMPLES?

If people other than the main researchers have access, please name who they are and explain for what purpose.
Named researchers only

6.3 IF DATA/SAMPLES ARE TO BE DISPOSED OF, PLEASE EXPLAIN HOW, WHEN AND BY WHOM THIS WILL BE DONE?

Note that simply deleting files is not sufficiently secure. The additional steps to be taken to maintain data security should be given. If data/samples are NOT being disposed of, please justify this decision.

Data collection sheets, electronic data and blood samples will be kept for 12 months from the date of publication of the research. The principal investigator will be responsible for disposal of the data. The original data sheets will be shredded by the principal investigator and the blood samples will be disposed using standard procedures in the School of Health and Human Performance for disposal of biohazardous material.

7. FUNDING

7.1 HOW IS THIS WORK BEING FUNDED?

National Dairy Council

7.2 PROJECT GRANT NUMBER (If relevant and/or known – otherwise mark as N/A)

N/A

7.3 DOES THE PROJECT REQUIRE APPROVAL BEFORE CONSIDERATION FOR FUNDING BY A GRANTING BODY?

No

7.4 HOW WILL PARTICIPANTS BE INFORMED OF THE SOURCE OF THE FUNDING? (e.g. will it be included in the Plain Language Statement)

Plain language statement

7.5 DO THE FUNDERS OF THIS PROJECT HAVE A PERSONAL, FINANCIAL OR COMMERCIAL INTEREST IN ITS OUTCOME THAT MIGHT COMPROMISE THE INDEPENDENCE AND INTEGRITY OF THE RESEARCH, OR BIAS THE CONDUCT OR REPORTING OF THE RESEARCH, OR UNDULY DELAY OR OTHERWISE AFFECT THEIR PUBLICATION?

YES

These above question has been addressed in the agreed contract between DCU and the National Dairy Council. (document attached)

Standard template for ethical justification for blood sampling associated with human studies conducted within DCU.

Completion instructions:

This document is intended to prompt responses to a number of standard questions which generally need to be answered to justify the sampling of blood associated with human studies.

The document is not meant to be an exhaustive exploration of the justification for such sampling and in specific situations. Additional information may be required/ requested.

Answers are expected to be brief but should also be informative. See a sample completed form at the end.

Queries should be directed to the Secretary of the Research Ethics Committee in the OVPR office.

1) Briefly explain why blood sampling is required

The blood samples will be used to measure biomarkers of muscle damage, circulating leukocytes and inflammatory cytokines

2) Outline the analyses, components or general applications to be investigated in subject blood (now and any future studies)

Circulating biomarkers (CK, LDH) will be determined using spectrophotometric assays, performed on an automated bench-top clinical chemistry system (ACE®, Alfa Wassermann B.V., Netherlands) using appropriate reagents, calibrators and controls (Randox Laboratories, UK). Cytokines (IL-1, IL-6 and TNF- α) will be determined using ELISA assays. Circulating leukocytes and erythrocytes will be determined using a bench-top Coulter Counter.

3) Are any alternatives available to substitute the venous sampling of blood? yes/no.

No

4) Will sampling require cannulation or direct vein puncture?

Direct vein puncture

5) Outline the minimum volume of original subject blood (i.e. not serum or plasma) required to measure the required components.

6.0 ml

6) Are steps being taken in the protocol to minimise the volume of blood samples being taken? Yes

Yes. We have taken the minimum volume of blood that will allow us to measure the circulating parameters

7) Are steps included to minimise the number of blood samples/vein puncture being taken? Yes

Yes. We have taken the minimum number of vein punctures that will allow us to measure circulating parameters at the time points of interest

8) Anticipated sampling methodology

Volume of blood to be taken per sample	6.0 ml
Maximum number of samples to be taken per "sitting"	1
Maximum number of samples taken per day	2
Maximum number of samples to be taken over the course of the full study (if long duration study indicate the amount taken in an active 1 month period)	5
Maximum anticipate number of vein puncture episodes	5
Total volume of blood that will be taken from subject.	30 ml

9) I certify that:-

- all persons sampling blood in this study are certified to do so through the school/unit where this work is being conducted
- that all those manipulating the resultant samples are fully trained in the safe practice of handling blood
- all persons handling this blood have received appropriate information according to current vaccination policy.

Signature of Study PI's

Niall Moyna

Date: 11 June 2015

An original signed copy must accompany electronic submissions. Alternatively, a PDF or other scanned version with a signature may be submitted

Appendix 1

Plain Language Statement

Project Title: COMPARISON OF TWO NUTRITIONAL INTERVENTIONS ON MUSCLE DAMAGE, RUNNING SPEED AND MUSCLE POWER FOLLOWING A GAELIC FOOTBALL GAME

The Research Study will take place in the School of Health and Human Performance, DCU.

The principal investigator is: Prof. Niall M. Moyna

(Tel: 7008802, Fax: 7008888, Email: niall.moyna@dcu.ie)

I. Introduction to the Research Study

During a game of Gaelic football our muscles fatigue and weaken temporarily. If the game is very intense, the muscles may be damaged to such an extent that it may take days for them to recover. This type of muscle damage has been named exercise-induced muscle damage. It results in the leaking of proteins from damaged cells into the blood and an inflammatory response to assist with healing. In addition, many individuals can experience muscle soreness and/or pain and poor performance in tests of speed and power. A number of interventions such as anti-inflammatory medications, massage and nutritional strategies have been used to limit muscle damage and attenuate the reductions in tests of speed and power following exercise-induced muscle damage. Milk contains electrolytes, and both carbohydrates and proteins, and has been proposed as a convenient, accessible, low-cost alternative to commercial powdered recovery supplements. This study will compare the effect of consuming 500 mL of milk or 500 mL of a carbohydrate drink on muscle damage, running speed and muscle power following a Gaelic football game. The study is being funded by the National Dairy Council.

II. What involvement in the Research Study will require

Your son will play a game of Gaelic football at the DCU sports fields and immediately after the game he will get one of two different 500 ml drinks to consume. Your son will wear a heart rate monitor and GPS tracking device during each game and the game will be recorded for analysis of player movements. Your son will have a blood sample taken 2-3 hours before the game, within 15 min after the game and 24, 48 and 72 hours after the game. Your son will rate his muscle soreness and undertake tests to measure his speed and power 2-3 hours before the game and 24, 48 and 72 hours after the game. In addition, his height, weight, muscle mass and percentage body fat will be measured and he will also undergo a fitness test on a treadmill to assess his cardiovascular fitness. With the exception of the post-game blood draw, all other measurements will be undertaken in the Human Performance Laboratory at DCU.

Your son's body weight will be measured using electronic weighting scales and his muscle mass and percentage fat mass will be estimated using skinfold calipers. His running speed will be measured by having him sprint 20 metres from a standing start. His explosive power will be measured by having him perform two simple jump tests. Your son's cardiovascular fitness will be assessed by having him run on a treadmill while wearing a special headgear that is attached to a mouthpiece.

III. Potential risks from your involvement in the Research Study

Your son may experience some muscle soreness in his legs or nausea following some of the exercise tests. Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks. The amount of blood drawn is not harmful. It is 30 mL which is equivalent to approximately two tablespoons.

IV. What are the benefits (direct or indirect) of taking part in the Research Study

Your son will be provided with his fitness test results, a copy of the overall study results and a pair of football boots.

V. Arrangements to protect the confidentiality of data

Your son's confidentiality will be guarded. Your son will be assigned an ID number under which all personal information will be stored in the secure locked filing cabinet and saved in a password protected file in a computer at DCU. The results of the study will be used for a postgraduate project and may be published in academic journals. Your son will not be identified, as his information will be presented as part of a group. You need to be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

VI. Data disposal

The data will be kept for a maximum of 1 year from the date of publication of the research. It will then be shredded.

VII. Withdrawal from the study

Involvement in this study is completely voluntary. Your son may withdraw from the research study at any point.

If you have concerns about this study and wish to contact an independent person, please contact: The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000

Appendix 2

Informed Consent

Dublin City University

I. Project Title. **COMPARISON OF TWO NUTRITIONAL INTERVENTIONS ON MUSCLE DAMAGE, RUNNING SPEED AND MUSCLE POWER FOLLOWING A GAELIC FOOTBALL GAME**

Principal Investigator Prof. Niall M. Moyna

II. Purpose of the research

This study will compare the effects of consuming 500 mL of milk or 500 mL of a carbohydrate drink on muscle damage, running speed and muscle power following a Gaelic football game

III. Confirmation of particular requirements as highlighted in the Plain Language Statement

Participant – please complete the following (Circle Yes or No for each question)

I have read the Plain Language Statement (or had it read to me)	Yes ☐	No ☐
I understand the information provided	Yes ☐	No ☐
I have had an opportunity to ask questions and discuss this study	Yes ☐	No ☐
I have received satisfactory answers to all my questions	Yes ☐	No ☐

IV. Confirmation that involvement in the Research Study is voluntary

Your son may withdraw from the Research Study at any point.

V. Advice as to arrangements to be made to protect confidentiality of data, including that confidentiality of information provided is subject to legal limitations

Your son's personal information will not be revealed, published or used in further studies. Your son will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password protected file in a computer at DCU. The named investigators will have access to the data. Data will be shredded by Prof. Moyna one year after publication of the study findings.

Confidentiality is insured, but I must be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

VI. Any other relevant information

If my son is in a dependent relationship with any of the researchers his involvement in the project will not affect ongoing assessment/grades/management or treatment of health at DCU.

VII. Signature:

I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to my sons taking part in this research project.

Participants Signature:

Name in Block Capitals:

Witness:

Date:

Appendix 3

DUBLIN CITY UNIVERSITY

INFORMED ASSENT

Study Title: COMPARISON OF TWO NUTRITIONAL INTERVENTIONS ON MUSCLE DAMAGE, RUNNING SPEED AND MUSCLE POWER FOLLOWING A GAELIC FOOTBALL GAME

1. My minor football team manager and my parents/legal guardian have talked to me about taking part in a research study.
2. I have been told that I will play a game of Gaelic football at the DCU sports fields and immediately after the game I will get one of two different 500 ml drinks to consume. I will wear a heart rate monitor and GPS tracking devise during each game
3. I have been told that game will be recorded
4. I have been told that I will visit DCU before the study to have my height, weight and body fat measured and that I will run on treadmill to see how fit I am
5. While running on a treadmill I will wear a nose clip on my nose and a mouthpiece in my mouth. The clip on my nose and the plastic tube in my mouth may feel a little bit uncomfortable.
6. I will have a small blood sample taken from a vein in my arm before the game, after the game and 24, 48 and 72 hours after the game.
7. I will rate my muscle soreness and undertake tests to measure my speed and power 2-3 hours before the game and 24, 48 and 72 hours after the game.
8. I have been told that with the exception of the blood sample after the game all the other tests will take place at DCU.
9. I will not do any exercise that makes me tired the day before the game.
10. I may feel tired or be out of breath when I am running on the treadmill and my legs may feel tired
11. If I wish, I can stop doing the tests at any time.
12. If I wish, I may choose not to take part in any of the tests.
13. I know that the people in DCU, my team manager and my parents/guardian will not be upset with me if I decide not to take part in this study, or if I wish to stop taking part in the study.

SIGNED: _____
(Participant's name)

DATE: _____

SIGNED: _____
(Witness name)

DATE: _____

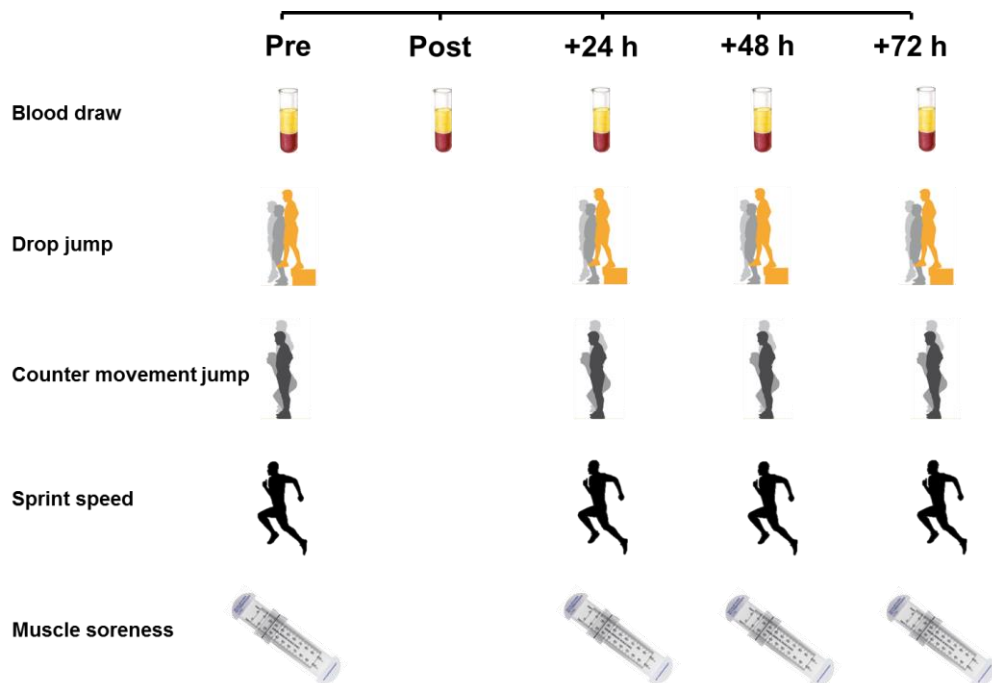
Appendix 4

Letter to the Coach

Dear:

I am an exercise physiologist and staff member in the School of Health and Human Performance at DCU. One of my Master's degree students, Kevin Reilly, is planning to undertake a study that will compare the effect of two nutritional interventions on muscle damage, running speed and muscle power following a Gaelic football.

Participation in the study will require your minor team playing a Gaelic football match at DCU Sports Ground. After the game they will consume 500 mL of milk or a 500 mL solution containing the same amount of carbohydrates as the milk. The football game will be recorded for analysis of skills performed and possession characteristics. Each player will wear a heart rate monitor and GPS tracking device during the game. The GPS device will be used to determine the distance covered and activity characteristics of each player. A small blood sample will be taken before the game, immediately post-game and at 24, 48 and 72 hours after the game (see figure). Sprint performance and muscle power will be measured and muscle soreness will be assessed before the game, and at 24, 48 and 72 hours after the game. Each player's body composition and aerobic fitness (VO_{2max}) will be measured. With the exception of the post-game blood draw, all other measurements will be undertaken in the Human Performance Laboratory at DCU (figure). The first visit to DCU will be 1 hour and the other five visits will be approximately 20 min in duration. Each player will receive a new pair of football boots for participating in the study.



We are contacting you to see if your minor football team would be interested in taking part in the study.

If you would like more information please feel free to me at – 087-9779633 or niall.moyna@dcu.ie

Kindest regards,

Niall M. Moyna, PhD
Professor

Appendix 5

Physical Activity Readiness Questionnaire

Please read the questions carefully and answer each one honestly: check YES or NO.

- | | |
|----------------------------|---|
| Yes / No | Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor? |
| Yes/ No | Do you feel pain in your chest when you do physical activity? |
| Yes / No
activity? | In the past month, have you had chest pain when you were not doing physical |
| Yes / No
consciousness? | Do you lose your balance because of dizziness or do you ever lose |
| Yes / No | Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity? |
| Yes / No | Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition? |
| Yes / No | Do you know of <u>any other reason</u> why you should not do physical activity? |

I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

NAME: _____

SIGNATURE: _____

DATE: _____

SIGNATURE OF PARENT: _____

WITNESS: _____

Appendix 2

Summary of studies examining the effect of milk supplementation and EIMD

	Gender	Muscle Damage	Supplementation dose	Nutrition Timing	Measurements	Summary
Wojcik et al., (2001)	27 healthy (23.5±0.7) untrained men	100 isotonic eccentric quadriceps contractions	CHO - 1.25 g/kg CHO-P (milkshake) – 0.875 g/kg CHO + .375 g/kg/protein Water	Post-muscle damaging exercise	Muscle glycogen, insulin, GH, testosterone, cortisol, IL-1, IL-6, TNF –α, 3-methyl-histidine	No effect on CK, DOMS, inflammatory cytokines or muscle function
Cockburn et al. (2008)	24 healthy men (21 ±3 yr) participating in a variety of team sports	Unilateral isokinetic dynamometry Eccentric-concentric knee flexions	1. CHO-P (milkshake) 2. Milk 3. CHO 4. Water	Post-muscle (500 mL) and +2h (500 mL)	Pre, 24h and 48h, and post EIMD Passive DOMS CK and Mb Peak torque	Attenuate decrements in peak torque and increases in CK and MB. No effect on passive DOMS
Pritchett et al., (2009)	10 cyclists & triathletes (27.1 ± 7.9yr)	High Intensity intermittent protocol	CHO-P Chol milk	Post exer and 2 h into recovery	Endurance performance CK	No effect of between the milk and CHO-P in markers of muscle damage and TTE in a subsequent exercise bout
Cockburn et al., (2010)	32 healthy men (20 ± 2yr) who participated in a variety of individual & team sports	Unilateral isokinetic dynamometry eccentric-concentric knee flexions	100 mL of semi-skimmed milk-based CHO-P supplement 707 Kcal, 34.5g protein, 118.2g CHO and 16.4g either before, immediately after or 24 h after exercise	Pre, post or 24h post muscle damaging exercise Comparisons only using post EIMD supplementation	Pre, 24h, 48h and 72h post EIMD CK (218 U/L) Peak Torque Active DOMS Reactive strength index (jump ht, contact time)	DOMS peaked at 48h Post EIMD milk supplementation attenuated the increase in CK, DOMS and decrements in peak torque and RSI over the first 48h
Ferguson-Stegall et al., 2011	10 healthy, trained cyclists and triathletes (5 men, 5 women) between the ages of 18 and 39 y	Cycle for 1.5 h at 70% VO ₂ max plus 10 min of intervals.	RPCDBT Choc milk CHO Placebo (flavoured water) Post exercise and	Blood: Immediately, 45, 120 and 240 min post exer and pre, 30,60 min and end of TT		

			2 h and 4 h into rec	Biopsy: Immediately, 45 & 240 min post ex		
Cockburn et al., (2012)	24 healthy men who participated in a variety of individual & team sports	Unilateral isokinetic dynamometry eccentric-concentric knee flexions	500 mL or 1000mL of semi-skimmed milk or 1000 mL of water	Post-muscle damaging exercise	CK, Mb, IL-6, DOMS (active & passive), peak torque were recorded pre, 24h, 48h and 72h post-EIMD	500 mL and 1000 mL of milk limited decrements in peak torque (dominant leg)and increases in CK and IL-6 500 or 1000 mL had no effect on active or passive DOMS or Mb
Cockburn et al. (2013)	Male soccer players	Isokinetic dynamometry Eccentric-concentric knee flexions	500ml of skimmed milk vs. 500mL water	Post-muscle damaging exercise	CK, Mb, DOMS, CMJ, RSI (jump ht/ contact time) 10 & 15m sprint and agility were recorded pre, 24h, 48, and 72h, post- EIMD. LIST performed 48h post.	Milk limited decrements in 10 and 15 m sprints, agility time, and mean 15-m sprint performance during the LIST (RSA) No benefit of milk consumption on active and passive muscle soreness, ↑ in CK or Mb decrements in RSI, CMJ, and RPE and HR measured during the LIST.
Rankin et al., (2015)	Male and female team sport athletes	Bilateral eccentric protocol-Ecc-concentric knee flexions using Isokinetic dynamometry	500mL of milk vs. 500ml of energy-matched CHO drink	Post-muscle damaging exercise	sTnl, CK, peak torque, CMJ height, 20m sprint & passive and active soreness were recorded pre, 24h, 48h and 72h post-EIMD	Milk consumption attenuated ↓ in peak torque and 20m sprint performance and ↑ in passive and active DOMS No benefit of milk consumption on

