A Comparison between the effects of a Whey Protein Drink and Trim Milk on Rehydration after Exercise in the Heat.

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Abstract

Background: Beginning an exercise session euhydrated is important for performance and health. Rapid and adequate rehydration is important for many athletes, especially those partaking multiple sessions of exercise each day, or those involved in weight category sports. The macronutrient and electrolyte concentration of the fluid ingested following exercise can affect the amount retained within the body and so can influence hydration status. However, the optimal rehydration beverage composition is currently unknown. Electrolytes and carbohydrates have been thoroughly studied, however the role of protein in rehydration is yet to be determined.

Objective: To compare the effect of a commercially available whey protein beverage against trim milk, in terms of rehydration after exercise induced dehydration.

Design: Ten healthy active males aged 23.1 (1.5) years provided written informed consent prior to participating in the study. All trials commenced between 17:30 and 18:00 hours and were separated by at least one week. For the two trials, participants cycled in the heat (35ºC and 65% RH) until 1.89 ± 0.36% of their body mass was lost. They then consumed either whey protein or trim milk in a randomised order replacing 150% of body mass losses in the hour post-exercise. Urine samples were collected pre-exercise, 1 hour post, 2 hours post, and first void of the following morning.

Results: Urine specific gravity values the following morning were not different between the whey trial (1.020 ± 0.004) and the milk trial (1.021 ± 0.005) ($p=0.684$). Total urine output was also not different between the whey trial (1498.0 ± 245.6mL) and the milk trial (1325.5 ± 426.4mL) ($p=0.150$). At the end of the study, compared to baseline, net fluid balance was negative for the whey trial (-733 ± 223mL) ($p<0.001$), and the milk trial (-544 ± 362mL) ($p<0.001$), and between the two drink trials, final net fluid balance was not different ($p=0.088$).

Conclusion: The main finding of this study is that a whey protein beverage is no better or worse at rehydrating than a trim milk beverage**.** Uniquely, the present study shows that athletes who exercise in the evening and follow the current rehydration recommendations of consuming 1.5L for every 1kg body mass lost during exercise, were likely to wake up the next day in a hypohydrated state. This would mean that more fluid would need to be ingested before beginning another bout of exercise. Previously such an overnight protocol has not been utilised in rehydration studies.

Preface

This research project was supervised by Dr Katherine Black from the Department of Human Nutrition, University of Otago, and co-supervised by Dr Nancy Rehrer from the Department of Physical Education, Sport and Exercise Sciences, University of Otago, and Dr Thomas Love from the Applied Sports Technology Exercise and Medicine Research Centre, Swansea University, Wales.

The Candidate was responsible for the following under supervision:

- Participant recruitment and screening
- Communicating with study participants
- Administering food records, and questionnaires to participants
- Data collection and data entry
- Compilation of results and carrying out statistical analyses
- Interpreting results and drawing study conclusions

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List of Abbreviations

American College of Sports Medicine	ACSM
Bioelectrical Impedance Assay	BIA
Carbohydrate	CHO
Centimeter	cm
Coefficient of Variation	CV
Degrees Celsius	°C
Grams	g
Gastrointestinal	GI
Kilograms	kg
Kilojoules	kJ
Litre	L
Milligram	mg
Millilitres	mL
Millimetre	mm
Milliosmol	mOsmol
Muscle Protein Breakdown	MPB
Muscle Protein Synthesis	MPS
Recommended Daily Intake	RDI
Relative Humidity	RH
Standard Deviation	SD
Urine Specific Gravity	USG

1 Introduction

The human body contains between 45-70% water, depending on body composition (Naghii, 2000). During exercise, especially in the heat, body temperature rises and sweat rate is increased. Sweating is the primary method for the body to remove heat (up to 80% of heat loss) and therefore sweat losses can be quite large (Rodriguez, 2009; Sawka et al., 2007). Dehydration by >2% body mass can impair thermoregulation, compromise cardiovascular function, lower exercise ability, decrease mental/cognitive ability, and increase risk of heat stroke (MacLeod & Sunderland, 2012; Naghii, 2000). Dehydration in athletes is a common problem, in situations such as weight category sports, athletes will purposefully dehydrate themselves in order to “make weight” (Clark, Bartok, Sullivan, & Schoeller, 2004). It is also not uncommon for athletes to train twice per day, putting them at risk of starting the next bout of exercise in a hypohydrated state, especially if their sweat losses were large during the first session. This is further compounded by the desire of some athletes to train or compete on an empty stomach to reduce gastrointestinal distress, meaning food and fluid consumption is limited (Stannard, Buckley, Edge, & Thompson, 2010). These athletes require a beverage which optimises rehydration in a short timeframe to ensure they are able to perform their best at their sport.

The American College of Sports Medicine (ACSM) recommends that athletes prevent excessive fluid loss (>2% body weight) and avoid large changes in electrolyte balance throughout exercise (Sawka et al., 2007). If rapid rehydration is required post-exercise, then it is recommended to consume 1.5L of fluid for every 1kg body mass lost during exercise (Sawka et al., 2007).

Drinking water alone dilutes blood (lowers osmolality), leading to diuresis and excretion of water, making it an inefficient rehydration beverage (Leser, 2011; Maughan & Leiper, 1995; Nose, Mack, Shi, & Nadel, 1988). The presence of sodium in the recovery drink has a large influence on rehydration. A linear relationship exists between sodium content of a drink and the level of fluid retention (Maughan & Leiper, 1995; Shirreffs, 1998). This is because it reduces the effect of water reducing plasma osmolality, therefore preventing diuresis (Nose et al., 1988). Carbohydrate is recommended post-exercise for replenishing glycogen stores. Carbohydrate has also shown a mild effect on fluid retention during rehydration (Osterberg, Pallardy, Johnson, & Horswill, 2010).

Protein is important for athletes for optimal recovery of muscle tissue. Milk protein (containing approximately 80% casein, 20% whey) in a rehydration beverage has shown to be at least as good as a carbohydrate-electrolyte beverage at rehydration (James, 2012; Shirreffs, Watson, & Maughan, 2007; Watson, Love, Maughan, & Shirreffs, 2008). This is likely due to milk having similar amounts of carbohydrate (39-64g/L) and similar or larger amounts of electrolytes compared with a carbohydrate-electrolyte drink. However new research is showing that milk protein, when matched for energy density and electrolytes, has a beneficial effect on rehydration when compared with a carbohydrate-electrolyte drink (James, Clayton, & Evans, 2011).

There have been mixed results in research so far, about the efficacy of whey protein in aiding rehydration. Initially an improvement in fluid retention was seen when adding 15g/L whey protein to a drink, however when matched for energy density of the drink, this effect was not seen (James, Gingell, & Evans, 2012; Seifert, Harmon, & DeClercq, 2006).

Therefore the aim of this thesis is to compare the effect of a whey protein beverage against trim milk, in terms of rehydration after exercise induced dehydration. While milk and its effects on rehydration have been studied a lot in recent years, there is yet to be a study looking directly at a whey protein beverage in comparison to a milk beverage meeting the guidelines for post exercise protein intake (20 g) as well as also meeting the fluid replacement guidelines.

2 Literature Review

2.1 Why hydration and therefore rehydration is important to athletes

As the human body contains between 45-70% water (with the water content of fat free mass being approximately 72 % whereas fat mass contains very little water) (Naghii, 2000; Sawka, 1992; Tzamaloukas, Murata, Vanderjagt, & Glew, 2003) it is of little surprise that fluid balance is important for health and performance. The lack of water in the body leads to cell dehydration, which if not corrected, can lead to death within a few days (Naghii, 2000). This could be of concern for athletes as during exercise, particularly in heat, body temperature rises and sweat rate is increased. This occurs as the body attempts to thermoregulate, as sweating is the primary method of the body to remove the heat (up to 80% of heat loss) and therefore sweat losses can be quite large (Rodriguez, 2009; Sawka et al., 2007).

Elite endurance athletes can lose more than 1.5L of sweat every hour of exercise which makes staying hydrated very difficult, especially when the gastrointestinal system can only absorb about 1L per hour (Naghii, 2000). Dehydration by >2% of body mass can impair thermoregulation, compromise cardiovascular function, lower exercise capacity and increase the risk of potentially life-threatening injury like heat stroke (Naghii, 2000; Rodriguez, 2009). Dehydration effects performance through increased glycogen utilization, distorted metabolic function and possibly altered central nervous system function (Sawka et al., 2007). Such levels of dehydration can also lead to a decreased mental/cognitive ability which can effect athletes performance, especially in team based technical sports (MacLeod & Sunderland, 2012).

In weight category sports it is common for athletes to purposely dehydrate in order to "make weight" (Clark et al., 2004; Sawka et al., 2007), this means that they are at risk of starting competitions hypohydrated. Furthermore, it is also not uncommon for athletes to train twice per day, if sweat losses are large in the first session of the day then this means that the athlete is at risk of starting the second session hypohydrated. Starting exercise hypohydrated has also been associated with impaired performance (Kalman & Lepeley, 2010). Therefore these athletes need to rehydrate as quickly as possible as the time period between the weigh in and competition is normally about 3 hours (Maughan & Leiper, 1995). This is further compounded by the desire of some athletes to train or compete on an empty stomach to reduce gastrointestinal distress, meaning food and fluid consumption is limited (Rodriguez, 2009; Stannard et al., 2010). Therefore, these athletes require a beverage which will optimise rehydration allowing them to perform at their best and reduce the risk of suffering from heat injury.

Despite knowing the negative effects of low body water, it is common for athletes to start training hypohydrated (Maughan & Shirreffs, 2010). This is shown to amplify the effects of the dehydration during exercise (Maughan & Shirreffs, 2010). This may also occur during days of multiple bouts of exercise (e.g. tournaments) as the thirst mechanism may not be enough to promote proper rehydration between games (Oppliger & Bartok, 2002). Hence it is important for these athletes to have hydration strategies in place to ensure they can perform at their peak (Oppliger & Bartok, 2002).

Athletes should aim for adequate hydration before, during, and after exercise for optimal performance and recovery (Rodriguez, 2009; Sawka et al., 2007). The American College of Sports Medicine recommends that athletes ingest 1.5 times their training body mass losses in the recovery period (Sawka et al., 2007).

In 2003, Keller *et al* conducted a study analysing the cell volume hypothesis (Keller, Szinnai, Bilz, & Berneis, 2003). This hypothesis shows that as the body dehydrates, plasma osmolality increases, drawing water out from the cells. This causes the cells to shrink, which in turn leads the cells to favour glycogenolysis (glycogen breakdown) and potentially protein breakdown (though this was only seen *in vitro* and not *in vivo*) (Keller et al., 2003). In contrast, a well hydrated swollen cell favours lipolysis (fat breakdown) and minimizes protein and glycogen breakdown. Thus adequate rehydration after exercise could potentially lead to desirable body composition and potentially spare glycogen stores, which could improve exercise performance (Leser, 2011).

2.2 Optimal composition of a recovery solution

Carbohydrates, electrolytes and water are the key nutrients for normal physiological function and optimal exercise performance (Rehrer, 2001). During exercise, water and electrolytes are lost through sweat. Muscle and liver glycogen stores are broken down with exercise and can rapidly be depleted. This is why during exercise, and especially afterwards (in preparation for the next exercise session), it is important for these to be replenished during recovery, particularly if more exercise will be undertaken within a short timeframe.

Rehydration is composed of 3 interrelated components: gastric emptying, intestinal absorption and fluid retention (Leser, 2011). When recovery time is short, it is recommended to drink 1.5L of fluid for every kilogram of weight loss to fully rehydrate and to choose drinks that replaces lost electrolytes with about 0.3-0.7g sodium per litre (Sawka et al., 2007; Shirreffs, 1998). Even in a dehydrated state, the body continues to create urine to remove waste products and water is also lost in small amounts through respiration (Shirreffs, Armstrong, & Cheuvront, 2004). In 1996, Shirreffs *et al* found that when adequate sodium was included, 1.5L per kg of body mass loss was enough to end the recovery period (6 hours) in a hyperhydrated state (Shirreffs, 1996). The additional half litre of fluid per kilogram lost is to compensate for the increased urine output from consuming a large volume of liquid in a short period of time (Sawka et al., 2007).

Consuming water alone dilutes blood (lowers osmolality), this leads to diuresis and excretion of water, thus making water by itself an inefficient rehydration beverage (Leser, 2011; Maughan & Leiper, 1995; Nose et al., 1988). Consuming only water after exercise resulted in only 53% retention of the fluid volume after a 3 hour period in one study (Seifert et al., 2006). The decreased plasma osmolality that results from water ingestion also lowers the athletes thirst which combined with low overall retention, can delay the entire rehydration process (Maughan & Leiper, 1995; Nose et al., 1988).

The presence of sodium in the recovery drink has shown time and time again its effectiveness in a recovery beverage. There is a direct relationship between sodium content in a drink, and level of fluid retention (Maughan & Leiper, 1995; Shirreffs, 1998). This is because sodium contributes significantly to plasma osmolality and therefore attenuates the decrease seen with water ingestion, preventing diuresis (Nose et al., 1988). Sodium also plays a role in glucose absorption in the small intestine via active transporters which promotes net water absorption (Shirreffs et al., 2004).

Carbohydrate is recommended in a recovery drink for replenishing glycogen stores post exercise. However, a study comparing drinks with equal electrolytes but differing amounts of carbohydrates showed that carbohydrates have only a mild effect on fluid retention during rehydration (Osterberg et al., 2010). Unfortunately this study only replaced fluid losses during exercise and did not follow the current recommendations so it is still unknown if this effect would be seen with fluid intake at 150% of weight loss. To optimise glycogen synthesis after exercise and rehydration, about 1.2g of carbohydrate (CHO) per kilogram of bodyweight should be consumed each hour for up to 6 hours after finishing the exercise (Spaccarotella & Andzel, 2011).

Protein is also important post-exercise especially for the optimal recovery of the muscle tissue. Dietary protein promotes muscle protein synthesis (MPS) and slows muscle protein breakdown (MPB) which aids recovery for further exercise. Currently, the recommendation for post-exercise protein intake is to consume 20g of protein within 30 minutes to maximally stimulate MPS (Moore et al., 2009).

It is likely the protein requirements for athletes could be up to twice the recommended daily intake (RDI) of 0.75-0.84g/kg/d (Ministry of Health, 2006; Phillips & van Loon, 2011). Further, in glycogen depleted athletes, when carbohydrate intakes are low, protein can enhance blood insulin levels and muscle glycogen synthesis, however this effect is negated as carbohydrate intake increases (van Loon, Saris, Kruijshoop, & Wagenmakers, 2000).

Given the need for both protein and carbohydrate post exercise, there is a growing amount of research, showing that milk is at least as good as a sports recovery beverage in rehydration (James, 2012; Roy, 2008; Shirreffs et al., 2007; Watson et al., 2008). The composition of milk compares favourably to sports drinks with amounts of CHO in concentrations similar to those found in typical carbohydrate-electrolyte sports drinks (39-64g/L) and contains similar or larger amounts of sodium and potassium than the sports drink which have shown to aid fluid retention (Maughan & Leiper, 1995; Nielsen, Sjogaard, Ugelvig, Knudsen, & Dohlmann, 1986). Leser concluded that milk has an ideal sodium level for rehydration and sodium levels above those in milk have only a limited effect on fluid retention (Leser, 2011). It also contains milk protein which has been shown to aid rehydration (James et al., 2011), therefore it potentially allows better recovery of muscle tissue post-exercise compared to the carbohydrate based sports drink.

As protein ingestion also plays a role in muscle protein synthesis, the addition of protein to a rehydration drink may accelerate recovery and improve subsequent performance (Lunn et al., 2012). Watson *et al* showed that after rehydration with skimmed milk (equivalent to trim milk), there was no difference in the time to exhaustion in a subsequent exercise bout compared with a sports drink (Watson et al., 2008). However Lunn *et al* found exercise time to exhaustion was significantly longer following the ingestion of chocolate milk than an isocaloric carbohydrate beverage (Lunn et al., 2012). Differences may be due to the carbohydrate difference between milk and flavoured milk or differences in the intensity of the exercise tests. However it must be cautioned that one limitation of using milk for recovery is that not all athletes can ingest large amounts of milk. Those with lactose or dairy intolerance would not be able to include milk in their rehydration plan (James, 2012). However, the research overall suggests that milk can be a viable option as a post exercise recovery beverage.

Palatability and its effect on the thirst mechanism are also an important aspect of a rehydration beverage. When a drink is more palatable, more fluid is likely to be consumed under voluntary conditions (this is more reflective of the real world situation than set volumes prescribed in most rehydration studies) (Shirreffs et al., 2004). Generally after dehydration in heat, with *ad libitum* fluid intake, the colder the fluid, the more that is consumed (Park, Bae, Lee, & Kim, 2012). The ideal beverage temperature would depend on the individual, but is likely to be between 10 and 21 degrees Celsius (Park et al., 2012). One study did find however, that maximal fluid intake was met with 15 degree Celsius water (Boulze, Montastruc, & Cabanac, 1983). Flavour of the drink has also been shown to effect palatability of the drink and therefore increase amount voluntarily consumed (Passe, Horn, & Murray, 2000).

2.3 Review of rehydration with protein and carbohydrate

It has been hypothesised that not only is protein important for muscle recovery but also with rehydration. Similar to the 'sodium-glucose co-transporters', there are 'sodium dependent amino acid co-transporters' (Leser, 2011). These in conjunction with the 'sodium-glucose co-transporter' can lead to a higher osmotic gradient, thus bringing over more water into the body from the intestine (Leser, 2011). The increased plasma protein (albumin) draws fluid into the vascular space. This creates an oncotic pressure which increases electrolytes and fluid retention in the intravascular space which in turn increases plasma volume (Leser, 2011; Okazaki et al., 2009).

In 2006, Seifert *et al* compared the level of rehydration with either water, a 6% CHO drink, or a 6% CHO and 1.5% whey protein drink (Seifert et al., 2006). The average fluid intake in this study was 1726mL which was given over the 20 minutes post-exercise and provided 25.9g of protein. This is higher than current recommendation to consume 20g protein within 30 minutes of exercise (Moore et al., 2009). This study showed fluid retention of water, CHO drink, and CHO and protein drink to be 53%, 75% and 88%, respectively. However a limitation of this study is that they only replaced the total weight loss with fluid, rather than the current recommendation of fluid intake 150% of total weight loss (Sawka et al., 2007). It also did not match for the energy content in the drinks, making it difficult to differentiate the effect from either the added protein, or the increased energy density, which has been shown to effect the rate of gastric emptying (Calbet, 1997; Leser, 2011).

However in 2011, James *et al* conducted a study comparing a 6.5% CHO beverage, and a 4% CHO, 2.5% milk protein beverage and their effectiveness on rehydration (James et al., 2011). They found that the drink containing the milk protein was more effective at lowering urine output, indicating more fluid retention during recovery. The drinks were matched for energy density, and therefore this data suggests that gram-for-gram, milk protein can increase fluid retention to a greater extent than carbohydrate alone (James et al., 2011). It is important to note that the average intake of the rehydration beverage was 2120mL which provided 53g of protein over a one hour period. This is far higher than the current recommendation, and is not applicable to a real world situation (Moore et al., 2009).

Conversely in 2012, James *et al* published a similar study comparing a 6% CHO beverage to a 1.5% whey protein, 5% CHO beverage (James et al., 2012). Again, this study provided a higher than necessary amount of protein, providing 34.8g of protein on average (James et al., 2012; Moore et al., 2009). Results from this study found that when matched for energy density and electrolyte content, CHO and whey protein held no benefit over a solution of CHO alone (James et al., 2012). The ratios of protein to carbohydrate were different between these two studies so makes direct comparison difficult.

There have been very mixed results regarding protein's role in rehydration. This is mainly due to the different methods used in each study, making it difficult to decipher the overall picture. Unfortunately, no study has replicated the practices of athletes following exercise whereby one protein bolus is ingested, followed by other types of drinks including water and food, in contrast nearly all the studies have provided multiple boluses of protein during the rehydration phase without any food. Despite this from the current literature, it would appear that whey protein may not have the same effect as milk protein on rehydration, potentially because the casein protein component clots in the stomach which slows digestion, compared to whey protein which is soluble and quickly emptied from the stomach (Bos et al., 2003; Roy, 2008). This leads to slower absorption of amino acids from casein protein and sustains blood amino acid concentrations, consequently aiding water retention.

2.4 Markers of hydration

Changes in body mass have a strong correlation with change in total body water during exercise because 1mL of water has a weight of 1g (Baker, Lang, & Kenney, 2009; Naghii, 2000; Shirreffs, 2003). No other body component can be lost in such a short time frame so this assumption is reasonable especially when there is energy balance (Oppliger & Bartok, 2002; Shirreffs, 2003). Body mass change is often commonly used in rehydration studies as acute changes in total body water in such a protocol can be determined by this method. This method is extremely cheap and easy to conduct and is very accurate (Baker et al., 2009).
Total body water and hydration can also be determined via measurements of deuterium dilution, blood and urine samples. Urine measures commonly used are urine specific gravity (USG), osmolality, and colour. Blood measures include osmolality, changes in plasma volume and electrolyte concentrations. All of these measures have their advantages and drawbacks, a full review of all these measures is beyond the scope of this thesis and has previously been described elsewhere (Armstrong, 2005; Shirreffs, 2003).
Urine osmolality is a measure of total solute present in urine. As an athlete becomes dehydrated, their kidney acts to conserve water by concentrating the amount of solute in the urine (Armstrong, 2005). Urine osmolality has shown to correlate well with body mass changes during dehydration (Armstrong et al., 1998; Shirreffs, 2003). Urine osmolality analyses require an osmometer and a trained technician to obtain, and using this equipment can be quite a time consuming process (Armstrong, 2005). Baseline urine osmolality values

have shown to range quite widely between countries and genders. For example, the mean 24 hour urine osmolality is approximately 900 mOsmol/kg in China and Japan, however it is less than half that value, of approximately 400 mOsmol/kg in Poland and Kenya (Manz & Wentz, 2003).

Urine specific gravity (USG) refers to the density of a urine sample compared with water (Armstrong, 2005). It has a strong correlation with urine osmolality and thus can be an effective measure of hydration status (Shirreffs, 2003).

However, some research is emerging which suggests that USG has a low specificity in some athletes, due to their high muscle mass and therefore is more likely to give false positive results (i.e. hypohydrated values) in this group (Hamouti, Del Coso, Avila, & Mora-Rodriguez, 2010; Oppliger, Magnes, Popowski, & Gisolfi, 2005). While the current cut off for euhydration/hypo hydration is 1.020 g/mL, in one study, 80% of athletes were correctly identified as dehydrated, however only 31.3% of euhydrated athletes were correctly identified as euhydrated (Oppliger et al., 2005). This leaves 68.8% of fully hydrated athletes, incorrectly reported as dehydrated. Hamouti *et al* found that USG can be artificially raised in athletes with high muscle mass such as rugby players, and may require a higher cut off for hypo-hydration than the current value of 1.020 g/mL (Hamouti et al., 2010). However, the equipment required to measure USG is relatively inexpensive and portable requiring minimal training this makes it a useful tool for the sports nutritionist or dietitian working in the field.

Urine colour has proven to be a strong indicator of hydration status and is widely used by athletes to measure rehydration post exercise because of its ease and carries no cost (Oppliger et al., 2005). The colour of urine is dependent on the amount of urochrome contained in the urine (Shirreffs, 2003). There is a scale from 1-8 with each level (or colour) reflecting the degrees of hydration with higher scores reflecting greater levels of dehydration (Armstrong, 2005). However, as it is a subjective measure, it should be measured by two investigators to prevent bias. Unfortunately, urine colour suffers the same downfalls as the other measurements of urine, such as a delayed response to acute changes in hydration, lagging behind plasma osmolality (Shirreffs, 2003).

Urine measures are easier to obtain than blood samples and are commonly used in the field as it is cheap and provides instant feedback. However, urine measures are often criticised due to a potential time lag when rapid changes in hydration occur as it takes the kidney time to react and produce more dilute or concentrated urine.

Blood measures tend to be seen as more robust but require sterile environments and trained staff to obtain and analyse them. They seem to be the most accurate measures of hydration, however are relatively expensive and invasive for the athlete (Oppliger & Bartok, 2002).

Plasma osmolality is the gold standard for measuring acute changes in hydration (Oppliger et al., 2005). It is sensitive to very small changes in hydration. The downfall of this method is the high cost, has high equipment needs including a sterile environment, and invasiveness to the athlete make this less feasible in the field setting, however works well in a research setting (Oppliger et al., 2005).

Plasma volume change can be calculated from changes in haematocrit and haemoglobin (Dill & Costill, 1974). It decreases as an athlete is dehydrated beyond 2-3% of their body mass (Naghii, 2000). It was suggested by Shirreffs in 2003, that the body attempts to maintain cardiovascular stability by maintaining plasma volume until a certain amount of water has been lost from the body, hence its lack of ability to detect small fluctuations in body water (Shirreffs, 2003). It is also affected by posture change therefore making it time consuming as the individual needs to be in a standardised position prior to each measure (Shirreffs & Maughan, 1994).

Finally, Bioelectrical Impedance Analysis (BIA) can provide a reliable total body water estimate most of the time (Shirreffs, 2003). While this may be the case for an absolute value of total body water, it has shown to have limitations of acute changes in hydration (Oppliger & Bartok, 2002; Shirreffs, 2003). For example, Saunders *et al* found that acute changes in body water were reported as body fat changes in endurance athletes (Saunders, Blevins, & Broeder, 1998). For this reason it is not very effective in practice as a hydration marker. Therefore, as each measure has its advantages and disadvantages, the marker of hydration status used depends on the situation and the number of measures required. For example in a research clinic where a one-off measure is required then a blood indices such as plasma osmolality would be the most appropriate. However, for a dietitian working in the field a measure of urine specific gravity would be the preferred choice. Similarly when multiple measures are required and in different locations such as the current study it seems most appropriate to use urine measures, thereby reducing the invasiveness to the participant but still providing reliable indices of hydration status.

2.5 Summary and Conclusion

There is a need for more research in the area of protein in rehydration. It appears milk is effective for rehydration, potentially through its high energy density, high electrolytes, and protein content (James, 2012; Roy, 2008). Studies looking at whey protein initially saw an improvement in fluid retention, however once matched for energy, this improvement was not seen (James et al., 2012; Seifert et al., 2006). It is thought the casein protein fraction in milk has the effect on rehydration through delayed gastric emptying (James et al., 2011).

The research of this thesis will compare a commercially available protein supplement beverage containing 37g/L whey protein and trim milk (which naturally contains about 37g/L protein). This study will be the first rehydration study to replicate a real world situation, whereby the recommended 20g protein is provided in a single bolus, followed by more fluid amounting to 150% of body mass losses and a meal. This protocol best reflects what athletes would actually do, and therefore will provide a unique addition to current literature. The intent of this research is to further understand the role of protein in a rehydration beverage for athletes.

3 Objective Statement

While milk and its effects on rehydration have been studied a lot in recent years, there is yet to be a study looking directly at a whey protein beverage in comparison to a trim milk beverage. No studies have investigated rehydration beverages meeting the current recommendation of 20g of protein post-exercise, while also meeting the fluid replacement guidelines of consuming 1.5L of fluid for every kilogram of body mass lost during exercise (Moore et al., 2009; Sawka et al., 2007). Also no rehydration study has conducted a protocol of dehydrating participants in the evening and assessed markers of rehydration right through until the following morning before.

The objective of this study is to compare the effect of a whey protein beverage against trim milk, in terms of rehydration after exercise induced dehydration. This study is looking to fill in these knowledge gaps of comparing these two drinks, meeting the guidelines for post-exercise protein intake (20 g) as well as also meeting the fluid replacement guidelines for athletes requiring rapid rehydration. The present study will also look at hydration status the following morning after dehydrating exercise in the evening after following current recommendations.

4 Participants and Methods

4.1 Participants

4.1.1 Study Design

This was a randomised cross-over intervention study investigating the effects of two drinks (whey protein and trim milk) on indices of rehydration.

4.1.2 Ethics

This study received ethical approval by the University of Otago Human Health ethics committee (13/169), and prior to any testing being undertaken all of the participants were provided with the opportunity to ask any questions and then gave written informed consent (Appendix A).

4.1.3 Recruitment

A total of 10 healthy male participants (Mean ± SD age 23.1 ± 1.5 years, height 177.88 ± 5.93 cm and body mass 82.62 ± 8.54 kg) volunteered to be in the study, with recruitment commencing in June 2013 and ceasing July 2013. Participants were recruited via word of mouth, email and posters.

4.1.4 Eligibility

In order for participants to be eligible for the study they had to complete a health screening questionnaire (appendix B) and report to comply with the following selection criteria: Healthy males aged 18-45 years who exercise on a regular basis without any food allergies, history of blood pressure disturbance or cardiovascular problem.

They were also screened by the following exclusion criteria: Anyone who has kidney problems, heart or other circulation problems, diabetes, food allergies, sleep disorders, asthma, high blood pressure, or problems with heatstroke.

4.2 Rehydration beverages

The current recommendations for post exercise protein intake is 20g (Moore et al., 2009) this equates to 540mL of trim milk. As the study was designed to replicate the real world setting whilst also meet the current guidelines 540 mL was utilised so that no manipulation of trim milks composition was required. The two beverages were matched for absolute protein content. The first was a 540mL whey protein beverage containing 37g/L of protein (Horley's Ice Whey Creamy Vanilla, Nutralac Nutrition Ltd., Mt Eden, New Zealand). The other was trim milk again containing 37g/L of protein (Pams Extra Slim Milk 0.5% fat, Pams Products Ltd., Auckland, New Zealand). For full macronutrient contents of the drinks see table 4.2.1.

Table 4.2.1. Macronutrient composition of the food and drink during each trial protocol

Food/drink	Amount (g)	Energy (kJ)	Carbohydrate (g)	Protein (g)	Fat (g)	Sodium (mg)
Whey Protein Trial						
Whey Drink	540	355	0.4	20	0.2	137
Spaghetti	420	946	45	7.2	2	1944
Bread	53	535	24.5	4.5	0.85	228
Cereal bar	20	342	14.2	0.5	2.1	227
Total	**1033**	**2178**	**84.1**	**32.2**	**5.15**	**2536**
Milk Trial						
Trim Milk	540	864	26.5	20	2.16	243
Spaghetti	420	946	45	7.2	2	1944
Bread	53	535	24.5	4.5	0.85	228
Cereal bar	20	342	14.2	0.5	2.1	227
Total	**1033**	**2687**	**110.2**	**32.2**	**7.11**	**2642**

4.3 Experiment protocols

Participants completed four different trials in total, however this thesis will only focus on two of these trials (trim milk and whey). All trials were separated by at least seven days. For the 24 hours prior to the first trial participants were asked to keep food and fluid records. They were then asked to repeat this diet before the three following trials. Participants were asked not to eat or drink anything in the four hours before the start of the trial, with the exception of 500mL of water two hours before.

All of the trials started in the early evening (5.30-6pm). Upon arrival at the clinic, the participants were asked to empty their bladder and provide a urine sample which was assessed for hydration status via USG prior to starting the exercise protocol. They were then weighed in minimal clothing (underwear only) (Digi DI-10, Wedderburn, Dunedin, New Zealand) to the nearest 10g and filled out their first questionnaire on subjective feelings (see appendix C) and gastrointestinal comfort (appendix D). After these measures were obtained, the participants moved into the environment chamber (mean ± standard deviation temperature of 35.17 ± 0.31°C and 61.71 ± 5.25% relative humidity over both trials) and cycled on a stationary cycle ergometer (Monark Ergometer, Cycleurope, Auckland, New Zealand) at a workload equivalent to two watts per kg body mass (low to moderate intensity), although this was adapted to suit the abilities of the participant. Participants cycled for 10 minutes followed by five minute breaks, where they towelled dry and body mass was obtained again in only underwear. This cycling to rest ratio continued until they lost 1.8% of their initial body mass. They then showered before another body mass was obtained in minimal (underwear) dry clothing. Fifteen minutes after completing the exercise protocol, participants were randomly assigned and given 540 mL of one of the two drinks –whey protein, or trim milk. They also completed another subjective feelings, gastrointestinal feelings and drink palatability questionnaire (appendix C). At 30, 45 and 60 minutes post-exercise participants were provided with further fluid (water) so that the total volume of fluid given was 1.5 times their body mass losses (i.e. a 1kg body mass loss would receive 1.5L of fluid).

One hour post-exercise, another urine sample was provided, another subjective feelings and gastrointestinal feelings questionnaire was completed, and a meal of spaghetti (Pams Spaghetti in Tomato Sauce, Auckland, New Zealand) and two pieces of toast (White sandwich sliced bread, Budget, Auckland, New Zealand) were given. This meal was intended to be low in protein and energy, to attenuate the effect it would have on rehydration. At two hours post exercise, participants provided another urine sample, and a subjective feelings and gastrointestinal comfort questionnaire was completed. Participants then went home with a cereal bar (Pams Choc Rainbow Bubble bar 20g, Auckland, New Zealand), a sleep questionnaire (appendix E), a large container for the collection of any overnight urine and two 20 mL urine sample tubes, one to provide a sample of the urine produced overnight and the other to provide a sample of the first void the following morning. Participants collected overnight urine up until their first void the following morning. They then completed a questionnaire about subjective feelings and sleep quality.

4.4 Sample Analysis

The total urine volume at each time point (upon arrival at the environmental chamber, at one hour post-exercise, two hours post-exercise, first urination the next morning, and their overnight urine container) were collected and weighed. Approximately 20 mL was initially retained from each sample and were later (within 24 hours) split into Eppendorf tubes and frozen at -20°C.

4.4.1 Urine Specific Gravity

Urine specific gravity was measured via refractometry (Atago Uricon-N Refractometer, Tokyo, Japan). The refractometer was calibrated using deionized water prior to sample analysis. Coefficient of variation (CV) for USG measures was 0.2%.

4.4.2 Urine Osmolality

Urine Osmolality was measured by freezing point depression using a Gonotec Osmomat 030 (Osmomat 030, Gonotec, Berlin, Germany). The machine was calibrated at 850mOsmol/kg prior to each batch of sample analysis, CV of 0.78%.

4.4.3 Urine Output

Participants collected their urine samples into a 20mL container, and the rest into a large plastic container from the end of exercise, until their first void the following morning. Scales were provided to the participants for weighing their urine container each time they urinated (Electronic kitchen scale with silicone platform, Salter Housewares Ltd, Tonbridge, England).

4.4.4 Subjective Feeling Questionnaires

Subjective feeling questionnaires were given at the following time points pre-exercise, post-exercise, one hour post-exercise and two hours post-exercise. These included feelings of thirst, hunger, tiredness, and nine gastrointestinal (GI) discomfort questions for headaches, flatulence, stomach cramping, belching, stomach ache, nausea, vomiting, diarrhoea, and stomach bloating. Also included in the post-exercise questionnaire were 3 aspects about the intervention drink; pleasantness, sweetness and saltiness. Participants were asked to mark the severity of their symptoms on a 100mm scale with 0mm being none to 100mm being severe. Mean ± SD were calculated for the feelings and drink results. The GI discomfort section was separated into positive for symptom (>10mm change from baseline) and negative for symptom (<10mm change from baseline).

4.5 Statistical Analysis

Data are presented as means and standard deviations. All data was analysed using Stata/IC 11.2 for Windows (StataCorp LP, College Station, Texas, USA). Given the protocol utilised in this study has not previously been utilised, there is no data available from other studies on which to base a power calculation, one of the aims of the study was to provide information for sample size needed for future studies. For the USG, urine osmolality data, and subjective feeling questionnaire results, mixed model regression analysis was undertaken to compare conditions across time, which accounted for random effects. Residuals were plotted to test for normality. Normality of variable distributions was also checked for using a Shapiro-Wilk test. For data that was normally distributed, paired two-tailed t tests were undertaken to test for differences between conditions at particular time-points and for data that was not normally distributed, Wilcoxon matched-pairs signed-rank tests were used.

For the subjective questionnaire gastrointestinal discomfort results, participants were categorized into either having the symptom or not based on a change from baseline that was >10mm on the 100mm scale. Proportion tests were undertaken on these to see if there were differences between trials and over time. The probability level for statistical significance was set at $p \leq 0.05$.

5 Results

5.1 Pre-exercise Measurements

Nine of the ten participants began the trials in a euhydrated state as shown by mean USG value below the hypohydration cut-off of 1.020 (Casa et al., 2000). There were no significant differences for any of the pre-exercise measures between trials (p=0.725) as shown in table 5.1.1.

Table 5.1.1. Mean ± SD pre-exercise body mass (Kg), urine specific gravity (g/mL) and urine osmolality (mOsmol/kg) for the whey and milk trials.

Measure	Whey Trial	Milk Trial	p-value
Body Mass (kg)	82.38 ± 8.98	82.64 ± 8.09	0.684
Urine Specific Gravity (g/mL)	1.011 ± 0.006	1.012 ± 0.006	0.725
Urine Osmolality (mOsmol/kg)	420.60 ± 222.78	443.00 ± 236.95	0.803

5.2 Dehydration and Rehydration Phase

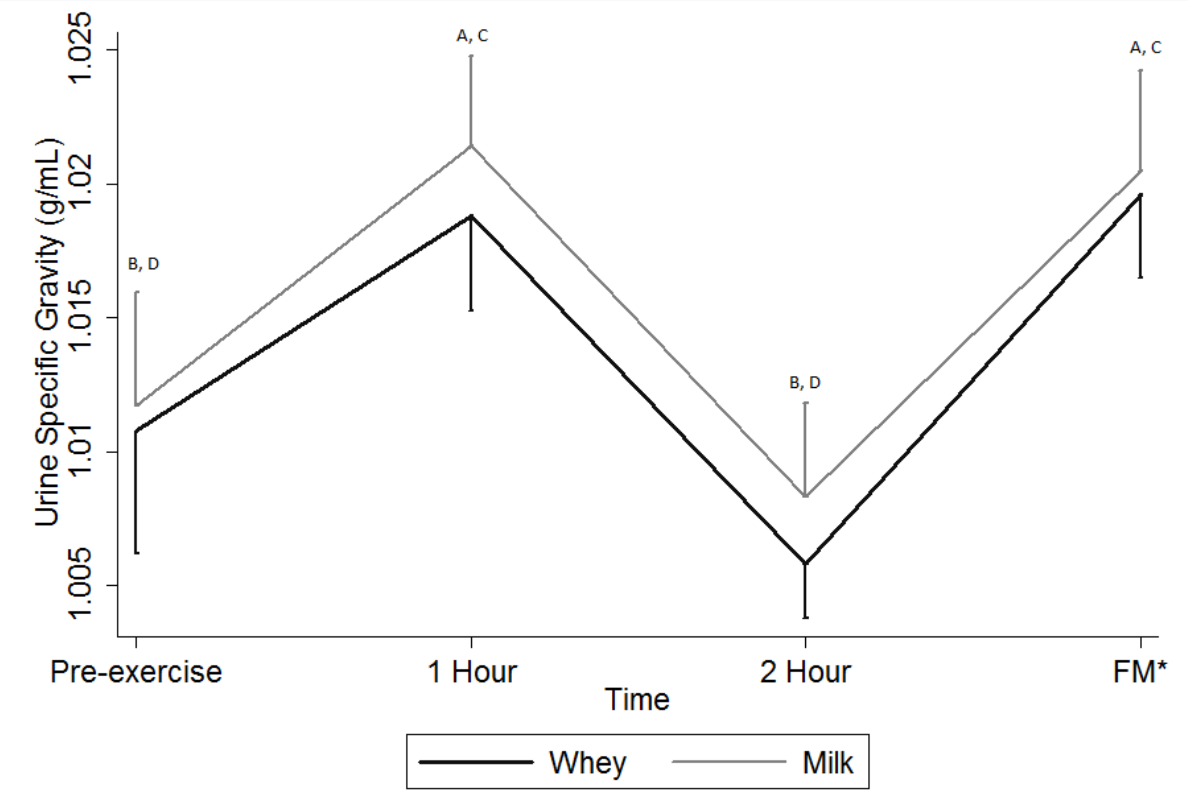
There were no significant differences in the environmental conditions between the trials ($p>0.05$), and participants lost the same absolute and relative body mass on both trials, ($P>0.05$) Table 5.2.1.

Table 5.2.1. Mean ± SD time to dehydration (min), relative humidity (%), temperature (ºC), mean weight loss (kg), weight loss (%), and drink intake (mL) for the whey and milk trials.

	Whey Trial	**Milk Trial**	**p-value**
Time to dehydration (min)	85.80 ± 19.30	80.45 ± 16.50	0.350
Relative humidity (%)	60.93 ± 6.76	62.50 ± 3.33	0.546
Temperature (ºC)	35.21 ± 0.34	35.14 ± 0.28	0.680
Mean weight loss (kg)	1.53 ± 0.30	1.56 ± 0.30	0.539
Weight loss (%)	1.87 ± 0.36	1.91 ± 0.38	0.473
Drink intake (mL)	2295 ± 451	2346 ± 446	0.539

5.3 Urinary Measures

5.3.1 Urine Specific Gravity



- * FM = Following morning

A. Significantly different from Pre-exercise for both whey and milk trials (p<0.01)
B. Significantly different from 1 hour for both whey and milk trials (p<0.01)
C. Significantly different from 2 hour for both whey and milk trials (p<0.01)
D. Significantly different from FM for both whey and milk trials (p<0.01)

Figure 5.3.1. Mean ± SD Urine Specific Gravity (g/mL) for trials whey and milk over the course of the protocol.

As shown in Figure 5.3.1, there was no significant difference between USG values over time between the trials ($p>0.05$). In the milk trial, the mean ± SD USG value for the following morning was 1.021 ± 0.005 with six of the ten participants recording a USG value > 1.020 representing a hypohydrated state the following morning. Conversely on the whey protein trial, the mean ± SD USG value for the following morning was 1.020 ± 0.004 also with six out of the ten participants recording a USG value > 1.020. Compared to the pre-exercise time point, for the whey trial, the one hour post-exercise and following morning USG values were significantly higher ($p<0.001$), and significantly lower at two hours post-exercise ($p<0.05$).

For the milk trial, the USG values were significantly higher at time points one hour post-exercise and following morning compared to pre-exercise ($p<0.001$). However the USG value at two hours post-exercise compared to baseline for the milk trial was not significant ($p=0.082$).

Table 5.3.1. Mean ± SD Urine Specific Gravity (g/mL) at time points one hour post-exercise, two hours post-exercise and following morning, accounting for baseline samples.

	Whey Trial	**Milk Trial**	**p-value**
One Hour Post-exercise (g/mL)	0.008 ± 0.008	0.010 ± 0.006	0.606
Two Hours Post-exercise (g/mL)	-0.005 ± 0.008	-0.003 ± 0.008	0.535
Following Morning (g/mL)	0.009 ± 0.007	0.009 ± 0.007	1.000

When baseline USG values were taken into account there were still no significant differences between drink trials for USG values, Table 5.3.1. ($p>0.05$).

5.3.2 Urine Osmolality

- * FM = Following morning

E. Significantly different from 1 hour for only the milk trial (p<0.01)
F. Significantly different from Pre-exercise for both whey and milk trials (p<0.01)
G. Significantly different from Pre-exercise for only the milk trial (p<0.01)
H. Significantly different from 1 hour for both whey and milk trials (p<0.01)
I. Significantly different from 2 hour for both whey and milk trials(p<0.01)
J. Significantly different from FM for both whey and milk trials (p<0.01)

Figure 5.3.2. Mean ± SD Urine Osmolality (mOsmol/kg) for trials whey and milk over the course of the protocol.

Urine osmolality followed a similar pattern to urine specific gravity as shown in Figure 5.3.2. Again there were no significant differences between drink trials for urine osmolality when accounting for baseline values ($p>0.05$), Table 5.3.2. Compared to the pre-exercise time point, for the whey trial, the one hour post-exercise and following morning urine osmolality values were significantly higher ($p<0.05$), and significantly lower at two hours post-exercise ($p<0.01$). For the milk trial, the urine osmolality values were significantly higher at time points one hour post-exercise and following morning compared to pre-exercise ($p<0.001$), and significantly lower at time point two hours post-exercise compared to baseline ($p<0.05$).

Table 5.3.2. Mean ± SD urine osmolality (mOsmol/kg) at time points one hour post-exercise, two hours post-exercise and following morning, accounting for baseline samples.

	Whey Trial	Milk Trial	p-value
One Hour Post-exercise (mOsmol/kg)	185.20 ± 270.86	239.90 ± 186.59	0.597
Two Hours Post-exercise (mOsmol/kg)	-203.10 ± 281.83	-146.00 ± 284.52	0.522
Following Morning (mOsmol/kg)	271.10 ± 245.35	279.20 ± 252.17	0.930

5.4 Urine Output and Net fluid balance

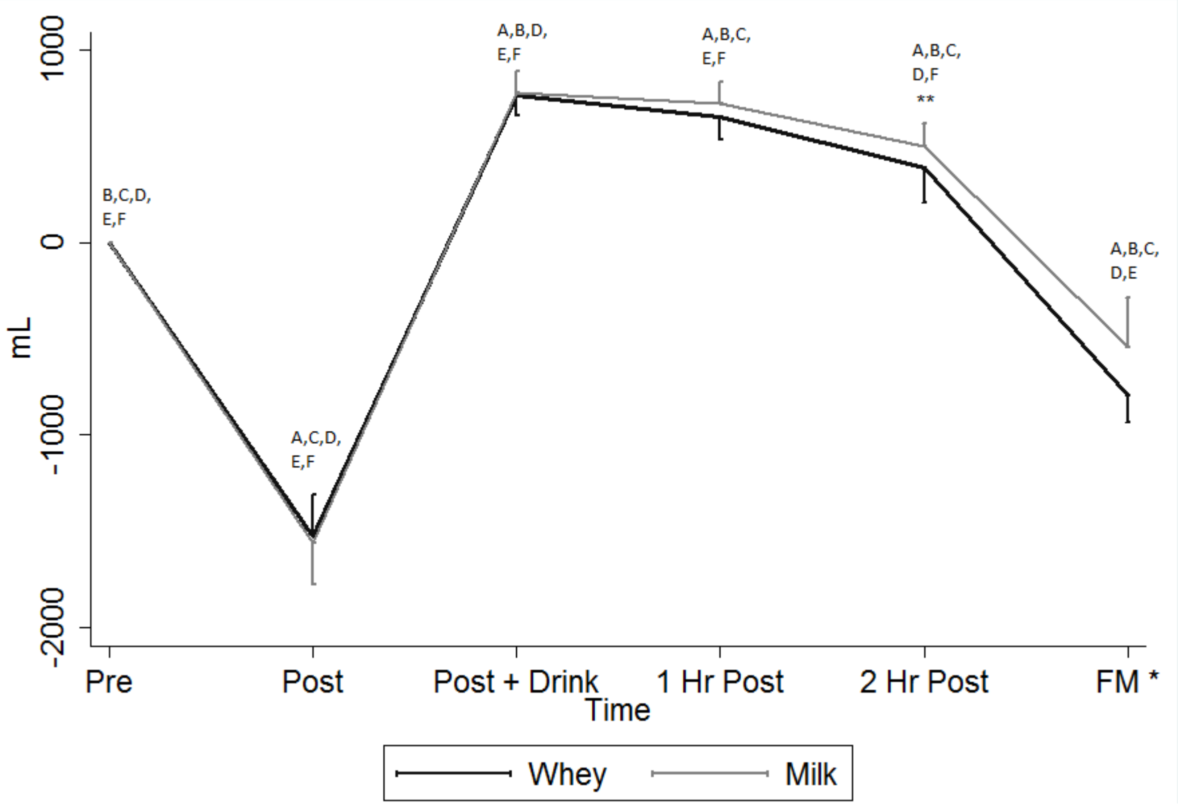
5.4.1 Urine Output

Urine output in the milk trial was significantly lower than the whey trial at the two hours post-exercise time point as shown in table 5.4.1 ($p=0.018$). At all other time points there were no significant differences in urine output ($p>0.05$). However for 7 of the 10 participants, total urine output was lower on the milk trial compared to the whey trial.

Table 5.4.1. Mean ± SD Urine output (mL) between trials whey and milk at time points one hour post-exercise, two hours post-exercise and following morning.

Time Point	Whey Trial	Milk Trial	p-value
One hour post-exercise (mL)	74.50 ± 39.66	60.88 ± 24.20	0.299
Two hours post-exercise (mL)	373.30 ± 201.68	284.00 ± 144.96	0.018
Following Morning (mL)	1498.00 ± 245.60	1325.50 ± 426.40	0.150

5.4.2 Net Fluid Balance



- * FM = Following morning
- ** Significantly different between whey and milk trials (p<0.05)

A. Significantly different from Pre for both whey and milk trials (p<0.01)
B. Significantly different from Post for both whey and milk trials (p<0.01)
C. Significantly different from Post + Drink for both whey and milk trials (p<0.01)
D. Significantly different from 1 Hr Post for both whey and milk trials (p<0.01)
E. Significantly different from 2 Hr Post for both whey and milk trials (p<0.01)
F. Significantly different from FM for both whey and milk trials (p<0.01)

Figure 5.4.1. Mean ± SD Net Fluid Balance (mL) at time points pre-exercise, post-exercise, post-drink consumption, one hour post-exercise, two hours post-exercise and following morning.

At two hours post-exercise, there was a statistically significant difference between net fluid balance for trials whey and milk (p=0.037), figure 5.4.1. At all other time points the difference between trials was not significant (p>0.05). However at one hour post-exercise, the difference was approaching significance (p=0.058). Over all time points, net fluid balance was significantly different from all other time points for both drink trials (p<0.01).

5.5 Subjective Feeling Questionnaire

Table 5.5.1. Mean ± SD Subjective Feeling Questionnaire measures (mm) on a 100 mm visual analogue scale for thirst, hunger and tiredness between trials whey and milk over time.

	Whey Trial	Milk Trial	p-value
Pre-exercise			
Thirst	47.80 ± 16.26	36.20 ± 24.50	0.166
Hunger	50.78 ± 19.86	42.56 ± 22.11	0.416
Tiredness	31.89 ± 13.30	28.67 ± 18.10	0.752
Post-exercise			
Thirst	57.20 ± 19.19	68.50 ± 21.31	0.154
Hunger	56.10 ± 29.39	62.50 ± 35.49	0.584
Tiredness	48.00 ± 23.62	59.20 ± 16.94	0.302
One Hour Post-exercise			
Thirst	9.90 ± 10.39	4.60 ± 7.50	0.267
Hunger	56.60 ± 31.48	52.00 ± 26.00	0.444
Tiredness	55.90 ± 24.22	60.10 ± 15.80	0.721
Two Hours Post-exercise			
Thirst	21.70 ± 17.21	18.00 ± 14.33	0.509
Hunger	45.40 ± 29.10	53.30 ± 23.54	0.358
Tiredness	64.40 ± 23.58	55.30 ± 18.25	0.368

There was no significant difference between the trials for participants rating of subjective feelings ($p>0.05$), Table 5.5.1. However both drink trials had significant differences over time for thirst and tiredness ($p<0.0125$). In the milk trial, participants were significantly more thirsty at post-exercise compared to pre-exercise, and significantly less thirsty at time points one hour post-exercise and two hours post-exercise ($p<0.0125$). The same trend was seen in the whey trial, however thirst was not significantly different in post-exercise compared with pre-exercise ($p=0.139$). In both trials, participants were significantly more tired at each time point compared to pre-exercise ($p<0.013$).

Table 5.5.2. Mean ± SD drink taste measures (mm) of pleasantness, saltiness and sweetness for the whey and milk trials.

	Whey Trial	Milk Trial	p-value
Drink Pleasantness	71.90 ± 14.40	82.90 ± 16.19	0.083
Drink Saltiness	20.00 ± 18.95	14.50 ± 10.66	0.213
Drink Sweetness	51.60 ± 18.10	47.20 ± 23.09	0.683

Table 5.5.2 shows the results from the ratings of the drinks. None of the measures of either drink were significant ($p>0.05$), however for drink pleasantness there was a tendency to prefer milk over the whey drink ($p=0.083$).

Table 5.5.3. Number (n) of participants reporting gastrointestinal discomfort at time points post-exercise, one hour post-exercise, and two hours post-exercise accounting for baseline for whey and milk trials.

	Whey Trial	Milk Trial	p-value
Post-exercise vs. Baseline			
Headache	1	1	1.000
Flatulence	3	1	0.264
Stomach Cramping	3	1	0.264
Belching	2	4	0.329
Stomach Ache	1	2	0.531
Nausea	0	1	0.305
Stomach Bloating	2	1	0.531
One Hour Post-exercise vs. Baseline			
Headache	2	2	1.000
Flatulence	3	0	0.060
Stomach Cramping	1	1	1.000
Belching	7	5	0.361
Stomach Ache	2	2	1.000
Nausea	1	0	0.305
Stomach Bloating	8	8	1.000
Two Hours Post-exercise vs. Baseline			
Headache	2	3	0.606
Flatulence	2	2	1.000
Stomach Cramping	0	1	0.305
Belching	3	2	0.606
Stomach Ache	1	0	0.305
Nausea	1	0	0.305
Stomach Bloating	1	4	0.121

Table 5.5.3 shows the results from the gastrointestinal measures over the three time points, post-exercise, one hour post-exercise, and two hours post-exercise compared to baseline. The questionnaire included diarrhoea and vomiting as options, however as no participant reported these symptoms at any time point, they are not included in the results. For the rest of the measures, there were no significant differences between trials whey and milk. There was however a tendency for the whey protein group to have more flatulence at 1 hour post ($p=0.060$). There were also significant differences between time points for stomach bloating, with both drink trials having significantly larger numbers of participants recording the symptom at one hour post-exercise compared with post-exercise ($p<0.008$). The whey trial

also had a significant decrease in number of participants recording the symptom of bloating between one hour post-exercise and two hours post-exercise ($p<0.002$).

6 Discussion

6.1 Main Findings

The main finding of this study was that a whey protein beverage is no better or worse at rehydrating than a trim milk beverage. To my knowledge, this is the first study to directly compare a whey protein beverage against a trim milk beverage, to see their effects on rehydration.

Milk's effect on rehydration has been thoroughly studied and has been shown to be at least as effective, if not more effective at rehydration as a sports drink (James, 2012; Watson et al., 2008). Currently in the literature, a whey protein drink made with water has not been compared with sports drink directly. However, whey protein has been added to a carbohydrate electrolyte drink which was matched for the energy density and electrolyte content of a standard carbohydrate electrolyte drink. In line with the present study they also found no difference in rehydration measures between the two drinks (James et al., 2012).

Uniquely, the present study shows that athletes who exercise in the evening and follow the current rehydration recommendations of consuming 1.5L for every 1kg body mass lost during exercise, were likely wake up the next day in a hypohydrated state (6 of the 10 participants on both trials). This would mean that more fluid would need to be ingested before beginning another bout of exercise. Previously such an overnight protocol has not been utilised in rehydration studies.

6.2 Urinary Measures

6.2.1 Urine Output

Urine output in the milk trial was significantly lower than the whey trial at the two hours post-exercise time point ($p=0.018$). However this trend had disappeared by the following morning, showing that the difference in net fluid retention was not different overall. In seven of the ten participants, total urine output was lower during the milk trial than the whey protein trial. Urine output at two hours post-exercise was likely lower in the milk trial due to multiple factors. The energy density of a beverage has shown to delay gastric emptying (Calbet, 1997). This delayed gastric emptying results in a reduced rate of water absorption into the blood circulation. This prevents the blood serum osmolality dropping which is beneficial because a low serum osmolality stimulates urine production in the kidneys (Maughan & Leiper, 1995). Gastric emptying can be delayed not just by total energy, but emerging research is showing that protein, in particular casein protein, can effect it also. Casein protein is the primary protein type in milk, making up approximately 80% of milk protein. The casein protein present in milk is thought to clot in the stomach in the presence of gastric acid, which in turn can delay the speed of emptying from the stomach (James et al., 2011). This delayed gastric emptying leads to slower absorption into the blood stream, therefore attenuating urine output and aiding fluid retention overall.

Another reason for the lower urine output could also be the higher amount of sodium and potassium present in the milk compared to the whey drink. Sodium is the primary electrolyte lost in sweat, and a linear relationship exists between sodium concentration and fluid retention (Maughan & Leiper, 1995; Shirreffs, 1998).

6.2.2 Urine Specific Gravity and Urine Osmolality

Across all time points, there was a trend for all of the whey protein trial USG values to be lower than the milk trial. However, there was no statistically significant difference between the whey and milk trials' USG values. These findings are somewhat surprising given that trim milk has a higher energy density than whey protein, which has shown to delay gastric emptying (Calbet, 1997). As a fluid is more quickly absorbed, diluted blood osmolality leads to an increase in urine production. As urine production is increased, urine becomes more dilute, which is represented by a low USG value. So although initially this would suggest that those on the whey protein trial are more hydrated. It is possible that the whey protein drink has entered the system quicker and is being excreted at a more rapid rate than the milk drink (which is still mainly within the gastrointestinal tract). Therefore this may explain why the milk USG values trended to be higher as it is taking longer for the fluid to enter the system. This suggests that if rehydration is required rapidly then whey protein may be the preferred choice as uptake into the body is rapid however, if fluid retention over a longer period is needed then trim milk maybe a better choice. However, caution must be taken as the results were not significant possibly in part due to the variability in gastric emptying and renal diluting capacity compounded by the small sample size. Alternatively the timing of the urine samples may have contributed to the non-significant findings. In 2011, James *et al* conducted a rehydration study using a similar protocol, however looked at rehydration markers for four hours following exercise (James et al., 2011). This study had only eight participants, but found a statistically significant result in USG values. It is interesting to note that these results were only significant at the three and four hour's post-exercise time points. This indicates that if we had continued to collect urine samples at the three and four hours post exercise time points, that significant results may have been found. The reason this was not done in this study was because of the lateness of completing the exercise protocol. This would have meant the participants would have had to stay until almost midnight. This would have impacted their quality of sleep, which was one of the investigations of the larger study and is an unreasonable demand on the participants.

In this study at the one hour post-exercise time point, a meal of spaghetti and toast and a cereal bar was given to the participants after they had finished their beverages and given a urine sample. This meal contained 1823kJ of energy and 2399mg of sodium, both of which would influence rehydration. The large amounts of energy and sodium from the meal may have minimised any potential differences in the rehydrating abilities of the two different beverages.

Similar to USG, there was no significant differences for urine osmolality values between trials whey and milk over all time points. There was an identical trend between USG and urine osmolality in the results because USG and urine osmolality correlate quite highly against each other as markers of hydration.

6.3 Net Fluid Balance

As shown in Figure 5.4.1, net fluid balance showed a significant difference between drink trials at the two hour post-exercise time point (p=0.037). This is consistent with the urine output at two hours post-exercise also being significantly different (p=0.018). At the one hour post-exercise time point the difference was approaching significance (p=0.058), however given the research by James *et al*. it would appear this was likely not long enough after having consumed the drink to have produced a significant difference. The following morning net fluid balance had a mean ± SD of -544 ± 362mL for milk, and -733 ± 223mL for whey. There was a tendency for the milk to have a more positive effect on net fluid balance the following morning, (p=0.088). This trend is likely due to the characteristics of milk explained above in section 6.2.1, which would lower urine production, and improve fluid retention and therefore net fluid balance.

6.4 Current Recommendations

Current fluid recommendations from the American College of Sports Medicine for rehydration post-exercise are to drink 1.5L for every kilogram of body mass lost during exercise (Sawka et al., 2007). This is the protocol followed in the present study, however it is interesting to note that following this recommendation, many participants awoke the next morning in a hypohydrated state. This would indicate that athletes may benefit from having more to drink the following morning before partaking in more exercise.

The recommendations do not state a time frame over which to consume this fluid, however it has been shown to be advantageous to consume the fluid over a longer time frame than the one hour used in this study (Jones, Bishop, Green, & Richardson, 2010). Jones *et al* compared drinking 100% of losses over one hour versus 12.5% of losses every 30 minutes over four hours, and found a 20% increase in retention in the four hour group versus the one hour group. Perhaps with a longer time frame for drinking, the participants in this study would not have awoken in a hypohydrated state.

6.5 Subjective Feeling Questionnaire

6.5.1 Gastrointestinal Discomfort

At each time point, there was no significant difference between either drink trials when accounting for baseline. There was however the tendency for participants to have more flatulence while on the whey drink trial. Other gastrointestinal discomforts have been seen after consuming a whey protein drink during another study where a whey protein beverage was given (Gentle, 2013). One possible reason that this study did not see the same gastrointestinal symptoms is that many of our participants were regular consumers of whey protein shakes to supplement their resistance training. This could mean that their gastrointestinal systems are more adapted to whey protein and may have attenuated any issues with discomfort.

Although there were no significant differences between trials, there were differences over time for each drink. Both drinks had significantly more stomach bloating at one hour post-exercise compared with post-exercise when baseline measures were accounted for ($p<0.008$). The whey drink also had a statistically significant difference between one hour post-exercise and two hours post-exercise ($p<0.002$) which was not seen in the milk drink ($p>0.05$). This means that on the whey drink trial, the stomach bloating that had occurred from the drink and food by one hour post-exercise, had gone by two hours post-exercise. It is possible that this is because whey protein emptied from the stomach quicker than the milk, leading to less distention in the stomach and intestines (Calbet, 1997). This faster absorption of the whey drink would lead to faster absorption into the blood stream and cause the trend of lower USG and higher urine output values seen in the results.

6.5.2 Drink Palatability

Drink palatability has been shown to affect voluntary fluid intake in dehydrated athletes (Passe et al., 2000; Shirreffs et al., 2004). As in most real world situations, fluid intake post exercise is ad libitum, which means that the rehydration beverage that the athlete is consuming must be highly palatable to promote adequate rehydration. In this study, although it was not significant, there was a tendency for the participants to prefer the milk over the whey protein drink in terms of overall pleasantness (p=0.083). This further adds to the case of milk being a suitable post-exercise beverage.

6.6 Strengths and Limitations

There were a number of strengths in this study. Firstly, the current recommendation for fluid intake post exercise of fluid amount equal to 150% of body mass lost during exercise was used (Sawka et al., 2007). The current recommendation for protein intake post exercise of approximately 10-20g of protein was also followed (Zoorob, Parrish, O'Hara, & Kalliny, 2013), while only drinking an amount of each drink type that is realistic to expect an athlete to drink in a real world situation, e.g. a 540mL whey protein shake, or 540mL of milk. This study also looked at rehydration markers for a long period of time. Most rehydration studies monitor responses for 4 hours after the dehydration phase, however for this study hydration markers were tracked through until up to approximately 12 hours post-exercise and feeding.

There were a few limitations in this study which need to be mentioned and kept in mind while critiquing this study. Firstly, the drinks were not matched for energy density or electrolyte content. The consequence of this is that we cannot differentiate whether any difference seen is from the different protein types, or if it was due to an increased calorie intake in the stomach which has been shown to aid rehydration as well. The reason this was done in this way was to make the study as applicable to real world situations as possible. For example, athletes are most likely to drink trim milk or a whey protein shake without modifying any of the sodium or energy levels in the drink first.

Another limitation is that an athlete with high muscle mass can have artificially increased USG readings which can increase the risk of incorrectly classifying these athletes as being in a hypohydrated state (Hamouti et al., 2010). As some of our participants were regularly engaged in resistance training and carried higher than average amounts of muscle mass, this could have influenced their USG values in the results. However the majority of our participants are not likely to be confounded by this as their body mass was lower than those reported by Hamouti *et al*.

In this study, only urine samples were used to measure participant hydration status. Urinary measures of hydration status are commonly criticised for the potential time lag of the kidneys to either concentrate or dilute urine samples after acute periods of dehydration or rehydration (Popowski et al., 2001; Shirreffs, 2003). However these urine measures are still valid for the determination of hydration status, especially when being used over a longer time frame (i.e. testing hydration status overnight) (Oppliger et al., 2005; Shirreffs, 2003). Blood plasma osmolality is the gold standard in measuring acute changes in hydration (Oppliger et al., 2005; Sawka et al., 2007). This was not done in our study due to relative cost of these measures, the invasiveness for the ten participants, and the need for a phlebotomist (Popowski et al., 2001).

6.7 Implications for Future Research

This is the first study to compare a plain whey protein drink with trim milk and their subsequent effects on rehydration. More research could be done however, accounting for some of the limitations of this study. Such as matching the drinks for energy density and electrolyte content, and using blood measures of hydration as well as urinary measures. Also the study could be conducted on more endurance oriented athletes as these athletes typically carry less muscle and thus could have more consistent USG values. As many of our participants awoke in a hypohydrated state, future research could look into the impact of this hypohydration on subsequent exercise performance measures. Dietary protein enhances recovery by increasing MPS and attenuating MPB. Protein intake before going to bed at night has shown to increase MPS rates overnight and provides a higher whole-body protein balance (Res, Groen, Pennings, Beelen, & LJ, 2012). Insulin response rate can effect rehydration also, by affecting glycogen replacement which in turn enhances fluid retention. By including measures of MPS, insulin response rate, and muscle glycogen replacement in future research, the role of protein in a rehydration beverage can be assessed from other aspects than only its effect on rehydration.

7 Application to Practice

The results from this thesis can be used by sports dietitians in practice that deal with athletes involved in weight category sports, or athletes who train multiple times per day. These athletes need to rapidly rehydrate in order to be able to perform optimally in their competition or subsequent training. The results from this thesis will help add to the pool of evidence regarding rapid rehydration and the effect that protein plays. This can be included in the current evidence used to determine the development of the ideal rehydration beverage composition.

The results from this thesis can also be used by sports dietitians to develop rehydration strategies for athletes on an individual basis. This study is the first to look at rehydration measures overnight, and finding that athletes can start the day in a hypohydrated state despite following current rehydration recommendations the previous evening means that dietitians will have to account for this when working with athletes.

In practice, rehydration strategies must be individualised relative to time of day, length of training, and time until next training, among other things. However in many instances I would recommend a milk beverage over other alternatives, due it its casein protein content, relatively high energy density, high electrolyte content, and overall protein content which all effect rehydration, and are also important for optimal recovery.

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Appendices

A. *Ethics*

B. *Health Screening/demographics Questionnaire*

C. *Subjective Feeling Questionnaire*

D. *Food Diary*

E. *Gastrointestinal Comfort Questionnaire*

F. *Sleep Questionnaire*

A. Ethics

[Reference Number *as allocated upon approval by the Ethics Committee*]
[*Date*]

Effects of different proteins on rehydration and sleep
INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the Aim of the Project?

Hydration is important to athletic performance, however, most athletes arrive at training sessions hypohydrated. Protein is important for post exercise recovery. It is at present unclear if the type of protein ingested after exercise effects rehydration or subsequent sleep quality. This project aims to answer the research question "does protein type influence rehydration and/or subsequent sleep quality?"

This project is being undertaken as part of the requirements for the Master of Dietetics

What Type of Participants are being sought?

- *Recruitment method*

  Posters, email, word of mouth.
- *Selection criteria (where relevant)*

  Healthy males and females aged 18-45 years who exercise on a regular basis

- *Exclusion criteria*

Anyone with a history of kidney problems, cardiovascular problems, diabetes, food allergy, sleep disorder, asthma, thermoregulatory disorder, hypertension.

Anyone who currently has musculoskeletal injury, and/or sickness in the 24 hours prior to the trials.

- *Number of participants to be involved*

  20

- *Description of any benefit or access to information which the participant will have access to as a result of participating in the research*

You will be provided with individual feedback on your sweat losses and dietary assessment.

What will Participants be Asked to Do?

Should you agree to take part in this project, you will be asked to ......

- Complete 4 trials in random order. For the 24 hours prior to your first trial you will be asked to keep a food diary and to repeat this diet prior to the remaining 3 trials.
- You should not eat or drink anything for the 4 hours prior to each trial with the exception of 500 mL of water 2 hour prior to arriving at the clinic.
- Upon arrival at the clinic you will be asked to provide a urine sample which will be analysed for hydration status and will then be weighed in minimal clothing and asked to complete a subjective feelings, gastrointestinal comfort and sleep questionnaire.
- Once these measures have been obtained you cycle on a stationary cycle ergometer in the heat (35^{o}C and 50% relative humidity), at a workload equivalent to 2 watts per kg body mass (low to moderate intensity). You will cycle for 10 minutes with 5 minute rest periods, during which time nude body mass will be obtained in private. You will continue cycling until they have lost 1.8 % of your initial body mass (usually around 60-90 minutes).
- You will then shower before another body mass is taken.
- Following this you will be provided with food and given one of four drinks –casein protein, whey protein, trim milk or sports drink. The volume provided will be 1.5 times your body mass losses ie a 1 kg body mass loss = 1.5 litres of drink.
- For the next hour you will sit at rest.
- At the end of exercise, 1, and 2 hours post exercise you will be asked to complete a subjective feelings and gastrointestinal comfort questionnaire, provide a urine sample and will be weighed in minimal clothing.
- At the end of the second hour post exercise you will be provided with a sleep monitor which you will wear around your waist and you will be asked to wear it during the evening and night.
- You will also be provided with a urine containers to collect any urine produced between leaving the clinic and the first urination the following morning, a diary to note the times of urination or any gastrointestinal distress and a sleep questionnaire to complete on waking the following morning.

The time commitment which involvement will entail

- Completing the food diary and then repeating your diet before each trial is likely to add about 5 minutes to each drinking and eating occasion.
- The trials are likely to require you to be in the clinic for 3.5 to 4 hours for each trial depending on your sweat rates.
- The urine samples from leaving the clinic to the following morning may take an additional 5 minutes.
- The questionnaire the following morning may take 10 minutes to complete.
- Total time per trial around 4.5-5 hours * 4 trials = 18-20 hours in total.

- *Description of discomforts, risks or inconvenience to participants as a result of participation*

Exercise always carries some degree of risk however, we will act to minimalise this. The intensity will be reduced to meet your fitness/abilities. Two investigators will be present at all times (at least one will hold a first aid certificate).

Please be aware that you may decide not to take part in the project without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

- *What raw data or information will be collected?*

We will collect data on your sweat rates and sleep quantity.

- *What personal information will be collected?*

We will collect information on your age, weight, height, sporting activities, training volumes, sex, and ethnicity.

- *What are the purposes for which the data or information is being collected?*

The data is being collected in partial fulfilment of the Master of Dietetics and will be used to form a thesis and potentially a peer reviewed publication.

- *Who will have access to the data or information?*

Only the investigators (Caleb Robinson, Thomas Chung, Dr Katherine Black, Dr Thomas Love and Assoc Prof Nancy Rehrer) will have access to the individual data.

- *How will data or information be securely managed, stored and destroyed?*

The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. Data obtained as a result of the research will be retained for **at least 5 years** in secure storage. Any personal information held on the participants such as contact details may be destroyed at the completion of the research even though the data derived from the research will, in most cases, be kept for much longer or possibly indefinitely.

- *What data or information will be reflected in the completed research?*

Data collected from this project will be used to calculate average values for the group and summary statements for the group as a whole.

The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve your anonymity.

- *Will the participants have the opportunity to correct or withdraw the data/information?*

Upon your completion in the study you will be provided with your data and will be allowed to correct or withdraw data or information if you wish.

- *Will participants be provided with the results of the study*

You will be provided with the results of this study upon its completion.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:-

Caleb Robinson email: robca402@student.otago.ac.nz

Thomas Chung email: chuth857@student.otago.ac.nz

Dr Katherine Black email: katherine.black@otago.ac.nz

Department of Human Nutrition, University Telephone Number:- 034798358

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

[Reference Number *as allocated upon approval by the Ethics Committee*]

[Date]

Consent form

PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. Personal identifying information contact details will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for at least five years;

4. Cycling in the heat will cause some discomfort

5. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve my anonymity should I choose to remain anonymous

6. At the end of the study, I consent to any remaining samples being disposed of using:

   ☐ Standard disposal methods, OR;

   ☐ Disposed with appropriate karakia

I agree to take part in this project.

....

(Signature of participant) (Date)

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

B. Health Screening/ Demographics questionnaire

1. Date of birth (day/month/year): ______________________

2. Sex: ___________________________

3. Which ethnic group do you belong to?

Mark the space or spaces which apply to you.

☐	New Zealand European
☐	Maori
☐	Samoan
☐	Cook Island Maori
☐	Tongan
☐	Niuean
☐	Chinese
☐	Indian
☐	Other (such as Dutch, Japanese, Tokelauan). Please State: ....

4. Have you <u>ever</u> had or been told you have …..

Kidney/renal disorder?	YES/NO
Cardiovascular/ heart problems?	YES/NO
Diabetes?	YES/NO
Food allergy?	YES/NO
Sleep disorder/ problems?	YES/NO
Asthma?	YES/NO
Thermoregulatory/sweat gland disorder?	YES/NO
Hypertension?	YES/NO
Told that you are unable to exercise?	YES/NO

5. At <u>present</u> or in the past 24 hours have you

A musculoskeletal injury (bone, muscle, cartilage)?	YES/NO
Been sick in (nausea, vomiting, diarrhoea, cold, flu)?	YES/NO

6. Have you ever suffered from heat stroke? YES/NO

C. Food Diary

Rehydration and sleep study

FOOD DIARY

Name:________________

Reminder
No eating or drinking 4 hours prior to each testing session.

Test is at _______pm
No eating or drinking after __________

Consume **500ml** of water 2 hours prior to the test
Test is at _______pm
Consume water at _______

<u>**Food Diary**</u>

<u>**How to fill in your diary**</u>

Below is a step-by-step guide on how to fill in your food diary. It is very important that you do not change what you normally eat or drink just because you are keeping a diary so that we get a true picture of what you eat and drink. Try to fill in the diary each time you have something to eat or drink rather than leave it until the end of the day so that you don't forget anything!

Step 1: When

The first thing to do is to find the right time slot in the first column of the diary (on the left) for when you ate or drank something. Then, in the column next to the time slot, write down the exact time you ate or drank something. So, for example, if you had breakfast at 7.30am, you would go to the first time slot in the diary (6am to 9am) and in the column next to it write in "7.30am".

Step 2: What

The next step in the food diary is to describe what you ate or drank, giving as much detail as you can. Include any extras like sugar and milk in your tea or cereal, butter or other spreads on your bread and sauces such as ketchup and mayonnaise. Do not forget to include drinking water.

If you know the cooking method used (e.g. roast, baked, boiled, fried) please write it down in this section. It would also help us if you can write down the brand name of any foods or drinks if you know it (e.g. Heinz, Robinsons).

For breakfast cereals, as well as the brand name, please write down the name of the cereal e.g. frosties, cocoa pops, corn flakes.

For sandwiches, please describe the type of bread used, how many slices of bread were used and give details of the filling.

For salad or mixed vegetables, please describe what is in it (eg. 1 lettuce leaf, half a tomato,6 slices of cucumber).

For pizza, please describe the topping (e.g. cheese and tomato, ham and pineapple).

Step 3: Portion size

In the next column, please write in the size of the portion of food or drink you had. Please use the scales provided when you can to give an accurate measure of the portion size. If food is left over at the end of a meal, weigh the remaining amount and subtract it from the initial amount to calculate the actual amount consumed.

Step 4: What to do with your food diary

Once you have completed your 24 hour food diary. Keep it and try to follow the same diet prior to each exercise test. Also try to keep to the same level of physical activity on days prior to testing, avoid any strenuous activities that you are unaccustomed to 24 hours prior to testing.

On the first page of the diary we have filled in a whole day to show you what to do.

Please put a circle around the day of the week for which you are writing about

Day 1	Day Monday Tuesday Wednesday Thursday Friday Saturday Sunday	Date

Time slot	When	What	Portion size
6am to 9am	8:00	Blackberries Sugar Toast + Flora Jam Apple Juice	10 ¼ teaspoon 1 Slice ½ teaspoon Glass
9am to 12 noon	9 to 10 10:00 11:00	Orange Squash (Robinson's High Juice) Kellogg's Fruit Winder Homemade Cup Cake	Sports Bottle 1 1
12 noon to 2pm	12:30	Baked Beans Ham Cheese Toast + Flora Robinson's High Juice	1 tbsp 1 slice 1 slice 1 slice Beaker

Time slot	When	What	Portion size
2pm to 5pm	2:00 5:30	Galaxy Chocolate Orange High Juice	5 small chunks Beaker
5pm to 8pm	7:00	Chicken Breast, with herbs, ham and cheese (homemade) Mini roast potatoes Green Beans Orange J20 Homemade Cup Cake	Small Small Medium Bottle 1
8pm to 10pm	8:00	Milk, semi-skimmed	Mug
10pm to 6am			

Day 1	**Day Monday Tuesday Wednesday Thursday Friday Saturday Sunday**	**Date**

Time slot	**When**	**What**	**Portion size**
2pm to 5pm			
5pm to 8pm			
8pm to 10pm			
10pm to 6am			

Time slot	When	What	Portion size
6am to 9am			
9am to 12 noon			
12 noon to 2pm			

If you have any questions about this diary please contact Katherine,
on 4798358,

D. Subjective Feeling Questionnaire

Subjective Feelings (questions 2, 3 and 4 only asked at hour 1 and 2)

Place a vertical mark (|) on the lines below to indicate HOW YOU FEEL AT THE MOMENT.

1) How thirsty do you feel now?

Extremely *Not at all thirsty*

2) How pleasant was the drink?

Extremely pleasant *Not at all pleasant*

3) How salty was the drink?

Extremely salty *Not at all salty*

4) How sweet was the drink?

Extremely sweet *Not at all sweet*

5) How hungry do you feel now?

Not at all hungry *Extremely hungry*

6) How tired do you feel?

Extremely tired *Not at all tired*

E. Gastrointestinal Comfort Questionnaire

This questionnaire asks you to rate the severity of any gastrointestinal (gut) symptoms you may be experiencing now.

1. If you are experiencing no symptoms, please circle the appropriate words eg no nausea.
2. If you are experiencing some symptoms, please indicate your overall rating by placing a vertical mark on the line eg ______|____________

Nausea

No nausea |————————————| Severe nausea
Moderate nausea

Flatulence

No flatulence |————————————| Severe flatulence
Some flatulence

Stomach Cramping

No stomach cramping |————————————| Severe stomach cramping
Moderate stomach cramping

Belching

No belching |————————————| Severe belching
Some belching

Stomach Ache

No stomach ache |————————————| Severe stomach ache
Moderate stomach ache

Bowel urgency

No bowel urgency |————————————| Severe bowel urgency
Moderate bowel

Diarrhoea

No diarrhoea |————————————| Severe diarrhoea
Persistent diarrhoea

Vomiting

No Vomiting |————————————| Severe vomiting
Persistent vomiting

Stomach bloating

No stomach |————————————| Severe stomach bloating
Moderate stomach bloating

F. Sleep Questionnaire

Sleep Questionnaire

How would you describe the way you <u>fell asleep</u> last night in comparison to usual?

1. More difficult than usual Easier than than usual

2. Slower than usual More quickly than usual

3. I feel less sleepy than usual More sleepy than usual

How would you describe the <u>quality</u> of your sleep compared to normal sleep?

4. More restless than usual Calmer than usual

5. With more wakeful periods With less wakeful periods

6. Did you have to get up to urinate during the night?

YES/NO If yes how many times? ____________

How would you describe <u>your awakening</u> in comparison to usual?

7. More difficult than usual Easier than usual

8. Requires a period of time longer than usual Shorter than usual

How do you feel <u>now</u>?

9. Tired Alert

How would you describe your <u>thirst upon awakening</u>?

10. More than usual Less than usual