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A Comparison of Sheep's Milk and Cow's Milk Beverages on Performance Recovery Following a Vigorous Eccentric Exercise Protocol

A thesis presented in partial fulfilment of the requirements of the degree of

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Abstract

PURPOSE: To compare the potential nutritional benefit of consuming a sheep's milk (SM) beverage versus a cow's milk (CM) beverage on acute performance recovery following strenuous eccentric (ECC) exercise.

METHODS: Ten healthy men volunteered to participate in the study which used a randomised, double-blind, cross-over design, using a one-legged model. Participants performed baseline (BL) tests for maximal voluntary concentric (CON), ECC, and isometric (ISO) muscle contractions for a leg extension movement, measuring peak torque (PT) of the quadriceps muscles of one leg before performing an exercise protocol designed to induce muscle damage. Following the exercise participants repeated the performance measures before consuming either a chocolate-flavoured SM or CM drink. Participants returned to the lab 24h, 48h, and 72h later to repeat the same performance measures. Subjective measures for muscle soreness were also recorded at BL, 0h, 24h, 48h, and 72h time points. Following a minimum 10-day washout period, the participants completed a second trial on the contralateral leg and consuming the other beverage.

RESULTS: A significant main effect for Time was observed for both performance and subjective measures when compared to BL measures, suggesting that muscle damage did occur. However, no significant Treatment effects or Treatment X Time interactions were observed.

CONCLUSIONS: The results of this study suggest that SM may offer similar benefits for recovery from exercise induced muscle damage (EIMD) as those which have previously been shown for CM supplements, providing some efficacy for its use in a sports recovery context. However, further research is warranted to better understand the differences between the two milks and the possible implications this may have in a sports nutrition setting.

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List of Abbreviations

A

A-DOMS Active delayed onset muscle soreness

B

BCAA Branched-chain amino acid

BL Baseline

C

CHO Carbohydrate

CJH Countermovement jump height

CK Creatine kinase

cm Centimetre

CM Cow's milk

CON Concentric

CR Carbohydrate replacement

CTRL Control group

D

$^{\circ}\cdot s^{-1}$ Degrees per second

DL Dominant leg

DOMS Delayed onset muscle soreness

E

EAA Essential amino acids

ECC Eccentric

EIMD Exercise induced muscle damage

F

F Female

FR Fluid replacement

I

IL-6 Interleukin-6

ISO Isometric

K

KF Knee flexion

L

LIST Loughborough intermittent shuttle test

M

M Male

Max Maximum effort

Mb Myoglobin

mg/mL Milligrams per millilitre

mmol/kg dw/h Millimole per kilogram of dry weight (dry muscle mass) per hour

N

Non-DL Non-dominant leg

P

%DI Percentage of daily intake

P Protein

P-CHO Protein-carbohydrate

P-DOMS Passive delayed onset muscle soreness

Post Post-exercise protocol group

Pre Pre-exercise protocol group

PS Protein supplement

PT Peak torque

R

RSI Reactive strength index

S

SD Standard deviation

SM Sheep's milk

sTnI Skeletal troponin I

T

TW Total work

Chapter One: Introduction

The sports industry is constantly evolving as it seeks and utilizes many forms of new, legal technologies and ergogenic aids which may provide athletes with a performance advantage. This includes a range of strategies to allow athletes to perform at their peak for as long as possible. Recovery from exercise-induced muscle damage (EIMD), in particular, is a critically important aspect of this, for both professional and recreational athletes, as EIMD can impair muscular strength and power (Marcora & Bosio, 2007) and general muscle function, which in turn affects athletic performance (Twist, & Eston, 2005). Many physical and external therapies have been used to try and reduce recovery time from EIMD, for example sports massage (Jonhagen et al. 2004) and cold-water immersion therapies (Eston and Peters 1999) have been investigated, among others, but results are often mixed. Post-exercise nutrition, however, can play a significant role in how well an athlete recovers between competition days and even between training sessions, and is often a focus for athletes (Beck et al., 2015).

During exercise, the body's muscle and liver glycogen stores are broken down and used as energy for muscle contraction. Muscle damage may occur as a result of exercise, particularly if the exercise bout is prolonged, or performed at a high intensity. Therefore, it is essential to promote the re-synthesis of glycogen and muscle recovery post-exercise (Levenhagen et al., 2001) through adequate intake of carbohydrates (CHO) and protein. Another important factor which needs to be considered, in terms of post-exercise recovery, is the replacement of fluid and electrolytes lost in sweat from the previous exercise activity (Burke, 1997). Often the first strategy to begin the replenishment of these key nutrients is via the intake of one of the many post-exercise recovery drinks which have emerged on to the commercial market. These include fluid replacement (FR) drinks, which aim to replace lost fluid and electrolytes and contain low levels of CHO; carbohydrate replacement (CR) drinks, which contain additional CHOs; and protein supplements (PS) which contain varying amounts of protein and CHOs (Karp et al., 2006).

Cow's milk (CM) products, including those that have been flavoured, have previously been investigated as potential recovery beverages, as they contain CHO and protein in amounts that lie within the ranges typically found in CR and PS drinks (Karp et al., 2006; Shirreffs et al., 2007; Cockburn et al., 2008; Gilson et al., 2010; Desbrow et al., 2014). In general, the research suggests that such beverages work well as rehydration and glycogen repletion beverages, while also providing sufficient protein to promote protein synthesis for muscle recovery (James et al., 2019).

With respect to these findings, sheep's milk (SM), while currently less available commercially, may also have similar, or perhaps greater, recovery effects than CM. SM is known to have a greater milk

solid percentage, including upwards of 60% more protein, 60% more leucine, an amino acid associated with the stimulation of protein synthesis (Nakai et al., 2018), and slightly higher amounts of CHO, when compared to the composition of CM (Haenlein & Wendorff, 2006). This nutritional profile suggests that a SM product could aid post-exercise recovery, which may be of benefit to athletes. This may be further extended when considering that SM naturally falls under the A2-type of milk. A2-type milks exclusively contain only the A2 beta casein proteins, and no A1-beta casein proteins which are said to have various health and digestive implications related to CM intolerance or CM allergy (Fernández-Rico et al., 2022), whose prevalence lies between 0.2-17% of the population (Flom & Sicherer, 2019). This could mean that athletes who are intolerant to the A1-type of milk, found in normal CM (Jianqin et al., 2015), may be able to consume and utilise the benefits of this type of sheep dairy product, instead, without any adverse health or gastrointestinal effects. Such benefits have previously been suggested in a study that compared A2 CM and normal CM for muscle recovery (Kirk et al., 2017), but no research exists for SM. Additionally, current consumer interest has been shifting towards products that are produced with a lower impact on the environment (Asioli et al., 2017), providing an additional premise for the use of SM, which has been deemed to be a more environmentally friendly product compared to CM (Lees & Lees, 2016; Boyd, 2015).

To date, there appears to be no research on the use of SM in a sporting context, or in comparison to CM and other sports beverages in a sports-related setting. Therefore, this study, therefore, aims to shed some light on the potential application of this type of milk as a recovery aid after exercise, and, tangentially, to further encourage additional research of this food in other contexts.

This thesis begins with a contemporary literature review which provides background information on the current nutritional recommendations for athletes following intense exercise, particularly for resistance trained athletes. Further background information will be reviewed regarding previous research that has looked at CM drinks and other supplements as recovery aids, and information related to the nutritional differences between CM and SM. Following the literature review, the specific aims and hypothesis of the current study will be outlined, followed by the methodology that was used. The results will then be presented and discussed, including an acknowledgement of the limitations of the study as well as recommendations for future research. And, finally, the thesis will end with the conclusions drawn from this study.

Chapter Two: Literature Review

2.1 Current Post-Exercise Nutrition Recommendations for Resistance Trained, Intermittent Exercise Athletes

The nutritional considerations related to recovery for resistance trained, intermittent exercise athletes following an intense exercise bout are important. The athlete's need to recover back to optimal, or near optimal condition before heading into their next activity is a key consideration in terms of providing them with the best opportunity to achieve their desired training adaptations, or competition goals. Failure to meet their nutritional needs, both acutely and chronically, can have detrimental effects on subsequent exercise bouts and long-term performance.

During intermittent exercise, such as team sport, the athlete experiences consistent high intensity, intermittent activity which is repeated for the duration of the training session or event. This type of exercise typically involves numerous acceleration and deceleration movements across multiple joints within the body. The eccentric (ECC) component of these high force movements, in particular, are typically responsible for causing damage to the athlete's muscles (EIMD). The symptoms of EIMD include ultrastructural muscular disruption, delayed onset muscle soreness (DOMS), increased intramuscular proteins released into the body's circulation, swelling, reduced force production capacity, and decreased range of motion (reviewed in detail by Owens et al., 2019). The severity of the muscle damage tends to be dictated by the exercise intensity and the duration of the activity, in conjunction with the individual's susceptibility to the damaging stimulus (Douglas et al., 2017). Furthermore, athletes experience a depletion in glycogen stores, in order to meet energy demands, as well as a loss of fluid and electrolytes through sweat and other modes of thermoregulation during exhaustive exercise (Knuiman et al., 2015; Shirreffs et al., 2004; Kessinger, 2018). It is important for the athlete to replace these losses before participating in their next bout of exercise, particularly if they wish to maintain a high level of performance.

Although nutritional recommendations should be both periodised and personalised for each athlete, based on their own circumstances and individual goals (Thomas et al., 2016), a vast amount of research now exists which provides robust guidelines for the appropriate use of nutritional practices for a range of sports, particularly in relation to exercise recovery. Energy balance, CHO, protein, fluid and electrolyte intake, as well as nutrient quality and timing are among the primary considerations (Thomas et al., 2016; Kessinger, 2018; Beck et al., 2015).

2.1.1 Energy Balance

Total energy intake determines the capacity for intake of macronutrients and micronutrients, which support optimal body function and adaptation to training when consumed in adequate amounts (Thomas et al., 2016). Energy intake and energy balance following exercise are important factors to consider, particularly if the athlete has a limited recovery window before their next bout of exercise. The depletion of energy stores, particularly glycogen stores, has been associated with fatigue, increased perception of effort, as well as impaired work rate, skill and concentration (Alghannam, 2011; Kessinger, 2018). Following exercise, athletes are likely to be in a negative energy balance and should, therefore, account for the endogenous energy they have just utilised ahead of their next bout of exercise in order to avoid any potential exercise impairments, as previously mentioned. It should be noted, however, that any musculoskeletal damage that has occurred from a bout of exercise could still impair subsequent exercise performance if the recovery window is not sufficient to achieve a complete recovery (Dupuy et al., 2018), separate to energy availability.

Energy balance is also important for skeletal muscle repair and adaptation to an exercise stimulus. Pasiakos et al. (2010) have shown that muscle protein synthesis is downregulated when athletes are in a negative energy balance. Muscle protein synthesis is an energetically expensive process, so it is easy to understand why such processes are down-regulated in times of low-energy availability. Additionally, skeletal muscle acts as an amino acid reservoir which can be readily catabolised to provide precursors for energy-generating pathways during periods of sustained negative energy balance (Carbone et al., 2019), suggesting that athletes could lose skeletal muscle mass, rather than gain it, if their energy needs are not met and they remain in an energy deficit. Decreases in skeletal muscle mass can have negative effects, including decreased protein turnover, decreased physical performance, and increased risk of injury (Carbone et al., 2012). Although consuming high-protein diets may spare muscle mass in response to a negative energy balance (Carbone et al., 2012), adequate energy intake is clearly important for an athlete's recovery, particularly when protein is known to have positive effects on recovery from EIMD, when consumed in sufficient amounts while in a positive energy state (Sousa et al., 2014).

2.1.2 Carbohydrate Intake and Timing

CHOs play important roles in performance and adaptation to training. They are used as a key fuel and substrate for the brain, nervous system, and skeletal muscle allowing the body to sustain physical activity across a large range of intensities via their use in both aerobic and anaerobic energy (ATP) production. When available for the working muscle, particularly as exercise intensity increases

beyond a moderate intensity, CHOs have been shown to be the preferentially used substrate over fat, since they can provide a greater yield of ATP per volume of oxygen consumed (Spriet, 2014), allowing the athlete to sustain a higher work rate for the duration of the exercise bout.

Endogenous stores of CHOs are limited and vary within the human body. This is largely dependent on the training status and habitual CHO intake of an individual (Hawley et al., 1997). CHOs, namely the monosaccharide glucose, are primarily stored in the body as both muscle and liver glycogen via the glycogenesis pathway, while limited amounts are freely available within the body's circulation. In general, an athlete's muscle and liver glycogen stores range between ~350-700g and ~100g, respectively, depending on sex, diet, body weight, training status and muscle fibre-type composition (Knuiman et al., 2015).

In terms of physical activity, an athlete's muscle glycogen stores can be significantly depleted by a single bout of strenuous exercise (Tesch et al., 1986; Robergs et al., 1991; Pascoe et al., 1993), with the level of depletion being determined by the duration, intensity, and volume of the activity (Knuiman et al., 2015). Furthermore, the depletion of muscle glycogen stores is linked with fatigue as it is known to impair the ability of skeletal muscle to perform exercise when stores drop below a certain level (Bergström & Hultman, 1967). For example, isometric (ISO) strength (Hepburn & Maughan, 1982) and isokinetic force production (Jacobs et al., 1981) have been shown to be reduced, and exercise induced muscle weakness accentuated with reductions in muscle glycogen concentration (Young & Davies, 1984). Additional studies have shown that CHO supplementation following glycogen depleting exercise can increase an athlete's performance or time to exhaustion in a subsequent performance test (Osterberg et al., 2008; Alghannam et al., 2016), further highlighting the need for their replacement between exercise sessions.

With the effects of glycogen depletion in mind, CHO intake and timing has become one of the most important considerations for an athlete following exercise, particularly in the context of having a short recovery window before their next bout of exercise, whether it be a key training session or as part of a competition event. The timing of CHO ingestion following exercise is an important consideration since muscle glycogen resynthesis occurs in two phases, as previously reviewed by Beelen et al. (2010) and Jentjens & Jeukendrup (2003). To summarise, the first phase of glycogen resynthesis occurs in the early post-exercise period which lasts for 30-60 minutes and is independent of circulating insulin. During this phase glycogen synthesis occurs at a high rate of between 30-45 mmol/kg dw/h (dry muscle), although it has been suggested that this may only occur if muscle glycogen concentrations have been reduced below a significant level of 128-150 mmol/kg dw. The second phase has been defined as the insulin-dependent phase which is characterised by an increase

in glucose uptake and glycogen synthase activity because of increased sensitivity of the muscle to circulating insulin levels (Beelen et al., 2010). During this phase the rate of glycogen synthesis is approximately 10-30% lower compared to phase one, however, this increased muscle insulin sensitivity can continue for up to 48h post-exercise, depending on CHO intake and muscle glycogen levels (Jentjens & Jeukendrup, 2003; Cartee et al., 1989). In general, the recommendations for efficiently replenishing energy stores in the form of muscle glycogen ahead of the next energy demanding session (for those within 8h of the first session) are to consume $1.0-1.2\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in the first four hours following the initial bout of exercise (Burke et al., 2011). Moderate to high Glycaemic Index (GI) foods are recommended during this short recovery period to promote a greater glycogen storage response (Burke et al., 1993). However, when the recovery period between the two bouts of exercise is extended (>8h), enhancing the rate of glycogen synthesis may be less relevant provided the athlete consumes sufficient amounts of CHOs within their recovery window (Beck et al., 2015).

2.1.3 Protein Intake, Quality, and Timing

Protein intake following exercise is an important consideration for athletes due to its role in skeletal muscle maintenance, repair, and growth, and, in-turn, adaptation to the exercise stimulus. In general, for muscle repair and adaptation to occur, it is widely accepted that an athlete needs to be in a positive muscle protein balance (i.e., when the rate of protein synthesis is greater than the rate of protein breakdown) for protein accretion to occur (Hawley et al., 2006; Sousa et al., 2014). Following a strenuous bout of resistance exercise, however, the athlete generally remains in a negative muscle protein balance, in the absence of nutrient intake (Børsheim et al., 2004), despite larger increases in the protein synthesis rate relative to the smaller increases in protein breakdown due to the effect of the training stimulus (Biolo et al., 1995). Therefore, it seems necessary to provide an exogenous source of nutrients to shift the balance to a positive state and achieve an anabolic environment which supports net tissue growth (Moore, 2019).

The nutrients required to achieve a net positive muscle protein state are the amino acids derived from protein in food or supplements, along with CHO and overall energy to help create the environment that facilitates and promotes protein synthesis, as previously mentioned.

In the context of post-exercise nutrient intake, it is currently recommended that an athlete consumes a high quality (highly bioavailable) protein source of between 15-25g, which provides them with approximately 8-10g of essential amino acids, to maximally stimulate the muscle anabolic response in the early recovery phase (0-2h post-exercise) (Phillips, 2011; Thomas et al., 2016; Moore, 2019). Protein ingestion doses beyond this level have been shown to provide no further

stimulus for muscle protein synthesis in young people (Phillips, 2014), however, it is argued that higher quantities may still be beneficial when a mixed protein source of fast and slower-acting proteins are used, and when whole-body exercise is taken into account (Schoenfeld & Aragon, 2018). In relative terms, athletes should aim for a protein intake of 0.25-0.4g/kg BW post-exercise, meaning that total protein requirements will vary depending on body size (Thomas et al., 2016; Schoenfeld & Aragon, 2018).

Protein quality is an important factor to consider, as well, as not all protein-containing foods are made up of the same array of amino acids, nor are they digested in the same way. A protein source that has high bioavailability and, subsequently, a higher delivery rate of amino acids into the blood and target tissues, should be preferentially considered over lower quality protein sources post-exercise in order to optimise the muscle protein synthetic response (Campbell et al., 2007). Protein quality is currently determined by a food or supplement's protein digestibility corrected amino acid score (PDCAAS), with milk derived proteins (whey and casein), whole egg, and soy protein isolate being among the highest scoring food sources (with a capped score of 1.0), in contrast to low-scoring foods such as lentils and wheat gluten (0.52 and 0.25 scores respectively) (Campbell et al., 2007; Gangurde et al., 2011; Phillips & Van Loon, 2011). However, there are some arguments that suggest this scoring method could be improved and that it potentially overestimates the protein quality of low-protein sources, compared to more modern techniques (Mathai et al., 2017). Regardless, the current literature suggests that milk proteins are superior to other tested proteins for stimulating muscle protein synthesis, which is largely attributed to the leucine content and favourable digestive and absorptive properties of the fluid-based food (Pennings et al., 2011; Thomas et al., 2016). Leucine, in particular, along with the other essential amino acids, is known to stimulate muscle protein synthesis (Drummond & Rasmussen, 2008) and it may even define the postprandial rate at which muscle protein synthesis occurs (Pennings et al., 2011), suggesting that a post-exercise protein source should contain these nutrients.

Further to this, in the context of recovery from EIMD, and reducing the signs and symptoms associated with it, there is a strong body of evidence that suggests that co-ingestion of protein with CHO may provide a beneficial recovery effect (Howatson & Van Someren, 2008; Sousa et al., 2014). One study compared the effects of the acute consumption of 500ml each of CM (a natural source of both protein and CHO), a milk-based protein-CHO (P-CHO) supplement, a CHO sports drink, or water (control (CTRL)), on attenuating EIMD over a 48h period, and found that the CM and milk-based P-CHO supplement had beneficial and significant effects on reducing losses in peak force production and total work (TW), as well as reducing serum levels of creatine kinase (CK) and myoglobin (Mb) (biomarkers for muscle damage), when compared to the CHO and CTRL groups (Cockburn et al.,

2008). Another study also showed reduced levels of serum biomarkers associated with muscle damage, as well as lower subjective indices for muscle soreness 24h-post-exercise in a P-CHO group compared to an electrolyte and artificial sweetener placebo group following a whole-body resistance training to volitional fatigue protocol (Baty et al., 2007). Again, suggesting that the P-CHO supplement had a beneficial effect on reducing muscle damage.

It is thought that these recovery effects may be related to an alteration in protein metabolism, where protein intake increases amino acid availability to promote muscle protein synthesis, while CHO synergistically optimises the hormonal environment by increasing circulating insulin levels (Cockburn et al., 2008). Additionally, insulin has been attributed to the reduction of exercise-induced muscle protein breakdown (Sousa et al., 2014) and, therefore, the co-ingestion of protein and CHO may result in net protein accretion.

In addition to this, studies have also shown that consuming a post-exercise supplement that contains a mix of both protein and CHO may promote maximum rates of insulin secretion and muscle glycogen replenishment, compared to the ingestion of a CHO supplement alone (Van Loon et al., 2000; Zawadzki et al., 1992), further suggesting that this combination may provide an advantage when recovering from EIMD, and exercise in general.

Looking beyond the acute post-exercise recovery period, it is now also known that the exercise induced- anabolic window extends beyond 24h (Burd et al., 2011). For this reason, it is currently recommended that an athlete's total daily protein intake is spread across the entire day to maximally stimulate muscle protein synthesis during the extended recovery period following a bout of exercise. The consumption of approximately 20-25g, or 0.3g/kg BW, of protein every 3-5 hours, or 0.4g/kg BW/meal (across a minimum of 4-meals/day), following a bout of resistance exercise is highlighted as being the optimal feeding pattern for promoting muscular adaptation and recovery (Areta et al., 2013; Thomas et al., 2016; Schoenfeld & Aragon, 2018). Further recommendations provide guidance to athletes in relation to their body size, where the total consumption of 1.2-2.2g/kg BW of protein per day is suggested to support metabolic adaptation, repair, and remodelling; this depends on the athlete's "trained" status, and the training intensity, while also considering that higher intakes may be necessary during periods of reduced energy intake (Campbell et al., 2007; Thomas et al., 2016; Schoenfeld & Aragon, 2018).

2.1.4 Fluid and Electrolyte Intake and Timing

Finally, the need for the athlete to restore their body's fluid and electrolyte balance following a session of exercise is another key consideration when wanting to optimise their recovery, particularly ahead of their next session.

Water plays various roles within the body, including the delivery of nutrients, hormones, and other substances to the cells, as well as the removal of metabolic by-products from the body, and it has a significant part to play in thermoregulation (Kessinger, 2018). The latter is particularly important for the continuation of exercise due to the heat that is generated as a by-product of muscular work and its need to be dissipated for exercise intensity to be maintained. Body fluid, in the form of sweat, assists with the dissipation of this heat to help maintain the body's temperature within acceptable ranges, however this results in a net reduction in body water, referred to as dehydration (Thomas et al., 2016), leading to a state of hypohydration.

Sweat not only contains water but also electrolytes and the amount of each that is lost during a single bout of exercise can be substantial, causing both hypohydration and electrolyte imbalance (e.g. hyponatremia) (Sawka et al., 2007). If these losses are not managed sufficiently then decreased plasma volume, increased submaximal heart rate and decreased maximal cardiac output increase the strain placed on the cardiovascular system which, along with the rise in core body temperature and functional changes to the metabolic and central nervous systems, can lead to life-threatening conditions such as heatstroke (Thomas et al., 2016). In terms of sport and exercise, water and electrolyte imbalances can have detrimental effects on athletic performance, particularly as levels of dehydration approach or exceed 2% of body weight depending on the athlete and exercise conditions (Sawka et al., 2007). For example, evidence suggests that muscular strength, power, and high-intensity endurance are impaired by ~2%, ~3% and ~10%, respectively, in a dehydrated state, suggesting that losses in total body water have an effect on force generation (Judelson et al., 2007). Furthermore, when plasma sodium levels fall below 125mmol/L, exercise-induced hyponatremia can cause confusion and disorientation, headaches, and vomiting, among other symptoms, with more severe and potentially life-threatening symptoms occurring when levels drop below 120mmol/L (Murray & Eichner, 2004).

The amount of these nutrients that an athlete loses is dependent on individual characteristics (including gender, body weight, genetic predisposition, heat acclimatisation state, and metabolic efficiency), factors relevant to the exercise being performed (duration and intensity), and the environment in which the exercise is being performed in (i.e., weather conditions and the type of clothing/equipment used) (Sawka et al., 2007). Sweat rates are therefore highly variable between

different athletes, as well as being largely dependent on the circumstances under which the physical activity is performed. Generally speaking, maximum sweat rates can reach levels as high as 2-3L/h which could feasibly cause body weight reductions of 2% or more (Shirreffs et al., 2004; Shirreffs, 2009). Additionally, typical losses in electrolytes have been reported to be in the range of 20-80 mmol/L of sweat for sodium, most notably, as well as 20-60 mmol/L, 4-8 mmol/L and <2.0 mmol/L of sweat for chloride, potassium, and magnesium, respectively (Maughan, 1991).

When considering these nutrient losses, the goal following exercise is to replace these nutrients back to normal levels during the recovery period between exercise sessions. The magnitude of the fluid-electrolyte deficit and the recovery period available between sessions or events will determine the aggressiveness of the rehydration protocol required to reach a euhydrated state (Sawka et al., 2007). For athletes who have a relatively short recovery window (<12h) following substantial fluid and electrolyte losses, it may be practical to undertake a more aggressive rehydration protocol. However, if the recovery period is extended, the consumption of normal meals and snacks that contain sufficient amounts of sodium, along with adequate amounts of water, will allow the athlete to reach a euhydrated state (Sawka et al., 2007). Current recommendations suggest that athletes should consume ~1.5L of water for every 1kg of body weight lost as this volume should also compensate for the continued sweat losses, as well as the increased urinary losses that occur due to the intake of large volumes of water during the post-exercise phase (Sawka et al., 2007; Thomas et al., 2016). To increase fluid retention and nutrient replacement, water should be consumed over time with sufficient electrolytes, instead of in large boluses (Wong et al., 1998; Kovacs et al., 2002). Electrolyte losses are more difficult to measure and are highly variable among athletes, however the consumption of electrolyte containing drinks (i.e. sports drinks or CM drinks) and foods is recommended to help restore losses (Sawka et al., 2007; Shirreffs et al., 2007). Furthermore, the addition of salt to post-exercise meals should not be discouraged when sodium losses have been significant (Thomas et al., 2016). Finally, post-exercise drinks containing multiple transportable CHOs (i.e. glucose, sucrose, etc) can have beneficial effects on fluid retention during the rehydration phase due to the enhancement of solute absorption through the stimulation of more transport mechanisms (Shi & Gisolfi, 1998). This is because water follows solute movement due to the osmotic gradient that is created when the solutes are absorbed from the intestinal lumen (Maughan et al., 1994). The optimal concentration of CHOs in a post-exercise drink is considered to be between 5-7% to promote high rates of gastric emptying and nutrient absorption (Shi & Gisolfi, 1998). Overall, a prescribed rehydration protocol that has been calculated to replace body fluid losses may be more beneficial to an athlete ahead of a subsequent bout of exercise (Wong et al., 1998).

2.2 Recovery Aids Post-Exercise: Cow's Milk vs Sports Supplements

A number of studies have compared cow's milk (CM) drinks with sports supplements such as fluid replacement (FR) drinks, carbohydrate replacement (CR) drinks and protein supplement (PS) drinks, as previously described. Such studies have suggested that CM performs just as well and, in some cases, better than currently available sports supplements in aiding recovery from a bout of exercise in relation to hydration status, perceived muscle soreness, and subsequent athletic performances. For example, one study (Shirreffs et al., 2007) showed that CM was more effective at replacing sweat losses and maintaining euhydration, following an exercise-induced dehydration protocol (~2% loss of body mass), compared to both water and a commercially available CHO-electrolyte sports drink. Interestingly, athletes who ingested the water and sports drinks experienced marked diuresis during the 2h following drinking, whereas those who consumed the milk drinks did not. Consequently, only the milk groups maintained euhydration until the end of the 4h recovery period. Similarly, Desbrow and his associates (2014) concluded that CM and milk-based drinks are more effective rehydration options when compared with traditional sports drinks. Furthermore, the ingestion of a CM drink was shown to be a more effective recovery aid than CR and FR drinks for cyclists who completed a glycogen depleting protocol, followed by a 4h recovery period (ingesting the recovery drink at 0h and 2h time points), and a cycle to exhaustion at 70% power at maximum oxygen uptake (Thomas et al., 2009). The results showed that the CM group cycled 51% and 43% longer when compared to the CR and FR groups, respectively.

In the context of an athlete recovering from EIMD, CM, again, appears to be a worthy recovery aid. By definition, EIMD is caused by unaccustomed ECC exercise which results in morphological changes within the muscle (Lauritzen et al., 2009), with damaging effects and symptoms occurring immediately after and in the days following the bout of exercise (Friden et al., 1983; Clarkson & Sayers, 1999). Such damage has been shown to cause muscle soreness, losses in muscular force production, and increased serum levels of muscle proteins, such as CK and Mb, among other things (McLeay et al., 2012; Kanda et al., 2013). These responses are known to negatively affect an athlete's ability to perform optimally in subsequent trainings or competition.

Evidence suggests that the consumption of CM following EIMD can have positive effects on athletic performance, as well as indices of muscle damage in the days following the bout of exercise (see table 1). Cockburn et al. (2008) examined the effects of acute CM and milk-based protein-carbohydrate (P-CHO) supplementation on attenuating EIMD in team sport athletes, compared to an equal volume of a commercially available CHO supplement and water (CTRL). The participants were split into four matched groups and baseline (BL) measures for muscle damage and isokinetic muscle

performance were recorded before participating in an exercise protocol designed to induce muscle damage. Participants then immediately consumed 500mL of their allocated nutritional supplement and again at 2h post muscle-damaging exercise, before returning to the lab 24h and 48h later for further blood sample collection, rating of muscle soreness, and isokinetic muscle performance measures. The results showed no difference between groups for passive-DOMS, but did show significantly lower increases in serum CK levels for the CM and milk-based P-CHO drinks compared to the CHO group at 48h, and only saw significant increases in CK levels for the CHO and CTRL groups from BL-48h. Additionally, serum Mb concentrations were significantly lower for the CM and milk-based P-CHO groups compared to the CHO group. Furthermore, measures of peak torque (PT) were significantly higher after 48h in the CM group compared to the CHO group, and for the milk-based P-CHO group compared to the CHO and CTRL groups. TW for the CM and milk-based P-CHO groups was also significantly higher compared to the CHO and CTRL groups. The researchers concluded that the ingestion of CM and milk-based P-CHO drinks are viable options for attenuating the effects of EIMD 48h following a muscle damaging exercise and suggested that athletes may benefit from choosing these types of supplements over CHO sports drinks in the context of recovering from EIMD.

Several other studies examined the effectiveness of CM for recovery from EIMD using probabilistic magnitude-based inferences about the true value of outcomes, as described by Batterham & Hopkins (2006). Cockburn et al. (2010) looked at the effects of timing of a 1000mL dose of a milk-based P-CHO supplement on the attenuation of EIMD and showed that the consumption of such supplements immediately, or 24h after muscle damaging exercise, can attenuate decrements in PT and dynamic muscle performance. They also showed a likely benefit in blunting increases in serum CK activity when consuming the milk-based P-CHO supplement compared to the CTRL group. In addition to this, and assuming that the benefit of CM or P-CHO supplementation is primarily derived from changes in protein metabolism, the same group looked to determine whether similar benefits were possible using a smaller dosage of CM (500mL containing 17g protein) (Cockburn et al., 2012); considering that the consumption of 20g of protein may be the upper-limit for maximal stimulation of muscle protein synthesis (Moore et al., 2009). The researchers concluded that following muscle damaging exercise, symptoms of EIMD, including decrements in isokinetic muscle performance and increases in CK levels, can be minimised to a similar degree with post-exercise consumption of a smaller 500mL amount of CM compared to 1L of CM. This may be worthwhile information for athletes since consuming a smaller volume of CM would be easier and, potentially, cheaper to implement and it could result in fewer instances of stomach discomfort.

To further understand how translatable these findings are when applied to a more applicable, dynamic sport setting, Cockburn et al. (2013) investigated the effects of a 500mL CM supplement

consumed after muscle-damaging exercise on performance tests specific to field-based team sports. In this study, the researchers observed a benefit for three out of the five performance measures, including improved time to cover 10 and 15m, agility test time, and mean 15-metre sprint performance for the Loughborough intermittent shuttle test (LIST). Since the muscle damage protocol primarily targeted the hamstrings muscle group, the researchers suggested that this may explain why benefits were evident in the sprint-related tests and not the jump-related tests, since the hamstrings are used to a greater extent during sprinting activities (Mann et al., 1986), while the quadriceps are the primary movers in jumping activities. If the consumption of CM does limit myofibrillar protein degradation, then an effect on the jumping performance tests would not be observed since the quadriceps were not damaged prior to the tests – a possible limitation of this study.

Further to this, the limitation of these studies was their lack of a placebo with equicaloric content, which makes it difficult to determine whether the benefits of the CM-based drinks on attenuating symptoms of EIMD were not just due to the additional calories consumed by the participants. Rankin et al. (2015) looked to provide clarity around this uncertainty by comparing the effects of consuming 500mL of CM compared to 500mL of a volume- and energy-matched CHO drink on EMID in both male and female team sport athletes. The study showed benefits for both male and female CM groups compared to their matched CHO groups, although the benefits were more profound in the female group, which may be due to a protective effect of oestrogen (Tiidus, 2001), and possibly a function of a slightly higher protein intake per kilogram of body mass compared to the male CM group. Both female and male CM groups also saw limited increases in indices for muscle damage compared to the CHO groups, which further supports the use of CM as an effective recovery aid from EIMD, even compared to an energy matched CHO supplement.

Table 1: Summary of cow's milk on EIMD studies

Study	Participants	Beverage(s)	Exercise Protocol	Indices of Muscle Damage		Force/Performance Recovery	
				Marker	Treatment Effect	Test	Treatment Effect
Cockburn et al., 2008	24 healthy, male team-sport athletes, split into 4 matched groups (n=6)	2x 500mL of: Milk-based P-CHO, CM, CHO, or water (CTRL) at 0h & 2h	6 x 10 max ECC-CON KFs at 1.05 rad·s ⁻¹ both legs	P-DOMS CK Mb	↔ - between groups ↑ - in CHO group vs milk-based P-CHO & CM groups at 48h, ↑ - from 0-48h in CTRL and CHO groups only ↑ - in CHO group vs milk-based P-CHO & CM groups	PT at 1.05 rad·s ⁻¹ KF TW across 6 max KF reps at 1.05 rad·s ⁻¹	DL: ↑ - for milk-based P-CHO group vs CTRL & CHO groups at 48h, ↑ - for milk group vs CHO group at 48h, Non-DL: ↑ - for milk-based P-CHO & CM groups vs CTRL group at 48h DL: ↑ - for milk-based P-CHO & CM groups vs CHO & CTRL groups at 48h, Non-DL: ↑ - for milk-based P-CHO group vs CTRL group at 48h
Cockburn et al., 2010	32 healthy male athletes, split into 4 matched groups (n=8)	1000mL of semi-skimmed milk-based supplement (Pre, Post, or 24-h Post exercise protocol), & 1000mL water (CTRL)	6 x 10 max ECC-CON KFs at 1.05 rad·s ⁻¹ both legs	A-DOMS CK	↓ - possible benefit for Pre & Post vs CTRL for BL vs 48h, ↓ - likely benefit for Pre vs CTRL and 24-h Post for BL vs 72h ↓ - likely benefit for Post & 24h-Post vs CTRL, & possible benefit for Pre vs CTRL for BL vs 48h, ↓ - possible benefit for Pre & Post vs CTRL for BL vs 72h	PT at 1.05 rad·s ⁻¹ KF RSI	↑ - likely benefit for Post vs Pre & CTRL for BL vs 48h, ↑ - likely benefit for Pre & Post, & possible benefit for 24h-Post, vs CTRL for BL vs 72h ↑ - likely benefit for Post vs CTRL & Pre for BL vs 48h, likely & very likely benefit for 24h-Post vs CTRL & Pre, respectively, for BL vs 48h, ↑ - likely benefit for 24h-Post vs CTRL & Pre, & Post vs Pre, for B vs 72h
Cockburn et al., 2012	24 active males, split evenly into	500mL or 1L semi-skimmed CM, or	6 x 10 max ECC-CON KFs at 1.05 rad·s ⁻¹ both legs	P-DOMS A-DOMS CK Mb	Unclear Unclear Very likely ↓ for 1L CM	PT at 1.05 rad·s ⁻¹ KF	DL: Likely benefit for 1L CM vs Placebo, ↔ between CM groups

	3 groups (n=8)	placebo (1L water)		IL-6	Almost certain ↓ for 500mL CM Likely ↓ for 1L CM, ↔ between CM groups		
Cockburn et al., 2013	14 semi-professional football male players, split evenly into two groups (n=7)	500mL semi-skimmed CM, or 500mL of water (CTRL)	6 x 10 max ECC-CON KFs at 60°·s ⁻¹ both legs	Passive soreness Active soreness CK Mb	Unclear Unclear Unclear Unclear	CJH RSI 15m-sprint Agility Test LIST	Unclear Unclear ↓ - possible benefit for CM group vs CTRL for BL vs 48h & 72h ↓ - likely benefit for CM group vs CTRL group for BL vs 72h ↓ - likely benefit in mean 15m-sprint time for CM group vs CTRL
Rankin et al., 2015	32 team sport players (16 F, 16 M), split evenly into 4 gender groups (2x F & 2x M groups)	500mL light CM or 500mL energy-matched CHO solution	6 x 10 max ECC-CON KFs at 60°·s ⁻¹ both legs	P-DOMS A-DOMS CK sTnI	↓ - very likely benefit for F & M CM groups vs CHO groups for BL vs 72h ↓ - very likely benefit for F & M CM groups vs CHO groups for BL vs 72h in both DL & Non-DL ↓ - possible benefit for M CM group vs CHO group for BL vs 72h, ↓ - most likely trivial benefit for F & M CM groups compared to CHO groups for 48 vs 72h ↓ - possible benefit in F & M CM groups compared to CHO groups	PT 60°·s ⁻¹ KF PT 180°·s ⁻¹ KF 20m sprint time CJH	↓ - for F & M CHO groups only for BL vs 48 & 72h, ↑ - very likely benefit for F CM group vs F CHO group from BL vs 48 & 72h ↓ - for M & F CHO groups only for BL vs 48 & 72h, ↑ - likely benefit for F CM group vs F CHO group for BL vs 48 & 72h ↑ - for M & F CHO groups for BL vs 24, 48 & 72h, ↓ - likely benefit BL-72h, 24-48h & 24-72h (F), & 24-72h (M) ↑ - possible benefit 24h-72h for F & M CM groups vs CHO groups

Abbreviations: CHO, carbohydrate; P, Protein; max, maximum effort, ECC-CON, eccentric-concentric; P-DOMS, passive - delayed onset muscle soreness; A-DOMS, active - delayed onset muscle soreness; CK, creatine kinase; Mb, myoglobin; IL-6, interleukin-6; sTnI, skeletal troponin I; PT, peak torque; KF, knee flexion; TW, total work; CJH, countermovement jump height; RSI, reactive strength index; LIST, Loughborough intermittent shuttle test; M, male; F, female; BL, baseline; CTRL, control group; Pre, pre-exercise protocol group; Post, post-exercise protocol group; DL, dominant leg; Non-DL, non-dominant leg; ↔, no significant difference/change; ↓, significant decrease; ↑, significant increase

2.3 Sheep's Milk Vs Cow's Milk

Fundamentally SM, as a food source, is very similar to CM, carrying all of the same key traits that you would expect to see in mammalian milk, i.e. protein, in the form of whey and casein subgroups, fat, CHOs, primarily in the form of lactose, as well as an abundance of vitamins and minerals, including calcium in particular. Despite these similarities, there are still a number of key compositional differences that set these two different milk types apart (see table 2), which could have implications for their functional use in specific populations or settings. CM has been researched extensively (for example, Bos et al., 2000; Haug et al., 2007; Roy, 2008; Pereira, 2014), however, very little research exists for SM when it comes to its potential use as a functional food, particularly in the context of the sports and fitness industry.

Table 2: Proximate nutrient quantities of cow's milk and sheep's milk^a

Nutrient	Unit	Cow's Milk		Sheep's Milk	
		Quantity per 100mL	%DI* per 100mL	Quantity per 100mL	%DI* per 100mL
Water	g	87.6	-	83.1	-
Total Solids	g	12.4	-	16.9	-
Energy	kJ	278	3	414	5
Protein	g	3.5	7	5.5	11
Fat, total	g	4.0	6	6.5	9
- Saturated	g	2.5	10	2.98	12
Carbohydrates	g	4.2	1	4.8	2
- Sugars	g	4.2	5	4.8	5
Dietary Fibre	g	0.0	0	0.0	0
Ash	g	0.7	-	0.9	

^aTable adapted from the "basic reports" for "Milk, cow, whole 4% fat, fluid, non-homogenised" & "Milk, sheep, whole, fluid, fresh" from New Zealand Food Composition Data (n.d). *Percentage Daily Intakes are based on an average adult diet of 8700 kJ as specified by Food Standards Australia and New Zealand (FSANZ).

In general, SM contains a higher percentage of total milk solids with approximately 16.9% compared to 12.4% for CM. From an energy and macronutrient standpoint, SM is more energy dense with significantly higher amounts of protein and fat (~60% more), while differences in CHO/lactose content are modest (~14% more) (see table 2). With similar levels of lactose between the two milk types, the proportion of lactose to total milk solids is lower in SM compared to CM (28.4% vs. 33.9%, respectively), with some researchers suggesting potentially greater differences still (22-27% vs. 33-40% for SM and CM, respectively) (Balthazar et al., 2017). This could have positive implications for

those who have sensitivities to lactose, however this is yet to be investigated. The nutritional value of SM is also considered to be higher than CM, as it contains greater amounts of almost all of the vitamins and minerals found in CM (see appendix 9.1) (New Zealand Food Composition Data (n.d); Balthazar et al., 2017). It should be noted that the composition of raw milk from both mammalian species is subject to seasonal variation based on physiology, environmental factors such as changes in season (climate changes) and animal diet, among other things (Li et al., 2022). However, typically, depending on the manufacturer and product type, milk is standardised to achieve a relatively consistent final product for the end consumer.

Table 3: Amino Acid content of cow's milk and sheep's milk^a

Amino Acid	Cow's Milk		Sheep's Milk	
	mg/mL	% of total amino acids	mg/mL	% of total amino acids
Essential Amino Acids				
Leucine	2.95	9.35	4.85	9.59
Lysine	2.50	7.92	4.12	8.15
Valine	1.94	6.14	3.17	6.26
Isoleucine	1.59	5.03	2.48	4.90
Phenylalanine	1.45	4.61	2.30	4.55
Threonine	1.40	4.43	2.25	4.44
Histidine	0.84	2.66	1.35	2.66
Methionine	0.67	2.12	1.13	2.22
Tryptophan	0.41	1.29	0.78	1.53
Non-Essential Amino Acids				
Glutamic acid*	6.32	20.02	9.64	19.04
Proline	3.04	9.63	4.90	9.68
Aspartic acid*	2.39	7.57	4.06	8.03
Serine	1.73	5.49	2.54	5.02
Tyrosine	1.41	4.45	2.24	4.42
Alanine	1.06	3.34	1.86	3.68
Arginine	1.06	3.37	1.61	3.18
Glycine	0.59	1.86	0.96	1.90
Cystine	0.23	0.72	0.37	0.73

^aTable adapted from: Milan et al. (2020). *Figures may include contributions of glutamine and asparagine, respectively, converted during hydrolysis.

When looking deeper into the nutritional quality of both types of milk, and in the context of the sport and fitness industry, protein composition is of interest. The casein-to-whey protein ratio of SM

compared to CM is slightly different at 76:24 and 82:18, respectively (Roy et al., 2020). Since whey protein is absorbed more rapidly compared to casein (Schoenfeld & Aragon, 2018), the combination of a higher total amount of protein per mL, with a higher proportion of whey protein content in SM, could mean faster delivery of a greater quantity of amino acids to the muscular tissue post-exercise, compared to using the same amount of CM. In addition, the greater concentration of protein in SM also provides significantly more amino acids per mL (see table 3), particularly for the essential amino acids, including leucine (4.85mg/mL vs. 2.95mg/mL for sheep and CM, respectively), which are known to be the key nutrients required to maximally stimulate muscle protein synthesis, as previously mentioned.

Furthermore, a recent double-blind, crossover and randomised control trial has shown that the consumption of SM results in a greater increase in circulating branched-chain amino acids (BCAAs; leucine, iso-leucine & valine; 641.1 ± 16.3 vs. $563.5 \pm 14.4 \mu\text{mol}\cdot\text{L}^{-1}$ in SM vs. CM, respectively; $p < 0.001$), as well as several other essential and non-essential amino acids, compared to the ingestion of the same volume of CM (portion-for-portion) in 30 “dairy-avoiding” women (Milan et al., 2020). In addition to this, circulating levels of the BCAAs remained significantly higher across each time point that was measured (1h, 2h, 3h & 4h), following the consumption of the treatment, in the SM group versus the CM group. The authors did note that despite the study population being “dairy-avoiders” it is unlikely that this influenced postprandial amino acid appearance, suggesting that similar responses would be expected in other population groups, which could include those in a sports recovery setting.

In addition to this, SM is said to be an “A2-like” milk (Bodnár et al., 2018), similar to the “A2” beta-casein CM variant which is suggested to be a better option for people who suffer from CM intolerance or CM allergy symptoms associated with the A1 beta-casein milk protein found in normal CM products (Park & Haenlein, 2021). This may provide some additional benefit to athletes who typically avoid normal dairy products due to these milk sensitivities, which are believed to affect between 0.2-17% of the general population, based on data from a number of studies that have attempted to estimate its prevalence (Flom, J. D., & Sicherer, S. H. (2019).

Chapter Three: Aims and Hypotheses

3.1 Introduction

Based on the literature review it is evident that CM, and the associated flavoured milk drinks, are some of the most suitable nutritional recovery options for exercise-induced muscle damage. When acknowledging this it is reasonable to consider that SM, potentially a nutritionally superior food source, may have the potential to elicit further recovery benefits above that of CM. Therefore, this study aims to compare the potential nutritional benefit of consuming a SM beverage versus a CM beverage on acute performance recovery following strenuous ECC exercise.

3.2 Aims

Specifically, the following aims were:

1. To compare the performance recovery capabilities of a SM beverage to that of a CM beverage following EIMD.
2. To gain new information on the potential efficacy of the use of SM as a performance recovery beverage.
3. And, thirdly, to identify potential differences in perceived satiety and gastrointestinal comfort between SM and CM.

3.4 Hypothesis

It was hypothesised that following exercise-induced muscle damage, measures for muscle performance and recovery for the SM trials would at least match, if not show superior results for recovery when compared to the same results from the CM trials.

Chapter Four: Methods

4.1 Experimental Overview

The current study used a randomised, double-blind, cross-over design using a modified version of the one-legged model described by Barnes et al. (2010). Ten healthy men volunteered to participate, and Leg exercised (dominant vs non dominant), and Treatment (CM vs SM) were allocated randomly. Briefly, participants first performed maximal voluntary leg extension movements in a CON, ECC, and then ISO fashion, measuring PT of the quadriceps muscles of one leg before performing 200 maximal ECC contractions using the same leg to induce muscle damage. Immediately after the muscle damage protocol, the performance tests were repeated again before the participant consumed either a chocolate SM drink or a chocolate CM drink. Participants returned to the lab 24h, 48h, and 72h later to repeat the same performance measures. Following a washout period of at least 10 days, the participants began their second trial which involved the same protocol on the contralateral leg and consuming the other beverage.

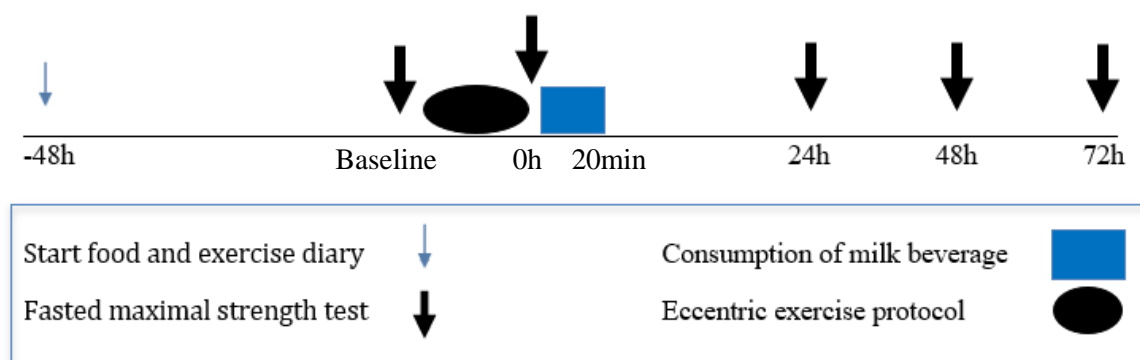


Figure 1: General overview of the testing protocol used for all trials.

4.2 Participants

Participants were recruited from local gyms, sports clubs and the student population. Ten healthy and physically active male volunteers (mean \pm standard deviation (SD) age 24.9 ± 4.3 years, body mass 82.8 ± 10.3 kg, height 179.1 ± 6.7 cm) agreed to take part in the study. All participants had at least two years of resistance training experience at a recreational level (minimum of two trainings per week). The study protocol was approved by the Massey University Human Ethics Committee: Southern A 19/22 and written consent was obtained from each participant.

Based on the work done by Barnes et al. (2010), from which the muscle damage and muscular performance protocols used here are derived, a statistical power calculation was used to determine that at least 7 participants were needed to reach a sufficient power (0.8) for muscle damage (a decrease in peak CON torque) at a 95% confidence interval and with a large effect size ($d=1.568$). From this we were able to determine whether SM has similar or different effects on performance recovery compared to CM.

Participants were familiarised with the protocol in the lab at least 7-days prior to the first trial and completed both a pre-exercise health screening questionnaire (see appendix 9.3), to confirm their suitability for participation, and an informed consent form (see appendix 9.4). Potential participants were excluded from the study if they had: any current or previous injury to their legs; any injury or medical condition that may affect their ability to sense pain or discomfort; any experience with persistent or regular lower back pain; any known heart or cardiovascular conditions; if they were taking prescribed medication, including over-the-counter pain relief or anti-inflammatories, that may affect the ability to exercise, or sense pain and discomfort; or if they were known to be lactose intolerant or have an intolerance to dairy products.

Anthropometric measures (height and weight) were also taken during this initial visit, along with the individual's specific adjustment settings on the Biodex® isokinetic dynamometer (Biodex Medical Systems, New York, USA) system which was used for this study. Participants were seated in the Biodex chair with the lateral femoral epicondyle aligned with the axis of rotation on the dynamometer, and the ankle strap of the knee attachment positioned slightly proximal (1-5cm) to the medial malleolus.

For both intervention trials, participants were required to refrain from any exercise (except for any necessary walking) and alcohol consumption in the 48h before day one of their trial, and for the duration of the trial period itself (72h). Participants were instructed to track their food intake (by estimating food quantity consumed) from 48h prior to day one of their trial until the end of the trial using the free Easy Diet Diary application on their own smart phones, or by recording their food intake on a paper version which was given to them prior to the start of each trial (see appendix 9.5). Participants were also asked to replicate their diets from trial 1 for trial 2 as best they could. Both food records were either uploaded or manually logged into a dietary analysis software (FoodWorks 10® Xyris Software (Australia) Pty Ltd.) for analysis. Additionally, participants were required to record all physical activity during the same time period on the Physical Activity Diary that was provided to them prior to the start of each trial (see appendix 9.5). Finally, participants were

instructed to arrive at the lab in the morning, after an overnight fast of at least 10h, on all data collection days.

4.3 Subjective Muscle Soreness Tests

Participants arrived at the lab and were asked to complete a series of subjective muscle soreness measures, as a BL measure for the leg being tested in the trial. These included a simple step-up to step-down using only the testing leg in both the raising and lowering phases on a 45-cm step, and a simple squat to 90-degrees with feet shoulder-width apart. Both exercises were performed twice before the participant marked how sore their leg felt on a visual analogue scale between 1-10 (1 = no soreness, 10 = unbearably sore/extremely sore) (see appendix 9.6).

Additionally, a pressure pain threshold test was used as a third and more direct measure of subjective pain. First, the approximate midpoint on the belly of the vastus lateralis muscle (halfway between the lateral edge of the patella and the top of the iliac crest) was marked before applying gradual pressure to that point using an algometer (Wagner FPX™ 25 Algometer, Greenwich, CT, USA), with the screen faced down, until the participant indicated for the pressure to stop (the point at which the pain gradually induced was no longer bearable). For this measure the participant was seated in an upright position on a bench with their legs comfortably relaxed over the edge at a 90-degree angle, and three measures were recorded. The highest value of the three measures was used for analysis.

All three of these tests were carried out at BL and at 0h-post the muscle damage protocol, and upon arrival to the lab on the performance testing days that followed (24h, 48h and 72h-post muscle damage protocol). In cases where visual bruising arose from the algometer test, between testing days, the testing sight was adjusted slightly on the belly of the vastus lateralis muscle to avoid potential sensations of pain that were unrelated to the muscle damage protocol.

4.4 Muscular Performance Tests

Following the perceived muscle soreness tests, participants were asked to warm-up on a cycle ergometer (Monark, Varberg, Sweden) for five-minutes at 100 watts. They were then seated on the Biodex chair and strapped in with belts fitted across the chest, hips and active leg to isolate movement to the quadriceps muscle group of the test leg. The ankle of the test leg was also strapped to the end of the knee attachment lever arm, which was connected to the dynamometer portion of the Biodex. A pre-set CON and ECC protocol was then loaded on the system and a 60°

range of motion was set for the participant's test leg. The range of motion for each participant was measured from a comfortably flexed knee position (0° start point) and extended out by 60° (end point). This range was saved and used in all subsequent performance measures. Once the participant was fully set up, understood the protocol, and verbally indicated that they were ready, the protocol was initiated. The CON and ECC protocol consisted of three consecutive repetitions of a maximal CON knee extension, followed by a two-minute passive recovery period, and then three consecutive repetitions of a maximal ECC knee extension. CON and ECC torque was measured at an angular velocity of $30^{\circ}\cdot s^{-1}$ for both tests. Following another two-minute passive recovery period, the ISO test protocol was loaded on the Biodex system and the participant's test leg was moved to, and fixed at, a 75° knee angle to measure peak ISO tension. For this test, participants were prompted to perform three repetitions of a three-second maximal ISO contraction of their quadriceps muscle group (attempting to extend at the knee), with a ten-second rest between each repetition. The absolute PT values were recorded for each test. Each performance test was repeated at 0, 24, 48 and 72h post the muscle damage protocol, and values were compared to BL measures.

4.5 Muscle Damage Protocol

Following the BL muscular performance tests on day one of testing, participants remained on the Biodex and performed two sets of 100 maximal ECC contractions using the quadriceps muscle group of their test leg. Each repetition was performed over the same 60° range of motion that was used in the performance test, and at an angular velocity of $30^{\circ}\cdot s^{-1}$. A five-minute passive recovery period was observed between the two sets. A display screen provided visual feedback to the participant giving an indication of their force output for each individual effort, and participants also received verbal encouragement to resist the downward movement of the dynamometer arm to ensure a constant maximal effort was given throughout the protocol. There was no significant difference between the SM and CM trials for TW completed during the muscle damage protocol (24.2 ± 6.5 kJ and 25.0 ± 5.1 kJ, respectively) ($P=0.52$).

4.6 Treatment

Upon arrival to the lab on day one of each trial, participants were asked to complete a BL gastrointestinal comfort and satiety questionnaire (see appendix 9.7) before beginning the performance testing and muscle damage protocol. Following the muscle damage protocol and the first re-test of the performance and muscle soreness measures (0h), participants were asked to

consume either a chocolate SM drink or the equivalent volume of a chocolate CM drink (450mL). To minimise the potential for bias, the treatment was double blinded; allocation of leg (dominant vs non-dominant) and treatment (SM vs CM) was randomised by an academic staff member who was not involved with the data collection process. Both milks were flavoured in the same way, including the addition of 1.5% sugar per volume. The drinks were provided to the participants in clear plastic bottles labelled either “1” or “2”. In an attempt to naturally offset the difference in total energy content between the SM (whole SM, Fernglen Farm, Masterton, New Zealand) drink and the CM drink, a full fat CM was used (silver top, full cream milk, Anchor, Takanini, Auckland, New Zealand). Proximate analysis tests were carried out independently by the Nutrition Laboratory at the School of Food and Advanced Technology, Massey University. Table 4 provides a summary of the energy and macronutrient content of both chocolate milk drinks following a proximate analysis, and the amino acid concentration of both drinks, following a standard amino acid profile, is also provided in table 5.

Table 4: Proximate analysis of the chocolate sheep’s milk and cow’s milk drinks.

Milk component	Chocolate sheep’s milk drink			Chocolate cow’s milk drink		
	Per 450mL treatment	% of milk (per 100mL)	% of milk solids	Per 450mL treatment	% of milk (per 100mL)	% of milk solids
Total milk solids (g)	86.5	19.2	100	66.3	14.7	100
Energy (kJ)	1828.2	(406.3)	-	1327.3	(295.0)	-
Protein (g)	26.7	5.9	30.9	15.9	3.5	24.0
Fat (g)	23.7	5.3	27.4	15.4	3.4	23.2
CHO (g)	27.1	6.0	31.4	26.4	5.9	39.8
- Lactose (g)	20.3	4.5	23.4	19.8	4.4	29.9
Dietary fibre (g)	4.41	1.0	5.1	4.95	1.1	7.5
Ash (g)	4.6	1.0	5.3	3.7	0.8	5.6
Water (mL)	363.6	80.8	0.0	383.9	85.3	0.0

Following the consumption of the allocated milk drink, the participants were asked to remain in the lab for a further 20-minutes before completing the same gastrointestinal comfort and satiety questionnaire, as well as indicating which milk drink they believed they had just consumed. Participants were then offered a non-obligatory standard breakfast (including muesli cereal, Weet-bix, almond milk, toast, spreads and fruit) before leaving the lab to carry on with their normal day.

Table 5: Amino acid profile of the chocolate sheep's milk and cow's milk drinks.

Amino acids	Sheep's milk (mg/100mg)	Sheep's Milk (g/450ml treatment) *	Cow's milk (mg/100mg)	Cow's Milk (g/450ml treatment) *
Aspartic Acid	0.44	2.05	0.26	1.20
Threonine [#]	0.26	1.21	0.16	0.74
Serine	0.29	1.35	0.17	0.78
Glutamic Acid	1.05	4.89	0.64	2.95
Proline	0.57	2.65	0.31	1.43
Glycine	0.11	0.51	0.07	0.32
Alanine	0.21	0.98	0.11	0.51
Valine [#]	0.38	1.77	0.22	1.01
Methionine [#]	0.16	0.74	0.09	0.41
Isoleucine [#]	0.29	1.35	0.17	0.78
Leucine [#]	0.56	2.61	0.33	1.52
Tyrosine	0.28	1.30	0.17	0.78
Phenylalanine [#]	0.27	1.26	0.16	0.74
Histidine [#]	0.15	0.70	0.09	0.41
Lysine [#]	0.49	2.28	0.29	1.34
Arginine	0.19	0.88	0.13	0.60
Total EAA[†]	2.56	11.92	1.51	6.95

Amino acid profile tests (acid stable) were carried out independently by the Nutrition Laboratory at the School of Food and Advanced Technology, Massey University. *values were calculated using density conversion factors for each milk using the lower value of the ranges documented by Park et al. (2007). [#]Essential Amino Acids (EAA). [†]Total EAA content of each sample excluding tryptophan which was not measured due to the test method used.

4.7 Statistical Analysis

The data were tabulated and analysed using the Statistical Package for the Social Sciences software (SPSS version 25.0, IBM, New York, USA). A general linear model, two-way, repeated-measures ANOVA (Treatment x Time) was used to compare treatment conditions over time for all performance, subjective muscle soreness, and gastrointestinal comfort and satiety measures. This showed the main effects of Time and Treatment, as well as the interactions between Treatment x Time. A Post-hoc analysis using Bonferroni adjustment was done to investigate any significant main effects. The data was also analysed as the absolute change in torque relative to the pre-muscle damage values since pre-muscle damage values were significantly different between treatments due to the trials being performed using different legs. A paired t-test was used to compare the TW done during the muscle damage protocol between the SM and CM trials. Paired t-tests were also used to compare dietary intakes between treatment groups for both the duration of the trial and for the treatment day alone, looking at energy, protein, fat and CHO. Results are reported as means \pm SD, and statistical significance was set at $P < 0.05$.

Chapter Five: Results

5.1 Sheep's and Cow's Milk Drink Compositions

Table 4 compares the composition of the chocolate SM and chocolate CM drinks, following proximate analysis tests. It shows that the SM drink had a higher portion of total milk solids which included higher amounts of protein and fat, but similar amounts of CHOs, compared to the CM drink. When looking at the contribution of individual macronutrients to the total solids of each milk, protein and fat components contributed a higher percentage in the SM drink compared to the CM drink (30.9 % protein, 27.4 % fat, and 24.0 % protein, 23.2 % fat, respectively). As such, the CHO, including lactose, contribution to total milk solids was lower in the SM drink (SM: 31.4 % CHO and 23.4 % lactose. CM: 39.8 % CHO and 29.9 % lactose).

Participants received approximately 1828.2 kJ of energy, 26.7 g of protein, 23.7 g of fat, 27.1 g of CHO, or 1327.3 kJ of energy, 15.9 g of protein, 15.4 g of fat, 26.4 g of CHO, per 450mL SM and CM treatment, respectively.

Table 5 compares the amino acid profile of both chocolate milk drinks, displaying the amino acid quantities as a milligram per 100 mg milk sample amount and as a total quantity, in grams, per 450 mL treatment. The SM drink was shown to contain higher amounts of all the amino acids listed in the table, compared to the CM drink. Notably, the branched chain amino acids, leucine, isoleucine and valine, were present in much higher amounts in the SM drink (2.61 g leucine, 1.35 g isoleucine, 1.77 g valine and 1.52 g leucine, 0.78 g isoleucine, 1.01 g valine per 450 mL drink for SM and CM, respectively).

5.2 Performance Measures

Completion of the muscle damage protocol resulted in significant decreases in peak CON ($P = 0.012$), ECC ($P = 0.001$) and ISO ($P = 0.011$) torque over time, compared to BL values. No significant Treatment effects (all $P > 0.097$) or Treatment X Time interactions (all $P > 0.318$) were observed for any of the performance measures. Similar results were observed for the percentage change values compared to BL values, with significant decreases in all performance measures over time (all $P < 0.007$), however, no significant Treatment effects or Treatment X Time interactions (all $P > 0.497$) (see table 6).

After 24h, significant decrements in both CON and ECC measures were observed in the SM trial ($P < 0.05$), but not in the CM trial. Peak ECC measures at 48h were significantly greater ($P < 0.05$) than 24h values in the SM trial, and 72h measures were significantly greater than 24h values in the CM trial, suggesting that some recovery from the muscle damage protocol had occurred across these timepoints, in both cases. Similarly, there was an improvement in 72h Peak ISO measures compared to 24h values in the CM trial ($P < 0.05$). Beyond these time differences, there were no significant differences observed between BL values and any of the subsequent measures in both trials.

Table 6: Changes in peak torque (Nm) over time, relative to Baseline scores, following the muscle damage protocol (mean \pm SD).

	Baseline	0h	24h	48h	72h
Peak CON					
SM	234.76 \pm 78.59	-65.30 \pm 63.53	-46.50 \pm 37.16*	-42.26 \pm 52.14	-17.74 \pm 41.84
CM	209.64 \pm 58.83	-50.21 \pm 47.51	-42.53 \pm 37.55	-31.07 \pm 70.52	-22.98 \pm 65.27
Peak ECC					
SM	287.23 \pm 103.23	-69.57 \pm 66.17	-68.03 \pm 52.17* ^a	-48.30 \pm 57.60 ^a	-12.42 \pm 79.83
CM	273.77 \pm 58.06	-44.34 \pm 47.62	-39.39 \pm 53.86 ^a	-34.80 \pm 65.66	3.92 \pm 58.98 ^a
Peak ISO					
SM	249.13 \pm 52.10	-34.51 \pm 49.32	-33.34 \pm 42.35	-18.77 \pm 40.41	9.30 \pm 30.42
CM	239.59 \pm 33.77	-43.81 \pm 49.23	-33.90 \pm 43.13 ^a	-12.23 \pm 55.46	1.39 \pm 38.15 ^a
% Change CON					
SM	0.00 \pm 0.00	-23.04 \pm 21.06	-18.43 \pm 10.94*	-16.86 \pm 17.49	-7.23 \pm 14.75
CM	0.00 \pm 0.00	-20.69 \pm 19.28	-19.20 \pm 15.30*	-13.65 \pm 26.82	-9.92 \pm 25.15
% Change ECC					
SM	0.00 \pm 0.00	-19.46 \pm 17.21	-21.17 \pm 14.46*	-12.92 \pm 18.13	4.46 \pm 51.62
CM	0.00 \pm 0.00	-14.49 \pm 15.40	-13.66 \pm 16.71 ^a	-11.46 \pm 19.83	2.59 \pm 18.84 ^a
% Change ISO					
SM	0.00 \pm 0.00	-11.79 \pm 17.84	-12.00 \pm 14.57	-6.22 \pm 14.44	-0.21 \pm 11.34
CM	0.00 \pm 0.00	-18.19 \pm 17.87	-13.95 \pm 15.81 ^a	-4.28 \pm 20.37	1.26 \pm 14.96 ^a

* Significantly different to PRE-muscle damage protocol (Baseline) values ($P < 0.05$). ^a Significant difference between values within respective trial ($P < 0.05$).

The percentage change was also significantly different ($P < 0.05$) from BL values for CON and ECC measures at 24h in the SM trial, and for the 24h CON measure in the CM trial, indicating that muscle damage had occurred as a result of the muscle damage protocol. Significant differences between 24 and 72h ECC and ISO measures in the CM trial were also observed ($P < 0.05$).

5.3 Subjective Measures for Muscle Soreness

Subjective measures of muscle soreness at BL and following the muscle damage protocol were recorded using a visual analogue scale, for the sensation of pain felt (if any) during a step-up and a squat, and using a pressure pain threshold meter (see table 7). There was a significant Time effect across all three measures (all $P < 0.002$), but no significant effects for Treatment or Treatment X Time interactions (all $P > 0.072$). Similar results occurred when looking at the percentage change values from BL scores with a significant Time effect for all three measures (all $P < 0.012$), but no significant effects for Treatment or Treatment X Time interactions (all $P > 0.62$).

Significant increases in perceived muscle soreness were observed at 0h and 24h in both trials, following the muscle damage protocol for both the Step-up and Squat measures ($P < 0.05$), indicating that muscle damage had occurred.

The 24h Pressure Pain Threshold Test was significantly different from the BL measure in the SM trial ($P < 0.01$) but not in the CM trial; nor at any other time points ($P > 0.05$) for this measure. There were, however, significant differences between 0 and 24h measures within both trials, for this measure, suggesting some muscle damage had occurred. Additionally, 72h muscle soreness scores were significantly lower than 24 and 48h scores in the SM trial ($P < 0.05$ and $P < 0.01$, respectively), suggesting that participants recovered somewhat over this time.

There was a significant percentage increase in the soreness scores for the Step-up measure, compared to BL values, at 0, 24 and 48h for the SM trial and 0 and 24h for the CM trial ($P < 0.05$), again indicating muscle damage had occurred. Significant percentage increases in Squat soreness were observed at 24 and 48h in the SM trial ($P < 0.01$ and $P < 0.05$, respectively), but no differences were detected in the CM trial for this measure ($P > 0.05$).

Table 7: Changes in perceived muscle soreness scores over time, following the muscle damage protocol, relative to Baseline muscle damage scores (mean \pm SD).

	Baseline	0h	24h	48h	72h
Step-up (1-10)					
SM	1.73 \pm 0.51	4.34 \pm 2.95*	3.19 \pm 2.16*	2.49 \pm 2.26	1.6 \pm 2.56
CM	1.63 \pm 0.45	5.57 \pm 2.73 [#]	4.06 \pm 2.75*	2.94 \pm 2.85	1.88 \pm 2.96
Squat (1-10)					
SM	1.63 \pm 0.49	3.66 \pm 2.89*	2.93 \pm 1.68 [#]	2.35 \pm 2.40	1.56 \pm 2.79
CM	1.75 \pm 0.70	4.34 \pm 3.05*	3.57 \pm 3.03*	2.57 \pm 3.08	1.7 \pm 3.04
Pressure Pain Threshold Test (N)					
SM	72.02 \pm 14.26	-1.82 \pm 19.54 ^a	-18.84 \pm 9.33 ^{#ab}	-17.00 \pm 15.65 [‡]	-9.44 \pm 14.02 ^{b‡}
CM	68.04 \pm 19.70	1.2 \pm 7.38 ^a	-9.58 \pm 8.34 ^a	-7.06 \pm 13.08	-2.92 \pm 17.63
% change Step-up					
SM	0.00	274.95 \pm 215.35*	182.88 \pm 116.10 [#]	141.59 \pm 99.79*	80.45 \pm 102.04
CM	0.00	367.07 \pm 233.80 [#]	270.66 \pm 182.44*	181.59 \pm 160.74	109.06 \pm 154.95
% Change Squat					
SM	0.00	257.97 \pm 235.38	179.47 \pm 68.84 [#]	138.66 \pm 100.80*	80.39 \pm 121.32
CM	0.00	285.66 \pm 259.61	237.03 \pm 215.54	162.21 \pm 181.25	100.02 \pm 168.20
% Change Pressure Pain Threshold Test					
SM	0.00	-0.80 \pm 31.25	-26.04 \pm 12.64 ^{#a}	-22.46 \pm 22.15 [‡]	-11.87 \pm 20.66 ^{a‡}
CM	0.00	1.55 \pm 12.44 ^a	-12.84 \pm 8.83 ^{*a}	-7.98 \pm 19.59	-0.10 \pm 30.51

* Significantly different to PRE-muscle damage protocol (Baseline) values ($P < 0.05$). # Significantly different to PRE-muscle damage values ($P < 0.01$). ^{a, b} Significant difference between values within respective trial ($P < 0.05$). [‡] Significant difference between values within respective trial ($P < 0.01$).

The 24h percentage change in Pressure Pain Threshold Test values significantly increased from BL values in both the SM ($P < 0.01$) and CM ($P < 0.05$) trials, suggesting significant muscle damage was present in both trials; no other timepoint values were significantly different. Within treatment differences were observed in both trials, with the 72h value showing a significant decrease from 24 and 48h values for SM ($P < 0.05$ and $P < 0.01$, respectively), and 24h values having increased significantly from 0h values in the CM trial.

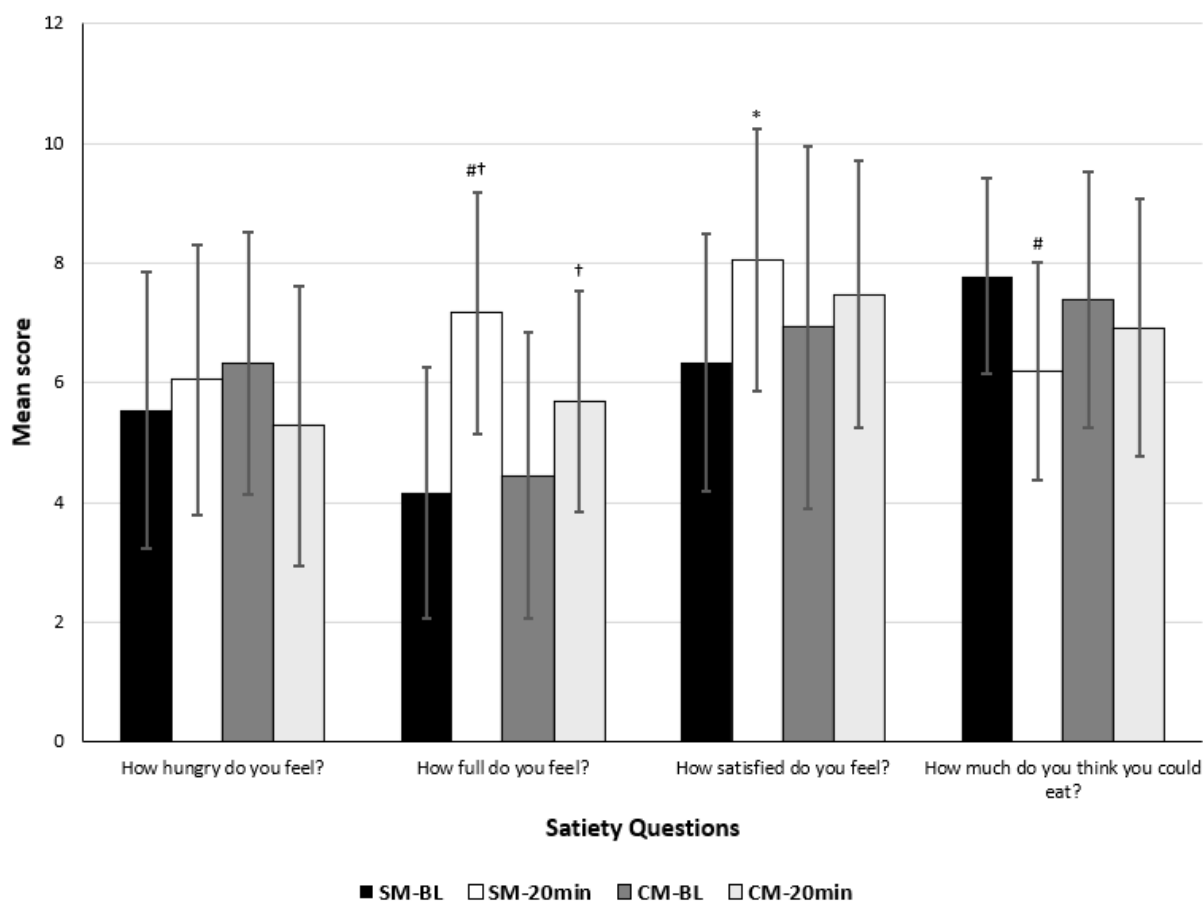
5.4 Satiety and Gastrointestinal Comfort Measures

The satiety questionnaire showed significant Time and Treatment X Time interactions for the “How full do you feel” question ($P = 0.005$ and $P = 0.048$, respectively), suggesting a difference in satiety effects between the two milk drinks. There were, however, no further differences detected for Treatment, Time, or Treatment X Time interactions for all the other questions (all $P > 0.107$). Percentage change for each of the scores, compared to BL values, were not significant for Treatment or Treatment X Time interactions (all $P > 0.136$). Time showed a potential effect, however, differences from BL values did not reach significance (all $P > 0.070$).

Figure 2 compares BL scores (following an overnight fast) with 20-min post treatment values for both treatment groups, for each question. The graph illustrates a significant increase in feelings of satiety in the SM trial, 20-min after consuming the drink, for three out of the four measures (all $P < 0.05$). No changes were detected in the CM trial (all $P > 0.05$). Additionally, the consumption of the SM drink was shown to provide greater feelings of fullness compared to the CM drink when participants were asked “How full do you feel?” 20-mins after treatment ($P < 0.05$).

Participants also filled in a gastrointestinal comfort questionnaire at BL and 20 minutes following the consumption of the allocated milk drink. Mild feelings of discomfort were felt by two participants in the SM trial and a single participant in the CM trial following the consumption of the drinks. All other participants were absent of any gastrointestinal discomfort.

Finally, immediately following consumption of the allocated drink, participants were asked to guess which treatment they believed they had received, for both trials. Six out of ten participants guessed correctly for the SM trial compared to four out of ten in the CM trial.



* Significantly different to baseline values ($P < 0.05$). # Significantly different to baseline values ($P < 0.01$). † Significant difference between treatments ($P < 0.05$).

Figure 2: Changes in perceived satiety scores before (BL) and after (20-min) treatment (mean \pm SD).

5.5 Food Diary Results

A comparison of the total average energy and macronutrient intake for the duration of each trial is displayed in Table 8. Participants recorded their food intake from 48h before the first trial day until the completion of the trial (5-days total). Data from two participants were excluded from the analysis due to having incomplete diaries; food entries were missing for entire days and for entire meals in both trials.

Total food intake was similar between both trials with no significant differences for energy, fat, and CHO intake (all $P > 0.417$). Protein intake appeared to be higher in the SM trial however differences were also not significant ($P = 0.078$). Similarly, there were no significant differences between energy

and macronutrient intakes between trials when comparing intakes from the Treatment day alone (all $P > 0.330$).

Table 8: Paired sample statistics for trial totals and treatment day totals of energy and macronutrient intakes (mean \pm SD).

	Treatment	Mean \pm SD	P value (2-tailed)
<i>Trial total</i>			
Energy (kJ)	SM	40939.23 \pm 6700.66	0.417
	CM	39041.54 \pm 4464.12	
Protein (g)	SM	539.08 \pm 108.82	0.078
	CM	451.03 \pm 55.88	
Fat (g)	SM	379.20 \pm 61.35	0.476
	CM	356.15 \pm 92.86	
CHO (g)	SM	977.09 \pm 307.49	0.738
	CM	1009.05 \pm 118.03	
<i>Treatment day total</i>			
Energy (kJ)	SM	10390.95 \pm 1756.89	0.520
	CM	9843.87 \pm 1530.83	
Protein (g)	SM	146.08 \pm 34.64	0.330
	CM	129.55 \pm 32.71	
Fat (g)	SM	104.14 \pm 21.19	0.491
	CM	95.05 \pm 28.06	
CHO (g)	SM	225.79 \pm 73.31	0.962
	CM	227.29 \pm 38.09	

Note: nutrient content of the intervention drinks were included in the analysis. Sample size, n=8.

Chapter Six: Discussion

6.1 Overview

The present study investigated the effects of a SM drink on recovery from EIMD by comparing it to an equal volume of CM drink, which is known to aid recovery after strenuous ECC exercise. Peak CON, ECC, and ISO torque, along with subjective muscle soreness was measured at BL, immediately following the muscle damage protocol, and 24, 48 and 72h later. The results from these tests were compared between treatment groups and across re-test days. The perception of satiety and gastrointestinal comfort was compared between treatment groups, as a secondary aim. The composition of both milk drinks was examined for macronutrient and amino acid content. To the author's knowledge, this study was the first to compare SM to CM in the context of recovery from EIMD.

6.2 Statement of Findings

The results from this study support the hypothesis. The results showed no significant difference between the SM group for attenuating decrements in muscular performance and perceived muscular soreness when compared to the CM group. This suggests that SM may present as a comparable and, therefore, viable alternative supplement for athletes in the context of recovering from strenuous exercise.

6.3 Sheep and Cow's Milk Drink Compositions

While SM and CM are made up of similar components, including water, whey and casein proteins, fat, and CHOs, primarily in the form of lactose, the composition of the two is different, both at the macronutrient and micronutrient levels. Interestingly, despite sheep being a smaller ruminant animal relative to cows, sheep produce a more energy and nutrient dense milk due to having a significantly higher percentage of milk solids, with differences varying depending on the time of season, breed of animal, genetics, and environmental factors (Wendorff & Haenlein, 2017). Some of the key nutrient differences of SM, relative to CM, include higher protein, fat (especially medium-chain fatty acids, mono-unsaturated and the essential polyunsaturated fatty acids), calcium, phosphorus, magnesium, and niacin, among others (Wendorff & Haenlein, 2017; Balthazar et al., 2017). In the current study, whole milk was used from both animal species, and both were flavoured

in the same way (1.5% added sugar and natural cocoa flavouring). Similar to previous summaries (Wendorff & Haenlein, 2017; Balthazar et al., 2017), the independent proximate analysis performed for this study showed higher total milk solids, protein and fat percentages, reasonably higher ash content, and almost identical CHO and lactose content, for the SM drink.

If current suggestions are correct, and the intake of a single 20g bolus of protein post-exercise will achieve maximal rates of muscle-protein synthesis (Moore et al., 2009), then it is possible that a SM drink may be a more efficient recovery aid, as a lower volume of SM can provide the required protein and all of the amino acids are present in higher amounts compared to the same volume of CM (see table 5). There may be trade-off, however, in the context of athletes who compete in a sport where body composition is important, for example when excessive fat and energy intake is undesirable. However, when considering the additional amount of CM, and therefore fat and CHO, required to achieve the same level of protein intake, then the energy differences are more likely to favour the SM beverage. Equally, the ingestion of calcium and proteins from the milk may help to attenuate the storage of adipose tissue (Hartman et al., 2007; Zemel, 2004).

Perhaps, unsurprisingly, with the SM drink containing nearly 70% more protein than the CM beverage (26.7g vs 15.9g, respectively), the SM group received a significantly higher amount of the BCAAs, which are known to play an important role in MPS, in particular leucine (Norton & Layman, 2006), as well as higher total EAAs. The leucine, isoleucine, and valine content were 2.61g, 1.35g and 1.77g for the SM drink, and 1.52g, 0.78g and 1.01g compared to the CM drink, respectively, which is a 71%, 73% and 75% greater amount for the SM. Moreover, the SM drink delivered approximately 11.92g of total EEA, compared to just 6.95g for the CM drink. Current research suggests that the ability of leucine to drive MPS occurs in a dose dependent manner, and plateaus at approximately 2g at rest (Glynn et al., 2010), and up to 3.5g when consumed during a bout of moderate intensity exercise (Pasiakos et al., 2011). However, it is important to understand that while an increased leucine concentration may stimulate increases in MPS, sustaining increased rates of MPS may require a higher total dose of all of the EAAs, since protein synthesis after resistance exercise appears to be limited by the availability of these substrates (Churchward-Venne et al., 2012; Jäger et al., 2017). The recommended dose of EAAs, to maximally stimulate MPS, is suggested to be between 8-10g (Phillips, 2011; Thomas et al., 2016; Jäger et al., 2017; Moore, 2019). Based on these recommendations alone, a SM drink, with its superior protein and EAA content, has the potential to be a more efficient post-exercise supplement for maximally stimulating MPS and recovery from exercise.

6.4 Recovery Outcomes

It is well known that EIMD is generally caused by high force ECC muscle actions that result in micro-structural damage to skeletal muscle, DOMS, impaired muscle function, and increases in intramuscular proteins in circulation (Owens et al., 2019). Therefore, in the current study, an eccentrically focused muscle damage protocol was used to achieve levels of muscle damage and decrements in muscular performance that would allow for measures of recovery to be accessed over subsequent test days. The same protocol has been extensively used elsewhere (MacIntyre et al., 1996; Barnes et al., 2010; Barnes et al., 2012; McLeay et al., 2012) and shown to bring about significant levels of muscle damage and soreness, although the number of sets was reduced by one here. Additionally, the use of a single-leg, cross-over model allowed for the minimisation of confounders including health and training status, genetics, and factors related to lifestyle. Abstinence from physical activity and alcohol consumption during the trial periods also helped to negate any possible positive or negative effects these may have had on the participants' recovery.

6.4.1 Muscular Performance

Voluntary CON, ECC, and ISO PT values significantly decreased following the completion of 200 maximal ECC contractions of the quadriceps (see table 6), showing that muscle damage occurred. The greatest decreases were observed immediately following the muscle damage protocol for CON, ECC, and ISO contractions in the CM group (-20%, -14%, and -18%, respectively), CON contractions in the SM group (-23%), and at 24h for ECC and ISO contractions in the SM group (-21% and -12%, respectively). These peak decreases in muscular performance were comparable to previous research that used a similar exercise protocol (Barnes et al., 2010; McLeay et al., 2012), and somewhat modest compared to others (Barnes et al., 2012). However, these performance measures were taken at different times between studies, so actual similarities, with reference to time, may be more or less pronounced. Including another set within the muscle damage protocol of this study may have produced greater decrements in performance, possibly bringing to light any differences between treatment groups that may have been present; this is a consideration for future research. It was evident, though, that 72h following the muscle damaging exercise participants had recovered back to within BL ranges for ISO and ECC measures for the CM group and peak ISO torque for the SM group, although mean CON, and CON and ECC scores were still slightly down for the CM and SM groups, respectively. Interestingly, peak ECC torque improved significantly at 48h vs 24h compared to 72h vs 24h values for the SM and CM groups, respectively, with no significant improvement

between 24h vs 48h tests for the CM group, which could suggest an earlier improvement occurred in the SM group. However, with no Treatment X Time effect this would only be a speculation.

There were no significant Treatment or Treatment X Time effects observed across all of the performance measures, which may indicate that neither the SM drink nor the CM drink was better at attenuating decreases in muscular performance than the other. Equally, since there is now a body of evidence that suggests that CM or CM-based drinks are useful nutritional supplements for athletes recovering from EIMD (Cockburn et al., 2008; Cockburn et al., 2010; Cockburn et al., 2012; Cockburn et al., 2013; Rankin et al., 2015), it might also be reasonable to suggest that a SM drink could provide similar benefits based on the results of this study.

6.4.2 Muscle Soreness

Similar to the muscular performance tests, a significant Time effect was observed for all three of the subjective measures for muscle soreness, when compared to BL levels; however, there were no significant Treatment or Treatment X Time interactions (see table 7). The worst level of perceived soreness was observed immediately following the muscle damaging protocol (0h) for both the step-up and squat tests, with muscle soreness ratings increasing by 275% and 258% for the SM, and 367% and 286% for the CM group, respectively. The participants' soreness progressively improved across subsequent measures and was not significantly different to BL values between 48-72h, indicating that the muscular tissue was recovering after being damaged. Similar improvements towards BL at 72h values were reported by Rankin et al. (2015), who showed a beneficial effect for blunting active muscle soreness from 0-72h for CM compared to a volume- and energy-matched CHO drink. Cockburn et al. (2010), also showed a likely benefit in reducing increases in muscle soreness at 48h compared to BL values when consuming a milk-based P-CHO drink compared to a CTRL. Perceptions of muscle soreness for the step-up movement in the present study, were also similar to what has been shown previously (McLeay et al., 2012) following a similar muscle damage protocol. Results from the pressure pain threshold test showed that peak soreness levels occurred 24h following the muscle damage exercise in both groups, with a reduction in tolerable applied force of 26% and 13% for the SM and CM groups, respectively, with improvements occurring over subsequent measures. Collectively, and in conjunction with measures of muscular performance over the same time points, these results indicate that significant muscle damage did occur.

The difference in time to peak perceptions of muscle soreness between the step-up and squat, measured on a visual analogue scale, compared to the pressure pain threshold test, may be explained by the nature of each of the tests, where both the step-up and squat measures were

based on functional movement of the damaged leg using the whole muscle, which equally experienced the worst decrements in muscular performance at 0h. Whereas the pressure pain threshold test is a more direct measure that only stimulates a small area of the whole muscle. The two styles of testing are said to measure different aspects of pain, with the pressure pain threshold test being a stimulus dependent method, compared to a response-dependent method for the visual analogue measures (Lau et al., 2013). Previous research that has assessed muscle soreness using similar methods has shown similar trends for the pressure pain threshold test, with peaks at 24h (Rice et al., 2008; Lau et al., 2013), as was shown here. However, their results from visual analogue assessments showed peak increases in muscle soreness 48h after exercise compared to 0h in the present study. It should be noted that these studies did not take any muscle soreness assessments at 0h following the exercise protocol, however, it is generally understood that muscle soreness tends to peak at around 24-48h due to the time course changes in DOMS (Cheung et al., 2003). It is hard to say why the visual analogue measures for ratings of perceived muscle soreness peaked earlier in the present study than would typically be expected, especially when muscle damage was clearly evident. It is possible that it was a function of fatigue from the exercise that caused an enhanced perception of pain in the leg at the 0h timepoint, since exercise-induced pain signals, received by the brain, can contribute to acute muscle pain in an attempt to manage the relative strain being placed on the body in order to avoid injury, or limit any damage that may occur (Mauger, 2013). One could also speculate that the recovery effects of the milk drinks played a role, but perhaps, again, greater increases in muscle soreness could have been achieved if the exercise protocol was extended, potentially eliciting greater muscular damage and feelings of muscular soreness at 24-48h like we might expect.

6.5 Satiety and Gastrointestinal Comfort Measures

When comparing groups for subjective measures of satiety using a visual analogue scale questionnaire, following the consumption of their respective milk drinks, and compared to BL feelings, SM appeared to potentially have a more satiating effect compared to the CM group with 1 out of the four questions reaching statistical significance ($P < 0.05$). No further differences were detected between groups for the other three questions, however, the SM group did show clear feelings of fullness compared to BL values, while no differences were evident for the CM group. Considering the stark differences in macronutrient composition between the two milk drinks, a possible increased satiating effect of the SM drink may be unsurprising since it contained significantly more protein and fat, and, in turn, energy compared to the CM drink. It is well known

that foods containing higher protein content can have greater satiating effects compared to other foods, even when they are matched for energy content (Morell & Fiszman, 2017). On the other hand, the consumption of higher fat foods is known to have little to no effect on increasing sensations of satiety (Blundell & MacDiarmid, 1997; Chambers et al., 2015), suggesting that if an increased feeling of fullness effect was provided by the SM drink, this was likely due to its higher protein content. Furthermore, Milan et al, (2020) recently demonstrated a greater circulating increase in the BCAAs, and several other amino acids, following the consumption of a portion-for-portion amount of SM compared CM in their randomised, double-blind, cross-over study. This increased appearance in circulation may reflect differences in protein structure, micronutrient interactions, and macrostructural effects which are known to influence digestion rates, as well as amino acid appearance, (Milan et al, 2020; Fardet et al., 2019) and could therefore play a role in the differences in satiety levels shown in the present study. It is possible that a higher feeling of satiety could have positive implications in terms of reducing athlete hunger after exercise, particularly in the context of preventing overeating for those athletes who need to manage their body weight. In addition, the sustained higher concentration of the BCAAs in SM compared to CM may have implications for stimulating MPS (Dreyer et al., 2008).

When considering GI comfort, just two participants reported feelings of mild discomfort for the SM group compared to one participant in the CM group. There was, however, no significant difference between treatment groups for GI comfort scores. This compares well to recent research that showed no difference in digestive symptoms between groups of “dairy avoiding” women who consumed an equal volume of SM and CM, despite seeing higher lactose malabsorption in the CM group (Shrestha et al., 2021).

6.6 Implications

Studies have already shown that there may be a “ceiling” effect in terms of the quantity of protein required to maximally stimulate MPS (Moore et al., 2009), so perhaps the significantly higher levels of protein that are provided by a SM drink are less important if the same amount of high-quality protein is consumed by an alternative means. In the present study, however, the CM drink did not reach the proposed “ceiling” amount of 20g, while the SM drink did. In the context of this study, however, participants diets were not administered, in order to simulate a real-world scenario, and although they were asked to eat a similar diet between trials as much as they could, it is possible that the deficits in protein and energy intake from the CM drink could have been made up across the subsequent meals (Griffioen-Roose et al., 2012); this could have negated any possible effect that the

additional protein of the SM drink may have had. There was some indication that treatment day protein intake was higher in the SM group, however this did not reach significance ($P=0.08$). Dietary analysis showed no significant differences between energy intake or any macronutrient between trials for both treatment day and trial total intakes.

Equally, there may be some merit to a SM drink if it means that the athlete does not have to consume as much of the food in order to reach their protein requirements following their session. A smaller bolus or volume of food consumed may have implications for improved digestion and absorption, particularly when SM has been shown to elicit a greater increase in circulating amino acids compared to an isovolumetric amount of CM (Milan et al., 2020). This may hold some value in the context of short recovery windows between training or competition bouts. However, with the ability of food manufacturers to increase specific nutrient contents of sports supplements, including CM-based drinks, and often for increased protein levels (Arenas-Jal et al., 2020), the increased protein load from a natural (i.e. no added dry milk powder components) SM drink may no longer have the edge over other drinks that are, in essence, fortified with additional protein. One would have to question whether there is a viable benefit of gaining these nutrients from natural and less processed sources, in the case of a less processed, lower ingredient, SM drink, when the same nutrients may be obtained by a similar drink that has been fortified with the desired nutrients. However, there is already evidence to suggest that not all sports supplements provide the nutrients that they advertise, with some ingredients not being nutritionally available to their consumer (Pellegrino et al., 2022), raising consumer concerns for the food safety of these supplements. Further research would be needed to identify whether or not there is greater benefit from a more “natural”, nutrient dense SM source compared to formulated sports supplements that are derived from CM sources. However, in the context of this study, SM does appear to contend well with whole, non-fortified CM that is flavoured in the same way.

6.7 Limitations and Recommendations for Future Research

A limitation was that the single-leg model employed here did not allow for a negative CTRL group since we were only able to damage two legs (two cross-over trials); one for the CM treatment (positive-control) and one for the SM treatment (test group). Future studies should look to compare both milk groups to a placebo CTRL group with no cross-over. In addition, the level of muscle damage and subsequent decrements in muscular performance were relatively modest in the present study which may have dampened any differences observed between interventions, further limiting the interpretability of the results. Therefore, future studies should ensure that the protocol used will

cause decrements in performance sufficient enough to provide a clearer indication on whether or not differences between treatments do exist.

Another limitation was the lack of control over the participants' diets during the trials. While there was no significant difference apparent between trials, in terms of energy and macronutrient intake, and the uncontrolled diet helped to replicate free-living conditions, future research that controls dietary intake may be able to make greater inferences about the results, since the test drinks would be the only nutrient source that would be different between trials.

Further research should also compare a CM drink matched for energy and protein (perhaps in two separate trials) to a SM drink to understand if there are any benefits prevalent from the milk itself, or if there may instead be benefits, if any, related more to a function of total energy, or protein intake at the macronutrient level.

In addition, when considering the ability of food manufacturers to create sports supplements with concentrated levels of sought-after nutrients, such as protein, it may be worth understanding if there is a benefit to consuming a minimally processed whole SM drink, with its evidently high nutritional properties, compared to sports supplements that have been fortified with additional dry ingredients for an improved nutritional composition, that may also cost more.

Chapter Seven: Conclusion

The purpose of this study was to compare the potential nutritional benefit of consuming a SM beverage versus an isovolumetric CM beverage on acute performance recovery following strenuous ECC exercise.

This study showed that SM provides significantly higher amounts of energy via a higher protein and fat content, which included over 70% more BCAAs for the same volume. Results for PT for CON, ECC and ISO contractions of the quadriceps muscle group from BL, 0h, 24h, 48h, and 72h measures showed no significant difference between treatment groups. Similar results were shown for subjective measures of muscular soreness across the same timepoints. This suggests that SM could be a viable alternative supplement for athletes, in the context of recovering from strenuous exercise, with the potential to offer similar recovery benefits to those that have already been discovered for CM supplements. However, further research is needed to better understand the differences between the two milks and the possible implications this may have in a sports recovery setting.

Chapter Eight: References

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Chapter Nine: Appendices

9.1 Nutritional Comparison of Cow's and Sheep's Milk

Table 9: Proximates, fat, carbohydrate, vitamin and mineral nutrient quantities of cow's milk and sheep's milk^a

Nutrient	Unit	Cow's Milk		Sheep's Milk	
		Quantity per 100mL	%DI* per 100mL	Quantity per 100mL	%DI* per 100mL
Proximates					
Water	g	87.6	-	83.1	-
Energy	kJ	278	3	414	5
Protein	g	3.5	7	5.5	11
Fat, total	g	4.0	6	6.5	9
- Saturated	g	2.5	10	2.98	12
Carbohydrates	g	4.2	1	4.8	2
- Sugars	g	4.2	5	4.8	5
Dietary Fibre	g	0.0	0	0	0
Ash	g	0.7	-	0.9	
Fats					
Fat, total	g	4.0	6	6.5	9
Saturated	g	2.50	10	2.98	12
Monounsaturated (MUFA)	g	0.85	-	1.09	-
Polyunsaturated (PUFA)	g	0.14	-	0.33	-
Trans fatty acids	g	0.06	-	0.27	-
Omega-3 fatty acids	g	0.04	-	0.12	-
Cholesterol	mg	13.0	-	19.0	-
Carbohydrates					
CHO available	g	4.2	1	4.8	2
Sugars, total	g	4.2	5	4.8	5
Lactose	g	4.2	-	4.8	-
Dietary Fibre	g	0.0	0	0.0	0
Vitamins					
A	µg	39	5	61	8
B1 (Thiamine)	mg	0.03	3	0.11	10
B2 (Riboflavin)	mg	0.18	11	0.41	24
B3 (Niacin)	mg	0.77	8	1.60	16

B5 (Pantothenic acid)	mg	0.30	6	N/A	
B6 (Pyridoxal phosphate)	mg	0.02	1	0.06	4
B7 (Biotin)	µg	N/A		N/A	
B9 (Folate)	µg	0.0	0	0	0
B12 (Cobalamin)	µg	0.0	0	0.48	24
C (Ascorbic acid)	mg	0.4	1	0.0	0
D	µg	0.0	0	2.8	28
E (Tocopherols)	mg	0.09	1	0.12	1
K	µg	N/A		N/A	
Minerals					
Calcium	mg	116	14	183	23
Copper	mg	0.0	0	0.02	1
Fluoride	µg	N/A		N/A	
Iodide (Iodine)	µg	9.7	6	170	113
Iron	mg	0.0	0	0.0	0
Magnesium	mg	11	3	17	5
Manganese	µg	3	0	8	0
Phosphorus	mg	88	9	150	15
Potassium	mg	168	-	133	-
Selenium	µg	1.2	2	2.7	4
Sodium	mg	42	2	39	2
Zinc	mg	0.33	3	0.61	5

^aTable adapted from the “basic reports” for “Milk, cow, whole 4% fat, fluid, non-homogenised” & “Milk, sheep, whole, fluid, fresh” from New Zealand Food Composition Data (n.d). *Percentage Daily Intakes are based on an average adult diet of 8700 kJ as specified by Food Standards Australia and New Zealand (FSANZ). N/A denotes no data available.

9.2 Participant Information Sheet

PARTICIPANT INFORMATION SHEET

Project Title: The effects of sheep's milk on muscle recovery.

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 19/22. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@massey.ac.nz.

The research team for this project is:

Mr. Ben Ravenwood **Masters Student**

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Prof. Jane Coad Professor in Nutrition, School of Food and Advanced Technology
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Introduction:

Thank you for your interest in this study. I am in my thesis year of my Masters of Science in Human Nutrition and this is the direction I have chosen for my research project. There is not a lot of scientific information about sheep milk in a sports and exercise setting so I hope to add something worthwhile to the literature.

The wider sports industry is constantly evolving in order to find new, legal technologies and ergogenic aids, which may provide athletes with a performance advantage. This includes a range of strategies to allow athletes to perform at their peak for as long as possible. Recovery from exercise-induced muscle damage (EIMD) is critically important for both professional and recreational athletes, since EIMD can impair muscular strength and power, and general muscle function, which in turn affects athletic performance. Post-exercise nutrition is often a focus for athletes as it can play a significant role in how well an athlete recovers between competition days and even between training sessions. Flavoured cow's milk products, such as chocolate cow's milk drinks, have previously been shown to work as well as, if not better than, a number of branded sports drinks as a recovery aid after strenuous exercise. Sheep milk, on the other hand, has not previously been investigated in an exercise recovery setting. With its unique characteristics, including significantly higher protein and energy contents, as well as potentially being easier to digest, there is reason to believe that sheep milk may be of some significant benefit to athletes for their recovery.

The purpose of this study therefore, is to compare the effects of a sheep milk drink with that of a cow's milk drink on muscle performance after eccentric exercise.

Participant Recruitment:

We are recruiting 10 healthy and active males aged between 18 and 40 years of age to participate in an experiment to investigate **how sheep milk may assist performance recovery** after eccentric lower limb exercise.

To participate, you must be physically active (strength train 2 x per week or more) and have at least 2 years of strength training experience. You must have a tolerance for lactose (dairy sugar). You must also be free from any injury that may affect your ability to perform the maximal knee extension exercise being used for this study.

All participation is voluntary, and you may withdraw from participating in the study at any time. If you agree to participate, you will be asked to attend a brief familiarisation session followed by 2 trials during which time your muscular strength will be tested. These sessions will take place under supervision in the Human Performance Laboratory (HPL) of the School of Sport, Exercise and Nutrition at Massey University.

Please do not hesitate to contact any of the researchers above if you have any questions about this project at any time.

Project Procedure:

At least 7 days before the first trial begins, you will be asked to come into the lab for a 40-50 minute familiarisation session. Firstly, you will be asked to fill in a pre-exercise health screening questionnaire so that we can be sure that it is safe for you to take part in this study. You will then have your height and weight measured, followed by an introduction to the Biodex isokinetic dynamometer machine. This machine will be used to measure the maximal performance of your quadriceps, as well as being used for the muscle damage protocol.

On the trial day you will be required to come into the Human Performance Lab (HPL) at an agreed upon time in the morning after an overnight fast of at least 10 hours. You will also be required to refrain from any exercise (except for any necessary walking and normal day-to-day work-related activity) and alcohol consumption in the 48 hours (2 days) before arriving at the lab.

On arrival, a perceived muscle soreness test and questionnaire will be carried out as a baseline measure for the thigh muscles (quadriceps group) of a specified leg. This will involve a squat, a step up, and the use of a pain testing tool on the front of the thigh. By applying pressure to the thigh, this tool measures the amount of force needed to elicit a sensation of pain. Following this, and after a short warm-up, you will perform 3 maximal concentric, eccentric and isometric efforts of the quadriceps on the Biodex machine. You will then be required to perform 200 maximal eccentric contractions across the knee joint. Finally, in order to measure fatigue after exercise, you will perform the 3 maximal effort tests again. This first trial should take about 50 minutes to complete.

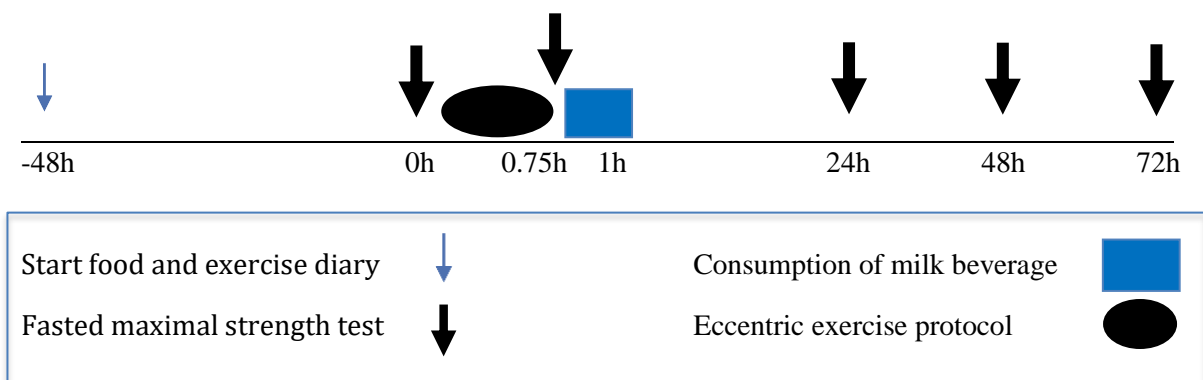
Immediately after the exercise you will repeat the perceived muscle soreness test and questionnaire and then be given a chocolate flavoured sheep milk or cow milk drink to consume. 20 minutes later you will be asked to fill in a gut symptom questionnaire. Following this you will be offered breakfast before leaving the lab and go about your day.

During the following 3 days you will be required to come back into the lab each morning, after an overnight fast of at least 10 hours, to repeat the measures for perceived muscle soreness, and muscular performance of the same leg. This should take no more than 20 minutes. During this 3-day period, you will be requested not to consume any alcohol and to not take part in any exercise, apart from necessary

walking and normal day-to-day work-related activity. At the end of each visit you will be offered breakfast as well.

Trial 2 will occur at least 10 days after the final performance measure of the first trial, and you will repeat the same protocol as described above, but using the other leg, after which you will consume the other drink (sheep or cow milk). Drink order will be allocated randomly.

During each of the trials, from 2-days before to 3-days after the first visit, you will be asked to record everything you eat and drink, and your activity in a diary which will be given to you on your familiarisation visit. To complete the food diary, you will need to specifically note what you eat and drink and in what quantity and note the time of the day you consume it. We also ask that you try to consume the same foods during your second trial, as you did during your first trial as much as possible.



Further Information:

For each visit to the lab we ask that you bring/wear appropriate clothing for exercise, specifically shorts and covered shoes. There is a private room at the HPL for you to get changed in and have a shower if you wish.

Possible Risks:

The procedures involved in this study are of low risk, however, as with all exercise there are small risks, and some discomfort may be experienced.

Eccentric exercise.

You are likely to experience fatigue, a decrease in muscle function and delayed on set of muscle soreness (DOMS) associated with strenuous exercise during and after the maximal eccentric contractions. Nevertheless, as in any physical activity, there is a very small possibility of injuries that include, but are not restricted to, muscle, ligament or tendon damage and dizziness. However, all protocols are commonly performed in exercise physiology laboratories and potential risks to participants have been minimized.

Exercising fasted.

In general, there is minimal risk in exercising during a fast, however you may experience feelings of hunger and a feeling of having slightly less energy than normal when you first arrive to the lab. There is a very small possibility that you may start to feel dizzy or slightly faint, in which case you should notify the researcher. However, as above, this protocol has been performed before and participant risk has been minimized.

Cardiac Arrest

You should be aware that even amongst healthy persons who undertake regular physical activity there is a risk of sudden death during exercise due to cardiac arrest. Although extremely rare, such cases can occur in people with an undiagnosed heart condition. In the event of cardiac arrest, the HPL is equipped with an automated defibrillator. Staff involved in this research are trained in the use of this apparatus and hold current first aid certificates.

Remuneration:

As a thank you for your time and participation you will be gifted \$50 at the conclusion of your final visit to the lab. You may also request to receive information related to your dietary intake after it has been analyzed.

Data Management:

The data will be collated into one document that refers to each participant by number, so that there is no association by name. This ensures that you will remain confidential.

Participant's Rights

- You can ask questions on any aspect of the project at any time, and we will do our best to answer them to your satisfaction.
- As a participant in the study you will provide information on the understanding that your name will not be used unless you give permission to the researcher.
- You have the right to view your own data at any stage and have it explained to you.
- You will also be given access to a summary of the project findings when it is concluded.
- You can withdraw from the project at any time, without giving any reason and without penalty.

Exclusion Criteria

If any of the following apply:

- *You have a known heart or cardiovascular condition or if a member of your family died below the age of fifty (50) as a result of a heart condition.*
- *You have any current or previous injury to your legs*
- *In the last six months you have suffered from any painful injury or condition that lasted more than one week.*
- *You have had an injury or medical condition that you think may affect your ability to sense pain or discomfort.*
- *You have ever had persistent or regular lower back pain.*
- *You are taking prescribed medication, including over-the-counter pain relief or anti-inflammatories, that may affect your ability to exercise, or sense pain and discomfort.*
- *You have cultural or religious sensitivities about human body measurements.*
- *You have any other reason to consider that you are not in good health and of average, or better than average, fitness.*
- *You are diabetic.*

- *You are lactose intolerant or have an intolerance to dairy products*

...you should **NOT** participate in this project.

Compensation for Injury:

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury. If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

9.3 Pre-Exercise Health Screening Questionnaire

Pre-Exercise Health Screening Questionnaire

Research topic: The effects of sheep's milk on muscle recovery

Name: _____

Address: _____

Phone: _____

Email: _____

Age: _____

Please read the following questions carefully. If you have any difficulty, please advise the researcher who is conducting the study. If you answered yes to any of the questions below more information may be requested to accurately assess your suitability to participate in this study.

This questionnaire has been designed to identify the small number of persons (18-40 years of age) for whom physical activity might be inappropriate. The questions are based upon the Physical Activity Readiness Questionnaire (PAR-Q), originally devised by the British Columbia Dept. of Health (Canada), as revised by ¹Thomas *et al.* (1992) and ²Cardinal *et al.* (1996), and with added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

Please answer all of the following questions by circling only one answer for each question, and providing further details where necessary:

- ***Do you have a known heart or cardiovascular condition and/or has a member of your family died below the age of fifty (50) as a result of a heart condition?***

Yes No

If "Yes", please specify:.....
.....

- ***Do you have any current or previous injury to your legs that may be aggravated by strenuous exercise?***

Yes No

If "Yes", please specify:.....
.....

- *In the last six months, have you suffered from any painful injury or condition that lasted more than one week?*

Yes No

If "Yes", please specify:.....
.....

- *Have you had or do you have an injury or medical condition that you think may affect your ability to sense pain or discomfort?*

Yes No

If "Yes", please specify:.....
.....

- *Are you taking prescribed medication, including any over-the-counter pain relief or anti-inflammatories?*

Yes No

If "Yes", please specify:.....
.....

- *Do you have any cultural or religious sensitivity regarding measurements of the human body?*

Yes No

If "Yes", please specify:.....
.....

- *Do you have any known allergies to foods?*

Yes No

If "Yes", please specify:.....
.....

- *Are you dairy or lactose intolerant?*

Yes No

- *Do you typically experience any gut/stomach discomfort after the consumption of any dairy products?*

Yes No

- **Have you been hospitalized recently?**

Yes No

If "Yes", please specify:.....
.....

- **Have you experienced medical complications as a result of soft tissue/contusion injury in the past?**

Yes No

If "Yes", please specify:.....
.....

- **Do you have any other reason to consider that you are not in good health and of average, or better than average, fitness?**

Yes No

If "Yes", please specify:.....
.....

You should be aware that even amongst healthy persons who undertake regular physical activity there is a risk of sudden death during exercise. Though extremely rare, such cases can occur in people with an undiagnosed heart condition. If you have any reason to suspect that you may have a heart condition that will put you at risk during exercise, you should seek advice from a medical practitioner before undertaking an exercise test.

I have read, understood and completed this questionnaire.

Signature: _____ Date: _____

References

1. Thomas S, Reading J and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 17(4): 338-345.
2. Cardinal BJ, Esters J and Cardinal MK. Evaluation of the revised physical activity readiness questionnaire in older adults. *Med Sci Sports Exerc* 28(4): 468-472

9.4 Informed Consent Form



Informed Consent Form

Title of project: The effects of sheep's milk on muscle recovery

Investigator(s): Ben Ravenwood, Masters student

Supervisor(s): Dr. Matthew Barnes, Senior Lecturer, Deputy Head in the School of Sport, Exercise and Nutrition

Prof. Jane Coad, Professor in Nutrition, School of Food and Advanced Technology

Please read the following statements, and if you agree, please check the corresponding box to confirm agreement:

	Check
I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	<input type="checkbox"/>
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason.	<input type="checkbox"/>
I understand that my data will be treated confidentially and any publication resulting from this work will report only data that does not identify me.	<input type="checkbox"/>
I freely agree to participate in this study.	<input type="checkbox"/>

Signatures:

Name of participant (block capitals) Date Signature

Researcher (block capitals)

Date

Signature

If you would like a copy of this consent form to keep, please ask the researcher. If you have any complaints or concerns about this research, you can direct these, in writing, to Matt Barnes by email at: M.Barnes@massey.ac.nz

9.5 Food and Activity Diary

5-Day Food and Activity Diary

Study title: The effect of sheep's milk on muscle recovery.

Food and Activity Diary Instructions

As part of your participation in this study we ask that you complete the food and activity diary as accurately as possible, to the best of your ability. You will record all of your food and activity in this diary from the 2 days (48 hours) before your first visit to the lab until your final visit to the lab which will be 3-days (72 hours) later. If after reading these instructions you have further questions regarding the food and activity diaries, please contact Ben Ravenwood: phone

██████████.

Food Dairy

It is important that:

- You please do not change your eating habits while keeping this food diary
- You record food as you eat it, do not wait to record later
- You only record the portion of food you actually consume
- When you record a food that consists of a combination of foods, please break it down into individual components: i.e.
Chicken sandwich:
 - o 2 slices of Tip Top white bread
 - o 100g deli chicken breast
 - o 15g fresh Leader Brand baby spinach
 - o 1 tablespoon Heinz mayo
- You record any beverages that you consume, e.g. water, juice, coffee
- You record any condiments you consume as well, e.g. tomato sauce, salt, pepper

The general information you will be asked to record on your food diary is:

- Time of eating, e.g. 9am
- Name and type of food/beverage, e.g. peanut butter, egg, latte
- The quantity/amount consumed of each item, e.g. teaspoon, tablespoon, ½ cup, small, medium, large, 2 x 50g servings, 100g, palm sized or fist sized portion, golf ball sized, 250ml etc. *It is important to use packet information as a guide, and to use measuring instruments (i.e. cups and spoons) where possible*
- The brand name, e.g. Pam's, Anchor, Wattie's, Cadbury
- Preparation method, e.g. boiled, fried, grilled, oven baked, roasted, microwave, including any oils and seasonings used
- Restaurant name, e.g. McDonald's, Café Cuba, Subway
- Details about each food and beverage you consume are crucial to us, so please include as many details as you can

Please start your diary in the morning, 2 days before you are scheduled to visit the lab for the trial, e.g. If your trial day is Monday morning, begin your food (and activity) diary from Saturday morning when you wake up.

Activity diary

- **Please remember that as part of this study, and during this activity recording period, you are asked not to participate in any physical activity, except for necessary walking**
- Please record your activity as often as you can, especially when you change from one activity to another, e.g. from sitting to walking to the bus stop
- Examples of activities: walking, sitting, getting ready for work, showering, sleeping etc.
- If more than one activity occurred in each half hour, please note each one indicating the rough duration of each e.g. sitting and studying 20mins, walking to carpark 10mins
- The activity diary for each day can be found behind the food diary for the same day

Activity Record – Day 1

(2 days before trial day)

Name: _____

Date: _____

Phone Number: _____

Trial Number: _____

Time	Activity - Morning
12:00-12:30am	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00pm	

Time	Activity – Afternoon/Evening
12:00-12:30pm	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00am	

Activity Record – Day 2

(1 day before trial day)

Name: _____

Date: _____

Phone Number: _____

Trial Number: _____

Time	Activity - Morning
12:00-12:30am	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00pm	

Time	Activity – Afternoon/Evening
12:00-12:30pm	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00am	

Activity Record – Day 3

(Trial day)

Name: _____

Date: _____

Phone Number: _____

Trial Number: _____

Time	Activity - Morning
12:00-12:30am	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00pm	

Time	Activity – Afternoon/Evening
12:00-12:30pm	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00am	

Activity Record – Day 4

(1 day after trial day)

Name: _____

Date: _____

Phone Number: _____

Trial Number: _____

Time	Activity - Morning
12:00-12:30am	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00pm	

Time	Activity – Afternoon/Evening
12:00-12:30pm	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00am	

Food Record – Day 5

(2 days after trial day)

Name: _____

Date: _____

Phone Number: _____

Trial Number: _____

Time	Food/Beverage Consumed	Quantity/Amount e.g. grams, cups, mL, teaspoon, tablespoon, Number/count, golf ball size, palm size	Brand Name	Preparation Method e.g. boiled, fried, grilled, oven baked, roasted, microwave, oil/salt used/added?

Activity Record – Day 5

(2 days after trial day)

Name: _____

Date: _____

Phone Number: _____

Trial Number: _____

Time	Activity - Morning
12:00-12:30am	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00pm	

Time	Activity – Afternoon/Evening
12:00-12:30pm	
12:30-1:00	
1:00-1:30	
1:30-2:00	
2:00-2:30	
2:30-3:00	
3:00-3:30	
3:30-4:00	
4:00-4:30	
4:30-5:00	
5:00-5:30	
5:30-6:00	
6:00-6:30	
6:30-7:00	
7:00-7:30	
7:30-8:00	
8:00-8:30	
8:30-9:00	
9:00-9:30	
9:30-10:00	
10:00-10:30	
10:30-11:00	
11:00-11:30	
11:30-12:00am	

9.6 Muscle Soreness Questionnaire

Muscle Soreness Questionnaire

Study title: The effects of sheep's milk on muscle recovery

Subject Name: _____

Date: _____

Trial no. + timepoint:
e.g. T1 - 0h Pre-trial, 0h Post, 24h, 48h or 72h

Leg being tested: _____

Participant's guess of milk drink consumed:

Sheep milk

Cow milk

Using a vertical line, please mark on the lines below the level of soreness experienced in your test leg when performing the following exercises.

Stepping Up

1 **10**

Squatting

1 **10**

1 = No soreness

10 = Unbearably sore/extreme soreness

Mechanical nociceptive threshold test (belly of vastus lateralis muscle)

Reading 1: _____ **Reading 2:** _____ **Reading 3:** _____

9.7 Gastrointestinal Comfort and Satiety Questionnaire

Satiety and Gastrointestinal Comfort Questionnaire

Study title: The effects of sheep's milk on muscle recovery

Subject Name: _____

Date: _____

Trial number: _____

Leg being tested: _____

For the following questions please place a mark on the line that fits best with how you are currently feeling.

How hungry do you feel?

Not hungry at all

Extremely hungry

How full do you feel?

Not full at all

Extremely full

How satisfied do you feel?

Not satisfied at all

Extremely satisfied

How much food do you think you could eat?

Nothing at all

A very large amount

Have you experienced any of the following since consuming the milk drink?
(please tick the boxes that apply)

	Absent	Mild	Moderate	Severe
Nausea				
Diarrhoea				
Flatulence/wind				
Rumbling/gurgling				
Abdominal cramps				
Abdominal pain				
Bloating				

Definitions:

Absent – No symptoms

Mild – Nagging or annoying

Moderate – Strong negative influence on your daily living

Severe - Disabling