Dairy Milk as an Ergogenic Aid: Assessing Athletic Performance and Perceptions

By

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ABSTRACT

Recent literature suggests that dairy milk may have the same effects as many ergogenic aids available today. Study 1 assessed the use and perceptions of dairy milk as an ergogenic aid among competitive athletes and recreational exercisers. Participants (n=294) completed a 14-question survey. Overall, 66.1% of athletes reported drinking dairy milk daily, 71.4% believed that milk will help with exercise performance and only 39.6% reported using milk as part of their exercise routine. Meanwhile, only 44.8% of football players reported consuming milk for exercise. A pilot study assessed the effects of chocolate milk (n=5) verses water (n=4) on attenuating symptoms of exercise induced muscle damage in collegiate football players. Muscle soreness, plasma creatine kinase and lactate dehydrogenase, countermovement jump and 15-m sprint were measured at various time points. No time*treatment effects were observed for all measurements. Given the gap between beliefs and actions, there is a need to provide nutrition education to athletes.

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AA: Amino Acids

ATP: Adenosine Triphosphate

BCAA: Branched chain amino acid

BCM-7: Bovine beta-cosmorphine7

BW: Body weight

$Ca^{2+:}$ Calcium

CHO: Carbohydrate

CE: Carbohydrate Electrolyte

CIS: Canadian interuniversity sport

CK: Creatine Kinase

CM: Chocolate Milk

CMJ: Countermovement jump

CPK: Creatine phosphokinase

CPR: Carbohydrate-Protein Replacement

CSA: Cross sectional area

DOMS: Delayed onset muscle soreness

EC: Excitation-contraction

EIMD: Exercise induced muscle damage

FA: Fatty acid

FBFM: Fat and bone free mass

FFST: Fat free soft tissue

FSR: Fractional synthesis rate

GLUT 4: Glucose transporter protein 4

IL-1ra: Interleukin-1 receptor antagonist

IL-6: Interleukin-6

IL-8: Interleukin-8

LDH: Lactate Dehydrogenase

Mb: Myoglobin

MLK: Milk

MLP: Mid-to late-pubertal

mTOR: mammalian target of rapamycin

MP: Milk protein

MVIC: maximal voluntary isometric contraction

NFB: Net fluid balance

NOLD: Non-oxidative leucine disposal

ORAC: Oxygen radical absorbance capacity

PCr: Phosphocreatine

PLP: Pre to late pubertal

PRO: Protein

PTH: Parathyroid hormone

Ra: Rate of appearance

RMR: Resting metabolic rate

rpS6: Ribosomal protein S6

RSI: Reactive strength index

sNtI: skeletal troponin I

SSC: Stretch-shortening cycle

SR: Sarcoplasmic reticulum

TG: Triglycerides

TT: Time trial

TTE: Time to exhaustion

USG: Urine specific gravity

VAS: Visual analogue scale

VO_2 max: Maximal oxygen uptake

WBPB: Whole body protein balance

WM: Whole milk

CHAPTER I. LITERATURE REVIEW

1.1 Introduction

Individuals engaging in exercise, both recreationally and competitively, experience metabolic and mechanical stresses, and if appropriate measures are taken, training adaptations will occur. Endurance athletes who are required to train for a prolonged period of time or those performing intermittent exercise (e.g. soccer, hockey, football, etc.) are at risk for impaired performance due to depletion of glycogen stores, dehydration, or damage to skeletal muscle, while those who are following a progressive resistance training program may also experience symptoms of muscle damage in addition to compromised strength gains and inability to achieve muscle hypertrophy. Indeed, optimal nutrition for recovery following strenuous endurance and/or resistance exercise is critical for athletes as their ability to perform at the following session may be compromised due to inadequate stored fuel and disruption of skeletal muscle. The ability to recover from exercise is extremely complex and requires sufficient fuel replenishment, the repair of skeletal muscle damage, and an improvement in training adaptations. The type of nutrient as well as nutrient timing and quantity are especially important when recovering from a strenuous bout of exercise. Prolonged, demanding endurance exercise relies heavily on body glycogen stores and performance may be hindered once depleted. Furthermore, high intensity exercise training can put stress on skeletal muscle and cause damage, by which symptoms may last several days. Therefore, if individualized energy and macronutrient targets are not met, athletes can experience severe decrements in their performance, resulting in reduced training adaptations.

As such, nutritional supplements are commonly used among recreational exercisers and elite athletes to promote recovery and enhance performance. However, these supplements are

often in the form of single nutrients and in order to effectively recover from an intense exercise session, one would need to replenish glycogen stores, repair/rebuild muscle tissue damage and consume adequate fluids and electrolytes to return to a positive fluid balance. Therefore, there is a recent interest towards dairy milk for post exercise recovery due to its abundance of high quality nutrients, which are rarely collectively found in commercially available dietary sport supplements. Thus, this review will discuss the mechanical and metabolic stress associated with exercise; specific nutritional interventions that can optimize recovery followed by a thorough evaluation of the current research of why dairy milk may be an effective nutritional strategy for post exercise recovery.

1.2 Substrate utilization during exercise

The ability for muscles to perform work relies on the body's energy systems using both endogenous and exogenous substrates. The major energy system used for muscular work during exercise lasting longer than 2 minutes is the oxidative (aerobic) pathway, which uses substrates such as muscle and liver glycogen, intramuscular triglycerides (TG) and adipose tissue TG and a minor amount of amino acids (AA), resulting in the production of adenosine triphosphate (ATP) via the Krebs cycle and the electron transport system (D. T. Thomas, Erdman, & Burke, 2016c). On the other hand, high intensity exercise lasting 10-180 seconds that require short bursts of energy seen in resistance training and sprinting use the anaerobic glycolytic pathway, which heavily relies on muscle glycogen and glucose (Rodriguez, DiMarco, & Langley, 2009). Furthermore, TG stored in adipose tissue, skeletal muscle and plasma contribute to the largest fuel reserve within the body and the amount that can be used for energy exceeds that of glycogen, which may contribute to an increase in fatty acid (FA) oxidation and decrease glycogen depletion during exercise (Hetlelid, Plews, Herold, Laursen, & Seiler, 2015; Horowitz

& Klein, 2000). The amount of carbohydrate and FA used by skeletal muscle depends on the exercise intensity and duration, substrate availability, and training status (Arkinstall et al., 2004).

In trained individuals, the substrates used for energy expenditure differ depending on exercise intensities and duration. Exercise intensity is often measured by an individual's maximal oxygen uptake (VO_2 max), which is a commonly used indicator of aerobic capacity. VO_2 max is defined as the maximal amount of oxygen which can be transported and used by the cells for cellular respiration during exercise done at increasing intensity (Midgley, McNaughton, & Wilkinson, 2006). When exercising at a low intensity (25% VO_2 max), there is minimal reliance on muscle glycogen for energy production thus the majority of energy is derived from plasma free fatty acids (FFA) and some from plasma glucose. When exercise intensity increases to moderate intensity (65% VO_2 max), there is an increase reliance on fat for energy in the form of intramuscular TG and plasma FFA whereas less muscle glycogen and plasma glucose are utilized (Romijn et al., 1993). Thus, the highest rate of fat oxidation occurs at this intensity. During moderate to high intensity (85% VO_2 max) prolonged endurance exercise muscle glycogen stores become the main contributor to energy production, despite greater endogenous fat stores (Knuiman, Hopman, & Mensink, 2015). Furthermore, in comparison to fat as a substrate, carbohydrates provide a greater yield of ATP per volume of oxygen that can be delivered to the mitochondria, thus greater exercise efficiency (D. T. Thomas, Erdman, & Burke, 2016b). Thus, specific nutritional strategies are recommended to ensure exercise performance can be improved and/or maintained at a given exercise intensity, which will be discussed later in the review.

1.3 Glycogen depletion

As previously mentioned, the body stores carbohydrates both in the liver and muscles, predominantly in the form of glycogen; an important and efficient energy source as exercise duration and intensity increases (Gonzalez, Fuchs, Betts, & van Loon, 2017). Exercise can stimulate the breakdown of liver glycogen, allowing this stored glucose to be transported to skeletal muscle and oxidized for energy, along with glucose-1-phosphate from skeletal muscle glycogen. Overall, body glycogen stores are limited, and the high fuel demand during moderate to high intensity exercise results in rapid depletion of glycogen stores, which can compromise exercise performance (C. Kerksick et al., 2008). Fatigue and muscular weakness experienced during prolonged, intense exercise can be associated with inability to maintain ATP synthesis due to depletion of muscle glycogen or hypoglycemia (Tsintzas & Williams, 1998). Furthermore, the ability for skeletal muscle to perform work is compromised with low glycogen availability, despite sufficient amounts of other fuel sources (Knuiman et al., 2015). Muscle glycogen stores are greater compared to liver, however liver glycogen plays a principle role in directly regulating blood glucose homeostasis and preventing hypoglycemia during exercise (Gonzalez, Fuchs, Betts, & van Loon, 2016). Muscle glycogen content varies depending on the training status of the athlete and their daily carbohydrate intake, whereas there is an observed upregulation of muscle glycogen concentrations in endurance trained athletes compared to untrained individuals (Knuiman et al., 2015). Furthermore, the accelerated rate of liver glycogenolysis that occurs as exercise intensity increases is reduced in trained versus untrained individuals, despite having similar starting liver glycogen concentrations (Gonzalez et al., 2016). Thus, at a given exercise intensity, endurance trained athletes would take longer to fully deplete glycogen stores, contributing to more prolonged exercise duration. Meanwhile, current

recommendations suggest daily carbohydrate intakes of 5-12 g/kg body weight to maximize endogenous glycogen stores, while training intensity and volume need to be considered to determine specific individualized carbohydrate targets (C. M. Kerksick et al., 2017). In general, nutritional strategies including nutrient type, amount and frequency have been sought to aid in adequate replenishment of body glycogen stores post exercise which will be discussed later in this review.

1.4 Exercise Induced Dehydration

Muscle contraction that occurs during exercise results in an increase in endogenous heat production and a rise in core body temperature. The primary mechanism to release this heat is through sweating, resulting in a loss of fluid and electrolytes from the body. If an individual loses sufficient fluids from the body, they would be in a hypohydrated state, indicating their body mass is less than ±0.2% normal in temperate environment and less than ±0.5% of normal in hot environments or during exercise (Evans, James, Shirreffs, & Maughan, 2017). Exercise induced dehydration can be a major concern for an athlete because a body water deficit as low as 2% body mass loss can significantly impair cognitive function and aerobic exercise performance, even more so in high temperatures (Nuccio, Barnes, Carter, & Baker, 2017). Exercise induced dehydration in the heat can lead to increased utilization of muscle glycogen stores, reduced cardiac output and blood flow to skeletal muscles contributing to reduced exercise intensity (Cheuvront, Carter, & Sawka, 2003). Meanwhile, impaired performance is commonly seen at 3-5% body weight loss in athletes who are involved in aerobic activities training in cool environments, athletes who are involved in anaerobic, high intensity exercise or those involved in sport specific skills training (D. T. Thomas et al., 2016b). Hence, adequate fluid and electrolyte intake should be consumed in order to overcome exercise induced dehydration, and

specific strategies should consider the type of exercise, environment and recovery time. These strategies will be discussed in further detail later in this review.

1.5 Exercise Induced Muscle Damage

Exercise induced muscle damage (EIMD) occurs as a result of unfamiliar or eccentric skeletal muscle contractions that are commonly performed during resistance, intermittent and endurance exercise. Eccentric movements involve elongation of skeletal muscle during concurrent muscle contraction, indicating that the muscle is active during stretch (Nosaka, Sakamoto, Newton, & Sacco, 2001). Concentric actions involve shortening of the muscle, thus natural muscle function is typically referred to as the stretch-shortening cycle (SSC), which involves a sequence of eccentric and concentric actions (Byrne, Twist, & Eston, 2004). The SSC occurs due to external forces such as gravity, which happens when the muscle is impacted or stretched during activities such as running, jumping and weightlifting (Byrne et al., 2004). The magnitude of damage that occurs to skeletal muscle differs depending on the intensity and duration and type of the exercise. Similarly, the time required to restore muscle strength depends on the severity of loss of muscle strength. EIMD can be increased following high repetitions of eccentric contractions, high vs. low eccentric torque, long vs. short muscle lengths, single joint vs. multiple joint exercises, exercising using arms compared to legs and knee flexors as opposed to knee extensors (Peake, Neubauer, Della Gatta, & Nosaka, 2017).

The mechanisms of how muscle damage occurs following exercise remains unclear, however there seems to be a general consensus among researchers that the initial event of damage to the muscle fiber can be due to physical or metabolic factors. It is believed that the damage that occurs to the muscle immediately following the eccentric exercise bout is due to the failure of the excitation-contraction (EC) coupling system and disrupted sarcomeres, however it is unclear

which of these events occur first (Proske & Morgan, 2001). The EC coupling system is a sequence of events that begins with the release of acetylcholine at the neuromuscular junction and ends with the release of calcium (Ca^{2+}) from the sarcoplasmic reticulum (SR) (Warren, Ingalls, Lowe, & Armstrong, 2001). Damage to the cell membrane leads to a disruption in Ca^{2+} homeostasis and elevated intracellular Ca^{2+} concentrations, resulting in the activation of phospholipase A_2 which activates inflammatory mediators. Inflammatory mediators such as prostaglandins and leukotrienes are responsible for the sensation of pain (Connolly, Sayers, & McHugh, 2003).

Although a bout of eccentric exercise may contribute to muscle damage, it has been suggested that repeating the same exercise over several weeks exhibits less damage as opposed to the magnitude of damage that occurs from the initial bout of novel eccentric movement (Nosaka et al., 2001). Furthermore, eccentric muscle contractions have been shown to be superior to concentric contractions, as they elicit greater hypertrophy and eccentric contraction-specific strength gains (Howatson & van Someren, 2008). However, muscle damage is still observed among trained athletes who repeatedly perform the same exercise, such as marathon runners (Hikida, Staron, Hagerman, Sherman, & Costill, 1983). Research also suggests that eccentric movements impair glycogen synthesis following exercise. Although this mechanism remains unclear, it is believed that impaired glycogen synthesis following eccentric exercise is due to the increased insulin resistance and decreased uptake of glucose into the muscle cell that occurs as a result of EIMD (Tee, Bosch, & Lambert, 2007). A possible explanation for this could be due to the down regulation of the insulin-mediated glucose transporter type 4 protein (GLUT4); found mainly on adipose and muscle, due to skeletal muscle damage or perhaps an

increased competition for glucose between inflammatory cells and muscle cells (Wojcik, Walber-Rankin, Smith, & Gwazdauskas, 2001).

It is important to note that the damage that occurs to the muscle following eccentric exercise is a normal part of muscle adaption and is not permanent (Armstrong, Warren, & Warren, 1991). However, the damage has been shown to cause impaired performance as a result of decreased power generation and a decrease in both isokinetic and isometric strength (Tee et al., 2007). Common indices of EIMD include delayed onset muscle soreness (DOMS), reduced range of motion, an increase of skeletal muscle enzymes in the serum and plasma, muscle swelling, inflammation, muscle stiffness, structural damage and, the most detrimental to an athlete is the inability to produce force. The measures of EIMD that are most commonly assessed in the literature are subjective feelings of muscle soreness, increases of muscle enzymes in the plasma (creatine kinase and myoglobin), indicators of inflammation and muscle function.

1.5.1 Biomarkers of EIMD

Muscle damage may be characterized by increases in serum or plasma intramuscular enzymes such as creatine kinase (CK) and myoglobin (Mb) (Tee et al., 2007). CK is an enzyme predominantly found in both the cytosol and mitochondria in skeletal muscle and is a key component of the regulation and maintenance of energy supply which is facilitated by the phosphocreatine (PCr) circuit. The PCr circuit involves CK in the mitochondria to use mitochondrial ATP to generate PCr which is then shuttled to the cytosol, allowing cytosolic CK to resupply ATP for muscle activity (Baird, Graham, Baker, & Bickerstaff, 2012). Therefore, the damage to muscle tissue following strenuous exercise facilitates the diffusion of this enzyme into the interstitial fluid (Cheung, Hume, & Maxwell, 2003).

Monitoring plasma CK levels may be used as an assessment of muscle overload or overtraining, which may be of interest to highly trained individuals in order to prevent injury. Plasma CK level varies depending on the level of training, duration of training, type of exercise and gender. For example, a strength training session can show peak CK values 8 hours following exercise, whereas more intense training such as multiple daily football sessions may lead to significant increases in CK on the fourth day following training (Brancaccio, Maffulli, & Limongelli, 2007). Females tend to have lower resting CK generation in skeletal muscle and less efflux into plasma following exercise compared to men, while the mechanism behind this is unclear, it could be due to less skeletal mass in females, thus less muscle protein turnover (Kendall & Eston, 2002).

1.5.2 Delayed onset muscle soreness (DOMS)

DOMS typically occurs following exercise when performing unfamiliar movements (e.g., downhill running, jumping) and is less likely to occur during regular training with commonly used movements, however it is still experienced during prolonged endurance exercise. DOMS can be reported by an individual experiencing pain or soreness in the exercised muscles within 24-48 hours following exercise, with relatively no pain in the first 8 hours (Byrne et al., 2004). DOMS is associated with muscle tenderness or stiffness and is assessed by a subjective measurement such as a Visual Analog Scale (VAS). A VAS allows individuals to rate their feelings of muscle soreness based on a scale rating from 0-10, wherein 0 indicates no muscle soreness and 10 indicates that muscles are too sore to move. There are many theories that seek to explain the onset of DOMS; however the exact mechanism remains unknown. While the proposed mechanism for EIMD has been explained earlier in this review, researchers seek to understand the mechanism behind why muscle soreness occurs days after the initial event. It is

believed that the inflammatory mediators responsible for the sensation of pain are produced by macrophages, which proliferate at 48 hours after the initial damage to sarcolemma (Cheung et al., 2003).

1.5.3 Effects of EIMD on exercise performance

For many athletes who are involved in intermittent team sports, their ability to produce power is an important factor for their performance. Team sports such as football, soccer and hockey may involve periods of short high intensity sprints followed by lower intensity periods of jogging or walking in addition to plyometric exercises. Several studies aimed to assess biomarkers of muscle damage and soreness by using study designs that imitates the exercises involved in intermittent team sports. It has been shown that when performing multiple 10 meter sprints following a series of maximal vertical jumps, peak running time significantly increased ($p < 0.05$) at 30 minutes (2.01s), 24 hours (2.02s) and 48 hours (2.02s) compared to baseline (1.96s) where values returned to normal at 72 hours (Twist & Eston, 2005). Plasma CK values were significantly higher at 28 (239 U/L) and 48 hours (245 U/L) compared to baseline (151 U/L) and peak values of perceived muscle soreness were detected 48 hours following muscle damaging plyometric exercises (Twist & Eston, 2005).

There has been mixed findings as to whether there is a relationship between biomarkers of muscle damage and DOMS. Muscle soreness has been shown to be greatest at 24 hours and 48 hours ($p < 0.05$) following a 90 minute Loughborough Intermittent Shuttle Test (LIST) which involved active males (23.8±1.7 yr) to walk and run intermittently which was designed to imitate multiple-sprint sports. Plasma CK was also elevated at 24 hours (774 U/L) and 48 hours (391 U/L) compared to baseline (202 U/L), however there was no correlation found between the elevations of these enzymes and the degree of muscle soreness (Thompson, Nicholas, &

Williams, 1999). On the contrary, Ascensao et al (2008) noted a correlation between a sprint time and Mb, CK, and DOMS in male soccer players (21 ± 1.1 yr) over a 72 hour recovery period following a 90 minute soccer match. Sprint time decreased by 5% compared to baseline until 72h, DOMS increased significantly until 48h ($p<0.05$) and plasma CK increased significantly until 72h compared to baseline ($p <0.05$). (Ascensao et al., 2008). As well, perceived muscle soreness was observed following eccentric actions of the elbow flexors, however impairment in muscle function (maximal isometric force) occurred prior to the peak in muscle soreness (Nosaka, Newton, & Sacco, 2002).

1.6 Nutritional strategies for post exercise recovery

As previously explained, there are many physiological limitations that are associated with strenuous exercise, with some similarities and differences that occur between different types of exercise. In order to overcome these limitations, athletes may benefit from the use of ergogenic aids. The purpose of an ergogenic aid is to ultimately promote greater training adaptations by improving exercise capacity, facilitating a prompt recovery and increasing the efficiency to perform work (Kreider, 2003). There are various types of ergogenic aids including physiological (e.g. blood doping), pharmacologic (e.g. androgenic steroids) and nutritional aids (e.g. creatine supplements) that are known to be used by athletes to enhance performance (Silver, 2001).

A wide variety of dietary sources of ergogenic aids are available to athletes that are unique in terms of attempting to facilitate glycogen synthesis, protein synthesis, and rehydration, and ultimately speed the recovery process. A recent study found that the main reported reasons for supplement use among elite athletes were to increase endurance, increase power and increase power and muscle mass (Giannopoulou, Noutsos, Apostolidis, Bayios, & Nassis, 2013). However, there is growing concern relating to the safety of these products, as athletes who

compete at an elite level may be subjected to random testing for illegal and banned substances which may not always be listed on the product label.

1.6.1 Post exercise glycogen resynthesis

Restoring muscle and liver glycogen stores are crucial in maintaining performance levels during prolonged high intensity exercise, specifically when executing multiple exercise bouts over a short time period (tournaments, stage races) or during periods of intensive training (Gonzalez et al., 2017). Despite glycogen synthesis rates occurring in the absence of carbohydrate intake, either through gluconeogenesis or lactate, resynthesis rates are maximized when carbohydrate is consumed following exercise (Burke, van Loon, & Hawley, 2017). Thus, immediate carbohydrate intake is recommended during a short recovery period (4-8h), as glycogen storage rates are highest within the first two hours following exercise which then fall to normal rates (Burke, Kiens, & Ivy, 2004). If sufficient carbohydrates are consumed and the level of muscle damage is minimal, glycogen stores can be restored within 24 hours (Barnett, 2006). However, if recovery period exceeds 8 hours, timing of carbohydrate intake is not as important as long as daily carbohydrate requirements are met (Burke et al., 2004). In order to restore muscle and liver glycogen stores, glucose is required either from the diet or from gluconeogenesis. The glucose derived from carbohydrate consumed following exercise must cross the skeletal muscle cell membrane, which is facilitated by glucose transporter proteins, specifically the isoform GLUT4, which is the predominant glucose transporter found in skeletal muscle and is regulated by insulin (Ivy & Kuo, 1998).

Research suggests that glycogen synthesis occurs in two separate phases; a rapid and slow phase. The rapid phase of glycogen synthesis occurs without the presence of insulin and occurs most rapidly if carbohydrates are consumed within 30 minutes to1 hour following

exercise. Glycogen synthesis rates are said to increase post exercise due to the activation of glycogen synthase, which is the rate limiting reaction in glycogen synthesis and increases post exercise. As well, muscle contraction increases the muscle permeability to glucose due to the increase in glucose transporters, thus those factors combined are responsible for the rapid phase of glycogen synthesis (Ivy & Kuo, 1998).The slow phase of glycogen synthesis is less understood, however it is believed that exercise training induces an increase in muscle insulin sensitivity which increases GLUT4 transporter proteins and promotes a rapid uptake of glucose into skeletal muscle (Ivy, 2004). Thus, since the slow phase is insulin dependent, delaying carbohydrate intake can reduce glycogen resynthesis.

Previous work has shown that co-ingestion of carbohydrate-protein supplement (CHO-PRO) immediately and 2 hours following exercise increases the rate of muscle glycogen synthesis compared to the intake of a high carbohydrate (HCHO: 108g CHO; 6g fat) and low carbohydrate (LCHO: 80g CHO; 6g fat) beverage (Ivy et al., 2002). These findings indicated that co-ingestion of a CHO-PRO supplement (80g CHO; 28g PRO, 6g Fat) resulted in a 22% recovery in glycogen storage at 40 minutes compared to HCHO (11.5%) and LCHO (5.5%), which further increased following the 2 hour supplement but was not observed in the HCHO or LCHO treatments. At the end of the 4 hour recovery period, CHO-PRO was able to replenish 46.8% of the glycogen utilized, thus these findings signify the importance of nutrient composition and nutrient timing following exercise (Ivy et al., 2002).

It has been suggested that an intake of carbohydrate at ≥1.2 g/kg body weight in intervals, such as every 30 minutes, contradicts any benefits of added protein, however, glycogen synthesis may be increased following ingestion of protein and carbohydrate combined if the carbohydrate intake is suboptimal (Gibala, 2007). Therefore, current recommendations suggest that

carbohydrate intake (1.0-1.2g/kg body weight/hour) immediately following exercise during the first 4-6 hours is highly recommended to enhance glycogen synthesis when there is limited time (<8 hours) between exercise sessions (Rodriguez et al., 2009). Similar glycogen synthesis rates can be achieved by combining carbohydrate (0.8 g/kg body weight) with protein (0.2-0.4 g/kg body weight) (C. M. Kerksick et al., 2017). Given that glycogen synthesis requires insulin and a rapid supply of glucose uptake, moderate to high glycemic index (GI) carbohydrates are recommended post exercise as they quickly raise blood glucose, which will promote a rapid uptake of glucose into the muscle (C. M. Kerksick et al., 2017). Furthermore, the type of carbohydrate has different effects on muscle and liver glycogen storage rates. Few studies have assessed the effects of nutrient intake on liver glycogen repletion following exercise. Recent studies have demonstrated that liver glycogen repletion is enhanced when co-ingestion of either fructose or galactose with glucose are consumed post exercise (Decombaz et al., 2011; Detko et al., 2013). Meanwhile, there is no added benefit to ingesting fructose and glucose mixtures on muscle glycogen repletion compared to glucose alone (Gonzalez et al., 2016). However, lower rates of gastrointestinal distress were associated with glucose-fructose co-ingestion which may be of importance for when glycogen stores need to be maximized and considerable amounts of carbohydrate need to be consumed over a short time period (Gonzalez et al., 2017).

1.6.2 Post exercise rehydration

Hydration is a key component of the recovery process for athletes performing endurance and intermittent exercise as increase sweat rate leads to dehydration, resulting from a negative fluid balance. In order to return to a positive fluid balance, fluids consumed post exercise must exceed the amount of fluid lost from the body. The main factors affecting rehydration is the volume and composition of the solution. It has been suggested that when the recovery period is

more than 24 hours, normal fluid intake is sufficient to replace the losses that occur from heavy training (Watson, Love, Maughan, & Shirreffs, 2008). However, when multiple training sessions are occurring within the same day, rehydration is crucial in order to effectively perform. Thus, a beverage containing adequate fluid and electrolytes to replace those lost in the sweat with additional carbohydrate to stimulate muscle glycogen synthesis would be ideal to enhance the rate of recovery. Furthermore, researchers propose that the addition of protein to a post recovery beverage will further promote sodium and fluid absorption into the intestine (Saunders, 2011a). Additionally, previous research suggests that the absorption of fluid is more rapid when fructose and glucose are ingested simultaneously compared to glucose alone (Casa et al., 2000). In order to return to a positive fluid balance, fluids consumed post exercise must exceed the amount of fluid lost from the body. Current recommendations suggest consuming fluids equivalent to 125-150% of body mass lost following exercise to accommodate for sweat losses and obligatory urine losses that continue throughout the recovery phase (D. T. Thomas et al., 2016b). In addition to fluid volume, fluid composition and rate of ingestion is critical in restoring fluid balance, particularly if the athlete is engaging in same day training sessions with short recovery time (Evans et al., 2017).

1.6.3 Post exercise muscle tissue repair

Muscular adaptations that occur from resistance exercise differ from those associated with endurance training. Protein supplementation is a popular practice among resistance-trained athletes to promote strength gains and enhance muscle recovery. For muscle hypertrophy to occur there needs to be a net positive protein balance. Therefore, muscle protein synthesis must exceed muscle protein breakdown, which would result in an increase in muscle mass if a regular training schedule persists. Resistance exercise has a profound effect on improving muscle protein

balance, however the balance remains negative in the absence of food. It is well known that AA in the free form is responsible for promoting muscle protein synthesis, more specifically the essential AA. Thus protein intake is crucial during the recovery period to repair and synthesize muscle proteins. As such, increases in the availabilities of AA and insulin, secreted from carbohydrate intake, should promote an anabolic environment for muscle protein synthesis. Resistance exercise may have an effect on muscle protein synthesis for up to 48 hours, however when the exercise intensity increases, the recovery of muscle protein synthesis is much slower (Rennie & Tipton, 2000).

On the other hand, a prolonged endurance session exhibits changes in whole body and skeletal muscle protein synthesis and breakdown, despite carbohydrate and fat being the main substrates used (Gibala, 2007). It has been previously shown that endurance exercise at moderate to high intensity decreases protein synthesis, increases protein breakdown and leucine oxidation (Miller et al., 2007). Skeletal muscle tissue damage has been observed in those who perform prolonged exercise as well as short bouts of high intensity exercise (Ferguson-Stegall, McCleave, Ding, Doerner Iii, et al., 2011). Although increasing muscle mass is not the main concern among endurance athletes, protein intake is equally important in addition to consuming sufficient energy from carbohydrate post exercise so that AA can be spared for protein synthesis and not oxidized to meet energy demands following a prolonged exercise bout.

1.6.4 Effects of nutritional supplementation on EIMD

Many strategies have been suggested for attenuating DOMS following strenuous exercise. These strategies include massage, contrast water immersion therapy, non-steroidal anti-inflammatory drugs and compression garments, to name a few (Barnett, 2006). However, recent research suggests possible benefits of nutrient ingestion on reducing feelings of muscle soreness

and attenuating markers of muscle damage (White et al., 2008). There is minimal research on the effects of PRO intake alone on attenuating markers of EIMD (Buckley et al., 2010) and most studies have only looked at energy intake prior or during (Saunders, Kane, & Todd, 2004) the exercise bout and not during the recovery period .

Milk protein solution has been shown to contribute to a more rapid skeletal muscle recovery, as measured by plasma levels of CK and lactate dehydrogenase (LDH), compared to a sucrose solution in trained male and female swimmers (Cade et al., 1991). However, Betts et al (2009) observed no differences in attenuation of biomarkers of muscle damage (CK, Mb, LDH) or inflammation (interleukin 6 [IL-6], IL-10, IL-1) and restoration of muscle function following a 90 minute intermittent shuttle run between highly trained males who consumed a carbohydrate (1.2g/kg body weight) beverage with added whey protein (0.4g/kg body weight) compared to an isovolumetric, isocarbohydrate beverage (Betts, Toone, Stokes, & Thompson, 2009). These findings are consistent with White et al (2008) who reported that consumption of a CHO-PRO beverage (23g PRO; 75g CHO) by sedentary males before or after maximal eccentric repetitions of the quadriceps had no effects on muscle soreness, isometric maximal voluntary contraction (MVC) and muscle enzyme activities (White et al., 2008). In general, further research is required to understand the effects of nutritional supplements in attenuating markers of muscle damage.

1.7 Dairy milk for post exercise recovery

Milk consumption for exercise performance has received some attention in recent years. In addition to its usual nutritional benefits, there is growing literature suggesting that milk may serve the same purpose as many ergogenic aids available today. Some studies suggest that ingestion of milk following endurance and resistance –type exercise could enhance athletic

performance by improvement in training time, hydration status and strength as well as a decrease in parameters of muscle damage.

Several studies have examined the influence of dietary sources of ergogenic aids either in the form of carbohydrate beverages or CHO-PRO for post exercise recovery. The addition of protein to a carbohydrate post exercise beverage have shown to increase muscle protein synthesis (Howarth, Moreau, Phillips, & Gibala, 2009), replenish muscle glycogen stores (Ivy et al., 2002), reduce muscle damage and improve time to exhaustion following prolonged exercise (Saunders et al., 2004). While many ergogenic aids are typically in the form of a single nutrient, whole foods such as dairy milk contain many nutrients that may enhance the recovery process following strenuous exercise. Milk contains a variety of nutrients with carbohydrate as the dominant nutrient, thus contributing to high rate of muscle glycogen replenishment. Milk contains similar quantity of carbohydrate as found in many CE sport beverages (Table 1), albeit milk carbohydrate is in the form of lactose compared to the glucose, maltodextrin, fructose or sucrose that comprised the carbohydrates in sports drinks (L. James, 2012). Additionally, depending on the brand, chocolate milk (CM) contains additional sugars such as sucrose or high fructose corn syrup, thus carbohydrate is the dominant macronutrient found in milk (Saunders, 2011a). Furthermore, milk contains high quality protein, which has important roles in muscle protein synthesis and metabolism, including the ability to attenuate various markers of muscle disruption after heavy endurance exercise (Saunders, 2011b).

Dairy milk is considered a high quality protein, meaning that it contains all 8 essential amino acids in appropriate portions required by the body. Furthermore, milk contains high quality proteins, casein (80%) and whey (20%), which contribute to muscle anabolism. Casein is considered a slow digesting protein, indicating that the gastric emptying time is slow, resulting in

a smaller increase in plasma AA compared to whey protein, which is digested quickly contributing to a more prominent rise in plasma AA (Hall, Millward, Long, & Morgan, 2003). Whey protein is believed to play a dominant role in whole body protein synthesis, whereas casein contributes to an inhibition of whole body protein breakdown (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). However, despite differences in blood AA responses following ingestion of either 20g of whey or casein proteins, both proteins have been showed to be responsible for promoting effects of muscle protein synthesis after heavy leg training (Tipton et al., 2004). In addition, milk has been shown to be a potent insulin secretagogue (Gannon, Nuttall, Krezowski, Billington, & Parker, 1986). Thus, an increase availability of AAs and insulin secretion should promote an anabolic environment for muscle protein synthesis when milk is consumed.

Milk is also fortified with Vitamin D, which is important for the promotion of bone health and is required for calcium absorption and regulation of serum calcium and phosphorus (Rodriguez et al., 2009).

The overall aim of this section of this chapter is to review the available literature to assess the effects of milk on exercise performance. Within this exploration, we will focus on some claims associated with milk and exercise performance, specifically, looking at whether milk can: 1) facilitate rapid recovery following endurance and intermittent exercise and improve subsequent exercise performance, 2) contribute favourably to hydration compared to carbohydrate-electrolyte beverages and other commercially available beverages 3) decrease exercise induced muscle damage following exercise, and 4) enhance muscle mass and strength and favorably change body composition, particularly when combined with resistance or endurance exercise.

1.7.1 Milk and Subsequent Endurance and Intermittent Exercise Performance

The physiological stresses that the human body experience from strenuous physical activity can be detrimental to an individual's performance, especially when having minimal time to recover before the next event. Several studies have examined the effects of milk consumption as a recovery beverage on various performance indicators in a subsequent exercise bout (Table 2). One of the earliest study to assess the effects of milk on exercise performance/recovery was conducted by Karp et al (2006) who examined the effects of low fat CM compared to a commercially available carbohydrate protein replacement drink (CPR), both with a carbohydrate content equivalent to 1g/kg body weight, and a CE drink as a post recovery beverage in endurance trained cyclists. The cyclists performed a glycogen depleting interval workout, followed by a 4 hour recovery period and a subsequent endurance trial to exhaustion at 70% VO_2 max. Despite similarities in protein and carbohydrate content between CM and CPR, time to exhaustion and total work performed were significantly greater in those who consumed CM (40.0 ±14.7 min) and a CE beverage (41.3 ± 15.0 min) compared to a CPR (26.8 ± 10.3 min), with no differences between CM and CE (Karp et al., 2006). However, these results were difficult to interpret considering all three beverages did not contain an equivalent amount of energy. Thus, a subsequent study by Thomas et al (2009) tested the same exercise protocol used by Karp et al (2006) with a group of trained male cyclists; however the CPR beverage contained carbohydrate equivalent to 1g/kg body weight, while CM was isocaloric. The results showed that participants cycled significantly ($p = 0.01$) longer when consuming CM (32 ± 11 min) compared to the CE (23 ± 8 min) and CPR (21 ± 8 min) treatments (K. Thomas, Morris, & Stevenson, 2009). Conversely, exercise time to exhaustion at 61% VO_{2peak} was not different between those consuming skim milk and CE following intermittent exercise in the heat (Watson et al., 2008).

Although glycogen synthesis was not measured in the previous study (Watson et al., 2008), it is likely that the initial exercise bout was insufficient to deplete glycogen stores (~37 minutes) which could be one explanation for the lack of significance between subsequent exercise performances. It is also important to note that although the milk group was in a net positive fluid balance prior to their second exercise bout, the difference in fluid balance between the groups (0.4 ± 0.5% body mass) was less than 2% body mass loss which has been associated with performance deficits (Watson et al., 2008). Meanwhile, 20 km cycling time trial performance 4 hours following a glycogen depleting protocol was similarly enhanced following consumption of various energy matched beverages compared to a low calorie placebo, despite differences in protein sources (dairy, hemp, soy) and carbohydrate to protein ratios (Upshaw, Wong, Bandegan, & Lemon, 2016). Furthermore, when regional level cyclists exercised at 85% VO_2 max, there was no difference in time to exhaustion between consumption of CM and a CPR (Pritchett, Bishop, Pritchett, Green, & Katica, 2009) . However their protocol used a higher cycling intensity and a recovery period of 15-18 hours compared to 4 hours used in the previous studies (Karp et al., 2006; K. Thomas et al., 2009; Upshaw et al., 2016). Therefore, the variability in results may be due to the differences in exercise intensities, recovery periods and treatment composition used between studies. More specifically, when matched for total energy, the fat content found in milk may have attributed to the improvement in moderate intensity subsequent exercise performance as fat has a slower digestion rate; thereby the increase in circulating free fatty acids was presumably used for energy. Furthermore, given the lengthy recovery time used by Pritchett et al (2009); it is likely that both beverages had sufficient time for complete digestion, resulting in a similar performance outcome.

Additionally, given the recent literature demonstrating favorable effects of pre sleep nutrition on overnight muscle protein synthesis and enhanced muscle recovery, Ormsbee et al (2016) investigated the effects of consuming chocolate milk within 30 minutes of sleep on next day endurance performance and metabolism among female endurance athletes. Ingestion of 355 ml CM increased next day resting metabolic rate (RMR) and carbohydrate oxidation as well as positively influencing hydration, as measured by urine specific gravity (USG) and reduced urine output. However there was no change in next day 10 km time trial performance (Ormsbee et al., 2016).

Although studies have reported improvements in subsequent exercise performance when recovery time is short, the mechanism remains unclear and warrants further investigation. A few studies have used more comprehensive laboratory procedures (muscle biopsies, stable isotopes) aimed to identify the role of dairy milk and exercise recovery on a cellular level. Lunn et al (2012) assessed the effects of low fat chocolate milk on measures of protein turnover, glycogen repletion and subsequent exercise performance while controlling dietary intake. Despite differences in carbohydrate composition between treatments (CHO: ~0.97g/kg; CM: ~0.76g/kg), glycogen content was equally maintained throughout 3 hours of recovery; however treadmill time to exhaustion was significantly improved following ingestion of CM. Meanwhile, CM enhanced muscle protein fractional synthesis rate (FSR) by 38%, attenuated leucine rate of appearance (Ra) and non-oxidative leucine disposal (NOLD) by 5% and 22%, respectively, and increased leucine oxidation by 77% throughout recovery compared to a carbohydrate beverage (Lunn et al., 2012). Furthermore, 40 km time trial performance was significantly improved 4 hours following a 1.5 hour endurance ride when CM (79.43 ± 2.11 min) was consumed compared to an isocaloric carbohydrate beverage (85.74 ± 3.44 min) and nonnutritive placebo

(86.92 ± 3.28 min). In agreement with Lunn et al (2012), muscle glycogen resynthesis was not different from CHO, although both were greater than placebo (Lunn et al., 2012); however phosphorylation of mammalian target of rapamycin (mTOR) and ribosomal protein S6 (rpS6), intracellular signaling proteins involved in protein synthesis, increased significantly throughout recovery (Ferguson-Stegall, McCleave, Ding, Doerner, et al., 2011). Thus, these results along with those from Lunn et al (2012) suggest that low fat CM can provide an anabolic environment throughout recovery from endurance exercise (Ferguson-Stegall, McCleave, Ding, Doerner, et al., 2011; Lunn et al., 2012). Moreover, most research has focused on the ergogenic effects of milk as a post exercise recovery beverage and its ability to limit decrements in performance. Typically, CE sports drinks are used during prolonged exercise to spare muscle glycogen and maintain blood glucose levels. However, there has been interest to determine whether milk consumption during exercise could be used to enhance exercise capacity and favourably affect whole body leucine kinetics due to its protein composition. Thus, Miller et al (2007) observed lower leucine Ra and NOLD and increased leucine oxidation throughout recovery from a 2 hour run at 65% VO_{2max} when skim milk was consumed in several intervals during exercise, however exercise capacity was not measured (Miller et al., 2007). The decrease in protein synthesis observed during recovery may be a result of increased oxidation of AA during exercise, therefore limiting AA availability for synthesis throughout recovery (Miller et al., 2007). Conversely, Lee et al (2007) found no difference in exercise time to exhaustion between skim milk, a CE solution, and skim milk with added glucose or water when consumed during exercise. It is important to note that the milk beverages were well tolerated, however given the limited research in this area, it is difficult to conclude whether milk should be recommended in replace of a commercially available sports drink during exercise.

There are several limitations that are presented in the current research when examining the effects of dairy milk on subsequent exercise performance. Most studies have participants perform their exercise following an overnight fast; however this is not always representative of a typical training day for an athlete. Additionally, most studies have used male participants (Karp et al., 2006; Pritchett et al., 2009; K. Thomas et al., 2009) and only one study have used female athletes, although gender differences were not reported (Ferguson-Stegall et al., 2011). Thus, Spaccarotella and Andzel (2011) study protocol tried to imitate a typical training program for soccer athletes. Therefore, 5 male and 8 female non-fasted collegiate soccer players consumed either CM (1.0g CHO/kg bodyweight) or an isovolumetric CE beverage immediately and 2 hours after their morning soccer practice. Following their afternoon practice they completed a 20m shuttle run to exhaustion alternating between 55 and 95% VO_2 max. When males and females were compared as one group, time to fatigue did not differ between both treatments. There was a trend (exact p=0.03; effect size 0.2; Spearman correlation coefficient 0.90) for an increase in time to fatigue for males only consuming CM (8.31 ± 6.53 min) compared to CE (6.24 ± 5.03 min). However, the carbohydrate content in the CE beverage was half of what was comprised in the CM treatment, therefore the difference in macronutrient composition and total energy between both treatments make these results difficult to interpret (Spaccarotella & Andzel, 2011). Furthermore, a previous study by Gilson et al (2010) observed no differences in intermittent sport specific performance tests (T-drill, vertical jump) in highly competitive male soccer players during a 4 day period of an increase in training duration following consumption of a high carbohydrate beverage or an isocaloric CM (Gilson et al., 2010). Therefore, given the influence of milk consumption post exercise on improving subsequent endurance exercise performance, in addition to increasing muscle protein synthesis and attenuating breakdown, milk can be of

benefit when allotted minimal time (≤4 hours) to recover before the next event. Although ingestion of milk did not provide additional benefits on exercise performance in some situations, it was equally as effective as many commonly used recovery beverages.

1.7.2 Milk and rehydration

Several studies (Table 3) have investigated the benefit of milk on markers of hydration due to its energy density, carbohydrate content and electrolyte concentration, all of which are key components for optimal rehydration. Shirreffs et al (2007) investigated the effects of low fat milk on restoring whole body net fluid balance (NFB) following exercise induced dehydration compared to a CE beverage, water and low fat milk with additional sodium. Low fat milk equivalent to 150% fluid loss following intermittent exercise in the heat resulted in a reduction of urine output and a positive NFB throughout a 4 hour recovery period, with no observed benefit of adding 20 mmol/L sodium. Meanwhile, fluid balance returned to and remained negative only after 1 hour of recovery following the ingestion of both water and CE (Shirreffs, Watson, & Maughan, 2007a). These findings are in agreement with a previous study which found subjects to be in a positive fluid balance throughout the recovery period from intermittent exercise in the heat following the ingestion of low fat milk equivalent to 150% body mass loss (Watson et al., 2008). These results indicate that consuming adequate fluid from a commercially available CE beverage is capable of restoring fluid loss in the short term; however this fluid balance is not maintained over time, which could have significant adverse impact on subsequent performance for athletes who have minimal time to recover prior to their next training session.

Although these results are in favor of milk at promoting hydration, it is unclear whether the protein itself had any effect on the hydration status of the participants. Therefore, a study by James et al (2011) examined the effects of the addition of milk protein (MP) to a carbohydrate

rehydration solution compared to energy and electrolyte matched carbohydrate beverage following exercised induced dehydration. The MP replaced an equivalent amount of maltodextrin found in the compared treatment, which resulted in reduced urine output and an increased fluid retention. Although NFB was negative at the end for both treatments (MP: -0.26 ± 0.27L; CHO: -0.52 ± 0.30L), it was significantly less negative ($p<0.05$) and not different from pre-exercise when consuming milk protein. The negative fluid balance could be explained by the difference in total energy, macronutrients and electrolytes in the MP beverage compared to fluid milk (L. J. James, Clayton, & Evans, 2011), thus, a follow up study was conducted to determine if an increase in MP solution has an effect on rehydration following exercise. Therefore, 8 males performed intermittent cycling in the heat until they lost 1.6% body mass and consumed either a CE beverage (60g/L CHO) or CE beverages with added MP differing in carbohydrate and protein composition (40g/L CHO;20g/L MP or 20g/L CHO;40g/L MP) which were equivalent to 150% loss of body mass. Despite differences in the amount of added protein, both beverages had similar effects of improving fluid retention compared to only consuming carbohydrate following exercise in the heat (L. J. James et al., 2013).

The study by Desbrow et al (2014) investigated the effects of 4 different commercially available beverages on rehydration following exercise induced fluid loss of ~1.8% body mass. Participants consumed either a CE sports drink, soy milk, cow's milk or a high carbohydrate, high protein liquid meal supplement which were all equivalent to 150% loss of body mass. Results of this study demonstrated milk consumption to be more effective at maintaining fluid retention compared to a CE solution, which is in agreement with previous findings (Shirreffs, Watson, et al., 2007a; Watson et al., 2008), while soy milk was equally as effective as dairy milk in promoting fluid retention following exercise induced fluid loss. Meanwhile, total urine output

was significantly less when consuming a liquid meal supplement compared to dairy and soy milks. Although, the beverages were not matched for macronutrients or total energy, the liquid meal supplement contained a considerable amount more energy, which may be associated with a slower rate of gastric emptying, thus more fluid retained (Desbrow, Jansen, Barrett, Leveritt, & Irwin, 2014). Due to the previous findings which have shown that both 20g and 40g of protein provided post-exercise equally improved fluid retention, these authors suggested that the source of protein may not be as important as the amount of protein for rehydration. However the addition of protein to a rehydration beverage seems to be safe and non-detrimental to performance and has the possibility of enhancing other aspects of recovery, which will be discussed later in this review.

While the majority of studies have demonstrated the effectiveness of milk and its rehydration abilities, it is important to consider the practical aspect of providing such large volumes of high energy density fluids post exercise. Consuming this volume (1.4-3.2 L) within a short time frame might be unrealistic for those competing in multiple day events as previous studies have reported tolerance issues (Desbrow et al., 2014; Seery & Jakeman, 2016). Unlike previous studies where treatments had to be consumed within 1 hour post exercise, Seery et al (2016) used a metered approach to rehydration, where participants consumed 1000 ml within the first 30 minutes of exercise followed by an additional 500 ml every 30 minutes until fluids ingested equated to 150% body mass loss, lasting about 2-3 hours. Overall, higher NFB was maintained following milk consumption compared to CE and water, although no differences were observed between milk and CE after 5 hours. However, post-exercise nutritional recommendations were not met throughout the 5 hour recovery which could have implications

on same day training performance (Seery & Jakeman, 2016). Thus, future research should investigate habitual dietary intake in addition to milk as a supplemental recovery beverage.

Furthermore, the studies discussed above have assessed the rehydration potential of milk following exercise induced dehydration in young adults over the age of 18 years. However, limited research exists for hydration recommendations for young, active children. It has been previously reported that young children involved in a variety of sports begin exercise in a hypohydrated state and remain hypohydrated following their training, when consuming fluid ad libitum (Arnaoutis et al., 2015; Rivera-Brown & De Felix-Davila, 2012). To date, only one study has assessed the effects of milk on rehydration in pre- to early pubertal (7-11 years) and mid- to late pubertal (14-17 years) children following exercise (Volterman, Obeid, Wilk, & Timmons, 2014a). Non-fat milk provided at 100% of body fluid loss was found to be more effective in promoting fluid retention compared to a CE sports drink and water of equal volume, however all groups remained dehydrated throughout the 2 hour recovery period (Volterman et al., 2014a). Thus, fluids equivalent to 100% body mass loss is insufficient to achieve a positive NFB throughout short term recovery. Given the current fluid recommendations of consuming a greater fluid volume than the final fluid deficit (125-150% or 1.25-1.5 L for every 1 kg body mass lost) (D. T. Thomas et al., 2016b) and previous research demonstrating favourable effects on hydration status following milk consumption equivalent to 150% body mass loss (Seery & Jakeman, 2016; Shirreffs, Watson, et al., 2007a; Watson et al., 2008), it is likely that these recommendations would be beneficial for young children as well, however future research should investigate the rehydration potential of various fluid volumes. Nonetheless, in agreement with previous findings, milk was more effective than water and a commonly used sports drink and would be a safe rehydration solution following exercise (Volterman et al., 2014a).

1.7.3 Milk and exercise induced muscle damage

Prolonged endurance exercise or exercise that consists of eccentric movements (running downhill, jumping) may result in muscle damage which can contribute to delayed onset muscle soreness (DOMS), reduced range of motion, efflux of muscle enzymes and decreased muscular performance (Peake et al., 2017). Nutritional interventions to diminish the response and effects of EIMD have been extensively researched over the years (Hennigar, McClung, & Pasiakos, 2017; Rahimi, Shab-Bidar, Mollahosseini, & Djafarian, 2017; Sousa, Teixeira, & Soares, 2014) and there has been some recent focus on the effectiveness of milk (Table 4) due to the CHO and protein composition. It has been suggested that the addition of dairy protein to a post recovery beverage may assist in reducing endogenous muscle protein breakdown and attenuate markers of EIMD. The earliest study to investigate the effects of dairy milk on attenuating symptoms of exercise induced muscle damage compared a CE beverage to a flavored milk beverage and a non-caloric placebo. Non-trained males completed a glycogen depleting exercise trial followed by 100 repetitions of eccentric exercises and consumed their assigned treatment immediately and 2 hours during the recovery period. Although the exercise protocol induced muscle protein breakdown, as indicated by excretion of urinary 3- methyl histidine, there was no difference observed in muscle function and inflammatory markers between the treatments. There was however a trend ($p < 0.08$) for a reduction in CK 6 hours following exercise in the milk group but was not different between groups throughout the next 5 days when CK was elevated (Wojcik et al., 2001).

The nutrient composition, along with the timing and volume of milk consumption has been studied by a research team to evaluate specific markers of muscle damage and soreness. In their first study, Cockburn and colleagues (2008) examined the effects of commercially available

semi-skimmed milk and milk-based supplement compared to a CE sports drink and water control on attenuating EIMD when performing resistance based concentric-eccentric exercise. Participants consumed isovolumetric beverages (500ml) immediately and 2 hours after exercise and markers of EIMD were assessed at baseline, 24 and 48 hours post exercise. The milk based supplement contained 2.5 times more carbohydrate compared to the milk group, however both groups showed significantly similar increases in muscle performance and lower CK and Mb values at 48 hours, whereas carbohydrate intake alone was unable to attenuate markers of EIMD (Cockburn, Hayes, French, Stevenson, & St Clair Gibson, 2008). In a follow-up study, Cockburn et al (2010) examined the timing of recovery beverage consumption on EIMD using a milk based supplement with the same nutrient composition used in the previous study (Cockburn et al., 2008). Consuming the milk based supplement immediately following exercise was more beneficial at reducing muscle soreness and impairments in muscle performance at 48 hours compared to consuming prior to exercise. Although the milk based supplement consumed before, immediately or 24 hours after exercise had more of an effect at blunting increases in CK compared to a water control group, these results were unable to reach significance (Cockburn, Stevenson, Hayes, Robson-Ansley, & Howatson, 2010). In a subsequent study, Cockburn et al (2012) acknowledged that the volume of the beverages used in the previous studies (Cockburn et al., 2008; Cockburn et al., 2010) may not be practical for an athlete, and although not determined in the studies, it was suggested that the macronutrient composition provided in such a large volume were not realistic in promoting any additional increases in protein synthesis. Thus, participants performed the same muscle damaging protocol and consumed 500 ml (17g PRO) or 1000 ml (34g PRO) of semi-skimmed milk or 1000 ml water immediately following exercise. Although milk consumption was beneficial at limiting reductions in peak torque and increases of

plasma CK compared to water, they were unable to observe clear differences between the volume of milk consumed (Cockburn, Robson-Ansley, Hayes, & Stevenson, 2012). Based on the findings by Cockburn et al (2012), the same group conducted another study using 500 ml of semi-skimmed milk compared to an equivalent volume of water and used an exercise protocol aimed to imitate typical exercise movements that are used in intermittent sports. Semi-professional male soccer players performed a series of performance tests including countermovement jump height, a 15m sprint and *t*-test at 24, 48 and 72 hours following a muscle damaging exercise and at 48 hours performed a 90 minute shuttle run test. Milk was beneficial at attenuating increases in mean 15-m sprint shuttle run performance and agility time. However, there were no significant differences in attenuating decrements in performance measures, DOMS, CK and Mb between treatments (Cockburn, Bell, & Stevenson, 2013). Additionally, these athletes were only consuming one single serving of each treatment immediately after exercise, perhaps the results would have been different if they were to ingest another serving 2 hours later, which was shown in their first study to attenuate markers of EIMD (Cockburn et al., 2013).

A previous study by Gilson et al (2010) aimed to assess the effects of milk on markers of muscle damage in highly competitive male soccer players during a 4 day period of an increase in training duration following consumption of a high carbohydrate beverage or an isocaloric CM. There were no differences between treatments on muscle function and DOMS, however serum CK levels were significantly lower in CM compared to carbohydrate after 4 days of increased training duration when performing short duration, high intensity skill based exercises (Gilson et al., 2010). This is in agreement with a previous study (Pritchett et al., 2009) in which there was a significantly lower serum CK values in cyclists consuming CM compared to those who

consumed an isocaloric, carbohydrate matched (1.0g/kg body weight) CHO-PRO beverage following high intensity cycling intervals. However, following a 15-18h recovery period, there was no difference in overall performance measured by time to exhaustion at 85% VO_2 max (Pritchett et al., 2009). Conversely, Ferguson-Stegall (2011) observed an improved time trial performance following CM ingestion, however they found no difference in plasma creatine phosphokinase (CPK), Mb and inflammatory cytokines (interleukin-6 [IL-6], IL-8, IL-1 receptor antagonist [IL-1ra]) throughout a 4 hour recovery between CM, an isocaloric carbohydrate beverage and a non-nutritive placebo (Ferguson-Stegall, McCleave, Ding, Doerner, et al., 2011).

Furthermore, Rankin et al (2015) assessed the effects of milk compared to an energy matched CHO beverage on markers of recovery following a laboratory based protocol intended to induce muscle damage in female and male team sport athletes. For females, 500 ml of milk post EIMD was beneficial at limiting decreases in peak torque and 20m sprint time, limiting increases in muscle soreness as well as skeletal troponin I (sNtI). Meanwhile, the effect of milk on muscle function in males was unclear; however there was a benefit of milk on attenuating muscle soreness and biomarkers of muscle damage (CK, sNtI) compared to carbohydrate alone (P. Rankin, Stevenson, & Cockburn, 2015). Overall, females demonstrated smaller decrements in performance; however differences in milk protein quantity for males (~0.21g/kg body weight) and females (~0.27g/kg body weight) could explain differences in muscle function.

Meanwhile, ingestion of 1 litre of CM following 5 days of judo training attenuated subjective feelings of muscle soreness and improved timed push up performance and judo specific performance by 14.6% and 6.8%, respectively. Interestingly, compared to consuming water, chocolate milk had no significant effect on pre competition intentional weight loss (Papacosta, Nassis, & Gleeson, 2015). Thus, considering that CM is an energy dense beverage,

these results are novel and of importance for athletes competing in combat sports as food and fluid restriction is a common weight loss strategy prior to competition (Artioli et al., 2010; Papadopoulou et al., 2017).

Additionally, significant reductions in muscle soreness was observed when lactobacillus helveticus fermented milk was consumed before, immediately and 2 hours following a leg and bench press protocol compared to unfermented milk. There were no differences between treatments in attenuating blood lactate or CPK; however carbohydrate oxidation and serum oxygen radical absorbance capacity (ORAC) was significantly reduced in unfermented milk but not in fermented milk. Thus, although the exact mechanism was not investigated, fermented milk was more effective at inhibiting glucose metabolism impairments associated with EIMD potentially related to increased antioxidant capacity (Iwasa et al., 2013b).

Although dairy milk contains many essential nutrients and may serve as a healthier alternative than many ergogenic aids available to the athletic population, certain individuals may experience gastrointestinal discomfort following consumption of dairy containing foods. A2 milk has identical nutritional composition to regular dairy milk, however lacks A1 beta casein and expression of bovine beta-cosmorphine7 (BCM-7), which has been thought to contributes to self-reported gastrointestinal related symptoms associated with dairy milk consumption (Kirk et al., 2017). Thus, a recent study explored the effects of A2 milk compared to regular dairy milk on attenuating various markers of EIMD following a repeated sprint protocol to simulate team sport training. Although there was no difference between treatments on attenuating increases in muscle soreness, which peaked at 48 hours, A2 milk was as effective as regular milk at attenuating decrements in 20-m sprint time and countermovement jump (CMJ) height compared to a carbohydrate only beverage at 48 hours following repeated sprint protocol (Kirk et al., 2017).

However, participants were not allowed to be included in this study if they had lactose intolerance/dairy allergy, therefore it is unknown whether A2 milk would have the same ergogenic effects on those athletes.

In summary, milk was effective at limiting increases in pro inflammatory cytokines, attenuating symptoms of muscle soreness and limiting decreases in muscle function following eccentric resistance based exercises and progressive team sport training. However, there are many inconsistences between studies on the above mentioned measures of EIMD. This is likely due to the exercise protocols used as well as the differences in milk volumes and control beverages. There is a need for more research assessing the impact of milk consumption compared to energy matched protein drinks on indices of EIMD. Furthermore, there is a need to explore the effects of milk consumption on attenuating symptoms of EIMD following progressive training, intermittent team sports as well as endurance exercise rather than single bouts of eccentric contractions, which is not always reflective of athlete's training.

1.7.4 Milk and muscle mass, strength, body composition and training adaption in resistance and endurance exercise

Based on the protein nutritional composition found in dairy milk, numerous studies have focused on its ability to stimulate muscle protein synthesis and determine whether milk can favourably impact muscle growth, body composition and muscular strength following resistance exercise (Table 1.5). One of the earliest studies to investigate this found that ingestion of whole milk (WM), fat free milk or fat free milk isocaloric to WM following a leg strength training exercise resulted in net muscle protein synthesis due to the stimulation of amino acids phenylalanine and threonine uptake across the leg (Elliot, Cree, Sanford, Wolfe, & Tipton, 2006). Meanwhile, uptake for threonine was greater for WM and a similar trend was seen for

phenylalanine, however it is unclear which component of WM had this effect (Elliot et al., 2006). Furthermore, Wilkinson and colleagues (2007) examined the effects of consuming fat free milk compared to an isoenergetic, macronutrient matched soy beverage on net muscle protein balance following an acute bout of resistance exercise in resistance trained males. Although blood total AA increased significantly 30 minutes following soy protein consumption, there was a greater uptake of amino acids across the leg and 34% greater increase in muscle protein FSR 3 hours after exercise following dairy milk consumption. This was likely due to the fact that milk has a slower digestion rate, resulting in a moderate rise and slower decline of total AA, thereby contributing to a greater post exercise stimulation of protein synthesis compared to a soy protein beverage (Wilkinson et al., 2007).

The above findings were observed following acute bouts of resistance exercise (Elliot et al., 2006; Wilkinson et al., 2007) thus, Hartman et al (2007) determined the effects of chronic supplementation of dairy milk throughout a 12 week (5d/week) resistance training program on muscle protein accretion (Hartman et al., 2007). Ingestion of 500 ml of fat free milk immediately and 1 hour following exercise resulted in a significant reduction in fat mass and greater increases in fat and bone free mass (FBFM) compared to an isoenergetic, macronutrient matched soy beverage and an isoenergetic carbohydrate beverage. Furthermore, there was a greater increase in Type II muscle fiber area in the milk group compared to soy and carbohydrate and a greater increase in Type I muscle fiber area in both milk and soy compared to carbohydrate, however all three groups showed similar increases in strength (Hartman et al., 2007).

An earlier study compared low fat CM to a CE beverage and its effects on body composition and strength in untrained males executing resistance based exercises over 10 weeks (J. W. Rankin et al., 2004). There were significant gains in muscular strength, increase in fat free

soft tissue (FFST) mass and reduction in body fat following the 10 week exercise protocol, however there was no significant difference observed between both groups, indicating both beverages demonstrated similar training adaptations. It is important to note that these participants were untrained and perhaps similar results were due to muscle adaptation to unaccustomed exercise (J. W. Rankin et al., 2004). Furthermore, the lack of significance in body composition between treatments in the previous study compared to that of Hartman et al (2007) may be related to the differences in exercise frequency, length and intensity between study protocols (Hartman et al., 2007; J. W. Rankin et al., 2004).

Additionally, consumption of 500 ml of low fat CM immediately post exercise throughout a 12 week resistance training protocol had no effect on muscle strength or muscle fiber area in young (22.4 ± 2.1 yr) or elderly males (74.4 ± 5.4 yr) compared to an isocaloric high carbohydrate beverage (Mitchell et al., 2015). Furthermore, consuming 200 ml of milk fortified with additional calcium and vitamin D_3 twice a day throughout an 18 month progressive resistance training program had no effect on muscle strength, muscle cross sectional area (CSA) and lean mass compared to exercise alone without supplementation in middle aged and older men (50-79 years) (Kukuljan, Nowson, Sanders, & Daly, 2009). However, muscle protein synthesis is maximally stimulated when dietary protein intake is 20-25g in young individuals and 40g for older adults (Phillips, 2014). The milk treatments in both above-mentioned studies only provided 13-14g of protein, thus protein intake may have been insufficient to stimulate muscle protein synthesis (Kukuljan et al., 2009; Mitchell et al., 2015).

Meanwhile, to date, only one study has examined milk consumption and its effects on strength gains and body composition in females. Following a 12 week resistance training program identical to that of Hartman et al (2007), non-fat milk was shown to be beneficial in

increasing lean mass, decreasing fat mass and demonstrating greater changes in 1 repetition maximum (1RM) strength with some upper body exercises in non-trained women compared to an isoenergetic carbohydrate beverage (Josse, Tang, Tarnopolsky, & Phillips, 2010). Furthermore, 1 litre of milk consumption post exercise over the 12 week training period resulted in a significant reduction in parathyroid hormone (PTH), which stimulates bone resorption; along with decreases in markers of bone turnover serum carboxyl terminal collagen cross-links and an increase in osteocalcin, and thus possibly improving bone heath (Josse et al., 2010). These results are of importance for the female population, as often they avoid dairy products and associate them as high fat foods (Mahon & Haas, 2013). Nevertheless, consuming milk post exercise tends to be more satiating, resulting in less energy consumed at the following meal (Rumbold, Shaw, James, & Stevenson, 2015) and increasing lean mass without increasing overall body weight (Josse et al., 2010).

As mentioned above, several studies have investigated the effects of CHO-PRO supplementation post exercise in the form of dairy milk on muscle protein accretion, muscular strength and body composition following resistance based exercises. However, limited research exists in regards to the impact of milk on body composition and training adaptations following aerobic endurance exercise. Consequently, a study by Ferguson-Stegall et al (2011) observed a significant 12.5% increase in VO_2 max following 4.5 weeks (5d/week) of aerobic exercise training at 75-80% VO_2 max when CM versus an isovolumetric, isocaloric carbohydrate beverage and a isovolumetric non-nutritive placebo was consumed immediately and 1 hour after each exercise session. The increase in VO_2 max occurred despite oxidative enzymes and lactate threshold increasing in all treatment groups. Furthermore, in agreement with previous findings following strength training (Hartman et al., 2007; Josse et al., 2010), CM was more effective in

improving body composition compared to carbohydrate alone (Ferguson-Stegall, McCleave, Ding, Doerner Iii, et al., 2011)

Nutritional recommendations for children differ to those of adults and should focus on strategies to promote optimal growth and development. Meanwhile, the large majority of the studies assessing the ergogenic effects of dairy milk have used individuals, both recreational and trained, over the age of 18 years old. Considering dairy milk is a whole food which provides essential nutrients including vitamin D and calcium for bone health and protein to support lean muscle tissue, it is of interest whether dairy milk possesses the same, if not superior benefits in active children. Very limited research exists in this area, however, a more recent study examined the effects of milk on whole body protein balance following an acute bout of moderate intensity cycling exercise in pre- to early pubertal (7-11 years) and mid- to late pubertal (14-17 years) children. Compared to water and a CE beverage, whole body nitrogen turnover, protein synthesis and whole body protein balance (WBPB) all increased with milk, however pre- to early pubertal girls were only able to achieve positive WBPB, while all other gender and age groups remained negative throughout 16 hours post exercise, even more so in mid- to late pubertal children (Volterman, Obeid, Wilk, & Timmons, 2014b). Thus, these results suggest that the protein intake (~0.4g/kg) was either insufficient or is required in several doses over time to maintain a positive WBPB. Future work by this group demonstrated WBPB to be maximized in young children when protein intake was provided in two boluses (~0.12-0.22g/kg) compared to one single bolus (~0.33g/kg) immediately following exercise (Volterman et al., 2017), thus protein timing rather than amount may be more important for promoting an anabolic environment in young children, however future research is warranted.

1.8 Conclusion and future implications

In summary, in the absence of sufficient energy and/or quality nutrients, individuals may experience performance deficits and reduced training adaptations. More specifically, high intensity or prolonged exercise can result in depletion of glycogen stores, dehydration and EIMD. Thus, nutrition recommendations are designed to ensure athletes are meeting daily energy requirements and macronutrient targets are established to maximize post exercise recovery. Current sport nutrition recommendations emphasize the importance of whole foods in the diet rather than reliance on low quality dietary supplements. In comparison to carbohydrate alone, dairy milk has the ability to improve subsequent endurance exercise performance. Furthermore, consumption of two separate boluses, one immediately following exercise and another two hours later seems to be advantageous to enhance short term recovery ($\leq$ 4 hours) and limit performance deficits in subsequent exercise. Although the mechanism remains unclear, it may be due to dairy milks' effects on rehydration, glycogen synthesis, protein turnover, attenuated EIMD, or a combination of these. However, these benefits are not consistent throughout the literature given differences in exercise protocols and treatment volumes. Although several studies were unable to detect differences between dairy milk and the control beverages, dairy milk contains a more complex nutrient profile than other commonly used beverages, and could potentially be a more suitable choice for both health and performance. However there is a need to determine specific recommendations of milk for exercise performance for young, active children as well as older adults.

Table 1.1 Nutrient composition of milk and commercially available sports drinks

Nutrient	Milk (1%) (250 ml)[1]	Milk (2%) (250 ml)[1]	Chocolate Milk (1%) (250 ml)[1]	Gatorade Thirst Quencher (250 ml)[2]	Gatorade G2 (250 ml)[2]
Energy (kcal)	108	129	188	56.34	20
Carbohydrates (g)	12.87	12.38	33.28	15.49	5
Protein (g)	8.69	8.51	8.56	0	0
Fat (g)	2.5	5.10	2.64	0	0
Sodium (mg)	113	121	161	112.68	115
Potassium (mg)	387	361	449	31.69	32.5
Vitamin D (IU)	103	103	105	0	0
Calcium (mg)	322	309	306	0	0

[1]Nutrition information retrieved from Canadian Nutrient File: https://food-nutrition.canada.ca/cnf-fce/index-eng.jsp. Information accessed on October 31, 2017.
[2]Nutrition information retrieved from: http://gatorade.ca/ on October 31, 2017

Table 1.2: Effects of milk on exercise recovery as measured by time to exhaustion in subsequent exercise performance

Reference	Subjects	Study design	Treatments (g/kg)	Protocol	Results (TTE)
(Karp et al., 2006)	9 males; endurance-trained cyclists; age 21.1 ± 2.0y, body mass 73.0 ± 4.6kg	Single-blind, randomized	**CHO Pro fat** CM:1.00 0.27 0.08 CE: 0.43 - - CPR: 1.00 0.26 0.02	**Ex:** High intensity bike interval workout (~1h) **Rc:** 4 h recovery, with treatment given at 0h and 2h post-exercise **F-Ex:** TTE at 70% VO_2max	CM: 40.0±14.7 min* CE: 41.3±15.0 min CPR: 26.8±10.3 min
(Watson et al., 2008)	7 males, active; age 23 ± 3y; body mass 75.6 ± 11.1kg	Randomized, crossover, at least 7 days between intervention	**CHO Pro fat** MLK: 1 .50 1.00 0.03 CE: 1.81 - -	**Ex:** Intermittent cycling at 55% VO_2peak (at 35C) until 1.8% body mass loss (~37 min) **Rc:** 3 h recovery, with treatment given at 0h post-exercise **F-Ex:** TTE at 61% VO_2peak	MLK: 39.7±8.1 min CE: 39.6±7.3 min
(K. Thomas et al., 2009)	9 males; trained cyclists; age 25.4 ± 8.0y; body mass 72.8 ± 8.4kg	Randomized cross-over	**CHO Pro fat** CM:0.90 0.20 0.13 CHO: 0.42 - - CPR: 1.00 0.26 0.02	**Ex:** High intensity bike interval workout (~1h) **Rc:** 4 h recovery, with treatment given at 0h and 2h post-exercise **F-Ex:** TTE at 70% VO_2max	CM: 32±11 min ** CE: 23±8 min CPR: 21±8 min
(Pritchett et al., 2009)	10 regional-level cyclists and triathletes, age 27.1±7.9 y, body mass 72.1±6.7 kg	Counterbalanced, crossover, repeated-measure, 1 wk between intervention	**CHO Pro fat** CM: 1.00 0.26 0.07 CPR: 1.00 0.27 0.03	**Ex:** High intensity bike interval workout (~48 min) **Rc:** 15-18 h recovery, with treatment given at 0h and 2h post-exercise **F-Ex:** TTE at 85%	CM: 13.0±10.2 min CPR: 13.5±8.9 min

				VO_2max	
(Gilson et al., 2010)	13 males; intercollegiate soccer players; age 19.5 ± 0.3y; body mass 79.4 ± 2.6kg	Randomized, cross-over, 2 wk between intervention	**CHO Pro fat** CM: 1.06 0.35 0.09 CHO: 1.54 - 0.03	**Ex:** 4-days of increased training duration (`~95 min/d). **Rc:** treatment given at 0h post-exercise each day **E-Ex:** T-drill on d2 and vertical jump after 4 day	T-drill: CM: 9.06±0.58 s CHO: 9.09±0.47 s Vertical-jump: inches CM: 26.7±3.6 inches CHO: 26.7±3.6 inches
(Ferguson-Stegall, McCleave, Ding, Doerner, et al., 2011)	10 (5 males/5 females) trained cyclists; age 31.8 ± 1.6; body mass 67.8 ± 2.6kg	Randomized, double blinded, placebo controlled, crossover design	**CHO Pro fat** CM: 1.90 0.60 0.30 CHO: 2.50 - 0.30 PLA: 0	**Ex:** Subjects cycled 1.5h at 70% VO_2 max plus 10 min intervals at 45 and 90% VO_2 max **Rc:** 4h recovery, with treatment given at 0h and 2h post-exercise, Biopsies at recovery times: 0, 45, 240 min. **F-Ex:** 40-km TT	CMK:79.43±2.11 min*** CHO: 85.74±3.44 min PLA: 86.92±3.28 min
(Spaccarotella & Andzel, 2011)	13 athletes (8 females, 5 males): college soccer team, age 19.5±1.1 y, body mass 70.9±11.5kg	Randomized, crossover, 2 d intervention separated by 2-day washout	**CHO Pro fat** CM:1.00 0.29 0.11 CHO: 0.51 - -	**Ex:** Morning and afternoon practice **Rc:** 4h recovery, with treatment given at 0h and 2h post-morning exercise **F-Ex:** Afternoon practice followed by a 20-m shuttle run TTE, alternating between 55 and 95%VO_2max	All participants: CM: 6.11±5.12 min CHO: 5.03±3.41min Males only: CM: 8.31±6.53 min CHO: 6.24±5.03min

(Lunn et al., 2012)	8 males runners, age 23.7±1.6y, body mass 76.0±3.8 kg,	Randomised, crossover, 7 d between intervention	**CHO Pro fat** CM: 0.76 0.21 0.0 CHO: 0.97 - -	**Ex:** 45min treadmill exercise at 65% VO_2peak **Rc:** 3h recovery, treatment given at **F-Ex:** TTE	TTE: CM: 250±43 s* CHO: 203±31 s Muscle FSR: CM: 0.11 ± 0.01 %·h-^{1}* CHO: 0.08 ± 0.01 %·h-^{1}
(Upshaw et al., 2016)	8 exercise trained male cyclists, age 21.8 ± 2.3y, body mass 73.4±10.5 kg	5 week, double blind, crossover, counterbalanced, repeated measures	CHO Pro Fat CM: 1.06 0.24 0.19 MLK: 0.74 0.54 0.19 SCM: 1.09 0.27 0.16 HCM: 0.97 0.16 0.27 PLA: 0.20 - -	**Ex:** 2 minute cyclin intervals between 90% and 50%, 80% and 50%, 70% and 50% maximal power output **Rc:** Treatment at 0h and at 30 min intervals over 2h, recovered for additional 2h **F-Ex:** 20 km cycling time trial	CM: 34.58 ± 2.5 min*** MLK: 34.47 ± 1.7 min SCM: 34.83 ± 2.2 min HCM: 34.88 ± 1.1 min PLA: 37.85 ± 2.1 min
(Ormsbee et al., 2016)	12 trained female runners and triathletes, age 29.8±6.5 y; body mass 58.2±4.4 kg	Crossover, randomized, double blind, minimum 72h between intervention	Cho Pro Fat CM 0.52 0.21 - PLA - - -	**Pre-Ex:** Treatment given at 2h after last meal and 30min before sleep the night before **Ex:** Intermittent exercise test; 5 min at 55%, 65% and 75% $VO_{2\ peak}$ **Rc:** 10 min **F-Ex:** 10km treadmill time trial	TT performance: CM: 52.8±8 min PLA: 52.8±8.4 min

(Miller et al., 2007)	5 endurance trained men; age 22 ± 1.0y; body mass 71 ± 3.0kg	Randomized, crossover design	Cho Pro Fat MLK 0.38 0.24 CHO 0.63 - - PLA - - -	**Ex:** 120 min run at 65% $VO_{2max.}$ Treatment given at 20, 40, 60, 80 minutes during exercise (200 ml) **Rc:** 3 hours, leucine kinetics assessed	Leucine Ra: MLK:125.0 ± 20.3 μmol·kg* PLA: 154.2 ± 17.2 μmol·kg Leucine non-oxidative leucine disposal: MLK:89.4 ± 20.5 μmol·kg* PLA: 129.7 ± 17 μmol·kg Leucine Ox: MLK: 35.6 ± 1.9 μmol·kg** CHO: 23.9 ± 3.7 μmol·kg PLA: 24.5 ± 2.9 μmol·kg
(J. K. Lee, Maughan, Shirreffs, & Watson, 2008)	8 males, active, age 24±3 y, body mass 68.9±9.5 kg	Randomized crossover; at least 7d between intervention	**CHO Pro fat** MLK:0.73 0.48 0.01 MLKC:0.87 0.48 0.01 CHO: 0.87 - - WAT: 0.00 - -	**Ex:** Cycling at 70% VO_2peak until volitional exhaustion Treatment consumed (1.5ml/kg) every 10 min during exercise	MLK: 103.3, range 85.7-228.5 MKC: 102.8, range 74.3-167.1 CHO: 110.6, range 82.0-222.7 WAT: 93.3, range 82.4-192.3min.

CM, chocolate milk; CHO, carbohydrate; CE, carbohydrate electrolyte; CPR, carbohydrate-protein replacement; MLK, milk; MLKC, milk with added glucose; SCM, soy chocolate milk; HCM, hemp chocolate milk; Ex, exercise; Rc, recovery; F-Ex, follow-up exercise; Pre-Ex, pre-exercise; Pro, protein; TTE, time to exhaustion; WAT, water; PLA, placebo; FSR, fractional synthesis rate.
*Significantly different than CPR
**Significantly different than CPR and CHO
***Significantly different than PLA

Table 1.3: Effects of milk on exercise induced dehydration

Reference	Subjects	Study design	Treatments (g/L)	Protocol	Results
(Shirreffs, Watson, et al., 2007a)	11 (5 males, 6 females), age 24 ±4 y, body mass 65.5±7.2 kg.	Randomised, crossover, at least 7 d between intervention	MLK MNa CE WAT Cho 50 50 60 0 Pro 36 36 0 0 Fat 3 3 0 0 Na 38.6 58 23 0	**Ex:** Series of 10min periods of cycling at a workload of 2W/kg body mass. Exercise continued until 1.7% body mass lost. **Rc:** Treatment consumed 20 mins post ex; provided in 4 equal bolus at 15min intervals. Recovery monitored for next 4 hours after consumption.	**Urine volume:** MLK: 611±207 ml* MNa: 550±141ml* CE: 1184 ± 321 ml WAT: 1205 ±142 ml
(Watson et al., 2008)	7 males, active, age 23 ± 3 y, body mass 75.6±11.1 kg	Randomized, crossover, at least 7 days between intervention	MLK CE Cho 50 60 Pro 33 0 Fat 1 0 Na 32 23	**Ex:** Intermittent cycling at 55% VO_2peak (at 35C) until 1.8% body mass loss (~37 min) **Rc:** 3 h recovery, with treatment given at 0h post-exercise **F-Ex:** TTE at 61% VO_2peak	**Urine volume:** MLK: 525 ± 118 ml** CE: 861 ± 396 ml **Fluid balance:** MLK: 191 ± 162 ml** CE: -135 ± 392 ml
(L. J. James et al., 2011)	8 males, age 21 ± 3y, body mass 75.7 ± 11.6 kg	Randomized, crossover, at least 7 days between intervention	MP CHO Cho 40 65 Pro 25 0 Fat 0.8 0.8 Na 7 7	**Ex:** Series of 10min periods of cycling at a workload of 2W/kg body mass. Exercise continued until 1.6% body mass lost. **Rc:** Treatment given at	**Urine output:** CHO: 1212 ± 310 ml MP: 931 ± 254 ml ** **Fluid retention** MP: 55 ±12% ** CHO: 43 ± 15% **Net fluid balance**:

								0h; provided in 4 equal bolus at 15min intervals. Recovery monitored for next 4 hours after consumption.	MP: -0.26 ± 0.27 L ** CHO: -0.52 ± 0.30 L
(L. J. James et al., 2013)	8 males, age 21.9 ± 2.0y, body mass 76.96 ± 8.73 kg	Randomised, double blind, crossover, each intervention separated by at least 7 days		MP20	MP40	CHO		**Ex:** Series of 10min periods of cycling at a workload of 2W/kg body mass. Exercise continued until 1.6% body mass lost. **Rc:** Treatment equivalent to 150% BM loss given at 0h; provided in 4 equal bolus at 15min intervals. Recovery monitored for next 4 hours after consumption.	**Fluid retention:** MP20: 59 ± 12%** MP40: 64 ± 6%** CHO: 46 ± 9% **Net fluid balance** CHO: -470 ± 154 ml MP20: -181 ± 280 ml** MP40: 210 ±126 ml**
			Cho	40	20	60			
			Pro	20	40	0			
			Fat	0.7	0.7	0.7			
			Na	20	21	21			
(Desbrow et al., 2014)	15 recreationally active males, age 24.9 ± 5.5 y, body mass 75.8 ± 6.6 kg	Randomized, crossover, each trial separated by at least 7 days		CE	SM	MLK	MRP	**Ex:** Cycled at 70-80% of age predicted maximum heart rate until ~1.8% BM loss was achieved. **Rc:** Treatment equivalent to 150% BM loss given at 0h; provided in 4 equal bolus at 15min intervals. Recovery	**Urine output:** CE: 1834 ± 427 ml SM: 1144 ± 446 ml ** MLK: 1338 ± 578 ml ** MRP: 771 ± 367 ml # **Fluid retention:** CE: 16.6 ± 16.5% SM: 46.9 ± 19.9% ** MLK: 40.0 ± 24.9% ** MRP: 65.1 ±14.7% #
			Cho	73	51	49	176		
			Pro	0	32	36	65		
			Fat	0	35	38	2		
			Na	12	20	18	29		

				monitored for next 5 hours after consumption	
(Volterman et al., 2014a)	38 (19 female, 19 male) PEP boys age 9.4 ±1.1y, body mass 32.6 ± 6.3kg; girls age 9.4±0.9y, body mass 28.7±5.5kg MLP boys age 15.4 ± 0.4y, body mass 59.3±8.7kg; girls age 14.6±0.6y, body mass 57.6±9.2kg	Randomised, repeated measures, crossover	MLK CE WAT Cho 52 80 0 Pro 32 0 0 Fat 0 0 0 Na 22.6 9.9 0	**Ex:** Cycled at 60% VO_2 max (2x20 min bouts) in heat (34.5 ± 0.3 °C) **Rc:** Treatment equivalent to 100% BM loss given at 0, 15 and 30 min. Recovery monitored for 2 hours after consumption	**Fluid retention:** MLK: 74 ± 18%* CE: 59 ± 20% WAT: 47 ± 26% PEP significantly more dehydrated than MLP
(Seery & Jakeman, 2016)	7 healthy men engaged in regular physical activity (>3h/week); age 26.2 ± 6.1 y; 86.4 ± 11.5 kg	Randomized, crossover, each trial separated by 7 days	MLK CE WAT Cho 50 39 0 Pro 33 0 0 Fat 1 0 0 Na 17.9 21.7 0.7	**Ex:** 50 min cycle in heat followed by 10 min intervals until ~1.8% BM loss was achieved **Rc:** 1000 ml treatment provided within first 30 min; 500 ml every 30 min until 150% of BM loss replaced	Urine volume: MLK: 794 ± 99 ml*** CE: 1314 ± 164 ml WAT: 1429 ± 131 ml Fluid retention: MLK: 71 ± 4%*** CE: 52 ± 16% WAT: 47 ± 15%

CM, chocolate milk; CHO, carbohydrate; MLK, milk; SM, soy milk; CE, carbohydrate electrolyte; MRP, meal replacement; MNa, milk with additional sodium; Na, sodium; Ex, exercise; Rc, recovery; Pro, protein; WAT, water; BM, body mass; MLP, mid- to late-pubertal; PEP, pre- to early pubertal
*Significantly different than CE and WAT
**Significantly different than CE
***Significantly different than WAT
Significantly different than MLK, SM and CE

Table 1.4: Effects of milk on exercise-induced muscle damage

Reference	Subjects	Study design	Treatments	Protocol	Results
(Wojcik et al., 2001)	26 untrained males; age 23.5 ± 0.7y; body mass 75.6 ± 2.3kg	Parallel MLK (n=9) CHO (n=8) PLA (n=9)	Cho Pro Fat MLK: 0.88 0.38 - CHO: 1.25 - - PLA: - - -	**Pre-Ex:** 40 min cycling at 70% VO_{2peak} followed by 5 bouts of 1 min max cycling sprints evening prior to Ex **Ex:** 10 sets of 10 reps of isotonic eccentric quadriceps contractions at 120% of 1RM **Rc:** Treatment given immediately and 2h post ex **F-Ex:** Blood taken before exercise, immediately, 3 and 6h post. DOMS, peak torque, Muscle biopsies immediately post, 24 and 72h	**Inflammatory markers (IL-6, TNF, cortisol), CK, Urinary 3MH** MLK: ↑ CHO: ↑ CON: ↑ **Glycogen resynthesis** MLK: ↔ CHO: ↔ CON: ↔ **Peak torque, total work of set** MLK:↓ CHO: ↓ CON:↓
(Cockburn et al., 2008)	24 healthy males, regularly competed in team sports; age 21 ± 3 years; body mass 80.2 ± 9.1kg	Parallel (n=6/grp)	**CHO Pro fat** MLK: 0.61 0.42 0.21 CPR: 1.45 0.42 0.20 CHO: 0.80 - - CON: - - -	**Ex:** 6 sets of 10 reps unilateral eccentric- concentrated knee flexion's **Rc:** Treatment given immediately and 2h following exercise **F-Ex:** DOMS, peak torque, CK, Mb were assessed immediately before and 24 and 48 h after EIMD.	**DOMS:** MLK: ↔ CPR: ↔ CHO: ↔ CON: ↔ **Peak torque at 48h:** MLK: ↑* CPR: ↑** CHO: ↔ CON: ↓ **CK at 48h:** MLK: ↓* CPR: ↓* CHO: ↑* CON: ↑*

						Mb: MLK: ↓* CPR: ↓* CHO: ↔ CON: ↔
(Cockburn et al., 2010)	32 healthy males, regularly competed in sports, age 20 ± 2y; body mass 78.5 ± 9kg	Single blind, independent group parallel design (n=8/grp)	**CHO** **Pro** **fat** CPR 1.51 0.43 0.21 CON - - -			**Ex:** 6 sets of 10 reps unilateral eccentric- concentrated knee flexion's **Rc:** Treatment given immediately before, after or 24h following exercise **F-Ex:** DOMS, peak torque, reactive strength index (RSI), CK, were assessed immediately before, 24, 48 h and 72 after EIMD.	**POST and TWENTY-FOUR** Benefit in limiting changes in active DOMS, peak torque, and RSI over 48 h compared with PRE. **PRE**: Possible benefit in reducing increases in CK over 48 h
(Cockburn et al., 2012)	24 healthy males, age 21 ± 3 y, body mass 79.7 ± 9.3kg	Parallel (n=8/grp)	**CHO** **Pro** **fat** MLK: 0.31 0.21 0.11 MLK2: 0.61 0.43 0.21 WAT: 0 - -			**Ex:** 6 sets of 10 reps unilateral eccentric- concentrated knee flexion's **Rc:** 500 ml (MLK) or 1L (MLK2) treatment given immediately after exercise **F-Ex:** DOMS, peak torque, CK, Mb, IL-6 at 24, 48, 72h	**<u>500ml vs 1L MLK:</u>** DOMS: possible benefit Peak torque: unclear CK: unclear Mb: almost certain benefit IL-6: unclear **<u>1L vs WAT:</u>** DOMS: unclear Peak torque: likely benefit CK: likely benefit Mb: unclear IL-6: likely benefit
(Cockburn et al., 2013)	14 healthy males, semi-professional soccer players.,	Parallel (n=7/grp)	**CHO** **Pro** **fat** MLK: 0.31 0.21 0.11 WAT: 0 - -			**Ex:** 6 sets of 10 reps unilateral eccentric- concentrated knee flexion's **Rc:** Treatment given	**<u>MLK vs WAT</u>** DOMS, RSI, CMJ, CK, Mb: unclear 15m sprint time: possible

	age= 24±4y, mass= 79.9±8.4kg			immediately after exercise **F-Ex:** DOMS, CMJ, RSI, 15-m sprint, agility time, CK, Mb at 24, 48, 72h, loughborough intermittent shuttle test (LIST) at 48h	benefit Agility/time to cover 15m in List:: likely benefit
(Gilson et al., 2010)	13 males; intercollegiate soccer players	Randomized, cross-over, 2 wk between intervention	**CHO Pro fat** CM: 1.06 0.35 0.09 CHO: 1.54 - 0.03	**Ex:** 4-days of increased training duration (`~95 min/d). **Rc:** treatment given at 0h post-exercise each day **E-Ex:** T-drill on d2 and vertical jump after 4 day	**T-drill:** CM: 9.06 ± 0.58 s CHO: 9.09 ± 0.47 s **Vertical-jump:** CM 26.7 ± 3.6 inches CHO: 26.7 ± 3.6 inches **CK:** CM: ↓* CHO: ↑
(Iwasa et al., 2013a)	18 healthy males, 21.6±0.8y; body mass 59.9±1.5 kg	Double-blind, repeated measures, counterbalanced, trials separated by at least 6 weeks	**Cho Pro Fat** FM: 0.36 0.11 - PLA: 0.36 0.11 -	**Ex:** Five sets of leg and bench presses at 70-100% 12 repetition maximum. **Rc:** 200 ml treatment consumed, before immediately and 2h after exercise **F-Ex:** DOMS, blood glucose, blood lactate assessed	**Blood lactate/CPK:** FM: ↑ PLA: ↑ **Muscle soreness:** FM: ↓*** PLA: ↔ **ORAC:** FM: ↔ PLA: ↔
(Papacosta et al., 2015)	12 trained male national level judo athletes; age 19 ± 4y, body mass 77.4 ± 7.9 kg	Crossover, trials separated by 14 days	Cho Pro Fat CM 1.4 0.5 0.4 CON - - -	**Ex:** 5 days of intensive judo training while making weight for weight category followed by simulated competition **Rc:** Treatment given immediately post exercise **F-Ex**: Counterbalanced horizontal jump test, timed	***Performance (D1vsD5):*** **Timed push ups (no):** CM: 48±7 vs 55±6* CON: 45±7 vs 46±6 **SJFT (index)** CM: 13.3±2.1 vs 12.4±1.1* CON: 14.2±1.6 vs 13.7±1.2 **SJFT (throws):**

				push up test, special judo fitness (SJFT) test on day 5	CM: 27±2 vs 28±2 CON: 25±3 vs 25±3 **Horizontal jump (m)** CM: 2.41±0.17 vs 2.43±0.17 CON: 2.32±0.16 vs 2.36±0.21 **DOMS** CM: ↓* CON: ↑ **Body mass:** CM: ↓ 1.1% CON: ↓ 1.9%
(P. Rankin et al., 2015)	32 team sport players 16 males; 81.1 ± 10.1 kg; 23.7 ± 3.4 y 16 females; 63.3 ± 5.8 kg; 21.9 ± 2.1 y	Randomized, independent group design	Cho Pro Fat FMLK: 0.40 0.27 0.08 FCHO: 0.85 MMLK: 0.31 0.21 0.06 MCHO: 0.66	**Ex:** six sets of 10 repetitions of eccentric–concentric contractions **Rc:** 500 ml treatment given immediately post ex **F-Ex:** Serum proteins, peak torque, CMJ, 20 m sprint and DOMS assessed at baseline, 24h, 48h and 72h post muscle damaging exercise	**<u>MLK vs CHO</u>** **Peak torque/20 m sprint:** FMLK: very likely benefit MMLK: unclear **DOMS:** FMLK: likely benefit MMLK: likely benefit **Skeletal troponin I:** FMLK: unlikely negative MMLK: unlikely negative
(Kirk et al., 2017)	21 male team sport athletes MLK: age 23 ± 1y; body mass 81.4 ± 13.1kg A2: age 23 ± 1y; body mass 79.4 ± 10.1 PLA: age 22 ± 1y; body mass 77.1 ± 7.8kg	Parallel, double blind (n=7/group)	Cho Pro Fat MLK: 0.30 0.23 - A2: 0.30 0.23 - CON: 0.63 - -	**Ex**: Repeated sprint protocol **Rc:** 500 ml treatment immediately after exercise **Fex:** DOMS, CMJ, MVIC, 20-m sprint assessed before, 24, 48 and 72h post repeated sprint	**20 m sprint (48h)** MLK: 3.3 ± 0.3*** A2 MLK: 3.3 ± 0.1* PLA: 3.6 ± 0.3 **CMJ (48h)** MLK: 33.1 ± 7.1* A2MLK: 32.4 ± 6.6* PLA: 29.2 ± 3.6 **MVIC and DOMS:** MLK: ↔ A2MLK: ↔

	PLA: ↔

CM, chocolate milk; CHO, carbohydrate, CPR, carbohydrate-protein replacement; MLK, milk; FM, fermented milk; FMLK, female milk group; MMLK, male milk group; FCHO, female carbohydrate group; MCHO, male carbohydrate group; Ex, exercise; Rc, recovery; F-Ex, follow-up exercise; Pre-Ex, pre-exercise; Pro, protein; TTE, time to exhaustion; WAT, water; PLA, placebo; FM, fermented milk; MJ, megajoule, CPK, creatine phosphokinase; MVIC, maximal voluntary isometric contraction, ORAC, oxygen radical absorbance capacity; CMJ, countermovement jump; CK, creatine kinase; Mb, myoglobin; DOMS, delayed onset muscle soreness; RSI, reactive strength index; SJFT, special judo fitness test; 3-MH,urinary 3- methyl histidine
*significantly different than CON
**Significantly different than CHO
***Significantly different than PLA
#Significantly different than CHO and CON

Table 1.5. Effects of milk on muscle mass, strength, body composition and training adaption in resistance and endurance exercise

Reference	Subjects	Study design	Treatments (g/kg)	Protocol	Results
(Lunn et al., 2012)	6 males runners, age 21.3±1.2y, body mass 71.3±2.7kg,	Randomised, crossover, 7 d between intervention	**CHO Pro fat** MLK: 0.76 0.21 0.0 CHO: 0.97 - -	**Ex:** 45min treadmill exercise at 65% VO_2peak **Rc:** 3h recovery -Protein FSR and whole-body protein turnover determined during the 3h recovery using muscle biopsies and D5-Phe and 13C-Leu	Mixed muscle protein FSR MLK: ↑ CHO: Plasma insulin concentration MLK: ↑ CHO:
(Ferguson-Stegall, McCleave, Ding, Doerner Iii, et al., 2011)	32 untrained cyclists (16 male; 16 female), age 22 ± 0.5y, body mass 71.7 ± 2.4kg	Randomized, double blinded, placebo controlled	**Cho Pro Fat** CM: 0.94 0.31 0.17 CHO: 1.25 - 0.17 PL:	**Ex:** cycled 60 min/d, 5 d/wk for 4.5 wks at 75-80% VO_2max. **Rc:** Treatment ingested immediately and 1 h after each exercise session	**VO_2 max** CM: ↑^ CHO:↑ PLA:↑ **Lactate threshold:** CM: ↑ CHO: ↑ PLA: ↑ **Whole body and trunk lean mass differential:** CM: ↑^ CHO: ↑ PLA: ↑
(J. W. Rankin et al., 2004)	19 untrained men; CM age 20.5 ± 0.62y; body mass 78.0 ± 5.2 kg CE age 21.0 ± 0.47y; body mass	Randomized, longitudinal design	**Cho Pro Fat**: CM: 0.92 0.21 0.06 CE: 1.25 - -	**Ex:** 10 week resistance periodized resistance training program (55-97% 1RM) **Rc:** Treatment given within 5 minutes post	**Muscular strength:** CM: ↑ CE: ↑ **FFST mass** CM: ↑ CE: ↑

	79.8 ± 4.9 kg			exercise	**Body fat:** CM: ↓ CE: ↓
(Wilkinson et al., 2007)	8 healthy males, age 21.6 ±0.3 y, body mass 81.7±5.9kg, regularly engaged in resistance training (>4d/wk)	Randomized, crossover, separated by >1wk	**Cho Pro Fat** MLK 0.28 0.22 0.02 SM 0.28 0.22 0.02	**Ex:** Standardized leg workout; 4 sets and 10 reps at 80% 1RM of each exercise for 3 sets, last set to exhaustion. **Rc:** Treatment given immediately following exercise	**Muscle FSR:** MLK: ↑** SM: ↑ **Amino acid uptake:** MLK: ↑** SM: ↑
(Kukuljan et al., 2009)	Men (n=180) , age 50-79 yr	2 x factorial design, 18 mo randomized controlled trial	Cho Pro Fat MLK: 11 6.6 2.2 g/200ml	**Ex:** 18 mo, 3 days/wk First 12wk training cycle: 15-20 reps at 50-60% 1RM Then 2 sets of 8-12 reps at 60-70% 1RM Last 8 wk training cycle: 80-85% 1RM **Rc:** 200 ml treatment consumed in the morning and in the afternoon or evening every day for 18 mo	**Lean mass:** FME: ↑ FM: ↔ EX: ↑ CON: ↔ **Muscle CSA:** FME: ↑ FM: ↔ EX: ↑ CON: ↔
(Elliot et al., 2006)	8 females; 16 males, had not participated in regular resistance training for at least 5 yr prior to study	Parallel (n=8/grp)	**Cho Pro Fat** FM 12.3 8.8 0.6 WM 11.4 8.0 8.2 IM 20.4 14.5 1.0 237 g FM 237 g WM 393 g of fat-free milk isocaloric with WM.	**Ex:** 10 sets of 8 reps of knee extensions of 80% 1RM **Rc:** Treatment was consumed at 60 min following exercise. Muscle biopsy taken before, 60, 120 and 300 min post exercise	**Threonine uptake:** FM: ↑ WM: ↑# IM: ↑ **Phenylalanine uptake:** FM: ↑ WM: ↑ IM: ↑

				.
(Hartman et al., 2007)	56 health young men	Parallel, 3-group, longitudinal design	Cho Pro Fat MLK 25.7 17.5 0.4 SM 17.5 CON g/500ml	**Ex:** 12 wk, 5 d/wk, rotating split-body resistance exercise training **Rc:** Treatment given immediately and 1h after exercise	**Type I muscle fiber area:** MLK: ↑* SM: ↑ CON: ↔ **Type II muscle fiber area** MLK: ↑*** SM: ↑ CON: ↑ **FBFM:** MLK: ↑*** SM: ↑ CON: ↑
(Josse et al., 2010)	20 healthy women MLK: age: 23.2 ± 2.8 y; BMI 26.2 ±kg·m^2, CON: age: 2.4 ± 2.4 y; BMI: 25.2 ± 3.8 kg·m^2	12 week, randomized	Cho Pro Fat MLK 24 18 0 CON 40	**Ex:** 12 wk, 5 d/wk, rotating split-body resistance exercise training **Rc:** Treatment given immediately and 1h after exercise	**Lean mass:** MLK: 1.9 ± 0.2 kg* CON: 1.1 ± 0.2 kg **Fat mass:** MLK:-1.6 ± 0.4 kg* CON: -0.3 ± 0.4 kg **25[OH]D Δ from baseline:** MLK: +6.5 ± 1.1 nM* CON: +2.8 ± 1.3 nM **PTH**: MLK:↓* CON: ↔ **EAA, BCAA and leucine**: MLK: ↑*

					CON: ↔ **Strength:** MLK: ↑ CON: ↑
(Mitchell et al., 2015)	16 healthy young men; age 22.4 ± 2.1 y; 23.2 ±3.6 kg/m^2 16 healthy older men; age 74.4 ± 5.4 y; 26.9 ± 3.2 kg/m^2	Randomized, parallel, 12 weeks	Cho Pro Fat CM: 54 14 5 CON: 66 0.4 5 g/500ml	**Ex:** Whole body resistance training 3x/ week for 12 weeks **Rc:** 500 ml treatment immediately after training and with breakfast on non-training days	**Muscle strength:** CM (young): ↑ PLA (young): ↑ CM (older): ↑ PLA (older): ↑ **Type 1 muscle fibre area:** CM (young): ↑ PLA (young): ↑ CM (older): ↑ PLA (older): ↑ **Type 2 muscle fibre area:** CM (young): ↑ PLA (young): ↑ CM (older): ↔ PLA (older): ↔
(Rumbold et al., 2015; Volterman et al., 2014b)	28 (15 female, 13 male) PEP boys age 9.4 ±1.0y, body mass 34.2 ± 7.7 kg; girls age 9.5±0.8y, body mass 29.6±5.7kg MLP boys age 15.6 ± 0.5y, body mass 59.4±9.0kg; girls age	Randomized, repeated measures, crossover design	Cho Pro Fat MLK 0.65 0.40 - CE 1.0 - - WAT - - -	**Ex:** Cycled at 60% VO_2 max (2x20 min bouts) at in heat (34.5 ± 0.3 °C) **Rc:** Treatment equivalent to 100% BM loss given at 0, 15 and 30 min.	Protein synthesis, nitrogen, WBPB increased with milk WBPB remained negative even more so in MLP, while PEP girls were only able to maintain positive throughout 16h

	14.8±0.4y, body mass 60.5±8.4kg				
(Rumbold et al., 2015)	9 female recreational exercisers, age 19.7 ± 1.3y; body mass 61.7 ± 4.4 kg	Within-subjects, randomised, cross-over design	Cho Pro Fat MLK 0.49 0.33 0.01 CON 0.81 0.04 -	**Ex:** 30 min continuous cycling (65% ± 4% $VO_{2\ peak}$) **Rc:** Treatment given immediately following exercise. Ad libitum pasta meal provided 60 min post treatment consumed (34% Fat, 14% Pro, 52% Cho)	**Absolute energy intake:** MLK: 2.39 ± 0.70 MJ* CON: 3.20 ± 0.84 MJ **Relative energy intake:** MLK: 1.49 ± 0.72* CON: 2.33 ± 0.90 MJ

FM: Fat-free milk; WM: Whole milk; IM: Isocaloric milk; CM, chocolate milk; CHO, carbohydrate, CPR, carbohydrate-protein replacement; MLK, milk; SM, soy milk; MJ, megajoule, IMTP, isometric mid-thigh pull; FSR, fractional synthesis rate; Ex, exercise; Rc, recovery; Pro, protein; WAT, water; PLA, placebo; EAA, essential amino acids; BCAA, branched chain amino acids; PEP, pre to early pubertal; MLP, mid to late pubertal; CSA, cross sectional area

*Significantly different than CON

**Significantly different than SM

***Significantly different than SM and CON

#Significantly different than IM and FM

^Significantly different than CHO and PLA

CHAPTER I I: STUDY RATIONALE, OBJECTIVES AND HYPOTHESES

2.1 RATIONALE – STUDY 1

Nutritional supplements are commonly used by athletes to enhance performance and promote recovery following strenuous exercise. However, athletes often lack sport specific nutrition knowledge (Andrews, Wojcik, Boyd, & Bowers, 2016; Magee, Gallagher, & McCormack, 2017), including knowledge regarding supplements (Kelly, Leveritt, Brennan, Slater, & Jenkins, 2017). Prolonged or high intensity exercise can result in depletion of glycogen stores, dehydration and symptoms associated with EIMD. These factors can impair an athlete's overall exercise performance, specifically when executing multiple exercise bouts over a short time period. Thus, for optimal post exercise recovery, one would have to consume sufficient carbohydrate to replenish glycogen stores, adequate protein for muscle tissue repair, as well as ample fluids and electrolytes for rehydration. Recently, interest has grown around dairy milk as a beneficial recovery beverage due to its nutritional composition, palatability, low cost and convenience. More specifically, dairy milk contains ample quantities of carbohydrates, in the form of lactose, and whey and casein proteins, along with high concentrations of electrolytes. Therefore, current research suggests that milk may be an effective ergogenic aid for post exercise recovery.

Several studies have observed that milk consumption can lead to a significant improvement in subsequent exercise cycling performance (Ferguson-Stegall et al., 2011; Thomas, Morris, & Stevenson, 2009), attenuated markers of muscle damage (Gilson et al., 2010; Pritchett, Bishop, Pritchett, Green, & Katica, 2009), improvements in hydration status (Desbrow et al., 2014; Shirreffs, Watson, et al., 2007a; Watson et al., 2008) and greater increases in muscle hypertrophy and lean body mass and decreases in fat free mass (Hartman et al., 2007; Josse et al., 2010).

Although there have been several intervention studies related to milk and exercise, there is no research to date conducted on athlete's perspective and use of milk for their personal athletic performance. Given the high prevalence of supplement use and lack of overall nutrition knowledge among athletes (Andrews et al., 2016; Magee et al., 2017), there is a need to understand whether athletes perceive milk to be a beneficial ergogenic aid and if they use it as part of their nutritional recovery program.

2.2 OBJECTIVES STUDY 1

2.2.1 Overall objective

The overall objective of this study was to assess the perceptions and usage of dairy milk as an ergogenic aid among competitive athletes and recreational exercisers.

2.2.2 Specific objectives

I. To determine if there are differences in perceptions and use of dairy milk for exercise between recreationally exercisers and competitive athletes.

2.3 HYPOTHESES STUDY 1

I. There will be a higher probability that athletes are consuming milk for sports performance.

II. Athletes will perceive milk to be beneficial for recovery in terms of glycogen replenishment, hydration, reducing muscle soreness and improving performance.

III. A higher percentage of competitive athletes will perceive milk to be a beneficial ergogenic aid and use it as a ergogenic aid compared to recreational exercisers

2.4 RATIONALE STUDY 2

Strenuous exercise is associated with various physiological disturbances resulting in muscle fatigue and compromised performance. The high demand for skeletal muscle to perform work may cause damage to the sarcolemma, which may result in muscle soreness and an increase

of intramuscular enzymes, making a subsequent exercise session more difficult to complete. EIMD has been observed among multiple sprint-type sports such as soccer, rugby and basketball. Multiple sprint-type sport involves high intensity, short bursts of power, which requires acceleration and changes in direction (Nightingale, Miller, & Turner, 2013). Overall, elite athletes participating in intermittent sports such as hockey and football require muscular strength, anaerobic fitness and aerobic endurance in order to be successful (Nightingale et al., 2013). However, due to the intense training schedules and minimal recovery time between training and/or games, elite athletes may experience EIMD in the absence of proper nutritional interventions, which may contribute to compromised performance.

Current research suggests that milk may be as effective as popular ergogenic aids for post exercise recovery. Previous studies have reported benefits of dairy milk for attenuating symptoms of muscle damage following intermittent activities such as soccer (Gilson et al., 2010), endurance exercise such as cycling (Ferguson-Stegall, McCleave, Ding, Doerner, et al., 2011) and eccentric exercises (Cockburn et al., 2013; Cockburn et al., 2012; Papacosta et al., 2015; P. Rankin et al., 2015). However, there is relatively little research on milk and EIMD for intermittent team sports. More specifically, there is currently no research examining the effects of milk on attenuating symptoms of EIMD in football players, and due to the heavy eccentric load involved in football they are known to experience symptoms of EIMD. Therefore, in addition to the gap in the literature regarding milk and EIMD for team sport athletes, the results from Study 1 were used to guide the development of Study 2. Based on our survey results, 82.8% of football players surveyed drink milk as part of their everyday nutritional intake, 89.7% believe it could help with exercise performance, however only 44.8% reported consuming it for exercise. Considering that milk is rich in many nutrients that have been shown to be beneficial for post

exercise recovery, it is of interest to determine whether the consumption of milk following a strenuous training session will attenuate indices of EIMD in football players.

2.5 OBJECTIVES STUDY 2

2.5.1 Overall objective:

The overall objective of this study was to assess the effects of dairy milk consumption on improving exercise recovery by attenuating biomarkers of EIMD in collegiate football players.

2.5.2 Specific objectives:

To determine if consuming low fat chocolate milk immediately and 2 hours post-exercise will:

I. Reduce CK and LDH, biomarkers of EIMD

II. Reduce delayed onset muscle soreness

III. Minimize the effect on exercise performance at 24 and 48 hours following muscle damaging exercise

2.6 HYPOTHESES STUDY 2

I. Low fat chocolate milk consumption immediately post exercise will lead to reduced biomarkers of EIMD at 24 and 48 hours as measured by CK and LDH in elite male collegiate football players compared to an isovolumetric water control beverage.

II. Consumption of low fat chocolate milk immediately post-exercise will reduce symptoms of delayed onset muscle soreness at 24 and 48 hours following muscle damaging protocol compared to the control beverage

III. Low fat chocolate milk consumption immediately and 2 hours post-exercise will minimize the effect on exercise performance as measured by countermovement jump height and power and 15m sprint time.

CHAPTER III: STUDY 1. USE AND PERCEPTIONS OF DAIRY MILK AS AN ERGOGENIC AID AMONG COMPETITIVE ATHLETES AND RECREATIONAL EXERCISERS

A version of this chapter will be submitted for peer-reviewed publication

3.1 INTRODUCTION

The use of dietary supplements as ergogenic aids is a common practice among athletes of various performance levels, where athletes have reported many reasons for using supplements, including to increase muscle mass/strength, increase endurance, increase energy, improve recovery, maintain health and boost immunity (Dascombe, Karunaratna, Cartoon, Fergie, & Goodman, 2010; Fraczek, Warzecha, Tyrala, & Pieta, 2016; Parnell, Wiens, & Erdman, 2016). However, incongruences are known to exist between self-reported rationales for supplement use and their established benefits, as well as adherence to diet trends which have yet to show evidence-based health and performance benefits (Kelly et al., 2017; Lis, Stellingwerff, Shing, Ahuja, & Fell, 2015; Parnell, Wiens, & Erdman, 2015; Wilson, 2016). Furthermore, the safety of nutritional ergogenic aids is often a concern as there is the possibility that a product may contain illegal or banned substances, and should be used with caution (Deldicque & Francaux, 2016). Thus, athletes would benefit from a quality diet focusing on whole foods to support recovery and training adaptations (D. T. Thomas et al., 2016b).

Meanwhile, nutrition recommendations differ depending on the intensity, frequency and volume of training as well as individual training goals. Typically, elite athletes train at a higher volume, however little research exists examining the dietary patterns between recreational exercisers and competitive athletes, specifically for ergogenic purposes.

Recently, dairy milk has garnered media attention and peaked interest among researchers as a beneficial post-exercise recovery beverage due to its nutritional composition, palatability, low cost and convenience. In addition to its usual nutritional benefits, there is increasing literature suggesting that milk may serve the same purpose as many currently available nutritional ergogenic aids (Gilson et al., 2010; K. Thomas et al., 2009; Watson et al., 2008). While most nutritional ergogenic aids are typically in the form of a single nutrient, dairy milk contains many nutrients that may enhance the recovery process following strenuous exercise. Milk contains carbohydrates in the form of lactose that contribute to muscle glycogen re-synthesis (Ferguson-Stegall, McCleave, Ding, Doerner, et al., 2011). Milk contains high quality proteins; whey and casein, which have principal roles in protein synthesis and limiting protein breakdown as well as the ability to attenuate muscle damage following exhaustive exercise (Saunders, 2011a). Milk also contains high concentrations of electrolytes, which are important for rehydration (Shirreffs, Watson, & Maughan, 2007b).

Overall, a growing literature suggests that dairy milk may have the same effects as many ergogenic aids in terms of improving time trial performance (Ferguson-Stegall, McCleave, Ding, Doerner, et al., 2011; K. Thomas et al., 2009), attenuating symptoms of muscle damage (Gilson et al., 2010), improving hydration status (Desbrow et al., 2014; Seery & Jakeman, 2016; Shirreffs, Watson, et al., 2007b; K. Thomas et al., 2009) and favourably changing body composition (Hartman et al., 2007) in both recreational and elite athletes performing endurance and resistance training exercises. Although there have been several intervention studies regarding dairy milk and its effects on exercise performance, there is no research on the use and perceptions of dairy milk as an ergogenic aid among athletes of various performance levels. Therefore, the objectives of this study were to assess athletes' perceptions of consumption of

milk for exercise performance and whether they use milk as an ergogenic aid for their personal training program, and determine if there are differences in these parameters between recreational exercisers and competitive athletes.

3.2 METHODS

3.2.1 Participants

The perceptions and use of milk for exercise performance was surveyed from a convenient sample of persons from various sport levels (recreational, provincial, Canadian Interuniversity Sport (CIS) and national), throughout Manitoba. Participants were recruited via email through various sport organizations including the Canadian Sport Centre Manitoba, University of Manitoba Bison Athletics, Sport Medicine and Science Council Manitoba, University of Manitoba gyms (Joe Doupe Recreation Centre, Active Living Centre), CrossFit Sublime and Running Room locations in Winnipeg, Manitoba. Provincial, CIS and National athletes were recruited from nine sports: ringette, hockey, wrestling, football, wheelchair basketball, volleyball, curling, synchronized swimming and soccer. On average 75% of team members from these sports responded to the survey. Ethics approval was obtained from the University of Manitoba Joint-Faculty Research Ethics Board. All participants signed an informed consent prior to completing the survey.

3.2.2 Milk & Exercise Survey Design

A pilot survey was first distributed to small groups of nutrition graduate students and dietitians. Survey questions were then modified for clarity based on feedback. A paper based, self-administered 14-question survey, which consisted of open- and close-ended questions, was distributed to competitive athletes from August 2014-July 2015. The survey consisted of the following areas:

1) Demographics: (age, gender, education, sport, level of sport, training volume)

2) Use and general perceptions regarding milk for exercise performance
3) Usage of milk for personal training program (amount, type, timing)
4) Usage of dietary supplements and type of supplements used
5) Sources of milk for exercise information

Close ended questions for demographics such as education level, gender and training hours were completed as pre-determined categories. Participants were permitted to leave questions unanswered.

3.2.3 Statistical Analysis

Statistical analyses were performed using SPSS software (version 20; SPSS Inc., Chicago, IL). When necessary, responses to open-ended questions were grouped into categories prior to data analysis and multiple response analysis was used to summarize responses. Responses to training level were grouped into recreational exercisers (cardio, weight training, yoga, amateur sports) and competitive athletes (athletes involved in sports competing at a provincial, CIS or national level). Use and perceptions of milk for everyday nutritional intake and use of milk for exercise performance were completed as yes/no. Beliefs associated with milk for exercise performance were combined into categories including; post exercise recovery (recovery, strength, hydration, restores energy), nutritional composition (protein, carbohydrates, calcium, vitamins,) and bone health (strong bones, prevents injury, healthy bones). Beliefs associated with why milk would not help with exercise performance were grouped into lactose intolerance/gastrointestinal issues (stomach upset, hard to digest, lactose sensitive/intolerant/allergy, congestion), able to perform without milk (haven't noticed a difference in performance, gotten to a high level without drinking milk), not part of diet (dislike taste, get nutrients from other sources, healthier alternatives/nutrients from other sources and not aware of benefits). Dietary supplement use was condensed into vitamin D, vitamins (vitamins, vitamin C, B vitamins, multivitamins), omegas

(cod liver oil, fish oil, omega 3, 6, 9), protein/amino acids (protein powder, whey protein, soy protein, hemp protein, BCAA, glutamine), sports drinks/electrolytes (Gatorade, Powerade, electrolyte gels/gummies), minerals (magnesium, calcium), other (probiotics, herbal supplements, pre-workout supplements, caffeine, meal replacements, CLA, creatine). Sources of information were amalgamated into trainer/coach (trainer, coach, physiotherapist), media (online and television advertisement, magazine article), dietitian/nutritionist, school (high school, university course) scientific article and other (family, friends, colleagues, teammates, word of mouth). Volume of milk were grouped into 250 ml or less (1 cup, 1 glass, 200 ml, 250 ml), 250-500 ml (1-2 cups, 325 ml, 250-500 ml), greater than 500 ml (750 ml, 1 litre, 1500 ml). Amount of milk was categorized into before exercise, after exercise and other timing (specified time of day, before bed, with breakfast/lunch/supper, if available). Descriptive data were calculated as frequencies and percentage, or mean ± standard deviation as appropriate. Differences between groups for categorical data were assessed by chi-square (χ^2) test of independence and fisher's exact test. Non-parametric data were assessed by Mann-Whitney U test. Independent samples t-test was used to analyze age difference. Statistical significance was set at p-value ≤ 0.05.

3.3 RESULTS

3.3.1 Demographics

Two hundred and ninety four participants completed the survey; comprising of 162 females and 132 males. **Table 3.1** summarizes the demographic characteristics of the participants. Participants were classified as recreational exercisers (35%; n=103) or competitive athletes (65%; n=191); competed at provincial, collegiate and national levels.

Table 3.1. Demographics characteristics of the participants

	Recreational (n=103)	Competitive (n=191)	Overall (n=294)	P-value
Gender†	**n (%)**	**n (%)**	**n (%)**	**0.760**
Female	58 (56.3%)	104 (54.5%)	162 (55.1%)	
Male	45 (43.7%)	87 (45.5%)	132 (44.9%)	
Age€				
Mean ±SD	32.9 ± 13.4	20.9 ± 4.2	25.2 ± 10.4	**<0.001**
Education†	**n (%)**	**n (%)**	**n (%)**	**<0.001**
Junior High/High School	7 (6.8%)	25 (12.0%)	32 (10.9%)	
Technical/Trade	12 (11.7%)	9 (4.7%)	21 (7.1%)	
Undergraduate	53 (51.5%)	152 (79.6%)	205 (69.7%)	
Graduate/Specialized Program	31(30.1%)	5 (2.6%)	36 (12.2%)	
Weekly Training Hours*	**n (%)**	**n (%)**	**n (%)**	**<0.001**
0-5 hours	22 (21.4%)	2 (1.1%)	24 (8.2%)	
6-10 hours	60 (58.3%)	22 (11.7%)	82 (26.7%)	
11-15 hours	16 (15.5%)	81 (43.1%)	97 (33.3%)	
16-20 hours	2 (1.9%)	46 (24.5%)	48 (16.5%)	
>20 hours	3 (2.9%)	37 (19.7%)	40 (13.7%)	

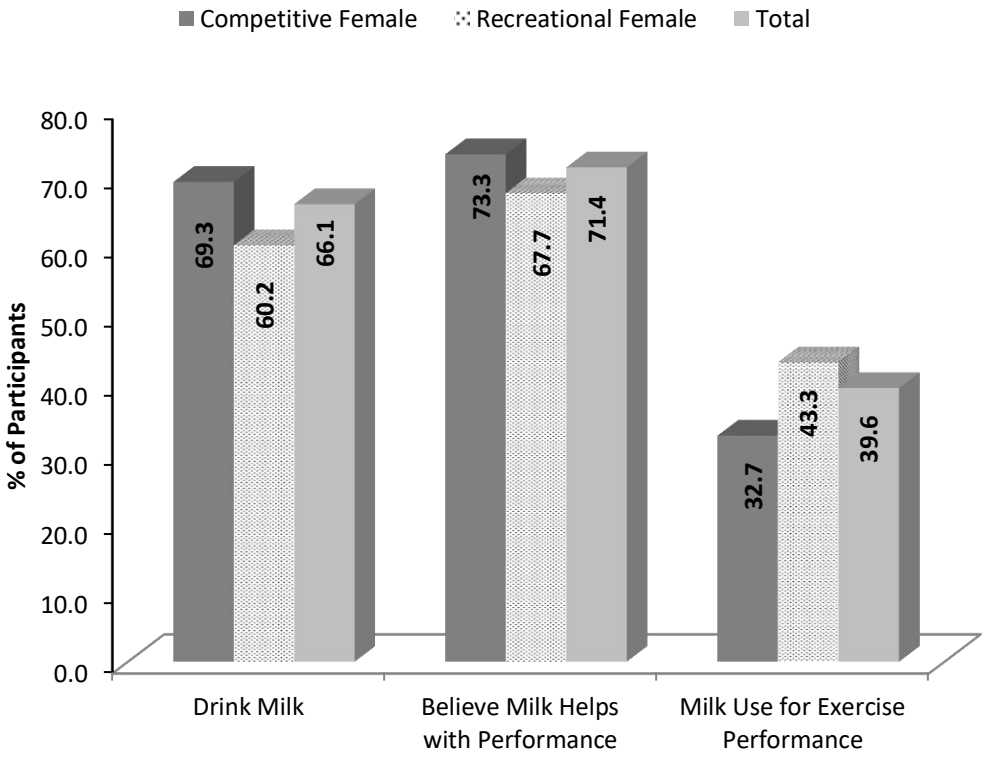
†P-value represents comparison of recreational exercisers and competitive athletes as assessed by chi-squared analysis.
€P-value represents comparison of recreational exercisers and competitive athletes as assessed by Independent samples t-test.
*P-value represents comparison of recreational exercisers and competitive athletes (Mann-Whitney U = 2598.50, Z = -10.684, p = 0.000).

3.3.2 Prevalence of dairy milk use and perceptions

Figure 3.1 shows that the majority of participants (66.1%; n=193) consume dairy milk as part of their everyday nutritional intake, with no differences between competitive (69.3%; n=131) compared to recreational exercisers (60.2%; n=62; $\chi^2 = 2.473$, p=0.116). Furthermore, a majority of the participants (71.4%, n=207) believed that dairy milk can be beneficial for exercise

performance. However, only 39.6% (n=114) of all participants reported routinely consuming dairy milk as part of their personal training program. These distributions were similar between recreational exercisers and competitive athletes ($\chi^2 = 3.106$, p=0.078). Although there were significant associations found between level of athlete and education, training hours and age (Table 3.1), these variables did not have a significant effect on perceptions of milk, daily milk consumption or milk consumption for exercise performance.

Figure 3.1. Prevalence of dairy milk use and perceptions as an ergogenic aid



3.3.3 Effect of gender on dairy milk use and perceptions

Table 3.2 displays the prevalence of daily milk use, perceptions for exercise performance and use of milk for exercise routine based on genders. Although not significant, an interesting trend was observed between gender and milk consumption, with more females reported not consuming milk as part of their everyday nutrition intake (38.3%) compared to males (28.50%). More males

(77.9%) believed milk could help with exercise performance compared to females (66.0%). However, no difference was observed between gender and those who reported consuming milk for exercise performance. Although, when expressed by level of athlete, a higher percentage of female competitive athletes reported drinking milk as part of their daily nutritional intake as well as using milk for exercise performance (68.3% and 46.1%, respectively) compared to female recreational exercisers (50.0% and 26.3%, respectively) (Table 3.3). Meanwhile, there were no significant association found between these parameters for males and level of athlete. No differences were observed between sport level and gender for perceptions of milk as an ergogenic aid.

Table 3.2. Prevalence of dairy milk use and perceptions among gender

		Gender		χ^2	P value
		Male	Female		
Drink Milk	Yes	93 (71.5%)	100 (61.7%)	3.097	0.078
	No	37 (28.5%)	62 (38.3%)		
Believes milk helps with performance †	Yes	102 (77.9%)	105 (66.0%)	4.916	0.027
	No	29 (22.1%)	54 (34.0%)		
Use milk for exercise performance	Yes	52 (40.3%)	62 (39.0%)	0.052	0.820
	No	77 (59.7%)	97 (61.0%)		

†P-value represents comparison of males and females as assessed by chi-squared analysis.

Table 3.3. Prevalence of dairy milk use and perceptions among female sport level

		Female Sport Level		χ^2	P value
		Recreational	Competitive		
Drink Milk †	Yes	29 (50.0%)	71 (68.3%)	5.260	0.022
	No	29 (50.0%)	33 (31.7%)		
Believes milk helps with performance	Yes	33 (60.0%)	72 (69.2%)	1.367	0.242
	No	22 (40.0%)	104 (30.8%)		
Use milk for exercise performance †	Yes	15 (26.3%)	47 (46.1%)	6.003	0.014
	No	42 (73.7%)	55 (53.9%)		

†P-value represents comparison of recreational exercisers and competitive athletes as assessed by chi-squared analysis.

3.3.4 Type, amount and timing of milk use for exercise

The type, amount and timing of milk use for exercise performance are displayed in **Table 3.4**. Out of those who responded, chocolate milk was the most identified milk choice (76.7%) for exercise performance, and the majority (84.9%) of participants reported milk to be consumed after exercise. There were significant effects ($\chi^2 = 41.73$, p=0.000) found between gender and milk amount consumed for exercise, where out of those who responded, more females (n=38; 45.8%) reported 250 ml or less, whereas males identified 500 ml or greater volume compared to females (n=28; 45.9% vs. n=21; 25.3% and n=18; 29.5% vs n=1; 1.2%, respectively). In regards to level of athlete, the majority of male recreational exercisers who responded (n=14; 66.7%) reported 500 ml for volume of milk, whereas 42.5% (n=17) of competitive male athletes compared to 4.8% of recreational exercisers indicated more than 500 ml athletes (χ^2 =9.86,

p=0.020). No differences were found between female level of athlete and amount of milk. No significant differences were observed between milk type choice and gender and level of athlete.

Table 3.4 Milk type, amount and timing for exercise

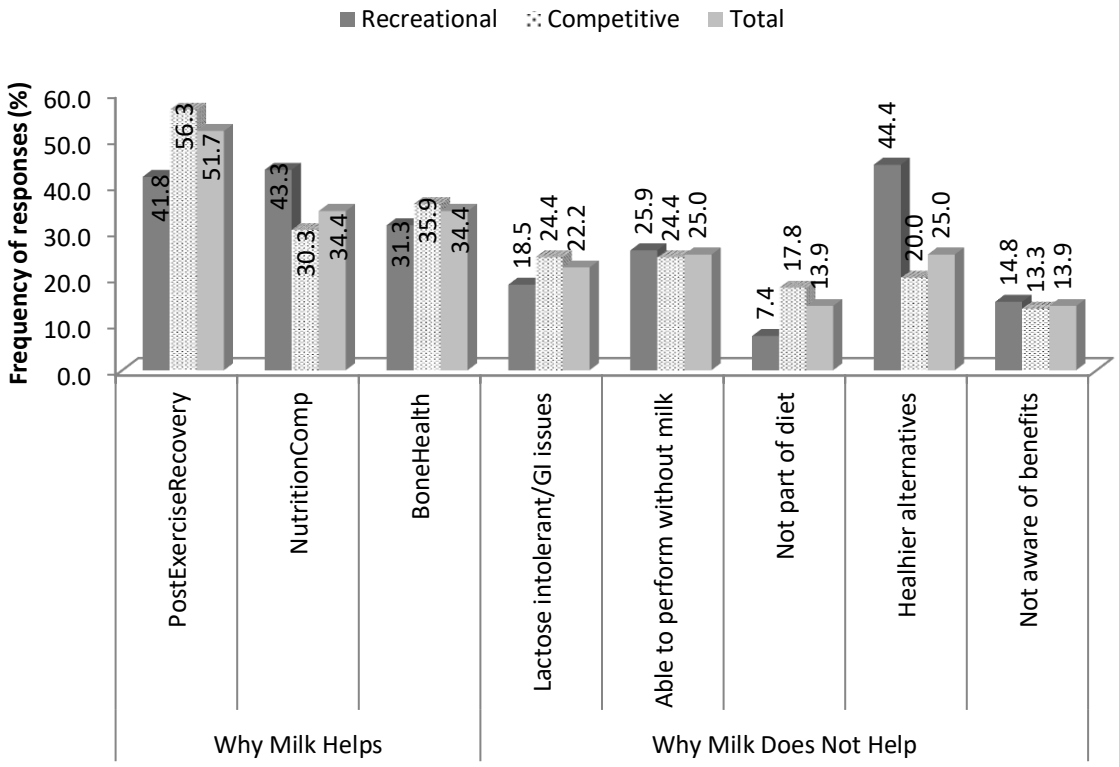
Milk Type	**Recreational**	**Competitive**	**Overall n (%)**	**P value** **0.199**
Skim	5 (13.5%)	23 (20.4%)	28 (18.7%)	
1%	10 (27.0%)	24 (21.2%)	34 (22.7%)	
2% or higher	5 (13.5%)	17 (15.0%)	22 (14.7%)	
Chocolate	23 (62.2%)	92 (81.4%)	115 (76.7%)	
Non Dairy	1 (2.7%)	10 (8.8%)	11 (7.3%)	
Milk Amount†			**n (%)**	**0.035**
250ml or less	12 (32.4%)	36 (33.6%)	48 (33.3%)	
250-500ml	4 (10.8%)	24 (22.4%)	28 (19.4%)	
500ml	19 (51.4%)	30 (28.0%)	49 (34.0%)	
>500ml	2 (5.4%)	17 (15.9%)	19 (13.2%)	
Milk Timing			**n (%)**	0.214
Before exercise	2 (5.0%)	8 (7.1%)	10 (6.6%)	
After exercise	31 (77.5%)	98 (87.5%)	129 (84.9%)	
Other Timing	13 (32.5%)	24 (21.4%)	37 (24.3%)	

†P-value represents comparison of recreational exercisers and competitive athletes as assessed by chi-squared analysis.
2% or higher category includes homogenized/whole milk

3.3.5 Perceptions of milk use for exercise performance

Figure 3.2 shows that the primary perceived benefits of milk for exercise performance that participants reported was its role in improving post-exercise recovery (n=108; 51.7%). Healthier alternatives/nutrients from other sources (n=21; 29.2%) was the main reason provided for why athletes did not believe milk would help with their exercise performance. Although significance was not reached ($\chi^2 = 7.69$, p=0.053), differences were observed between level of athlete, where a higher percentage of competitive athletes (n=80; 56.3%) perceived post exercise recovery to be of benefit for exercise performance whereas nutritional composition was the most often perceived benefit for recreational exercisers athletes (n=29, 43.3%). There was no significant association between level of athlete and reasons for why milk would not help with performance **(Figure 3.2).**

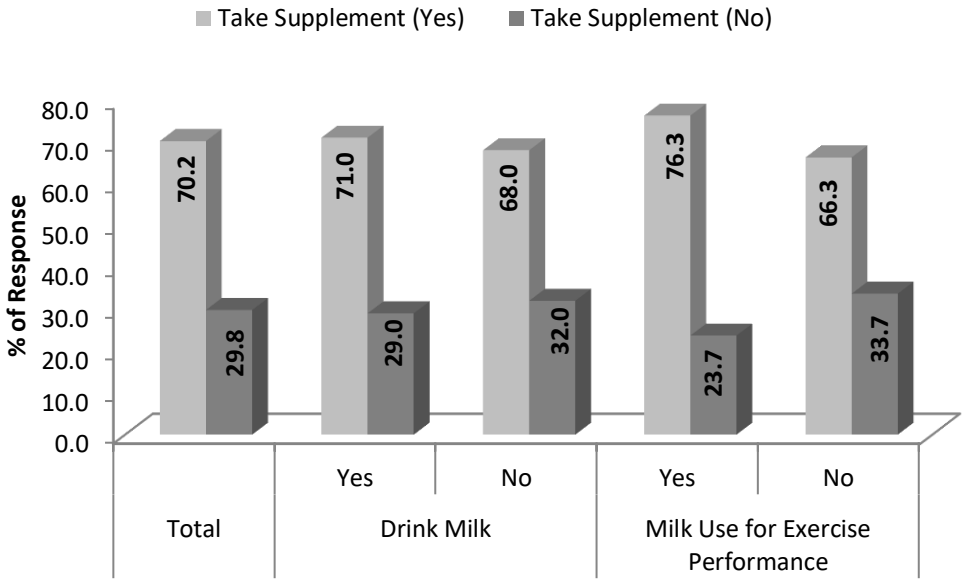
Figure 3.2. Perceptions of milk use for exercise performance.



3.3.6 Prevalence of dietary supplementation and use of milk for exercise

Figure 3.3 shows that most participants (70.2%; n=205) reported taking at least one dietary supplement, with no difference between recreational exercisers (n=72; 69.9%) and competitive athletes (n=133; 70.4%; $\chi^2 = 0.007$, p=0.933). There was however significant association between genders and supplement use, with more males (78.6%; n=103) reportedly taking dietary supplements compared to females (63.4%; n=102) ($\chi^2 = 8.05$, p=0.005). Among those who reported consuming milk for exercise performance, the majority (76.3%; n=87) also indicated using at least one nutritional supplement. The most common dietary supplements reported were: protein/amino acids (n=105; 51.2%), vitamins (n=78; 38.0%), sports drinks/electrolytes (n=73; 35.6%), other (pre-workout supplements, caffeine, creatine, herbal supplements; n=45; 22.0%), Vitamin D (n=41;20.0%), minerals (n=21;10.2%) and omega fatty acids (i.e. omegas; n=21; 10.2%). The most frequent dietary supplements reported for competitive athletes were protein/amino acids (n=64; 48.1%), sports drinks (n=60; 45.1%) and vitamins (n=45; 33.8%) whereas the most frequent dietary supplement reported for recreational exercisers were protein/amino acids (n=41; 56.9%), vitamins (n=33; 45.8%), vitamin D (n=18; 25.0%) and other (n=18; 25.0%).

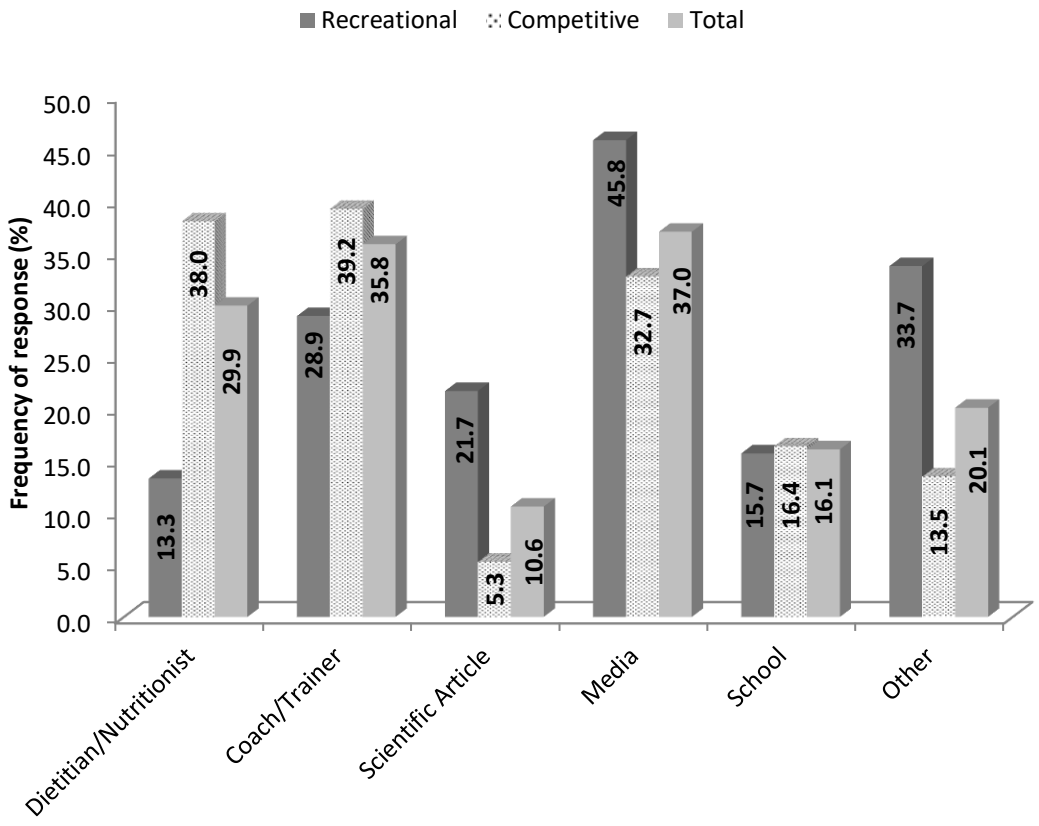
Figure 3.3. Prevalence of dietary supplement use



3.3.7 Source of information

Figure 3.4 shows that overall, coaches and trainers (35.8%), media (37.0 %) and dietitians/ nutritionists (29.9%) were the most often identified sources of information regarding dairy milk for exercise performance. The most often identified sources of information for competitive athletes were coaches/trainers (39.2%), dietitians/nutritionists (38.0%) and media (32.7%). The sources of information that recreational exercisers most often identified were media (45.8%), other sources (35.7%) and coach/trainer (28.9%). There was a significant association between level of athlete and source of information ($\chi^2 = 53.183$, $p<0.001$). There were more competitive athletes (38.0%) who reported receiving information from dietitians/nutritionists compared to recreational exercisers (13.3 %;). Furthermore, recreational exercisers were more likely to obtain information from scientific articles (21.7%) and other sources (33.7%), including friends, family and teammates, compared to competitive athletes (5.3% and 13.5% respectively).

Figure 3.4. Source of information about milk for athletic performance



Significant association between recreational exercisers and competitive athletes assessed by chi-square analysis ($p \leq 0.05$).

3.4 DISCUSSION

The main findings from the present study are that two-third (66.1%) of the athletes consumes milk as part of their daily nutritional intake, and even more (71.4%) believe that milk can help with their exercise performance. However, there is gap between athlete's beliefs and their actions, as only 39.6% of the athletes reported drinking milk as part of their exercise routine. The majority of the athletes in this study believed that milk can help with performance and indicated that its role in improving post-exercise recovery, bone health and its nutritional composition are the main benefits. Participants who reported that milk does not help with

exercise performance stated that they were able to perform at a high level without milk, they were unaware of the benefits, and/or that there are healthier alternatives than milk. These findings concur with other studies that indicated that athletes are able to identify some, but not all, supposed claims of nutritional ergogenic aids (Kelly et al., 2017; Parnell et al., 2015; Wilson, 2016). In general, several studies have demonstrated benefits of milk consumption compared to alternative beverages on various markers of recovery in both trained athletes and recreational exercisers. For example, post-exercise milk consumption has been shown to favourably improve body composition in recreational exercisers and elite athletes (Hartman et al., 2007; Josse et al., 2010). Milk can also improve post-exercise recovery in endurance trained athletes by increased skeletal muscle protein synthesis and decreased whole body protein breakdown during recovery (Lunn et al., 2012). Furthermore, milk has been shown to attenuate symptoms of exercise-induced muscle damage (EIMD) in recreationally active individuals as well as team sport and endurance athletes (Gilson et al., 2010; Papacosta et al., 2015; Pritchett et al., 2009; P. Rankin et al., 2015). The majority of competitive athletes that were surveyed in this study were involved in sports that involve short burst of exercise and would likely be involved in strength and conditioning with eccentric movements, which has often been related to symptoms associated with EIMD (Leeder et al., 2014; Wiewelhove et al., 2016). Recreationally active individuals performing unaccustomed and/or eccentric exercise are more likely to be susceptible to EIMD and would also benefit from understanding the role milk plays in attenuating associated symptoms (Peake et al., 2017).

We hypothesized that the higher the training volume, the more likely that athletes would use milk as part of their exercise routine; similar to what has been observed for dietary supplement (Giannopoulou et al., 2013). However, although most competitive athletes reported

training at a higher volume, the survey demonstrated no significant differences between athlete training level and milk consumption for exercise performance. Despite some individuals indicating that they do not use milk as part of their exercise routine, there were still some participants who responded to the type, amount and timing of milk use for their exercise routine. Although not determined in our study, it is possible that athletes do not drink milk routinely for exercise, rather they use it occasionally. There were no differences in the type of milk reported for exercise performance wherein athletes more often identified chocolate milk as the preferred beverage. In regards to the amount of milk consumed for ergogenic purposes, recreational exercisers and competitive athletes reported volumes ranging between 200 ml and 1000 ml and greater. These volumes are in accordance with studies reporting recovery benefits and improvements in exercise performance with milk (Cockburn et al., 2012; Elliot et al., 2006; Ferguson-Stegall, McCleave, Ding, Doerner Iii, et al., 2011; Gilson et al., 2010; Josse et al., 2010; Kirk et al., 2017; Papacosta et al., 2015; Shirreffs, Watson, et al., 2007a; K. Thomas et al., 2009). Furthermore, the majority of athletes reported consuming milk post-exercise, which concurs with the aforementioned literature to promote optimal recovery. Thus, of those who reported consuming milk as an ergogenic aid, their practices concurs with the current literature in terms of appropriate timing and amount of milk use for exercise recovery and performance enhancement.

The literature indicates that men consume more servings of dairy (Larson et al., 2009), while many females avoid dairy and are resistant to change these habits (Gulliver & Horwath, 2001). Our survey results indicated a trend towards a higher percentage of females who reported not consuming milk as part of their daily nutritional intake. Interestingly, when expressed by level of athlete, we found that competitive female athletes were more likely to consume milk as

part of their daily nutritional intake and as part of their exercise routine compared to recreationally active females. Although not determined in this study, other studies suggest females may be engaging in exercise to facilitate weight loss (Craft, Carroll, & Lustyk, 2014) and therefore avoid consuming milk, which they perceived as a high-fat food (Mahon & Haas, 2013).While more males believed that milk consumption would benefit exercise performance, there were no significant differences between gender and the use of milk for exercise performance. There was however differences between gender and the use of dietary supplements, with more males reported taking at least one dietary supplement compared to females. These findings on supplementations are in agreement with the literature (Fraczek et al., 2016; Giannopoulou et al., 2013), and although not determined in our study, male athletes often perceive dietary supplements to improve speed and agility, strength and power, and increase muscle mass (Erdman, Fung, Doyle-Baker, Verhoef, & Reimer, 2007; Froiland, Koszewski, Hingst, & Kopecky, 2004). Considering dairy milk has been effective on such outcomes (Desbrow et al., 2014; Hartman et al., 2007; Josse et al., 2010; Karp et al., 2006; Papacosta et al., 2015), further research should investigate the reason for the gap between male and female athlete's perceptions and usage of milk for exercise performance.

The majority of athletes surveyed indicated using at least one dietary supplement, with no differences between recreational exercisers and competitive athletes. This is not surprising considering the abundance of research demonstrating the popularity and use of supplements among all athletes (Knapik et al., 2016). Moreover, the present study demonstrated a high use of dietary supplements among athletes while using milk as an ergogenic aid. This result is aligned with other studies that have shown that supplement users are also likely to consume more whole food (Cantarow, Livermore, McEntee, & Brown, 2015). The main dietary supplements that

athletes in this study reported consuming were protein/amino acids, sports drinks/electrolytes and vitamins, all of which are prominent nutrients in dairy milk. Chocolate milk consumption following a glycogen depleting interval exercise improved time to exhaustion 4 hours later compared to a carbohydrate protein replacement beverage in male endurance trained cyclists (Karp et al., 2006; K. Thomas et al., 2009). Furthermore, athletes who consumed non-fat milk equivalent to 150% of their fluid losses displayed improved fluid retention compared to a commercially available carbohydrate electrolyte beverage (Desbrow et al., 2014; Shirreffs, Aragon-Vargas, Keil, Love, & Phillips, 2007; Watson et al., 2008). Therefore, considering the emerging research demonstrating the potential risks of consuming supplements containing banned or illegal substances (Deldicque & Francaux, 2016), athletes could benefit from selecting milk for their post-exercise recovery beverage as demonstrated by many studies.

The use of milk for exercise performance may be influenced by where the athletes are receiving their information. Overall, athletes were most likely to obtain their information from coaches/trainers, media and dietitians/nutritionists. The high percentage of competitive and recreational exercisers who reported seeking advice from coaches and/or trainers in the present study concurs with other research demonstrating the strong influence of these individuals on dietary supplement advice and information among high performance athletes (Burns, Schiller, Merrick, & Wolf, 2004; Torres-McGehee et al., 2012). Indeed, as shown by Wilson (2016), athletes were more likely to use a coach as the main source of nutrition advice despite ranking scientific journals as the most reliable source of information. However, it has often been reported that coaches and/or trainers have insufficient nutrition knowledge and therefore are not the best source of advice (Couture et al., 2015; Danaher & Curley, 2014; Torres-McGehee et al., 2012). Conversely, in the present study, there were a higher percentage of recreational exercisers who

indicated retrieving their information from scientific articles compared to competitive athletes. Meanwhile, competitive athletes were more likely to receive their information from dietitians/nutritionists compared to recreational exercisers who seek their information from other sources, as determined in our survey. Dietitians and nutritional professionals are more likely to recommend whole foods to their clients rather than dietary supplements or diet trends that are not always supported by evidence-based research (D. T. Thomas et al., 2016b). Indeed, research has demonstrated an improvement in dietary intake in athletes following nutrition education by a dietitian (Valliant, Emplaincourt, Wenzel, & Garner, 2012). Thus, results from this survey highlight the need for dietitians to provide nutrition education, specifically in regards to health and performance among recreational exercisers. Given the strong influence of coaches and trainers on dietary supplement information for athletes, this could be an opportunity for nutrition professionals to provide education to members of the athletic staff that would result in better information available to high performance athletes (Torres-McGehee et al., 2012).

Our survey was only able to capture the responses from a convenient sample of participants within the province of Manitoba and may not be representative of the majority of the athletic population. Additionally, competitive athletes that were surveyed comprised mostly of team sport athletes who could have opposing beliefs and nutritional patterns compared to those competing in individual endurance-based sports. Given that there are numerous studies assessing the effects of milk for post exercise recovery in endurance athletes such as cyclists and runners, it would be of interest to determine whether those results have impacted the nutritional practices of that specific athletic population. Our survey did not assess the reasons for why athletes choose not to incorporate milk for their performance and future research is warranted. Within the context of this province, many recreational exercisers and competitive athletes do not consume

dairy milk as an ergogenic aid despite believing it could help with their performance. Therefore, given the high percentage of athletes who consume milk as part of their daily nutritional intake, further research is needed to explore the reasons for the gap in athlete's beliefs compared to use regarding milk for exercise performance, which may lead to future educational opportunities for dietitians and nutrition professionals to offer more focused advice to their clients.

3.5 ACKNOWLEDGEMENTS

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CHAPTER IV: STUDY 2. THE EFFECT OF CHOCOLATE MILK ON THE ATTENUATION OF EXERCISE INDUCED MUSCLE DAMAGE IN COLLEGIATE FOOTBALL PLAYERS: A PILOT STUDY

4.1 INTRODUCTION

Eccentric muscle contractions are a major component of resistance exercise, sprinting with hard deceleration and plyometric movements, all of which are prominent in training and competition for those involved in team sports such as football. Exercise induced muscle damage (EIMD) is often a result of unaccustomed eccentric skeletal muscle contractions (Schoenfeld, 2012). Although eccentric (lengthening) muscle contractions is a stimulus for increased training adaptations, eccentric muscle contractions can lead to ultrastructural disruptions in the muscle, an increase in intramuscular enzymes, muscle soreness and loss of strength/power leading to a slower recovery compared to concentric (shortening) muscle contractions (Peake et al., 2017). These symptoms usually peak 24-48 hours after exercise and have been shown to last for several days following the initial exercise bout (Ascensao et al., 2008; Baird et al., 2012). These symptoms, especially the reduced ability to generate force can be detrimental for athletes who perform multiple exercise bouts in a short time period (Byrne et al., 2004). Additionally, strenuous exercise increases the rate of protein synthesis and breakdown, while a positive protein balance is necessary for muscle repair and adaptation to exercise (Sousa et al., 2014).

Numerous studies have assessed the ability of various nutritional interventions to attenuate symptoms associated with EIMD (Hennigar et al., 2017; Rahimi et al., 2017; Sousa et al., 2014). Post exercise ingestion of protein containing essential AA, particularly leucine, can stimulate muscle protein synthesis (Phillips, 2017). Co-ingestion of carbohydrates and protein have received considerable attention in regards to attenuating EIMD, as the carbohydrate can increase plasma insulin levels and attenuate muscle protein breakdown (Sousa et al., 2014). More

recently, dairy milk has been investigated for its ability to promote exercise recovery due to its protein and carbohydrate combination. Consuming dairy milk post endurance exercise has increased plasma insulin levels, facilitated glycogen resynthesis and activated intracellular signalling proteins responsible for activating protein synthesis (Ferguson-Stegall, McCleave, Ding, Doerner, et al., 2011; Lunn et al., 2012). Previous studies have demonstrated attenuation of symptoms of EIMD, such as increases in biomarkers, muscle soreness and reduced muscle function when dairy milk was consumed as a recovery beverage following eccentric exercise (Cockburn et al., 2013; Cockburn et al., 2012; Gilson et al., 2010; Kirk et al., 2017; Papacosta et al., 2015; P. Rankin et al., 2015).

While several studies have assessed the recovery potential of dairy milk using endurance athletes, very few studies have assessed the effects of dairy milk for muscle recovery in team sport athletes (Cockburn et al., 2013; Gilson et al., 2010). More specifically, there have been no studies to date examining the effects of dairy milk on attenuating symptoms of EIMD in team sports athletes such as football players. Football is a physiologically demanding sport, involving acceleration and deceleration, high load exercise with eccentric and plyometric movements (Kraemer et al., 2013). High repetition eccentric skeletal muscle movements in addition to blunt force trauma associated with high impact collisions stimulates skeletal muscle tissue damage (Stone et al., 2017). Biomarkers of EIMD, including CK, Mb and LDH have been shown to be elevated after a game and throughout a season (Hoffman et al., 2002; Hoffman et al., 2009; Kraemer et al., 2013). An earlier study observed significantly elevated serum CK in Division 1 football players on the fourth and seventh day of participating in 2-a-day practices over 10 days of preseason training (Ehlers, Ball, & Liston, 2002). Serum CK and Mb increased at 18-20 hours post game in starters when observed in the 9th game of the season but returned to pre-game

values by 40-42 hours (Kraemer et al., 2013; Sterczala et al., 2014). A more recent study observed elevations in CK over an entire season, wherein starters experienced greater increases compared to non-starters (Stone et al., 2017). Competitive football players require strength, power and speed in order to be successful (Kraemer et al., 2013), thus focusing on recovering strategies is especially important for these athletes in order to prevent injury and maximize performance. Although previous research has examined the biochemical changes and level of muscle damage effects during a single game, pre-season and in season football training (Hoffman, Kang, Ratamess, & Faigenbaum, 2005; Hoffman et al., 2002; Kraemer et al., 2013; Kraemer et al., 2009), to date, there is currently no research assessing the effects of a nutritional intervention and its ability to blunt increases in biomarkers of muscle damage and limit decrements in performance. Thus, the objective of this pilot study was to assess the effects of low fat chocolate milk on attenuating symptoms of EIMD in Canadian Interuniversity Sport (CIS) male football players.

4.2 METHODS

4.2.1 Participants

Fourteen CIS football players from the University of Manitoba Bisons Football Team were recruited to take part in the study. Of the 14 original participants, three dropped out of the study and were not included in the analysis. The reasons for withdrawal included the following: injury unrelated to the study (1) and dissatisfaction with blood draw (2). A total of 11 participants completed the study until 24 hours and 9 participants completed until 48 hours. However, there were missing data for different time points in 2 participants, therefore only 9 participants were included in the final analysis. Ethics approval was obtained from the University of Manitoba Joint-Faculty Research Ethics Board. All participants signed an informed consent and completed a pre-screening questionnaire (medical and health history, allergies, use of ergogenic aids,

current exercise routine) prior to the study. Individuals were eligible to participate in the study if they were at least 18 years of age, in good physical health, determined by the physical activity readiness questionnaire (PAR-Q) and had no existing lactose intolerance or dairy allergy.

4.2.2 Study design

This study followed a randomized, parallel design where participants were randomized to a chocolate milk recovery drink group or control (water) group. Participants were required to visit the laboratory on four separate occasions. On the first visit, participants completed baseline performance tests (countermovement jump height and 15-m sprint). During the baseline performance tests, perceived muscle soreness (assessed by visual analogue scale; VAS) was recorded and a 20 ml blood sample was drawn. Participants then reported to the laboratory on 3 consecutive days. Baseline performance testing were conducted at 24 hours and 48 hours after the muscle damaging intervention (see section 4.2.4) while muscle soreness and blood samples were measured before, immediately, 2 hours, 24 hours and 48 hours following the muscle damaging protocol. All of the blood samples were obtained at the same time each day (within 1 hour) to account for diurnal variations. All testing was conducted during the athlete's recovery week during their summer off-season conditioning training.

4.2.3 Nutritional Intervention

The nutritional interventions used in the study were the consumption of chocolate milk (CM) or control (water consumption at the same volume). Participants were provided their allocated treatment immediately after the muscle damaging protocol and consumed a second bolus of the beverage 2 hours later. Participants were instructed not to consume any other nutrients over the 2 hour period. Energy and macronutrient composition of is the study treatments are shown in **Table 4.1**. The volume of treatment was stratified according to body weight range. Participants weighing <86.36 kg received 700 ml of treatment, totalling 1,400 ml during the recovery period.

Participants weighing between 86.4 kg and 95.5 kg received 800 ml per treatment, totalling 1,600 ml during the recovery period. Participants weighing between 95.5 kg and 104.6 kg received 900 ml per treatment, totalling 1,800 ml during the recovery period. Participants weighing between 104.6 kg and 113.6 kg received 1,000 ml per treatment, totalling 2,000 ml during the recovery period. Participants weighing between 113.6 and 122.7 kg received 1,100 ml per treatment, totalling 2,200 ml during the recovery period. Participants weighing >122.7 kg received 1,200 ml per treatment, totalling 2,400 ml during the recovery period. These doses were based on post exercise nutrition recommendations that suggest a carbohydrate intake of 1.0 g/kg body weight immediately following exercise and again in over 4 hours (D. T. Thomas, Erdman, & Burke, 2016a).

Table 4.1. Macronutrient content per 500 ml supplement.

Treatment	Energy (Kcal)	Carbohydrate (g)	Protein (g)	Fat (g)
Chocolate Milk	349	58	18	5
Water	-	-	-	-

4.2.4 Muscle damaging protocol

The athletes each completed a 10 minute warm-up on a stationary bike, between 80-90 revolutions per minute. Following warm-up, the athletes in this study each completed 15 maximal speed sprints over a distance of 15 meters with a 5 meter distance given for hard deceleration from the maximum sprint, allowing for a 45 second rest between each sprint (15 x 15 meters with a 5 m deceleration phase). Out of the 15 sprints, they performed 10 hard decelerations on a verbal cue. The hard decelerations were intended to induce significant eccentric muscle action (i.e. the muscle is lengthening as it contracts) in the leg musculature and induce mild skeletal muscle damage. They then completed a total of 40 drop landings from a

specified height (60 cm for linemen, 80 cm for non-linemen), where each set of 10 was separated by a 2 minute rest. They also complete an eccentric bench press exercise, completing 10 sets of their pre-determined 1 repetition maximum, allowing for 30 seconds of rest in between each set. Verbal encouragement was given throughout. It was estimated that the muscle damage that would be experienced from this protocol would not be any greater than competing in a football game or practice. The muscle damage was expected to induce an inflammatory reaction to initiate the healing process of the skeletal muscle (which is a very plastic and pliable tissue) and will encourage positive adaptations in athletic performance in these athletes after a recovery period.

4.2.5 Muscle soreness measurement

Participants were required to rate the level of perceived muscle soreness using a visual analogue scale (VAS). A VAS allows individuals to rate their feelings of muscle soreness based on a scale rating from 0-10, whereby 0 indicates no muscle soreness and 10 indicates that muscles are too sore to move.

4.2.6 Countermovement jump

A countermovement jump test on a force plate (Quattro Jump 9290AD, Kistler Instrument Corporation, Amherst, USA) was used to measure force production and jump height. The force plate has piezoelectric sensors that detect when forces are applied to them and can measure jump height (hf, hc), instantaneous force (Fi) and average power (Pavg). Hf represents the rise in height of the centre of gravity from the bottom of the jump to the peak of the jump. Hc represents the fall of centre of gravity from the peak of the jump to the landing. Fi represents the force exerted on the platform in the transition from eccentric to concentric, relative to body weight (Lee, Huang & Wu, 2012). Pavg represents the average watts produced during the jump, relative to body weight. Each participant was originally given 3 attempts to jump, however recording

errors occurred at baseline testing thus 4 attempts were given and the best of 4 jumps was used in analysis. Participants were instructed to jump with the hands on their hips to perform each jump. Each attempt was given a five second window to be completed. Sampling rate of the force platform was set at 500 hertz.

4.2.7 15-m sprint

The best time of 4 maximal speed sprint tests over a distance of 15 meters from a three point starting position with a minimum of 3 minutes break between sprints (to ensure full recovery of ATP stores in the working skeletal muscle). Sprint time was measured using a photo-electric timing system (SmartSpeed Pro, Fusion Sports, Chicago, USA). Time recording began when the participant crossed the first pair of gates and ended when they crossed the second pair of gates, placed 15 metres away.

4.2.8 Diet and exercise

Participants were asked to provide a diet and training log for the 4 days of the study. Participants were instructed to maintain their diet consistent with their baseline diet and refrain from vigorous training outside of the study protocol. Food records were entered and analyzed using The Food Processor Nutrition and Fitness Software (ESHA Research Professional Nutrition Analysis Software and Databases; Salem, OR 97309 USA). Participants were also instructed to report any side effects associated with the experimental beverages.

4.2.9 Urine specific gravity

On each morning of the study, participants were asked to provide a urine sample upon waking. Urine specific gravity (USG; Atago digital hand held pocket refractometer; Tokyo, Japan) was used to determine hydration status by measuring the concentration of solutes in the urine. The average of 3 samples were recorded and participants were classified as euhydrated if USG reading was <1.020.

4.2.10 Blood sample collection and analysis

Participants provided a non-fasted blood sample on six occasions following the completion of their perceived muscle soreness ratings. Blood sample (~20 ml) was drawn from the antecubital vein under sterile conditions in ethylenediaminetetraacetic acid (EDTA) tubes. Collected blood samples were centrifuged for 20 min at 520 x g, aliquots separated and stored at -80^{o}C for later analysis which was used to analyze biochemical substances in the plasma including creatine kinase and lactate dehydrogenase. Prior to analysis, plasma was brought to room temperature and mixed through inversion. Plasma CK and LDH was determined by automated methods on Vitros 350 Analyzer (Ortho-Clinical Diagnostics, Markham, ON, Canada) utilizing enzymatic reagents (Vitros Chemistry Products, Ortho-Clinical Diagnostics, Markham, ON, Canada).

4.3 Statistical Analysis:

Statistical analyses were performed using SPSS software (version 20; SPSS Inc., Chicago, IL, USA). Basic descriptive characteristics (height, age, weight) were calculated for participants and mean differences were compared between groups using independent t-test. Performance (countermovement jump, 15-m sprint) and recovery variables (CK, LDH, muscle soreness) between both treatment groups were analyzed using two factor (treatment*time) analysis of variance (ANOVA) for repeated measures. In the case that Mauchly's Test for Sphericity was significant or Epsilon value was low, Greenhouse–Geisser corrections were conducted for unequal variance data. Significant effects were adjusted using the Bonferroni method. Partial eta squared (ηp^2) effect size was calculated to determine the magnitude of effect between groups: small (0.01), medium (0.06), large (0.14) (Murphy & Myors, 2004). Mean nutrient intake was compared between treatment groups using independent t-test. Data were expressed as mean ± standard deviation when appropriate. Statistical significance was set at p-value $\leq$0.05.

4.4 RESULTS

4.4.1 Participant characteristics

Mean height, age, weight and body mass index (BMI) of the participants is displayed in **Table 4.2.** There were no significant differences in age, weight, height, or BMI between treatment groups. Dietary analysis revealed there were no differences in mean energy, protein, carbohydrate or fat intake between groups across the four days **(Table 4.3).** There were no side effects reported for consumption of treatments between groups.

Table 4.2. Participant characteristics

Variables	CM (n=5)	CON (n=4)	p-value
Age (years)	22.60 ± 1.52	22.25 ± 2.22	0.786
Weight (kg)	99.68 ± 19.33	104.60 ± 20.66	0.739
Height (cm)	181.56 ± 6.89	185.42 ±7.48	0.447
BMI (kg/m^2)	30.13 ± 4.06	30.32 ± 3.97	0.946

CM, chocolate milk; CON, control
Values presented as mean ± SD. Mean participant characteristics between groups assessed using independent t-test.

Table 4.3. Mean dietary intake between treatment groups over 4 days

	CM (n=5)	CON (n=4)	P-value
Kcal	2680.28 ± 850.81	2830.40 ± 872. 22	0.802
Carbohydrate	307. 75 ± 138.28	274.17 ± 108.64	0.704
Protein	170.47 ± 24.26	188. 47 ± 58.13	0.546
Fat	85.37 ± 46.03	110.98 ± 29.22	0.368

CM, chocolate milk; CON, control
Values expressed as mean ± SD. Mean energy and macronutrient intake using independent t-test not statistically significant between groups ($P<0.05$).

4.4.2 Biomarkers

The results of CK and LDH are displayed in **Table 4.4** There were no significant differences ($p=0.705$) for baseline CK levels between treatments (CM: 282.40 ± 163.85 UL; CON: 227 ± 257.60 UL). Mauchly's test of Sphericity indicated that the assumption of sphericity has been

violated, $\chi^2 = 25.294$, $\varepsilon = 0.504$, $p = 0.048$. Greenhouse-Geisser correction revealed no significant differences for time ($p=0.116$, $\eta p^2=0.250$), main effects for treatment ($p=0.179$) or interaction effects between time and treatment ($p=0.557$, $\eta p^2=0.087$). There were no significant differences ($p=0.963$) for baseline LDH levels between treatments (CM: 466 ± 50.73 UL; CON: 469.25 ± 144.49 UL). Mauchly's test of Sphericity indicated that the assumption of sphericity has been violated, $\chi^2 = 22.516$, $\varepsilon = 0.401$, $p = 0.095$. Greenhouse-Geisser correction revealed a significant main effect for time ($p=0.04$, $\eta p^2=0.369$), however there were no significant main effects for treatment ($p=0.474$) or time and treatment interactions ($p=0.194$, $\eta p^2=0.209$). Post hoc tests using the Bonferroni correction revealed that LDH level increased an average of 63.70 ± 11.87 (SEM) UL/L from pre EIMD to immediately post EIMD ($p=0.016$) and increased an average of 64.70 ± 12.56 (SEM) UL/L from pre EIMD to 2 hours post EIMD ($p=0.020$).

Table 4.4. Mean plasma CK and LDH over 4 days

Biomarker	Group	Baseline	Pre	Post	2hr	24hr	48hr
CK (U/L)	CM	282.40 ± 163.85	529.40 ± 333.103	589.40 ± 331.42	530.00 ± 265.03	570.40 ± 297.16	481.00 ± 268.95
	CON	227.00 ± 257.60	284.25 ± 99.90	341.25 ± 68.34	290.25 ± 166.21	336.00 ± 55.61	353.50 ± 135.01
LDH* (U/L)	CM	466.00 ± 50.73	463.00 ± 70.81	518.40 ± 75.30	532.40 ± 85.91	531.00 ± 55.00	459.60 ± 56.51
	CON	469.25 ± 144.50	409.25 ± 108.21	481.25 ± 148.47	469.25 ± 159.00	427.00 ± 105.44	432.25 ± 108.40

Values expressed as mean ± SD. CK, creatine kinase; LDH, lactate dehydrogenase; CM, chocolate milk; CON, control
*Indicates significant main effect for time ($p<0.05$).

4.4.3 Performance variables

The results for performance are displayed in **table 4.5**. No significant differences were observed for baseline performance variables between treatments ($p>0.05$). Mauchly's test of Sphericity indicated that the assumption of sphericity was violated for Hf, Hc and Fi, $\chi^2 = 4.522$, $\varepsilon = 0.654$, $p = 0.104$; $\chi^2 = 3.464$, $\varepsilon = 0.696$, $p=0.177$ and $\chi^2 = 4.864$, $\varepsilon = 0.643$, $p = 0.088$, respectively. Greenhouse-Geisser correction revealed a significant main effect ($p=0.026$, $\eta p^2=0.499$) for time for Fi, although no significant differences between treatment ($p=0.280$) or interaction effects between time and treatment ($p=0.589$, $\eta p^2=0.051$) were present. Post hoc tests using the Bonferroni correction revealed that Fi was reduced by an average of 0.182 from baseline to 48h ($p=0.029$). A significant main effect for time was observed for Pavg, although no significant effect for treatment or interaction effect between time and treatment. Post hoc tests using the Bonferonni correction did not reach significance. No significant differences were observed for time, treatment or time and treatment interaction effects for 15-m sprint, Hc and Hf ($p>0.05$).

Table 4.5 Performance tests results.

Variable	Group	Baseline	24h	48h	Time	Treatment	Time by Treatment
15-m sprint (s)	CM CON	2.47±0.09 2.50±0.16	2.45±0.14 2.51±0.14	2.42±0.10 2.47±0.14	0.230	0.550	0.839
Pavg (W/kg)	CM CON	30.46±2.18 29.50±1.99	29.42±4.57 26.30±3.28	28.58±3.47 26.9±1.23	0.033	0.260	0.450
Fi (BW)*	CM CON	1.38±0.39 1.15±0.23	1.26±0.40 0.95±0.34	1.20±0.36 0.97±0.13	0.026	0.280	0.589
Hf (cm)	CM	60.66± 7.12	57.62 ± 3.51	53.22±5.10	0.145	0.568	0.858

	CON	62.53±17.89	60.98 ± 10.82	57.2±54.99			
Hc (cm)	CM CON	-24.7±5.97 -19.33± 14.19	-27.14±9.36 -17.18 ± 3.86	-30.98±12.28 -28.50±6.06	0.060	0.291	0.447

Values presented as mean ± SD. S, seconds; Pavg, average power; Fi, instantaneous force; W, watts; hf, jump height rise; hc, jump height fall; BW, body weight; CM, chocolate milk; CON, control

*Significant main effect for time ($p<0.05$).

4.4.4 Muscle soreness

There were no significant differences ($p=0.369$) for baseline DOMS level between treatments (CM 3.60 ± 2.191; CON 2.00 ± 2.828). Mauchly's test of Sphericity indicated that the assumption of sphericity has been violated, $\chi^2 = 29.328$, $\varepsilon = 0.462$, $p = 0.016$. Greenhouse-Geisser correction revealed no significant differences for time ($p=0.607$, $\eta p^2=0.074$), main effects for treatment ($p=0.574$) or interaction effects between time and treatment ($p=0.622$, $\eta p^2=0.071$).

4.4.5 Hydration results

Mean USG are displayed in table 5. There were no significant differences for mean USG results between treatments at any time point ($p>0.05$).

Table 4.6. Mean USG results over 4 days.

Treatment	Baseline	Pre	24h	48h
CM (n=4)	1.023 ± .005	1.026 ± 0.004	1.023 ± 0.005	1.023 ± 0.009
CON (n=4)	1.019 ± 0.009	1.017 ± 0.012	1.021 ± 0.002	1.019 ± 0.008

Values presented as mean ± SD. CM, chocolate milk; CON, control; USG, urine specific gravity.

Mean USG between groups not significant at any time point, assessed using independent t-test.

4.5 DISCUSSION

While dairy milk as a recovery beverage has been investigated in team sport athletes, such as soccer and rugby (Cockburn et al., 2013; Cockburn et al., 2008; Gilson et al., 2010; Kirk et al., 2017; P. Rankin et al., 2015), this is the first study to use football players. This study used a whole body muscle damaging protocol intended to simulate muscle movements used in football training. Despite high amounts of total energy and macronutrients provided in the CM treatment, which are in line with current post exercise nutrition recommendations (D. T. Thomas et al., 2016c), the CM and non-caloric control beverage provided similar effects on markers of recovery following a muscle damaging protocol with high repetition eccentric muscle contractions. No significant treatment by time interactions was observed for performance outcomes (CMJ height, power and force; 15-m sprint time), muscle soreness or biomarkers (CK, LDH).

In regards to CMJ performance, we observed a significant decrease in instantaneous force which decreased over 48 hours compared to baseline values. Although the remaining performance variables were unable to reach significance (CMJ height, 15-m sprint time), we observed medium to large effect size on these parameters, indicating that performance potentially decreased over time. However, due to the small sample size these results should be interpreted with caution. Nonetheless, consuming low fat chocolate milk immediately and 2 hours post EIMD had no notable effect on attenuating the slight decline in performance at 24 and 48 hours. Although previous studies have reported an attenuated decrease in exercise performance following eccentric exercise when milk was consumed as a post exercise recovery beverage (Cockburn et al., 2013; Cockburn et al., 2012; Kirk et al., 2017; Papacosta et al., 2015),

not all findings have been able to detect treatment differences (Gilson et al., 2010; P. Rankin et al., 2015). The non-significant effect of milk consumption on performance in this study is in agreement with previous studies. For example, Gilson et al (2010) observed similar performance tests results (T-drill, vertical jump) between CM and carbohydrate only treatments following 4 days of soccer training (Gilson et al., 2010). Furthermore, a study by Rankin et al (2015) was unable to detect differences in 20-m sprint time and peak torque in male team sport athletes when 500 ml of milk was consumed post EIMD (P. Rankin et al., 2015).

In the current study we observed a small but statistically significant increase in LDH from immediately and 2 hours post muscle damaging exercise compared to baseline; however these values were not different between treatments. This result provides little relevance to the study as LDH returned to baseline values at 24 and 48 hours when performance outcomes were measured. Although Kraemer et al (2009) found a small but significant increase in LDH 20-22 hours following a regular season football game, it was undetermined whether the rise in LDH would have any effect on next day exercise performance (Kraemer et al., 2009). In the present study there were also no statistical increases in CK over time; and no treatment effect was present. Previous studies have also reported no significant effects of milk on CK (Cockburn et al., 2013; Cockburn et al., 2010; Wojcik et al., 2001). Whereas Gilson et al (2010) observed significantly lower CK in those who consumed CM following four days of intensive soccer training however found no beneficial effect on sport specific exercise performance (Gilson et al., 2010). Meanwhile, in the present study we observed large individual variations in plasma levels of CK, consistent with previous literature (Baird et al., 2012; Kraemer et al., 2013). Aside from the small sample size, there are a few possible explanations for the nonsignificant results between treatments. It has been well established that when an individual has performed

subsequent bouts of exercise following the initial muscle damaging exercise, symptoms of EIMD are less intense and recovery is enhanced (Bridgeman, Gill, Dulson, & McGuigan, 2017; Coratella, Chemello, & Schena, 2016; Peake et al., 2017). This phenomenon is referred to as the repeated bout effect. A previous study examined the effects of hormonal changes and the CK and Mb response following the ninth game of a football season and compared to results from starters the year before. Serum CK values returned to pre-game values by 42-48 hours after the game and did not differ from the season before (Sterczala et al., 2014). Meanwhile, Hoffman et al (2009) found a significant increase in CK following preseason training camp; however CK values were minimized over time throughout the regular season (Hoffman et al., 2005). The researchers above also suggested the term 'contact adaptation', which proposes that the muscle tissue adapts over time to the high velocity contact and blunt force trauma experienced during a game of football and as the season progresses. The participants in the present study were on a week break from their off season summer training and had minimal recovery time prior to entering the study. Thus, it is possible that the progressive training of the participants in the present study provided a protective effect, either by the repeated bout effect, contact adaptation or both. Muscle tissue breakdown associated with the large eccentric component of football training results in an injury stimulus that can result in greater muscular mass and strength if given sufficient recovery time and adequate nutrition is provided. Considering we did not see any significant reductions in 15-m sprint, CMJ height and average power over time, it is likely that the athlete's muscles were adapted to the eccentric exercise and the muscle damaging protocol used in the study was not enough to elicit a negative response. Further, while CK has been commonly used across various studies as a marker of muscle damage, it has been recently suggested to look at additional biomarkers to determine muscular status during recovery. CK has

been previously correlated with high impact collisions and may not always be reflective of tissue damage from eccentric exercise (McLellan & Lovell, 2012; Twist, Waldron, Highton, Burt, & Daniels, 2012). A recent review suggests measuring myoglobin, which leaks into circulation and peaks at 1-3 hours post, urea nitrogen which measures protein synthesis vs. breakdown in addition to CK would be optimal in assessing the overall muscle recovery status of an athlete (E. C. Lee et al., 2017).

Muscle soreness, which typically presents at 48 hours post EIMD is a highly subjective measurement. Considering the lack of significance observed for CK and performance over time, it is not surprising that there were no differences over time or between groups for muscle soreness. Given that the treatments could not be blinded in this study, these results easily could be biased. However, although increases in muscle soreness were present, multiple studies using milk as a post exercise recovery beverage were unable to detect differences between treatments (Cockburn et al., 2013; Gilson et al., 2010; Kirk et al., 2017).

Further, current literature suggests that if given >8hours between exercise sessions, post exercise nutrition is not as crucial as long as sufficient carbohydrates and protein are consumed throughout the day. Based on the participant's dietary records, protein intake did not differ between groups and they were consuming an average of 1.7g/kg bodyweight over the 4 days of the study. This is in agreement with current recommendations which state that daily protein intake of 1.4-2.0 g/kg body weight is optimal for building/maintaining muscle mass through a positive muscle protein balance (Jager et al., 2017). Although not measured in the present study, it is possible that participants in both treatment groups consumed sufficient protein to remain in a positive protein balance, allowing for adequate muscle tissue repair. However, dietary food

records/food recall should be used with caution due to misreporting or underestimating/ overestimating actual food intake.

The low sample size and therefore lack of statistical power make these results difficult to interpret. Although previous studies have used a sample size similar to the present study, a crossover design was used which increases statistical power as participants act as their own control. Due to the time frame of this study, a crossover design was not feasible which presented as a major limitation. Again due to the time frame of the study, we were unable to control for supplement intake and other modalities that have been used to minimize symptoms of EIMD. The majority of the participants in the study were using dietary supplements within 3 months of the study and also throughout the 4 days of the study which could have largely impacted these results.

In conclusion, consuming low fat CM immediately and 2 hours post EIMD had similar effects on exercise performance, muscle soreness and blood biomarkers compared to an equal volume of water. Future studies are warranted using a larger sample size and crossover design to determine a more accurate response to milk for post exercise recovery in football players. It would be of interest to assess the effects of milk on symptoms of EIMD throughout an entire training session, given the differences observed in the literature during summer training, pre-season and in-season. Although no treatment effects were present, dairy milk contains a range of nutrients which are not typically found in supplements and could provide essential nutrients for a high performance athlete. However, future research is needed to determine whether CM would be a suitable choice to attenuate symptoms of EIMD.

CHAPTER V: OVERALL CONCLUSIONS, LIMITATIONS AND FUTURE DIRECTIONS

5.1 SUMMARY AND IMPLICATIONS

Numerous studies have assessed the prevalence of ergogenic aids among athletic and recreationally active populations. More specifically, studies have assessed the type of supplement use, reasons for supplement use and source of information for supplement use. It is often found that athletes' reported beliefs for dietary supplement use are inconsistent with suggested claims of such supplements. In addition, the safety of ergogenic aids is often a concern, especially for those who compete at a high level and are subjected to testing for illegal and banned substances. Results from our study showed gaps between competitive athletes and recreationally exercisers individuals' belief and practices, as athletes are drinking milk as a beverage as part of their daily intake, however they are not using it as a recovery beverage despite believing it could help with performance. Given the high prevalence of dietary supplement use among both competitive athletes and recreational exercisers in the present study, there is a need for nutrition education to recreational exercisers and competitive athletes. Furthermore, differences between sources of information for milk and exercise advice highlight the need for registered dietitians to provide nutrition education to recreational exercisers as well as coaches and trainers to ensure evidenced based nutrition information is being delivered and dietary needs are being met first by whole foods rather than supplements.

The effects of acute milk supplementation on attenuating symptoms of EIMD in football players remain unclear in this pilot study. Given the heavy training schedule of the athletes at the time of the study, it is possible that their previous training provided a protective effect, suggested by the lack of significance changes in biomarkers, muscle soreness and performance over time.

However no definitive conclusion can be made given the small sample size. Thus, considering that dairy milk has an abundance of nutrients which have also been shown to promote post exercise recovery, it could be a safe option to promote health, recovery and training adaptations, although future studies using football players is warranted. Therefore, registered dietitians should work with both recreational exerciser and competitive athletes to determine if dairy milk would be a suitable choice, taking into consideration their exercise intensity/duration, recovery time, overall nutritional intake and dietary preferences.

5.2 LIMITATIONS

5.2.1 Study 1 limitations:

1) Although we found a gap between those who believe milk helps with exercise and use milk for exercise performance, we did not ask why athletes do not use milk for exercise performance. Therefore, the reasons for the gap between beliefs and behavior are unknown.
2) This study surveyed a convenient sample of Manitoba athletes and is not reflective of the entire athletic population. Additionally, the recreational exercisers were surveyed from two university gyms and running room locations and may not be representative of all recreational exercisers who train out of private gyms or larger fitness clubs (e.g. GoodLife Fitness, Shapes Fitness).
3) The majority of athletes surveyed in this study were team sport athletes and did not include endurance athletes such as cyclists, triathletes or endurance runners at the competitive level. Considering there are numerous studies assessing the effects of dairy milk for recovery in endurance athletes, inclusion of these athletes would have been beneficial to gain an understanding of a wider range of athletes.

4) A limitation for this survey was that validity and reliability were not established and assumptions were made. Therefore, it is unknown whether these results would be similar if distributed to other athletic groups. More specifically, food frequency questions should have been included to determine a more accurate picture of how often/when athletes are drinking milk and using it for performance. It is possible that the athletes were in their off season and were not routinely using milk at that time.

5.2.2 Study 2 limitations

1) The main limitation to this study was the small sample size. Originally, a sample size of 14 was chosen as the minimum number of participants required for statistical analysis. However, due to dissatisfaction with blood draw and an injury unrelated to the study, the sample size was reduced. Additionally, errors in the software used for performance tests resulted in missing data points which further reduced the total sample size used for analysis making it difficult to interpret results and reach statistical significance.
2) Given the time frame and availability of the participants we were unable to use a crossover design; therefore a parallel design was used. This design may have been appropriate for a larger sample size, however given the small sample size and large individual variations in plasma CK observed between participants, it was difficult to detect any statistical differences between treatments using the parallel design.
3) The athletes involved in the study were involved in a summer training program, which involved rigorous resistance training multiple days per week in addition to football skill specific training. Therefore, they were entering the study highly conditioned which could explain the lack of significance of between treatment groups on biomarkers, muscle soreness and performance over time.

4) Although participants provided dietary recalls and exercise logs, we were unable to provide education or control for these factors. Thus, participants were still consuming dairy products and protein powders in both treatment groups and engaging in exercise outside of the study protocol which may have influenced the results.

5.3 FUTURE DIRECTIONS

1) Future studies evaluating the use and perceptions of milk for exercise performance among competitive endurance athletes is necessary as well as looking at different sports among different sporting institutes.

2) Given the high prevalence of athletes who seek advice from their coach/trainer, future studies should consider assessing nutrition knowledge and dietary recommendations coaches/trainers are making to their athletes. This could allow for educational opportunities for registered dietitians to ensure coaches are providing evidence based nutrition information to their athletes.

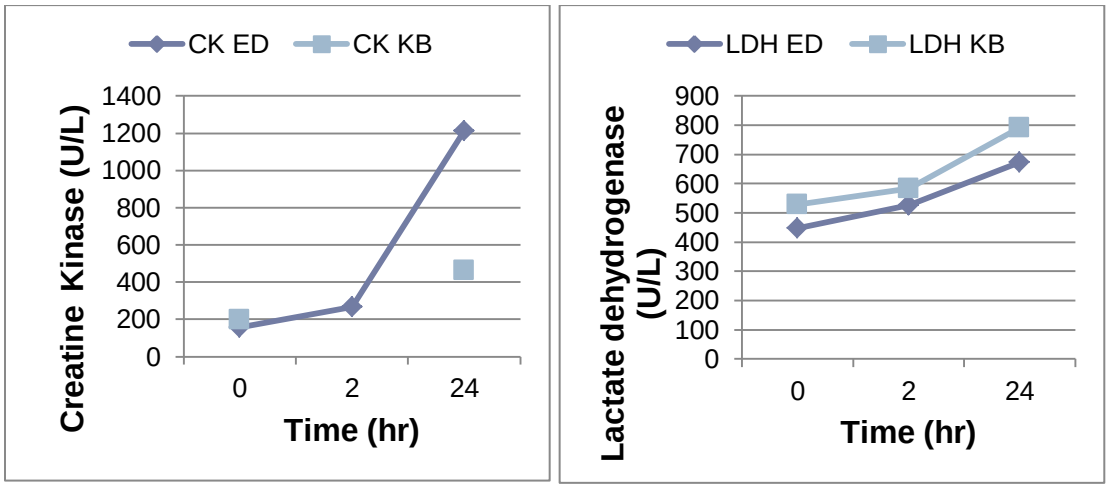
3) Given the differences in body composition and physical requirements between lineman and non-lineman, future studies should evaluate the magnitude of EIMD between positions and determine if there is a benefit of milk consumption on attenuating symptoms of EIMD between positions.

4) From a practical perspective, assessing dietary habits and nutrition knowledge of collegiate football players over the course of their football training (summer training, pre-season camp, in-season, off season) in addition to looking at additional biomarkers (CK, Mb, urea nitrogen) would not only provide valuable information to coaches and trainers about muscle recovery in their athletes but also provide education opportunities for registered dietitians to provide periodized nutrition strategies for optimal recovery and

training adaptations. Recent studies assessing the changes in biomarkers over a football season have not analyzed dietary patterns.

5) Prior to this pilot study we initially tested the muscle damaging protocol in two moderately trained recreational exercisers. We saw increases in biomarkers from baseline to 24 hours, indicating the protocol was sufficient to induce muscle damage response **(Figure 5.1).** Given that the athletes in the present study were well trained and exposed to this type of exercise regularly, we decided to increase the volume of exercises for the football players. However, future studies using this protocol with recreationally active individuals would be of interest to determine the effects of milk on attenuating symptoms of EIMD and enhancing training adaptations.

Figure 5.1 Plasma CK and LDH following a muscle damaging protocol in recreational exercisers



Plasma CK and LDH between two participants (ED, KB) before (0hr), 2hr and 24hr following a muscle damaging protocol. CK, creatine kinase, LDH; lactate dehydrogenase.

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