Milk as a rehydration fluid following exercise-induced loss of body mass.

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UNIVERSITY of LIMERICK
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Department of Physical Education and Sport Sciences

Milk as a rehydration fluid following exercise-induced loss of body mass.

Suzanne Seery, BSc, Pg. Dip Nutrition and Dietetics

Supervisor: Professor Phil Jakeman

University of Limerick

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Master of Science by Research

Author's Declaration

Declaration

I hereby declare that this thesis is entirely my own work and was completed without collaboration or assistance from others other than the counsel of my supervisor, Professor Phil Jakeman, of the Department of Physical Education and Sport Sciences, University of Limerick. This work has not been submitted to any other university or higher education institution, or for any other academic award with this university.

Suzanne Seery **Professor Phil Jakeman**

Dedication

To my hardworking, supportive parents, I am forever indebted to you for all you have done for me. Thank you for supporting me in my decision to take a year out of my dietetic career to pursue a research masters.

Acknowledgements

I would like to thank Professor Phil Jakeman for his supervision and support during the year. To all in the P-1039 office, especially those of you who started in UL back in November 2013, it has been a great experience getting to know you all and I look forward to maintaining the friendships long into the future.

A very special thanks to Beate the phlebotomist whose role was so important in this study, your support throughout the long days of testing was very much appreciated and you also helped greatly to keep the spirits of the participants up when they were feeling dehydrated! I would also like to thank the research assistants Rob and Katie for all those crazy statistics conversations we had, it has been a steep learning curve and I feel I have learned a lot. To Lexi, thank you for your positivity throughout the year and support in the lab during testing, it was very much appreciated. To Orla and Will, your sound advice and help in all things lab related was very much appreciated. A big thank you to Gavin, the final year student in sports and exercise science, who assisted on the project and was a pleasure to work with. A huge thank you to all of the participants, without you there would not have been a study. Your enthusiasm, commitment and willingness to return for each of the three trials was impressive and greatly appreciated.

Thank you to the National Dairy Council for funding the project and giving me the opportunity to explore this interesting research area.

Abstract

Milk as a rehydration fluid following exercise-induced loss of body mass

The effectiveness of 0.1% fat milk (M) at restoring fluid balance after exercise and heat induced hypohydration was compared to a commercially available carbohydrate-electrolyte (CE) sports drink and water (W) using a metered rate of fluid ingestion. After losing 2.1 (0.2) % body mass, participants (n = 7) consumed a drink volume equivalent to 150% of their body mass loss, over a period of 2.5-3 hours. A metered rate of fluid ingestion was chosen as it is widely acknowledged that rapid ingestion (< 60 min) of a large volume of fluid (>1000ml) can over-stimulate diuresis. Blood and urine samples were collected before and for 5 hours after exercise-induced loss of body mass. Mean plasma osmolality was higher in the M trial 289 (3) mOsmol/kg compared to W 286 (3) mOsmol/kg and CE 287 (3) mOsmol/kg, during this 5 hour period (p = 0.021). Indicative of a reduced diuretic response, urine volume was lower and urine osmolality higher in the M trial compared with CE and W. Total urine volume during the M trial was 774 (92) mL compared to CE 1314 (434) mL and W 1429 (345) mL (p = 0.023). A net positive fluid balance from 2h to 5h was achieved in the M trial, whereas the CE and W trials returned to net negative balance by the end of the 5h rehydration period. Final net fluid balance in the M trial was 117 (122) mL compared to CE -381 (460) mL and W trials -539 (390) mL (p = 0.049). This represents a final relative net fluid balance of 5.9 (5.9) % in the M trial compared with CE -22.7 (23.3) % (p = 0.048) and W - 30.9 (22.7) % (p = 0.012).

Table of Contents

Table of Contents

Index of Figures

Index of Tables

Index of Appendices

Abbreviations and definitions

AVP	Arginine Vasopressin
BM	Body Mass
CE	Carbohydrate-electrolyte drink
CHO	Carbohydrate
CHO3-	Bicarbonate
Cl-	Chloride
ECW	Extracellular water
ICW	Intracellular water
K^+	Potassium
M	Milk (0.1% Fat)
Mg	Magnesium
Na^+	Sodium
Posm	Plasma Osmolality
Pvol	Plasma Volume
TBW	Total Body water
Uosm	Urine Osmolality
W	Water

Definitions

Euhydration: Normal state of body water balance

Hypohydration: Body water deficit

Dehydration: A state of net loss of hypotonic body fluids > 2%BM loss

Chapter 1
Introduction

Maintaining adequate hydration during exercise is a challenge for most athletes. It is widely acknowledged that the active sports person generally finishes training or competition in a hypo-hydrated state (Broad et al 1996, Maughan and Shirreffs 2010b). For a person undertaking regular exercise a fluid deficit incurred during one exercise session can potentially compromise the next if timely, adequate replacement of fluid does not occur. Optimum rehydration post-exercise is therefore essential in order to restore body water loss and aid recovery.

The consensus opinion regarding rehydration post-exercise is that it is necessary to consume a fluid volume of 150% of body mass loss with sufficient sodium content, to account for on-going renal and other water losses, when rehydration is required within 4-6h (Sawka et al 2007). Recent studies are limited in the area of post-exercise drinking patterns of sports people but it is widely accepted that people fail to drink sufficient volumes of fluid to restore fluid balance, even when drinks are made freely available (Burke 1997, Greenleaf 1992). In laboratory studies assessing the effectiveness of rehydration solutions, it is common to employ a protocol that involves a prescribed volume equivalent to 150% of body mass loss within 60 min, which does not generally reflect field-based practice. Ingesting a large volume of fluid (≥ 1-2L) in a short period has the potential to cause gastro-intestinal discomfort due to over distension of the stomach. It can also cause an over-stimulation of the diuretic response of the kidneys, resulting in a return to negative fluid balance (Casa et al 2005). Recently it has been acknowledged that rapid ingestion may also mask the benefits of the rehydration solution under investigation (James et al 2014).

The sports drink industry has developed considerably in recent decades with reported combined sales of sports and energy drinks in Ireland worth approximately €186 million (Euromonitor 2014). New product development is now focused on health and wellness with increasing demand for natural rehydration beverages with a ‘clean’ ingredients label. A ‘clean’ ingredients label is generally accepted as being the removal of chemical-sounding ingredients such as artificial food additives and E-numbers to create a simpler ingredients’ list that also includes natural origin ingredients or a healthier nutrient profile (Euromonitor 2014). As a result, the spot light has focused on re-branding natural plant based and household beverages that are free from artificial ingredients. Milk naturally has a high content of electrolytes, particularly potassium (K^+) (~45mmol/L) and also contains carbohydrate (5%) in a similar concentration to many commercially available sports drinks (4-8%).

It is thought that the composition of milk, in particular the energy density, protein and electrolyte composition, influence the gastric emptying rate and intestinal water absorption, thus slowing the appearance of water in the circulating blood volume leading to improved fluid retention (Shirreffs et al 2007b, Watson et al 2008). However, the consumption of milk in a volume equivalent to 150% of body mass loss within 1h has been demonstrated to cause gastro-intestinal discomfort as indicated by high bloatedness scores in the case of some individuals during the Shirreffs et al (2007b) study and significantly higher levels of reported stomach fullness during recovery in the Watson et al (2008) study.

The study design adopted in this thesis followed the standardised procedure of exercise and thermal induced dehydration to -2% of body mass followed by ingestion of rehydration fluids of varying composition i.e. water, carbohydrate-electrolyte solution and milk. In consideration of the requirement to optimise fluid retention and minimise gastro-intestinal discomfort, a metered rate of ingestion was applied, to compare the effectiveness of milk (0.1% fat), a proprietary carbohydrate electrolyte solution and water, in restoring fluid balance within a 5h period post-exercise and thermal induced dehydration to -2% of body mass.

1.1 Hypothesis

Null Hypothesis (H_O) - Following a body water loss equivalent to -2% of body mass achieved by exercise in a hot environment (30°C) and a metered rate of oral fluid replacement equivalent to 150% of the body mass loss, the retention of fluid replaced, measured over 5h, *will not be* affected by the composition of the rehydration solution (i.e. 0.1% fat Milk (M), Water (W), proprietary carbohydrate electrolyte solution (CE).

Alternative Hypothesis (H_1) – Following a body water loss equivalent to -2% of body mass achieved by exercise in a hot environment (30°C) and a metered rate of oral fluid replacement equivalent to 150% of the body mass loss, the retention of fluid replaced measured over 5h, *will be* affected by its composition (i.e. 0.1% fat Milk (M), water (W), proprietary carbohydrate electrolyte solution (CE). The composition of the rehydration solutions is provided in Chapter 3 Table 3.

Chapter 2
Literature Review

This review of the literature seeks to present an understanding of the physiology of body fluid balance and homeostatic regulation. Fluid replacement guidelines are reviewed. The conditions required for optimal fluid replacement and key ingredients and characteristics of a rehydration beverage are explored. Finally, it presents the evidence regarding milk and its potential benefits as part of a rehydration strategy.

2.1 Body water

Body water is required to sustain the cardiovascular and thermoregulatory systems and to support cellular homeostasis. It has a high specific heat capacity which allows it to absorb and dissipate body heat. Daily water intake must be balanced with losses in order to maintain body water homeostasis, otherwise known as a state of ‘euhydration’.

Water accounts for approximately 60% of total body mass in the average adult male (equates to 42L in a 70kg male) (Figure 2.1) but can range from 45-75% of body mass depending on body composition, sex and age. Variation due to body composition relates to lean and fat mass. The water content of lean body mass is relatively constant (~73%) across age, sex, and race (Wang et al 1999, IOM 2005). Fat tissue is composed of ~ 10% water (Sawka and Montain 2000). Individuals with a higher percentage fat mass therefore have lower relative total body water content. Trained athletes have relatively greater total body water than sedentary individuals due to lower percentage body fat and a higher lean tissue mass. The concentration of skeletal muscle glycogen is also a factor; the amount of water stored with glycogen ranging from 1 to 4g of water per gram of glycogen (Sherman et al 1982).

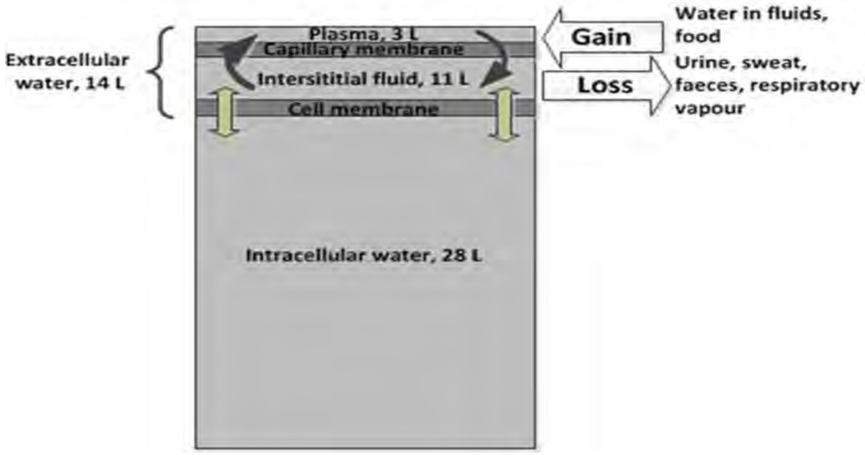


Figure 2.1 - Body fluid compartments that comprise 42 L of total body water in a 70 kg human, and sources of fluid gain or loss. (Armstrong 2007).

Total body water can be subdivided into two compartments, intracellular, which accounts for 55-65% of total body water and the extracellular compartment which accounts for the remaining 35-45%. The extracellular compartment can be further divided into the interstitial fluid and plasma (~7.5% total body water) (IOM 2005). Intracellular water (ICW) is mainly composed of potassium (K^+) and magnesium (Mg) and the extracellular water (ECW) is mainly composed of sodium (Na^+) and its associated anions chloride (Cl^-) and bicarbonate ($CHO3^-$). To maintain homeostasis between fluid compartments water moves across a concentration gradient by osmosis between the intracellular and extracellular space either passively or actively by the $Na\text{-}K^+$ pump. Maintenance of this distribution of electrolyte balance between the intracellular and extracellular compartments is vital for cell function and electrical signalling throughout the body.

Water loss from the body occurs via respiration, through the skin (sweating), urine and in faeces. Evaporative water loss through respiration and water diffusion through the skin are termed insensible water losses. Water loss in faeces is typically 100 to 200mL/day (IOM 2005). Water loss through respiration is approximately 250 to 350 mL/day but can increase as respiration rate increases during exercise. Water loss in urine, faeces and thermoregulatory sweating are termed sensible water losses as the person is aware of the loss. The loss of water via urination ranges from a minimum of approximately 500mL/day to an average of 1 to 2L/day and can vary considerably as renal fluid output is a function of fluid intake. Obligatory urine loss occurs because of the physiological need to remove metabolic waste products mainly urea and uric acid which are generated during protein and nucleic acid catabolism. Other substances which need to be eliminated from the body include; food additives, drugs and an excess solute load, in response to ingestion of food, fluids and medications Urine output volume represents the primary avenue to regulate net body water balance and is variable depending on daily fluid consumption and activity. Minimum outputs of ~20 mL/hr and maximal volumes of ~1000 mL/hr are possible (IOM 2005).

Water is also produced by the body as a by-product of the oxidation of metabolic fuels (mainly carbohydrate and fat). Oxidation of 1g of carbohydrate will result in the formation of 0.6ml of water and oxidation of 1 g of fat will result in the generation of 1.13mls of water (Maughan et al 2007). The rate at which water is produced via oxidation depends on the rate of energy turnover and on the substrate mixture being oxidised (Maughan et al 2007). Respiratory water loss and metabolic water produced are difficult to measure during exercise but can be estimated using predictive calculations (King et al 2008, Maughan et al 2007). During physical exercise and exposure to heat stress, sweat loss accounts for the largest source of water loss from the body as a result of the thermoregulation mechanism (Sawka et al 2005)

2.2 Body water and electrolyte flux during exercise

When exercise is performed metabolic rate increases resulting in the production of heat by contracting muscle. Core body temperature set-point of ~37°C is maintained via sensors in the preoptic area of the hypothalamus which are sensitive to changes in circulating blood temperature and also receive signals from thermoreceptors located in the skin. When body temperature starts to increase the hypothalamus triggers heat losing mechanisms including; vasodilation to increase blood flow to the surface of the skin and the sweat response which removes heat from the body via evaporation of sweat produced by eccrine glands in the skin. Other mechanisms of body heat loss include; radiation and conduction, however they are only effective when the temperature of the environment is lower than body temperature. When the surrounding environmental temperature is greater than 37°C, the body relies solely on evaporation for heat loss because water continues to evaporate even when it is cooler than its surroundings.

Sweat losses vary considerably among individuals; heat acclimatization enhances an individual's ability to achieve higher and more sustained sweating rates. Electrolytes lost through sweat are highly variable due to the mode of sweat loss (thermal *vs.* exercise alone) (Fukumoto et al 1988) biological variability and the methodological issues of sweat collection (Maughan and Shirreffs 1997). Sweat is mainly composed of sodium with typical concentrations of 20–80 mmol/L and its associated ion chloride (20-60mmol/L). Sweat also contains potassium (K^+) (4-8mmol/L) and to a lesser extent magnesium (Mg) (0.2-1.5mmol/L) and calcium (Ca) (0.3-2mmol/L) (Sawka et al 2007).

Sweat is hypotonic relative to blood plasma due to active reabsorption of Na^+ and Cl^- within the coiled duct of the eccrine gland. This reabsorption of electrolytes helps to promote evaporation at the skin surface, since a more dilute sweat will have a greater water vapour pressure at the same skin temperature. With training and acclimatisation more Na^+ is reabsorbed and sweat is more dilute, due to the sweat gland becoming more sensitive to the hormone aldosterone which stimulates Na^+ reabsorption (Taylor et al 2013)

The effects of sweat loss during exercise can be described as inducing a hypertonic hypovolaemic state resulting in intracellular dehydration (Figure 2.2) (Cheuvront and Kenefick 2014). The term hypertonic refers to a higher concentration of solutes (electrolytes) in the extracellular space compared with the intracellular space. The osmotic gradient increases as the total volume of body water is reduced (hypovolaemia) via sweat and the ECW becomes more concentrated relative to the ICW. This causes water to shift by osmosis from the intracellular space to the extracellular space until the osmotic pressures are equal, thereby redistributing the Na^+ evenly. The volume of fluid shifts from the intracellular space to the extracellular space in dehydration is determined by the Na^+ content of sweat (Nose et al 1988b)

2.3 Fluid deficit and sports performance

The effects of a body water deficit on sports performance are well documented (Cheuvront et al 2003, Shirreffs and Sawka 2011, Cheuvront and Kenefick 2014). A ≥ 2% body mass loss in warm environments mainly affects aerobic capacity by increasing physiological strain on both the cardiovascular and thermoregulatory systems. It has been demonstrated that for every 1% body mass loss there is an increase in core body temperature of 0.15-0.2°C (Sawka et al, 1985; Montain and Coyle, 1992). This impairment in thermoregulation occurs as sweat rate reduces due to plasma volume deficit which results in impaired skin blood flow and vasodilation which normally have a cooling effect on the body (Sawka and Coyle, 1999). The reduction in plasma volume causes cardiovascular strain due to reduced stroke volume causing heart rate to increase.

Dehydration to the extent of 3-4% loss of body mass has been reported to elicit an even greater degradation in aerobic performance and muscle endurance especially in warm environments. However, dehydration to the extent of 3-5% of body mass does not appear to degrade strength (Greiwe et al 1998, Evetovich et al 2002), anaerobic performance (Cheuvront et al 2006) or jumping ability (Cheuvront et al 2010).

Decrements in strength performance appear to be observed when greater than a loss of 5% body mass occurs (Sawka et al 1996). In relation to intermittent activities/sports, dehydration to the extent of 2-3% body mass has been associated with a decrement in high-intensity endurance activities (Judelson et al 2007) and the performance of sport -specific skills (Baker et al 2007). The environment also plays a part, as in the heat, aerobic performance decrements are exaggerated (Sawka et al 1996) and dehydration to 3% body mass has little effect on degrading aerobic performance in a cold environment (Cheuvront et al 2005).

There is inconsistency in the literature relating to effects of dehydration on cognition and completion of sports specific tasks (Cheuvront and Kenefick 2014). Effects on cognition appear to relate more to the symptoms of dehydration such as changes in mood state, dry mouth and thirst which distract the athlete and result in higher levels of concentration required to achieve the same level of performance. Kempton et al (2011) and Baker et al (2007) demonstrated a progressive decline in timed skills and shooting skills as the level of dehydration increased (1-4% BM loss) in male basketball players.

In summary, physiological factors that contribute to dehydration-mediated aerobic exercise performance decrements include: increased body core temperature, cardiovascular strain, glycogen utilization, altered metabolic function, and possibly altered central nervous system function. It is thought that each of these factors interact to have an overall degrading effect on aerobic exercise performance (Sawka et al 1985, Sawka and Coyle 1999).

2.4 Mechanisms of body water regulation

Total body water volume is tightly controlled by two sensitive inter-related mechanisms that are stimulated via changes in plasma concentration (osmolality) and changes in plasma volume. The primary and most efficient mechanism of water conservation begins with osmoreceptors located in the hypothalamus (also present in the oropharyngeal and gastric regions) that are highly sensitive to changes in the concentration of extracellular water (plasma). The second mechanism involves baroreceptors, located in the walls of large systemic veins and the right atrium of the heart. Baroreceptors are sensitive to pressure changes only when a plasma volume loss of 10% occurs (≥ 4% body mass loss) (Cheuvront et al 2013) (Figure 2.2).

The renin-angiotensin aldosterone system (RAAS) is activated in response to a decrease in mean arterial pressure as a result of blood volume loss. Angiotensin II stimulates the posterior pituitary to release the anti-diuretic hormone arginine vasopressin (AVP) and increases secretion of the sodium retaining hormone, aldosterone, in order to preserve blood pressure. Both the osmoreceptors and the baroreceptors therefore have a role in stimulating AVP which has a dual role in the stimulation of water conservation by the kidneys and thirst via the hypothalamic region of the brain (Germann 2005).

Thirst sensation is detected by the organum vasculosm of the lamina terminilas (OVLT), located outside the blood-brain barrier in the antroventral part of the third ventricle of the brain. Changes in peripheral Posm associated with changes in body water or Na concentration are sensed by neurons in the OVLT, transmitted to the hypothalamus and stimulate thirst sensation, drinking and AVP release. The second pathway initiating drinking is via the median preoptic nucleus (NM) also in the hypothalamus, which responds to volumetric changes sensed by the atrial baroreceptors (Stachenfeld 2014). The sensation of thirst is activated when a total body water loss equivalent to 1-2% of body mass has occurred (Greenleaf et al 1996). Once drinking commences the osmoreceptors down regulate the thirst sensation despite incomplete body water restoration. This is known as involuntary dehydration and is a safety mechanism to prevent fluid overload, for this reason thirst is not a sensitive measure of body water balance (Stachenfield 2014).

Drinking behaviour is subject to a range of environmental, cultural and psychological factors as well as the availability and organoleptic properties of the fluid (Passe 2001). Typical intake patterns of water/fluids vary amongst populations, for example, lower total water intakes are observed in European populations (~2.5L) compared with North America (~3.18L) (Gibson and Shirreffs 2013), however methodological differences in data collection may also be a factor. The current European guidelines for daily adequate intakes of water are based on observed intakes in population groups with desirable urine osmolarity values and desirable water volumes per energy unit consumed. They are more conservative, 2.0 (females) - 2.5L (males) (EFSA 2010) when compared with the American guideline of 2.7 L (females) and 3.7 L (males) (IOM 2005).

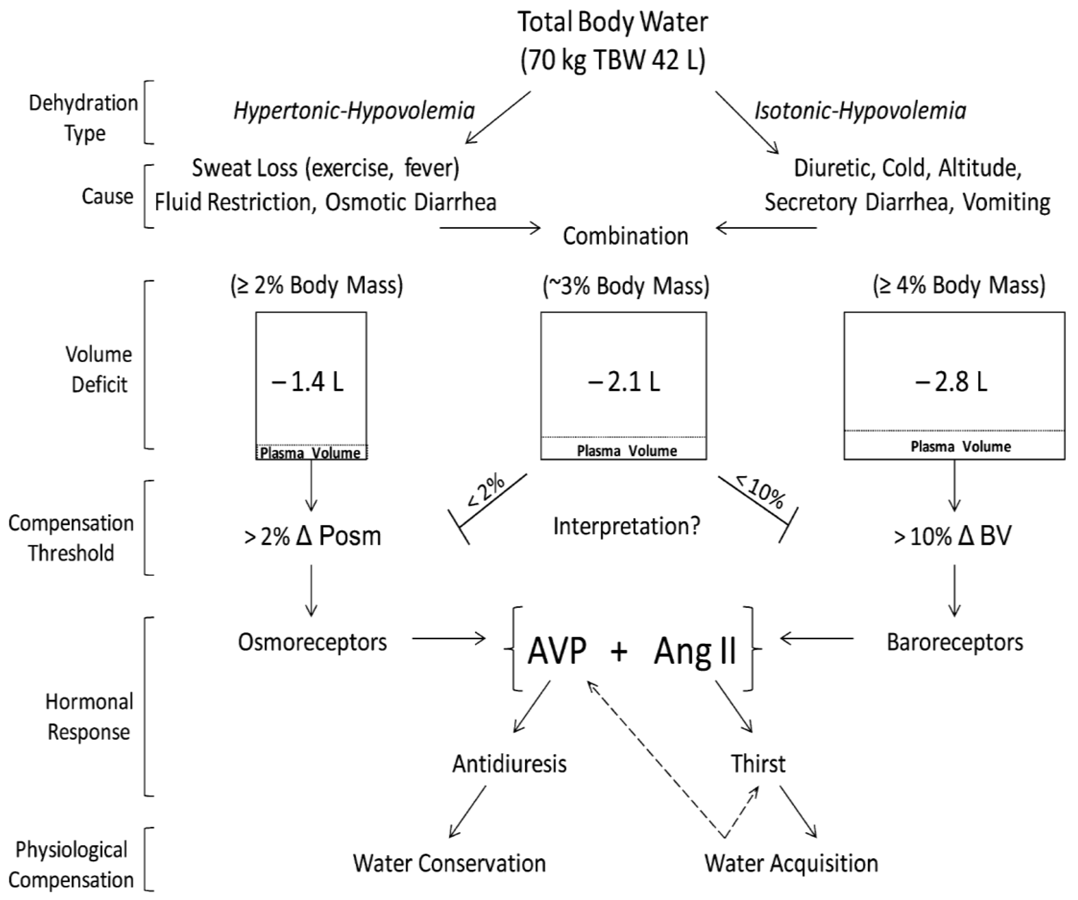


Figure 2.2 Illustration of body water regulation in response to two different types of dehydration (Cheuvront et al 2013)

2.5 Osmoregulation of body water

The osmotic regulation of body water can be considered the first response by the body to conserve water during periods of exercise induced water loss (Figure 2.2) The osmoreceptors are sensitive to small plasma volume losses associated with sweat losses during exercise of ≥ 2% body mass loss (equates to ~ 0.14L of plasma loss in a 70kg male). Posm in a euhydrated state can range between 279 – 291mOsmol/kg but is subject to biological variation (IOM 2005). Within an individual, day to day flux of 1-2% around a basal set point is considered normal biological variation (Cheuvront et al 2013). An increase above this threshold range of > 2% has been shown to stimulate a compensatory secretion of AVP, the anti-diuretic hormone (Cheuvront et al 2013).

AVP stimulates the insertion of aquaporin-2 water channels into the apical membrane of the late distal tubules of the kidneys enabling increased reabsorption of water. This retention of water results in a decrease in extracellular fluid osmolality as total water volume is retained.

2.6 Plasma osmolality and AVP relationship

AVP is a nine amino acid peptide. It is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus, and stored in the posterior pituitary. The reference range for circulating AVP is 1-5pg/mL in healthy adults (0.9-4.6 pmol/L using conversion factor of 0.926 to convert from pg/mL to pmol/L) (NIH 2013). It has been demonstrated that at plasma concentrations of 0.3-0.5pmol/L there is maximum water diuresis consistent with minimal biological activity of AVP and a maximal anti-diuresis occurs as plasma AVP approaches ~ 4pmol/L (Baylis 1987).

Studies assessing the relationship between Posm and AVP secretion were first conducted in the 1970's by Robertson and Athar (1976) who showed a close correlation between Posm and plasma AVP in healthy individuals infused with hypertonic saline. The slope of the regression line was 0.41 pmol/L per mOsmol/kg i.e. for every 1 unit rise in plasma osmolality AVP increased by 0.41pmol/L. Zerbe et al (1991) investigated the reproducibility of the plasma osmolality-AVP relationship in seven healthy subjects, again with an infusion of hypertonic saline. The results confirmed that the slope or sensitivity of the relationship differed widely between subjects but was reproducible within subjects. The individual values for osmotic threshold, i.e. the initial increase in Posm above baseline which stimulates compensatory AVP release, appear to be less variable with a range of ~ 8mOsmol/kg but less reproducible. Zerbe et al (1991) concluded that the sensitivity for AVP secretion as well as the osmotic thresholds for thirst and AVP is subject to significant polygenetic variance among healthy adults.

2.7 Bio-markers of hydration

A range of biomarkers are used in the assessment of hydration and there is on-going debate surrounding the most accurate measure of hydration status and the merits and limitations of the many techniques available (Cheuvront et al 2010, Armstrong et al 2010, Cheuvront and Kenefick 2014). Body water turnover is complex and dynamic and subject to significant biological variation, therefore it is difficult to assign numerical values across a range of parameters for euhydration and dehydration (Cheuvront et al 2011). A list of hydration assessment techniques and the recommended cut-offs for euhydration in the athletic population are presented in Table 2.1. Measurement of total body water using isotope dilution which involves ingestion of a volume of doubly labelled water using deuterium oxide tracer, is deemed to be the most accurate in the research community. Measurement of the concentration of the tracer in the body fluid sampled allows calculation of the total volume of fluid into which the tracer has been diluted. The smallest detectable change in TBW is 2% (0.8-1.2L) (Armstrong 2007). However, this method is not suitable during acute rehydration studies due to the dynamic and rapid turnover in dehydration and the complexity and expense involved (Armstrong 2005).

The choice of biomarker and timing of hydration assessment depends on the study design and whether acute or chronic changes in hydration status are being measured (Armstrong 2007, Stachenfield 2014). In this study the bio-markers used to assess hydration include: body mass change, plasma osmolality, urine osmolality and plasma volume change. The electrolytes Na^+ and K^+ were also measured.

Posm was chosen to assess change in hydration status as it is one of the most widely used haematological indices of hydration status. In studies of acute changes in hydration where a series of Posm measurements are taken, it has been shown to correlate well with changes in BM when hydration is controlled at baseline (Popowski et al 2001) and has shown a positive linear regression relationship with TBW losses (IOM, 2005). However, debate still surrounds its use as a single measurement of hydration due to its regulation around a homeostatic set-point and biological variability in response to dehydration and fluid loading (Armstrong et al 2013, Cheuvront et al 2011). Plasma Na^+ and K^+ concentrations contribute to Posm, with Na^+ concentration being the most important contributor (95%) as it is the main electrolyte found in the ECW (Shirreffs et al 1998).

Uosm is a measure of the concentration of solutes (electrolytes) in urine. The first void morning urine sample has been shown to be an accurate indicator of day to day hydration status among athletes, especially in hot weather conditions (Shirreffs et al 1998). The concentration of urine indicates the concentrating ability of the kidneys as influenced by Posm, which is influenced by the volume and osmolality of the fluid ingested. For example, ingestion of a hypotonic fluid such as water results in a reduction in Posm and an increase in dilute urine production with a low osmolality (≤ 200 mOsmol/kg) (Shirreffs et al 2008). The reference range for urine osmolality is 50 – 1200mOsmol/kg (Germann 2005). When assessing hydration status during periods of rapid body water turnover such as in rehydration studies, Uosm represents the response to the fluid loading rather than the true hydration status. Serial measurements of Uosm in conjunction with Posm will therefore provide an indication of change in hydration status during the period of rehydration and the response of the kidneys to the rehydration solution ingested.

Pvol change is a useful measure of ECW loss that occurs as a result of sweat loss (IOM 2005). It is calculated using measurements of haemoglobin and haematocrit concentrations as per the method described by Dill and Costill (1974). Dehydration increases haematocrit (Packed cell volume (PCV) as the proportion of red cells in whole blood increases when plasma volume decreases. The red blood cell protein (haemoglobin) concentration also increases in concentration as plasma volume decreases. The Dill and Costill (1974) method avoids the distortion of Pvol change results due to altered cell volume by inclusion of haemoglobin concentration in the calculation. Other pre-analytic factors such as postural changes can alter Pvol results (Shirreffs et al 1994) therefore positioning of participants during sampling needs to be controlled for accuracy. Heat acclimatisation and physical fitness influence sweat rates and the level of plasma volume loss for a given dehydration level and should be factored into study design (IOM 2005).

2.8 Use of body mass (BM) to assess hydration status

Acute changes in body mass are used widely in both field and laboratory based studies of dehydration and rehydration as an index of change in total body water. A > 2% loss of BM is accepted as the functional threshold at which body fluid balance is perturbed and an impairment in sports performance is observed, especially in warm environments (Maughan 2003, Sawka et al 2007, Cheuvront et al 2013). Using body mass loss as an indicator of total body water losses is based on a number of assumptions. Firstly, the specific gravity of sweat is assumed to be equal to that of water, however there is a small but variable difference depending on sweat rate and concentration, sweat specific gravity can range from 1.001- 1.008 and water has a specific gravity of 1.0. When sweat rates are high during exercise in the heat it is acknowledged that the precision of measurement of body mass is less than any error introduced by this assumption (Maughan et al 2007). Other methodological issues which may impair accuracy of BM as an indicator of hydration status are faecal losses and incomplete drying of the skin prior to measurement.

Correction for substrate oxidation and respiratory water loss is not usually deemed necessary in acute dehydration and rehydration studies (Maughan et al 2007). The majority of studies involving a dehydration and rehydration protocol control for air temperature, humidity, and exercise intensity which are all factors that influence water losses via respiration and substrate loss and water produced via metabolism. It has been shown that the volume of metabolic water produced during cellular metabolism (~0.13 g/kcal) is approximately equal to respiratory water losses (~0.12 g/kcal) which results in no net change in TBW (Mitchell et al 1972, IOM 2005).

Table 2.1 - Hydration assessment techniques

Hydration Assessment Technique	**Outcome Variable**	**Cut-off for Euhydration**
Isotope Dilution	TBW volume	<2%
Bioelectrical impedance analysis / spectroscopy	TBW, ECW and ICW volume	<2% TBW
Body mass change	Body water loss or gain	<1%
Plasma Osmolality	ECW concentration	<290mOmol/kg
% Plasma Volume change	Haematocrit and haemoglobin*	Relative to baseline Pvol
Urine Osmolality	Urine concentration	<700 mOsmol/kg
Urine Specific gravity	Relative density of urine versus water	<1.020g/mL
Urine Colour	Urine concentration	Colour code: 1-3**
24h Urine Volume	Daily flow rate	1.3-1.6L /day (healthy male)
Saliva osmolality	Osmolality	Unavailable due to high biological variability

*(Dill and Costill 1974, Armstrong et al 1994) **(Sawka et al 2007, Armstrong 2007)

2.9 Fluid and electrolyte replacement – Guidelines and practice

Fluid and electrolyte replacement guidelines vary depending on the level of intensity, duration and recovery time available. For recreational exercise performed for shorter periods (<90min) consumption of normal meals and snacks with a sufficient volume of plain water should restore euhydration, provided the food contains sufficient sodium to replace sweat losses (Sawka et al 2007). Food ingestion during recovery post exercise attenuates fluid retention due to its effect on slowing the rate of gastric emptying and compared to rehydration with water or a CE drink alone, results in greater fluid retention (Maughan et al 1996). Various national sports institutes and the IOC (2010) provide practical and sport specific guidelines for athletes post-exercise, which include the consumption of protein, carbohydrate and sodium containing foods to optimise recovery and rehydration (AIS 2014).

While it is not typical for athletes to abstain from eating for several hours after the end of exercise there are occasions when rehydration within a short time-frame (4-6h) may be required and consumption of solid food may not be tolerated or possible. Many of the published fluid and electrolyte replacement guidelines for athletes (Sawka et al 2007, Casa et al 2000, Shirreffs and Sawka 2011, Maughan 2012) (Table 2.2) are aimed at the athlete who needs to rehydrate within a short time-frame (4-6h) or when there are shorter recovery periods (<24h) between training or competition. The aims of these guidelines are to ensure the athlete commences each exercise session in a euhydrated state and replaces losses during exercise to prevent dehydration (<2% BM loss) and maximise sports performance. However, many guidelines acknowledge that fluid and electrolyte replacement recommendations should be individualised. Rehydration strategies should consider the biological variability in sweat rates as well as variability due to sport type, intensity, access to fluids during play and environmental conditions, especially at a competitive level when multiple bouts of training and competition are taking place within a 24h period (Maughan 2012).

Typically athletes replace only 30-70% of fluid lost via sweat during exercise (Noakes et al 1988). Fluid replacement is an issue for all sports and there are many reasons for athletes failing to drink enough to replace fluid losses. The rules of the sport and sport type may limit fluid replacement opportunities, athletes may be so focused during competition they forget to drink or avoid drinking due to fear of gut discomfort (Burke 2007). When athletes exercise at higher intensity for longer duration (> 1h) or perform in a hot environment dehydration can be > 2% BM loss despite ingestion of fluids during exercise (Maughan and Shirreffs 2010a). It has also been shown that many athletes may begin both training and competition hypohydrated (Shirreffs et al 2006).

The rationale for consuming a volume greater than the body mass loss is to compensate for the continuation of sweat loss during rehydration and respiratory water loss, but predominantly the ongoing urine loss that persists despite individuals being in body-water deficit (Shirreffs et al 1996). The 150% guideline originates from studies that involved systematically administering varying volumes of fluid and varying sodium levels (Shirreffs and Maughan 1998, Shirreffs et al 1996). It was found that when a volume equal to twice the sweat loss was ingested, subjects did not remain in a positive fluid balance when a low-sodium solution was ingested (23mmol/L) however fluid balance was achieved with less volume (150% BM loss) when the sodium content was increased (61mmol/L) (Shirreffs et al 1996). Sodium appears to be the only electrolyte for which a recommendation for replacement is available in published guidelines (Sawka et al 2007). Commercial rehydration beverages typically contain 20-50mmol/L sodium (EFSA. 2011).

Table 2.2 - Fluid replacement guidelines for athletes

Source:	**Quantity**	**Timing**	**Fluid Composition Guidelines**
American College of Sports Medicine (2007)	~1.5 L /kg of body weight lost when there is a relatively short recovery period (<12h) and dehydration is substantial	Fluids should be consumed over time rather than being ingested in large boluses to maximize fluid retention	20 to 30 mmol/L Na^+ ~2 to 5 mmol/L K^+
National Athletic Trainers' Association. Position statement: Fluid Replacement for athletes (Casa et al 2000).	25-50% more than sweat losses.	Should be completed within 2h to assure optimal hydration 4-6h after the event.	Rehydration solution should contain water, carbohydrates and electrolytes.
International Olympic Council (2010)	1.2-1.5 litres of fluid for each kg/BM loss in training or competition	Not specified	Sodium replacement can be achieved via sodium-containing fluids such as sports drinks and pharmacy oral rehydration, or salt containing foods.

2.10 Factors influencing fluid replacement

The main factors that influence effective retention of a rehydration fluid are the rate, volume and composition of the beverage. Recent research has re-emphasized the importance of avoiding rapid rehydration after exercise if a diuresis is to be avoided and euhydration is to be achieved and maintained (Shirreffs and Sawka 2011). The slowing of the appearance into the circulation of the rehydration fluid can be achieved by the drinking pattern, for example 500mL aliquots ingested every 20-30mins (Kovacs et al 2002) or by delaying the gastric emptying of the drink from the stomach into the intestine by modifying the osmolality and energy density of the drink (Evans et al 2009).

2.11 Gastric emptying

Gastric emptying of liquids is regulated by the interaction of gastric volume and feedback inhibition related to the nutrient content in the small intestine. The main factors influencing gastric emptying rates are rate and volume of fluid ingested, energy density and osmolality of the fluid (Maughan et al 2004, Martinez Gonzalvez et al 2005, Vist and Maughan 1995).

Generally, the gastric emptying rate of liquids follows an exponential curve, the greater the volume of fluid ingested the greater the rate of gastric emptying, up to a volume of approximately 600 mL (Costill and Saltin 1974). Beyond this point a further increase in volume may not increase the rate of gastric emptying. Gastric emptying can be maintained at a high rate of >20mL/min by ingestion of either water or a dilute carbohydrate solution (Costill and Saltin 1974).

When a large bolus of pure water or hypotonic fluid is consumed rapidly, it enters the blood stream quickly, resulting in the kidneys producing a large volume of dilute urine before the intracellular and extracellular fluids equilibrate (Armstrong et al 1998). This appears to be a protective mechanism to defend against fluid overload even when complete restoration of fluid deficit is not achieved. Relatively few studies have compared different rates of fluid ingestion post exercise. Kovacs et al (2002) study involving cyclists rehydrating with a carbohydrate-electrolyte solution (120 % replacement) found that plasma volume and net fluid balance increased more rapidly in the high compared to low consumption rate group. However total urine outputs and plasma volume were not significantly different after 6h. The high rate of ingestion in their study consisted of consuming fluids over a period of 3 h post dehydration versus 5 h in low rate. The apparent “metering” of fluid ingestion in both groups, may have led to the similar results reported between the two treatments. Jones et al (2010) studied fluid retention post rehydration comparing a large water bolus, equal to 100% of fluid lost consumed within 60 min versus metered consumption of 12.5% boli consumed every 30 min over 4h. Mean urine production was significantly lower in the metered consumption trial versus the bolus trial. A limitation of these studies was the inclusion of a meal during the rehydration phase which is likely to have influenced gastric emptying and confound results. Studies rehydrating with a volume equivalent to 150% of BM loss comparing a range of fluids of differing composition in a metered approach is lacking.

2.12 Energy density

The energy density of the fluid also impacts on the gastric emptying rate. It has been demonstrated that the higher the energy density, the slower the rate of gastric emptying and fluid absorption (Murray 1987, Vist and Maughan 1995). The proportion of macro-nutrients will determine the energy density of solution, however it appears that the energy density determines the rate of gastric emptying more than the nature of the calories (Calbet and MacLean 1997). In a study comparing solutions with the same energy density composed of different macro-nutrients (protein *vs*. CHO), gastric emptying rates appear similar. Shirreffs at al (2007b) estimated that the gastric emptying rate of milk was ~ 14% slower than a 6% carbohydrate-electrolyte solution which contained 31% less calories than milk.

2.13 Osmolality of ingested fluid

The osmolality of a solution is dependent on the energy density and electrolyte concentration. The higher the CHO content of the solution the higher the energy density and osmolality. The effects of osmolality independent of CHO content on gastric emptying has been investigated using glucose polymers to control for energy density of solutions. It was found that both osmolality and CHO content influence gastric emptying of liquids but the effect of osmolality is more marked at high concentrations of carbohydrate (Vist and Maughan 1995).

2.14 Composition of a rehydration beverage

The optimum rehydration beverage should be formulated to facilitate renal water reabsorption, promote retention of fluid and stimulate the drive to drink. Depending on the intensity of the exercise, replenishment of substrates used during exercise is also an important consideration as part of a recovery programme, particularly for more competitive athletes. The compositional range proposed to aid rapid delivery of fluid and fuel and maximise gastric tolerance and palatability is 4–8% (4–8 g/100 ml) carbohydrate and 23–69 mg/100 mL (10–30 mmol/L) sodium (Sawka et al 2007). In order for a glucose-electrolyte solution to be able to make a claim in relation to hydration, the solution should have an osmolality 200-330mOsm/kg, sodium concentration of 20-50mmol/L, energy level 80-350kcal/L of which 75% should be derived from carbohydrates characterised by a high glycaemic index (EFSA 2011).

2.14.1 Sodium

As discussed sodium is the major electrolyte lost in sweat and therefore replacement is essential in order to maintain extracellular fluid volume through increasing plasma sodium concentration and osmolality which stimulates water reabsorption by the kidneys and the drive to drink (Nose et al 1988a, Maughan et al 1997). Sodium also stimulates glucose and water uptake in the small intestine. It has been shown consistently in rehydration studies that urine output is inversely related to sodium content (Shirreffs and Sawka 2011). However, there appears to be no significant added benefit of increasing the sodium composition of a fluid above 50 mmol/L and such high sodium concentration may decrease drink palatability (Maughan and Leiper 1995, Baker and Jeukendrup 2014, Wemple et al 1997).

2.14.2 Potassium

Potassium is often included in fluid replacement beverages in relatively small amounts (2-5mmol/L), corresponding to the amount generally lost in sweat. The varying concentration of potassium and sodium in rehydration beverages may influence how the fluid is redistributed among body fluid compartments. When the primary electrolyte in the beverage is potassium, slower rates of plasma volume recovery than beverages with lower electrolyte content overall or those with predominantly sodium have been observed (Maughan et al 1994, Nielsen et al 1986). Beverages with potassium as the primary electrolyte may preferentially replace intracellular fluids during rehydration (Yawata 1990) but further research in this area is needed.

2.14.3 Magnesium

There is a lack of evidence to support the inclusion of Mg in rehydration sports drinks, although it does tend to be added in small amounts (~ 0.6mg/100mL). Mg deficiency has been implicated in the development of muscle cramps (Liu K. 1983) The slight decrease in plasma magnesium which has been reported during exercise (Consolazio et al 1963) may be a result of the redistribution of Mg in fluid compartments rather than an actual loss of Mg through sweat.

2.14.4 Carbohydrate

The presence of varying amounts of carbohydrate (CHO) in rehydration beverages has been studied (Osterberg et al 2010, Evans et al 2009, James et al 2013). It has been shown that the type and amount of CHO impacts on the rate of fluid absorption and retention. At a cellular level the co-transport of glucose and sodium facilitates the passive absorption of water across the intestinal mucosa. Increasing the CHO content of the beverage increases the energy density and osmolality. Hypertonic high CHO fluids (10%) have been shown to cause net secretion of fluid into the intestinal lumen thus reducing circulating plasma volume (Gisolfi et al 1990). High CHO > 8-10% containing fluids are not generally advisable when rapid rehydration is required due to the delay in intestinal absorption rate and the potential for gastrointestinal disturbance which is more commonly seen when rehydrating during exercise (Pfeiffer et al 2012).

A study varying the amount of glucose ingested during post exercise rehydration using 0, 2 and 10% glucose solutions standardised for electrolytes, showed that the 2% solution was found to increase plasma volume more rapidly compared with a 10% solution. However, the rapid increase in plasma volume led to a greater diuresis and an overall negative fluid balance compared with the 10% solution at the end of the rehydration period (G. H. Evans et al 2009). The standard CHO content of a sports drink ranges between 4-8g/100mL, this is based on studies to optimise endurance performance and minimise gastric discomfort (Jeukendrup 2010, Sawka et al 2007)

Carbohydrate in a rehydration solution also has a role in replenishing depleted glycogen stores especially when all intake post exercise is in liquid form. This may increase the amount of work that can be completed in a subsequent bout of exercise in situations with a short recovery time (Wong et al 2000, Fallowfield et al 1995). The choice of rehydration fluid based on CHO content will therefore depend on the rehydration goals; whether it is rapid (< 6h) versus longer duration (24h) and whether food intake is planned/tolerated post exercise.

2.14.5 Protein

The influence of protein on rehydration is not completely understood and there are a number of possible mechanisms. Perfusion and absorption studies of the intestine of animals have shown that amino acids enhance sodium and water absorption in the intestine which creates a greater osmotic gradient to attract and retain water (Wapnir et al 1997). The increase in energy density of the protein containing fluid and the clotting of casein (milk protein) in the acid environment of the stomach has been shown to result in delayed gastric emptying (Boirie et al 1997, Hall et al 2003) which delays absorption in the intestine. This delayed uptake of fluid may play a favourable role in fluid retention by maintaining a higher plasma osmolality thus prolonging the stimulus for water reabsorption by the kidney.

A range of milk based protein ingredients have been investigated including milk in its natural form (Shirreffs et al 2007b) as well as whole milk protein or whey protein added to various water or CE solutions. It can be difficult to interpret the findings of some of these studies as compositional factors which influence fluid retention, such as energy density and osmolality are not always controlled. In a study by Seifert et al (2006), although a 15% greater retention was observed in the 6% CE drink with added protein (1.5%), it also had a greater electrolyte content, higher osmolality and energy density compared with the non-protein CE drink, which likely influenced the result. When energy density and osmolality are controlled, fluid retention appears to be greater in a 2.5% protein + 4% CE drink versus a 6% CE drink (James et al 2011). It does not appear however, that protein alone in solution can achieve restoration of fluid balance. Recently, James et al (2014) assessed rehydration with water compared to a 2% whey protein water solution with low osmolality (~14mOsmol/kg). Even with 150% of loss replacement, a positive fluid balance was not achieved and fluid retention was similar and relatively low 37% (water) and 40% (whey protein drink) for both drinks. Therefore it appears that, protein content in addition to electrolyte composition and energy density are all part of a matrix in optimising rehydration.

2.15 Milk – a natural rehydration solution?

The popularity and prevalence of sports and energy drinks consumption in both the athletic and non-athletic population has been associated with adverse health consequences, such as obesity, type 2 diabetes, increased risk for cardiovascular diseases, and dental caries (Park et al). In contrast milk has drawn favourable attention as a post exercise ‘sports drink’ in recent years due to its high quality protein content and its electrolyte composition. Milk is a natural nutrient dense food containing carbohydrate (lactose), proteins, fats, minerals (calcium, phosphorous, zinc, iodine) and vitamins (A, E, B group vitamins) as well as electrolytes (sodium, potassium, magnesium) (Appendix A).

International institutes of sport now advise athletes of the potential negative effects on dental health as a result of over-consumption of sports drinks and provide guidance on healthy drinking techniques (AIS 2014). The acidity and sugar content of sugar sweetened beverages such as sports drinks, lower the plaque and salivary pH resulting in enamel erosion. Milk is less acidic with a pH of between 6.5-6.9 compared with a pH of 5.5 typically found in sports drinks. Consuming milk after intake of sugary foods may help reduce tooth decay in young adults, as it neutralizes acid in the mouth resulting from sugary foods intake (Telgi et al 2013).

Milk meets two of the three criteria for the European Food Safety Authority (EFSA) claim regarding enhanced fluid absorption, as it typically contains 4.6-5% carbohydrate and 44-45mg/100mL (~19mmol/L) sodium. However the carbohydrate source is low glycaemic as opposed to high glycaemic which is the third criteria (EFSA, 2011) Milk as a post exercise drink has been shown to be more effective than a CE drink (with similar CHO content) in promoting post exercise recovery and performance capacity when multiple bouts of exercise are undertaken in close succession (Karp et al 2006). There are also a number of studies that have shown benefits of consuming milk after resistance exercise in stimulating muscle protein synthesis. Gains in fat free mass over a 10 week resistance training program have been demonstrated (Elliot et al 2006) as well attenuating exercise induced muscle damage and delayed onset muscle soreness after exercise (Cockburn et al 2010). Milk contains all the essential amino acids including leucine which is one of the key amino acids involved in muscle protein synthesis (Norton and Layman 2006).

In addition to protein, milk also provides other nutrients essential to the diet of an athlete including minerals such as calcium for bone health, zinc for repair of muscle tissue and immune function and iron for synthesis of haemoglobin. It is also an important source of B vitamins for the athlete. Adequate intake of B vitamins is important to ensure optimum energy production (thiamin, riboflavin) and maintenance of a healthy central nervous system and red blood cells (Vitamin B12). Vitamin B12 can be lacking in the diets of vegetarians so milk can be an important source of this nutrient in the diet of a vegetarian athlete.

One potential drawback to milk as a rehydration drink, for a small percentage of the northern European population (5%-15%) (BNF, 2009) is intolerance to lactose, the sugar found in milk. It is caused by a deficiency or decreased level of activity of the enzyme lactase which breaks down the disaccharide sugar in the digestion process Drinking milk in the large volumes that may be required for rehydration post-exercise may result in gastrointestinal disturbance for individuals who are affected by mild lactose intolerance that does not present itself when consuming more moderate amounts of milk. Those of asian ethnicity may also be limited in choosing milk as a rehydration solution given the greater incidences of lactose intolerance (90–100 %) (de Vrese et al 2001).

This study seeks to build on the evidence that milk, consumed post exercise can enhance fluid retention and fluid balance restoration, compared with a carbohydrate electrolyte solution and water as demonstrated by Shirreffs et al (2007b). The milk drink being investigated in this study was chosen due to its lower calorie content compared with full fat milk. It was felt that using milk with a minimum fat content (0.1% fat) was preferable in order to prevent an over-extension of the gastric emptying rate associated with energy dense liquids which may not facilitate rehydration within the 5h monitoring time-frame. The nutrient and osmolality profile of the 0.1% fat milk chosen for this study is also relatively similar to that used by Shirreffs et al (2007b) and Watson et al 2008 and therefore facilitates comparisons between the metered versus rapid bolus ingestion rates. By utilising a metered approach it is proposed that gastro-intestinal discomfort in the initial stages of rapid fluid ingestion in the Shirreffs et al (2007b) study may be reduced. By measuring the osmotic response to each of the three test fluids via plasma osmolality, urine volume and net fluid balance, the difference in osmotic compensatory water conservation response by the kidneys can also be interpreted.

The aim of this study was therefore to investigate the effectiveness of low-fat milk (0.1%) at restoring whole-body net fluid balance within a 5h period following exercise and thermal-induced dehydration to -2% body mass loss using a metered drinking approach.

Chapter 3
Methodology

3.1 Recruitment

Healthy, physically active, young adult males aged between 18-35 years were recruited from the University of Limerick campus. Based on the variance reported for the mean response in the restoration of fluid balance following rehydration with either proprietary carbohydrate electrolyte sports drink or Milk (Watson et al 2008) >80% statistical power is achieved at a probability of $p<0.05$ by the study of 8 subjects. Interested participants were requested to attend an information session, where they were provided with a subject information sheet (Appendix B) and verbal details outlining their involvement in the study. All participants provided written informed consent and the study was approved by the University of Limerick Research Ethics Committee (2014_02_14_ EHS).

3.1.1 Inclusion criteria

Participants had to be routinely engaging in moderate intensity activity a minimum duration of one hour, three to five times per week. Participants were screened via completion of a physical activity readiness questionnaire (PAR-Q) which verified that they were not taking any medications that would affect measured physiological variables and were free from any known cardiovascular, metabolic or respiratory disease or lactose intolerance (Appendix C).

3.1.2 Exclusion criteria

Those who did not meet the PAR-Q requirements or had known lactose intolerance were excluded. Females were also excluded to limit potential biological variability in fluid retention due to the menstrual cycle (Armstrong et al 2012).

3.2 Study Design

Each subject completed two pre-trial habituation days (A, and B) and three trials (C,D,E) which were randomised and separated by a minimum of 7 days (Figure 3.1).

3.2.1 Pre-trial protocol

The researcher met with each participant prior to the first baseline assessment and provided full details of the study protocol. Guidance was given on completing the food and physical activity diaries. Participants were requested to avoid intake of protein supplements and caffeine intake in excess of their habitual consumption pattern in order to avoid excess diuresis which may compromise hydration status prior to testing (Maughan and Griffin 2003). Food diaries were completed 24h prior to pre-trial habituation days A and B and 48h prior to trial days C, D and E (Appendix F). Participants were requested to replicate the dietary pattern prior to trial day C as closely as possible in the two subsequent test days

A text message was sent by the researcher to participants the day before each trial reminding them to comply with the protocol and to report to the lab at the designated time. Pre-trial habituation days (A, B) involved measurement of body composition (Section 3.5) and biomarkers of hydration (Section 3.6). The researcher also reviewed the dietary records with each participant to ensure accuracy of reporting for the remaining trial period.

On pre-trial habituation day B the work rate at which each participant would exercise during the three experimental trials was determined by an incremental cycle ergometer test. The Karvonen formula (see below) was used to determine the target heart rate (THR) during steady state exercise of an intensity of 60% of age predicted max HR. Participants resting heart rate (HRRrest) was obtained using a HR monitor (Polar RS, Polar electro, Finland). The subjects HRrest was then applied to the Karvonen formula (Karvonen et al 1957) as follows:

- HRmax = 220-Age
- HRrest = Resting Heart Rate
- HRR (heart rate reserve) = HRmax - HRrest
- HR60% = (HRR * 0.6) + HRrest = THR during exercise
- e.g. Age 20y with HRrest = 70
- HR 60% = (200-70)* 0.6 + 70 = 148bpm

The participant then commenced exercising at a work rate of 120 watts (W) (60 rpm). This work rate was incrementally increased until participants reached their steady state THR as calculated above.

3.2.2 Trial Protocol

On each trial day (C,D,E) participants reported to the laboratory at staggered times between 7.00 - 7.20am on each of the three experimental test days, the duration of each test day was 7.5h. On arrival at the laboratory participants were requested to be seated in the laboratory and to complete a visual analogue scale of subjective feelings of thirst. Baseline blood samples were obtained (Section 3.6). Participants then voided their bladder and a sample was retained for analysis. Subject's baseline nude body mass and body composition was measured by bioelectrical impedance analysis (BIA) (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.). Participants were then provided with a controlled breakfast (Appendix D).

One hour post-breakfast participants entered a climatic chamber at an ambient temperature of approximately 30 °C and 38 ± 1.4% relative humidity (measured by AMIS 2000). Participants completed 60 min of steady state sub-maximal exercise (Section 3.2.1), on a cycle ergometer (Monark Ergomedic 874E; Monark Exercise AB, Vansbro, Sweden). A continuous exercise protocol was used to expedite the dehydration process and the timeframe was chosen for the initial trial based an exercise times reported to achieve 2-3% BM loss in studies of similar design and participant profile (Evans et al 2009, Kovacs et al 2002). Three samples (3min duration) of expired air was collected and analysed by indirect calorimetry (AMIS 2000; Innovision A/S, Odense, Denmark) to provide an estimate of energy expenditure and substrate utilisation over the duration of exercise.

Participant's towel dried and nude body mass was measured on completion of 60 minutes of exercise. If a subject had achieved close to the 2% loss of initial body mass, they ceased exercise as it was expected that there would be on-going sweat losses in the time period of showering. If not, their sweat rate was calculated and they returned for the estimated time with intermittent body mass checks, until the target 2% loss from baseline body mass was achieved. After exercise ceased, they were permitted ten minutes to shower and dry before a final nude body mass measurement was obtained. The 2% BM loss target was chosen in this protocol as it is similar to losses observed on completion of a marathon, or similar endurance events and also observed in football players post match play in warm environments (Sawka et al 2007, Shirreffs and Sawka 2011). It is also the physiological threshold at which perturbations in fluid balance are observed (Cheuvront et al 2013)

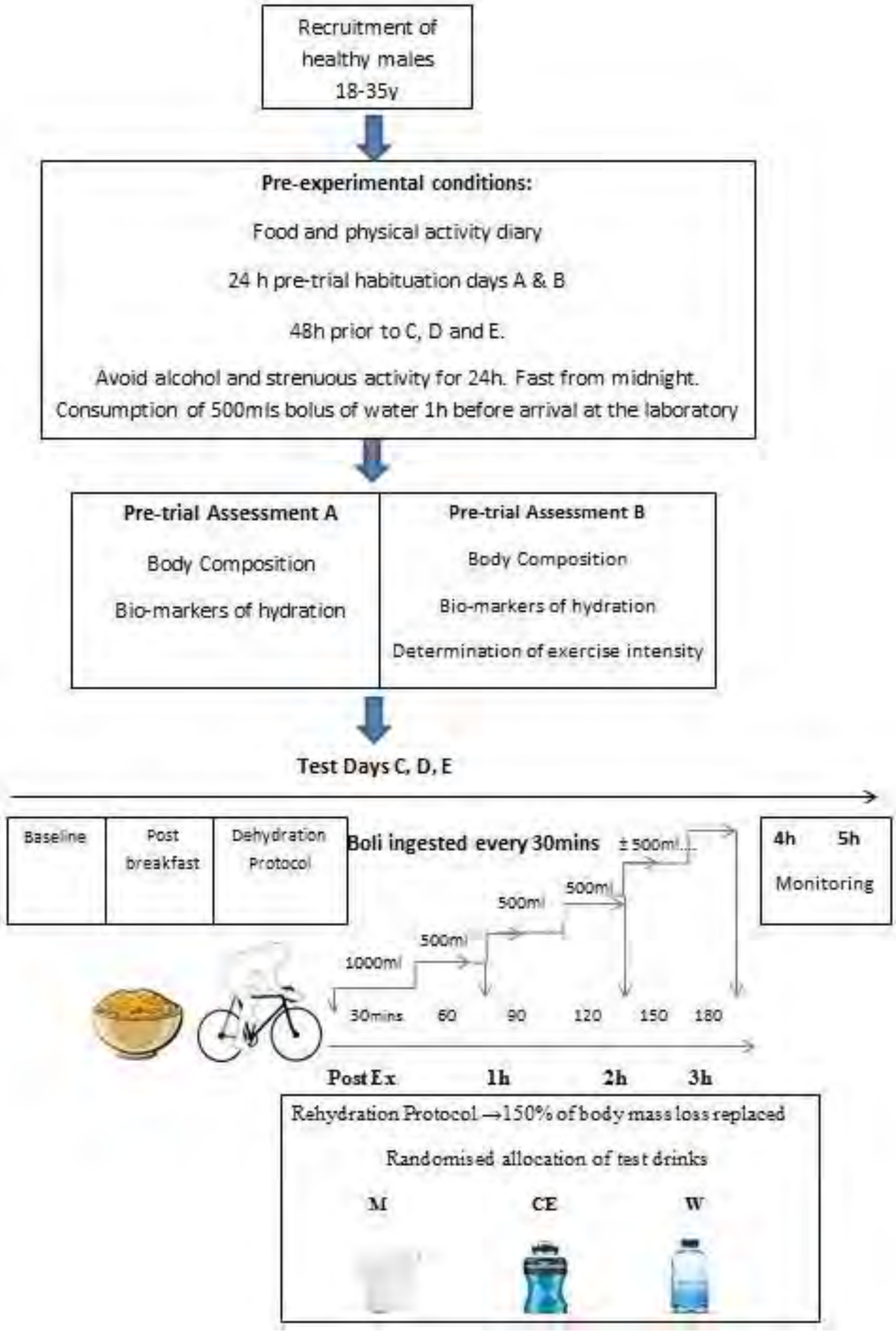


Figure 3.1 – Illustration of trial protocol

3.2.3 Rehydration protocol

Subjects commenced the rehydration protocol twenty-five minutes post exercise. Utilising a metered approach subjects were required to ingest one litre of the test fluid in the first 30 minutes, followed by 500 ml boli (or remainder) every 30 minutes thereafter, until a volume of fluid equivalent to 150% of body mass was ingested. Participants remained in the laboratory for monitoring (room temperature of 22 (2) ºC), for a duration of five hours beginning from the time rehydration commenced. No food or other fluid was consumed during this period. Participants remained in a seated upright position during monitoring. A blood sample, total void urine volume, urine sample, and a measurement of nude body mass were all collected at the end of each hour following the commencement of rehydration (1, 2, 3, 4 and 5h). A final measurement of TBW, ICW and ECW was taken at the end of the fifth hour post commencement of rehydration.

3.3 Test Rehydration fluids

One of three test drinks was randomly assigned to each participant during each of the three trials. Test drinks were not blinded as it was impossible to do so due to the colour and taste differences which could not be masked completely during the study. The test drinks were 0.1% fat Milk (0.1% fat; Tesco Ireland Ltd; trial M), a commercially available carbohydrate-electrolyte drink (Powerade, Coca Cola Ltd, London, UK; trial CE) and water (Centra, Musgrave Retail Partners Ireland, Cork, Ireland; trial W) (Table 3.1). Drink volumes were calculated by multiplying mass lost in kg by 1.5. All test drinks were served at room temperature.

Table 3.1 - Nutritional composition of test drinks*

Nutrient per 100mls	**M**	**CE**	**W**
Energy (kcals)	34	16	0
Energy (kJ)	145	70	0
Carbohydrate (g)	5	3.9	0
Fat (g)	0.1	0	0
Protein (g)	3.3	0	0
Na^+ mg (mmol/L)	41.2 (17.9)	50 (21.7)	1.5 (0.7)
K^+ mg (mmol/L)	176 (45.2)	12.5 (3.2)	0.11 (0.03)
Mg mg (mmol/L)	12.5 (5.2)	0.6 (0.3)	1.1 (0.5)
Ca (mg)	120	1.3	24
Osmolality (mOsmol/kg)	280 (2)	299 (2)	11 (1)

*Macronutrient content and energy density were obtained from the manufacturers as detailed in section 3.3. Milk (M), carbohydrate-electrolyte drink (CE) and water (W). The Na+, K+ and Mg composition of milk was obtained by an external laboratory on behalf of the manufacturer.

3.4 Blood sampling

A phlebotomist took blood samples from all participants. Each participant had an 18G cannula placed at the anti-cubital fossa vein at the beginning of each of the three test days which remained in position for the duration of the trial. Cannulas were placed in accordance with the University of Limerick approved procedure EHSREC_10_RA02. All blood samples were collected after 15 minutes of being seated to avoid postural changes in plasma volume. The exception to this was a blood sample taken at 10 min prior to the estimated finishing time of exercise. This sample was collected for observation purposes and is not included in the overall analysis of results. Samples were collected without stasis and the venous line was kept patent by flushing with 5mls of saline (0.9%) between samples. At each blood draw the first two millilitres were discarded to avoid saline contamination and a total of 7mls of blood were drawn at each time point. Blood samples drawn were divided between lithium heparin (x2) and EDTA (x2) vials (Starstedt Ltd, Waterford, Ireland). Blood samples were collected in lithium heparin for the purposes of blood glucose, haemoglobin and haematocrit measurements as per manufacturer instructions. EDTA tubes were used for future analysis of arginine vasopressin as recommended by the manufacturer assay protocol. One vial of the lithium heparin whole blood sample was used for analysis of haemoglobin, haematocrit, glucose, Na and K^+ concentration. All remaining vials were centrifuged (5000rpm, 4°C for 10 minutes) and approximately 1ml of the supernatant was aliquot and stored at -80°C. Blood samples were taken at the following time-points; baseline, during exercise, post exercise, and at 1,2,3,4 and 5h post commencement of rehydration.

3.5 Body composition

3.5.1 Body mass and height.

Nude Body mass (BM) was measured to the nearest 0.05kg using an electronic scales (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd, Middlesex, UK). Height was measured to the nearest 0.1cm using a stadiometer (Seca 213, Seca, Birmingham, UK).

3.5.2 Total, intracellular and extracellular body water

Total body water (TBW), intracellular (ICW) and extracellular water (ECW) were indirectly measured to the nearest 0.05kg using the bioelectrical impedance analyser (BIA). Subjects were instructed to remove their clothing and stand barefoot in contact with the metallic electrodes. When prompted participants grasped the hand grips and held them by their sides with metallic electrodes in contact with the palm and thumb. Arms were extended and abducted away from the body according to the manufacturer’s instructions (Tanita 2005). For privacy and ethical purposes, the subjects performed this behind a portable screen.

The Tanita MC-180MA is a four frequency body composition analyser consisting of low and high frequencies of 5, 50, 250 and 500 kHz. The lower frequencies measure the impedance external to the cell membrane. The higher frequencies penetrate the cell membrane. By measuring impedance at both the lower and higher frequencies it is possible to estimate ECW, ICW and TBW. The GMON software (Tanita, v1.7.0) generated values for TBW, ICW and ECW. Repeated measurement using the Tanita MC-180MA has a coefficient of variance of 0.4% (Leahy et al 2012). The standard operating procedure was followed at baseline, to ensure reliability of measurements. (Tanita, 2005). The final BIA measurement at 5h post-exercise was not within the conditions recommended by the manufacturer. It is acknowledged that there are limitations in the use of BIA in the measurement of TBW, ECW and ICW during acute changes in hydration status (Utter et al 2012, O Brien et al 2002) with BIA tending to overestimate TBW (Moon et al 2014). Further validation studies on the use of BIA in the measurement of hydration status and body composition are needed, this was outside of the scope of this study.

3.5.3 Lean and Fat tissue mass

Dual energy x-ray absorptiometry (DXA; Lunar iDXA^{TM}, GE Healthcare, Chalfront St. Giles and Bucks., UK) was used to measure total body fat mass (kg) and total lean tissue mass (kg) at baseline on test day A. The exposure dose is 0.4µGy ionising radiation. The subjects were required to lie on the DXA bed for the duration of the scan (7 minutes). The DXA was performed and results reported by a trained operator within the research unit.

3.6 Analytical methods

3.6.1 Urine Volume

All urine was collected in a specified urine container assigned to each participant. If a subject needed to void their bladder during the time period in between collections, the volume of urine was collected and added to the volume at the end of the next hour. Total urine volume for each hour was measured using a volumetric cylinder (Azlon Plastics, Staffordshire, UK) which measured volume to the nearest 10ml.

3.6.2 Osmolality of urine and blood plasma

Both urine and plasma osmolality were measured by freezing point depression using the Advanced Micro-osmometer Model 3220 (Advanced Instruments Inc. Norwood, MA, USA). Duplicate 2ml urine samples were stored in air-tight Eppendorfs at -80°C, on day of testing and analysed once full testing was complete. Urine samples were defrosted in small batches at room temperature (19°C) for one hour before analysis as described by Sparks and Close (2013). Plasma samples were defrosted in a water bath at a temperature of 37°C and defrosted within 3 minutes as per method described by Seifarth et al (2004). Urine samples were defrosted at room temperature for one hour as per the method used by Sparks and Close (2013). Both plasma and urine samples were vortexed for 10 seconds prior to analysis to ensure any sediment was redistributed evenly within the sample.

Instrument calibration was performed twice weekly during batch analysis. A two stage calibration was conducted in triplicate by using standards within the dedicated ranges for biological fluids; saliva (50 mOsmol/kg), and urine (850 mOsmol/kg). A Clinitrol™ 290mOsmol/kg control solution was also run in triplicate every day of testing according to the manufacturer's instruction (Advanced Instruments Inc. 2014).

An analysis of fresh versus frozen urine and plasma samples was conducted to assess the variance in measurement as it was not possible to analyse samples during testing. Urine and plasma samples obtained from three participants were tested in triplicate (Table 3.2). Frozen samples had a variance from fresh of -1 (0.3) % for Posm. The variance between fresh and frozen Usom samples was 1.2 (0.9)%. The variance between fresh and frozen samples was accepted for Posm as it was within the limits of intra-individual biological variation of 1.3% for Posm and therefore below the threshold of a clinically significant change in Posm (Cheuvront et al 2010). The variance in Usom between fresh and frozen samples was accepted as it was considerably less than 95mOsmol/kg which is the change in Uosm associated with a clinically significant 1% change in Posm (Robertson et al 1976).

Table 3.2 - Mean variance between frozen and fresh samples for Posm and Uosm*.

Plasma osmolality				Urine osmolality			
Sample	Fresh	Frozen	% Variance	Sample	Fresh	Frozen	% Variance
1	298	294	-1.30	1	894	906	1.3
2	299	297	-0.70	2	1088	1090	0.2
3	299	296	-1.00	3	233	238	2.0
Mean Variance: -1.0 (0.3) %				Mean Variance: 1.2 (0.9) %			

*All samples tested in triplicate and values presented as mean (SD).

All urine samples were tested in triplicate and the mean value was taken as the final value (CV ≤2%). Plasma samples were tested in duplicate if ≤ 1mOsmol/kg difference between samples, in triplicate if greater than 1mOsmol/kg but less than 1% difference and if any of the intra-sample triplicate measures differed by > 1.0%, the median of 5 samples was used, this is a modified version of the method used by Cheuvront et al (2010). This approach was based on instrument resolution and the potential physiologic importance of small fluctuations (>1.0%) in Posm to hormonal fluid regulation. The median value was taken as the final value. Unit of measurement of osmolality for urine and plasma is reported as milliOsmol per kilogram (mOsmol/kg).

3.6.3 Haemoglobin

The spectrophotometric method using the modified azidemethemoglobin reaction with the HemoCue Hb ® 201+ device (HemoCue AB, Ängelholm, Sweden) was used to measure haemoglobin. The absorbance is measured at dual wavelengths: 570 nm to quantify azidemethemoglobin and 880 nm to compensate for sample turbidity. The absorbance is directly proportional to the haemoglobin concentration.

In order to minimise error, manufacturer's instructions were followed and samples were processed within one minute of filling the micro-cuvette and within 20 minutes of blood sample collection. The expected reference range stated by the manufacturer for males is 13-17g/dl (HemoCue AB, 2014)

According to the manufacturers the HemoCue Hb ® 201+ is factory calibrated and needs no further calibration. Quality control checks were performed prior to each day of testing in order to monitor the performance of the analyser. Two level control solutions recommended by the manufacturer (R&D Glu/Hgb control, Bio-techne Corporation, USA) were used (lot number GH024) and values obtained were within the expected ranges supplied by the manufacturer.

An analytical CV of 1.9% was obtained by repeated testing of one individual sample 10 times using the same batch of micro-cuvettes and the same analyser. This was within accepted clinical limits of 2% (Bain 2006). Duplicate measurements were taken and if the acceptable CV of 2% was exceeded then a third measurement was taken. The mean of the values was taken as the final value. Haemoglobin is reported as g/dL.

3.6.4 Haematocrit

Haematocrit was measured directly by micro-haematocrit centrifugation using the Hawksley Haematospin 1400 micro-haematocrit centrifuge method. Sodium heparinised capillary tubes were filled directly through capillary action with 75µl of whole blood from the lithium heparin collection tube, to within 15mm from the end. The capillary tubes were then sealed and centrifuged at a set speed of 11,800rpm for 5 minutes as per manufacturer's instructions. Samples were then read using a micro-haematocrit reader. To read the haematocrit value the centrifuged microhematocrit tube was placed vertically on the heamatocrit reader (Figure 3.2) with the bottom edge of the clay sealant just touching the red line below the “0" percent line. The bottom of the column of the blood was aligned to the “0" % line. The heamatocrit tube is then positioned by sliding the tube along the chart until the meniscus of the plasma intersects the “100" % line. The height of the packed red cell column is then read as a percent value.

Intra-sample CV of 1.7% was determined by the researcher by repeated measurement of one whole blood sample 10 times on the same day at the same time. The accepted biological and analytical variation is 3% (Thirup 2003). Duplicate measurements were taken and the mean value taken as the final value. Sources of error were minimised as much as possible through appropriate sample handling and processing as per manufacturer’s instructions. Haematocrit is reported as percentage packed cell volume (% PCV).

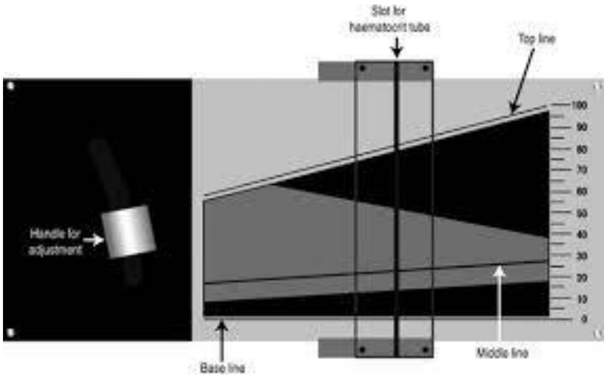


Figure 3.2 Haematocrit reader

3.6.5 Blood glucose

Blood glucose was quantified using the HemoCue Glucose 201 (HemoCue AB, Angelholm, Sweden) based on the spectrophotometric method. A fresh 4 µL blood sample is drawn up by capillary action into the cavity of the micro-cuvette which is immediately placed in the HemoCue Glucose 201 analyzer. The transmittance is measured and the absorbance and glucose level calculated. According to the manufacturers the HemoCue is factory calibrated and needs no further calibration. The calibration of the analyzer is traceable to the Isotope Dilution GC-MS method as per manufacturer. Quality control checks were performed using three levels of control solutions of known blood glucose concentration, prior to each day of testing, in order to monitor the performance of the analyser. Values obtained were within the expected ranges supplied by the manufacturer. The reported analytical CV by the manufacturer is 1.6-3.5% across a range of blood glucose concentration levels (Hemocue, 2014). Blood glucose was measured in duplicate with an accepted CV of 3% and the mean value taken as the final value. Results are reported in mmol/L

3.6.6 Blood electrolyte concentration

The I-STAT portable clinical analyser and EG6+ cartridges (Abbott Inc., Illinois, USA) were used to measure sodium and potassium concentrations of venous whole blood. The I-STAT employs an electro-analytical method of ion-selective potentiometry using the Nernst equation. Potentiometry passively measures the potential of a solution between two electrodes, the potential is then related to the concentration of the analyte being measured.

A 95 μL blood was pipetted into a port in the EG6+ cartridge within 15 minutes of blood draw, as recommended by the manufacturer. The cartridge is immediately inserted into the I-STAT hand-held analyzer. A motor in the analyzer moves the calibrant across the electrodes to perform a single-point calibration, and then pushes the test sample forward for contact with the electrodes. The test cycle is completed and results displayed in two minutes. In order to confirm conductivity performance of the I-STAT™ analyser an external electronic simulator test was conducted prior to each day of testing as recommended by the manufacturer. The I-STAT™ analyser is a unit-use testing system. The cartridge sensors are exposed to sample once only so there is no protein build-up, which is a major cause for deterioration of sensor slope and the need to calibrate and/or verify calibration on a frequent basis in multi-use analysers. Calibration was conducted as per manufacturer's guidelines using a level three control solution for each new batch of cartridges. The lot numbers were recorded and results fell within the manufacturers assigned values for each batch of cartridges.

The reportable range stated by the manufacturer is 100-180mmol/L for Na+ and 2-9mmol/L for K^+. The normal reference range for Na+ is 135-145mmol/L and K^+ is 3.7-5.2mmol/L. Studies evaluating the I-STAT against validated clinical laboratory methods have reported values obtained with the I-STAT within 1.2mmol/L for Na^+ ($r = 0.897$) and 0.21mmol/L for K^+ ($r = 0.991$) (Mock et al 1995 and Abbott Inc. 2013). This deviation was deemed acceptable for the requirements of this study. The I-

STAT^{TM} analyser is a point of care system and therefore one measurement was taken as the final value unless it fell outside of the normal reference range, in which case, a repeated measure was taken as final if within the normal range. Results are reported as mmol/L

3.6.7 Plasma Volume

Blood and plasma volume changes were calculated from haemoglobin concentration and Haematocrit (packed cell volume) values according to the method of Dill and Costill (1974). Plasma volume change is expressed as a percentage (%).

3.7 Nutritional intake and substrate utilisation

3.7.1 Evaluation of dietary records

Nutritional analysis of the food diaries was conducted using WISP dietary analysis software package (Tinuviel Software V 4.0, Warrington, UK). Food records were inputted into WISP by the researcher and an assistant. Any incomplete or unclear entries were resolved using a 24-hr recall on the day the participant returned to the laboratory or by emailing or phoning the subjects thereafter. The purpose of the dietary record was to ensure that dietary patterns remained consistent as much as possible prior to each trial day. Dietary intake was evaluated for total energy (kcal and kJ), macronutrients (fat, protein and carbohydrate (g)) and sodium and potassium (mg) content.

3.7.2 Substrate Utilisation and replenishment

Gas samples were measured for the rate of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) by indirect calorimetry (AMIS 2000; Innovision A/S, Odense, Denmark). The ratio of carbon dioxide produced to oxygen consumed gives the respiratory exchange ratio (RER). Non protein respiratory quotient (NPRQ) was then calculated to determine the rate of energy expenditure and the rate of carbohydrate and fat metabolised using the following equations by Consolazio et al (1963) and modified by Peronnet and Massicotte (1991).

- Energy (kJ/min) = (15.8 * VO2) + (4.86 * VCO2)
- Carbohydrate oxidation (g/min) = (4.585 * VCO2) - (3.226 * VO2)
- Fat oxidation (g/min) = (1.695 * VO2) - (1.701 * VCO2)

Replenishment of substrate utilised during exercise by the test drinks M and CE is calculated as a percentage using the following formula:

> Replenishment (%) = CHO ingested from test drink / CHO oxidised during exercise x 100

3.8 Assessment of Fluid Balance

3.8.1 Net Fluid balance

Net fluid balance was calculated using the following equation:

> Net fluid balance = Fluid intake during rehydration – sweat loss during exercise + urine loss during rehydration

Relative net fluid balance was calculated as a percentage using the following equation:

> Relative Fluid Balance (%) = Final Net Fluid balance (mL) / total body mass loss during exercise (mL) x 100

3.8.2 Fluid Retention (%)

Fluid retention was calculated as a percentage of the amount of fluid ingested post-exercise that was retained in the body at the end of the five hour monitoring period. The calculation used is as follows;

$$Fluid\ Retention\ (\%) = \frac{Total\ Volume\ ingested - volume\ of\ urine\ excreted}{\text{Total Fluid ingested}} \text{ x } 100$$

3.9 Visual Analogue Scales

Subjective feelings related to hydration and perceived taste characteristics were assessed using visual analogue scales with responses logged on a continuous scale from 0mm to 100mm. The following subjective feelings were assessed regarding hydration status: thirst, hunger, bloatedness, mouth taste, alertness, headache, tiredness and refreshment. The perceived taste characteristics assessed were; visual appeal, palatability, aftertaste, sweetness and saltiness (Appendix E).

3.10 Statistical Analysis

All data were checked for normality of distribution using Shapiro-Wilk test. All data containing two variables were then analysed using a two-way repeated measures ANOVA. Variables containing one factor were analysed using a one-way repeated measures ANOVA with Bonferroni adjustment. Wilcoxon signed-rank test was performed within trials between two time-points, as appropriate for non-parametric data. Friedman test was performed on dietary intake variables to analyse differences between the three trials as data was non-parametric. Paired sample t-test was used to compare two time points (baseline with 5h measurement) within a trial. Data are presented as mean and standard deviation (SD) unless otherwise stated. Differences were accepted as being significant when $p < 0.05$. Results were analysed using SPSS 18.0 (SPSS, Inc).

Chapter 4
Results

4.1 Participants

Eight males were recruited from the student population at the University of Limerick. One of the participants did not meet the 2% body mass loss requirements on each of the experimental days and therefore was excluded from the final analysis. Table 4.1 outlines the characteristics of the sample population studied.

Table 4.1 - Age profile and body composition of participants

	Unit	Mean (SD)	Range
Age	y	26.1 (5.9)	19.4-34.0
Height	m	1.79 (0.04)	1.7-1.9
BM	kg	86.4 (11.5)	69.8-100.2
BMI	kg/m^2	26.9 (3.3)	22.3-31.3
BF	%	21.4 (4.5)	19.5-28.1
BFM	kg	18.8 (6.1)	11.7-28.1
LTM	kg	64.7 (6.6)	55.4-75.5
TBW	kg	51.0 (5.0)	42.7-58.8
ECW	kg	19.7 (1.6)	17.0-21.5
ICW	kg	31.3 (3.5)	25.6-37.3

BM = Body mass, BMI = Body mass index, BF = Body fat, BFM = Body fat mass, LTM = Lean tissue mass, TBW = Total body water, ECW = Extracellular water, ICW = Intracellular water. Values are mean (SD) (n = 7).

4.2 Pre-trial conditions

Overall dietary intake was consistent among participants prior to each trial. There were no statistical differences found in fluid intake 3401 (1519) mL (M), 2607 (1025) mL (CE), 3078 (1229) mL (w) between trials (p = 0.156) (Friedman test, values are median (IQR)). Sodium intake also did not differ between trials 3005 (2294) mg (M), 3074 (2247) mg (CE), 3018 (2407) mg (W) (p = 1.000, median (IQR)). Potassium, protein, CHO and energy intake prior to each trial were also consistent (Appendix G). Of note, participant five had a median habitual fluid intake of 2085 (217) mL prior to each trial which is less than the recommended adequate intake of 2500mL for adult males (EFSA 2010). Baseline body mass, TBW, ECW, ICW were also similar between trials (Table 4.2).

Table 4.2 - Summary of total body mass and body water at baseline for each trial

Mass (kg)	**M**	**CE**	**W**	***p*-value***
BM	85.60 (11.5)	86.16 (11.6)	85.71 (11.0)	0.559
TBW	49.98 (4.46)	49.77 (4.23)	49.75 (4.17)	0.909
ECW	19.35 (1.49)	19.42 (1.53)	19.37 1.49()	0.853
ICW	30.64 (3.11)	30.36 (2.80)	30.40 (2.79)	0.837

*One way repeated measures ANOVA. Values are mean (SD). BM = Body Mass., TBW = Total Body water, ECW = Extracellular water, ICW = Intracellular water.

4.3 Baseline Plasma Osmolality

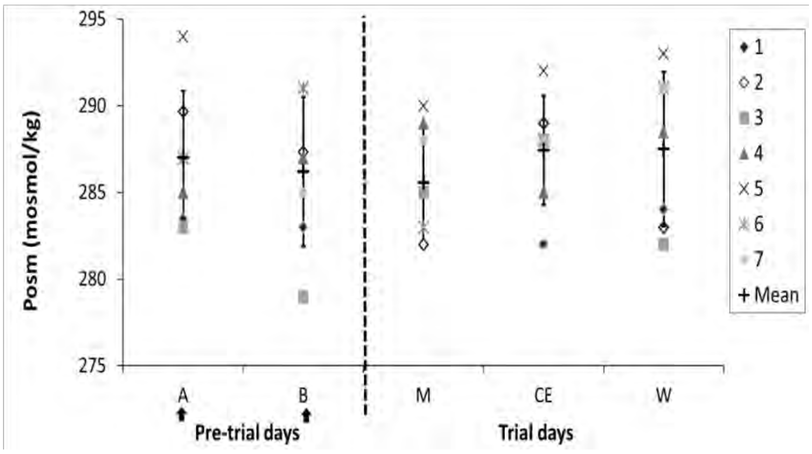
Baseline plasma and urine osmolality are presented for the pre-trial habituation days (A and B) and each of the trial days (M, CE and W) in Figure 4.1 (a) and (b) and Table 4.3. The within-participant biological coefficient of variance for Posm during the trial days M, CE and W was 0.4-1.4% which is similar to findings reported in the literature (1.3%) (Cheuvront et al 2010). The mean baseline Posm over the five baseline measures was 287 (3.7) mOsmol/kg, and for the three trial days (M, CE, W) was 287 (3.6) mOsmol/kg. The range in Posm between participants across the 5 baseline measures was 15 mOsmol/kg. The highest range in baseline Posm for an individual participant at presentation for trial M, CE and W, was 290-293mOsmol/kg (participant five) (Figure 4.1(a)). This participant consistently had a fluid intake lower than the recommended adequate intake as stated in section 4.2. The recommended cut-off for euhydration for athletes is 290mOsmol/kg (Sawka et al 2007). However, similar values for baseline Posm have been reported 292 (3) mOsmol/kg) in studies that have controlled for euhydration using prescribed fluid intakes (Cheuvront et al 2010).

4.4 Baseline Urine Osmolality

The range in baseline Uosm among all participants (Figure 4.1 (b)) was 186-1087mOsmol/kg on pre-trial habituation days (A, B) and between 132 mOsmol-926 mOsmol/kg on experimental days (M, CE, W) (Figure 4.1 (b)). The cut-off for euhydration for athletes is <700mOsmol/kg Sawka et al (2007). This cut-off was exceeded in the case of two participants (5 and 6) who presented at baseline with a range of 540-884mOsmol/kg (CV=19%) and 846-1087mOsmol/kg (CV=10%)

respectively. Although Uosm was > 700mOsmol/kg, the CV attained was below the accepted biological CV of 28.3% reported in the literature (Cheuvront et al 2010) indicating that this represents normal biological variation range for these participant's.

(a)



(b)

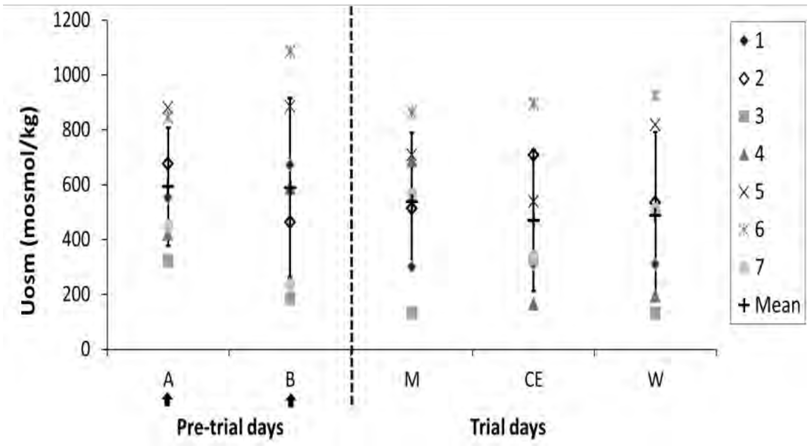


Fig.4.1(a) Baseline Plasma osmolality (Posm) and (b) Baseline Urine osmolality (Uosm) for each participant (n=7) on presentation at pre-trial habituation days (A,B) and trial days MILK (M), Carbohydrate-electrolyte (CE) and Water (W) trials. Values are mean (SD). Legend indicates subjects 1-7.

There was a moderate association between Uosm and Posm ($r^2 = 0.341$) ($p = <0.01$), indicating that 34% of the change in Usom is associated with the change in Posm (Figure 4.2)

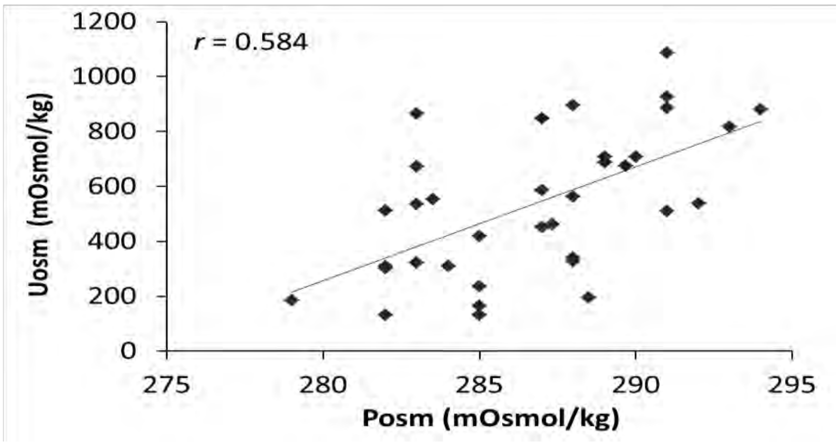


Figure 4.2 - Pearson correlation co-efficient of mean plasma and urine osmolality using five baseline measurements from each participant (n = 7).

4.5 Baseline electrolytes and blood glucose

There was no difference in participants sodium, potassium or blood glucose at baseline on each trial day (Table 4.3) and all values were within normal reference ranges (Blann 2007).

Table 4.3 - Blood and urinary markers at baseline for pre-trial and trial days

Hydration Biomarker	**A**	**B**	**M**	**CE**	**W**	***p*-value*** $F_{2,12}$
Uosm (mOsmol/kg)	539 (216)	469 (328)	539 (251)	469 (258)	489 (302)	0.680
Posm (mOsmol/kg)	286 (3.8)	287 (4.3)	286 (3.4)	287 (3.2)	288 (4.4)	0.330
Sodium (mmol/L)	140.7 (2.1)	141.8 (2.5)	140.7 (1.7)	140.6 (2.9)	139.9 (2.0)	0.552
Potassium (mmol/L)	4.1 (0.3)	4.2 (0.4)	3.9 (0.1)	4.0 (0.3)	3.9 (0.3)	0.947
Blood Glucose (mmol/L)	4.9 (0.5)	4.8 (0.4)	4.9 (0.5)	4.8 (0.4)	4.9 (0.5)	0.678

* One way repeated measures ANOVA of baseline blood and urinary markers for the M (Milk) CE (Carbohydrate-electrolyte) and W (Water) trials only. Values for A and B pre-trial habituation days also provided as reference. Values are mean (SD).

4.6 Dehydration phase

4.6.1 Body mass loss

The dehydration protocol resulted in similar total reductions in BM during each trial. BM loss during exercise in heat was 1.82 (0.28) kg (M), 1.86 (0.37) kg (CE) and 1.84 (0.28) kg (W) ($p = 0.844$) which translated to a percentage BM loss of 2.13 (0.15) % (M), 2.16 (0.21) % (CE), 2.15 (0.20) % (W).

4.6.2 Exercise duration and substrate utilisation

All subjects exercised at a steady state at 60% VO_2max. The exercise and heat exposure time required to reach the desired 2% body mass was not statistically different between trials with a mean duration of 100.9 (15.8) min for the M trial, 93.1 (10.5) min for the CE and 94.3 (17.2) min for the W trial ($p = 0.162$).

Energy expenditure and substrate utilisation data were calculated as detailed in section 3.7.2. There was no difference in energy expenditure ($p = 0.440$) or carbohydrate oxidation between trials ($p = 0.361$). Fat oxidation was also similar between trials ($p = 0.603$) (Table 4.4). Energy expenditure and substrate oxidation values for each participant are provided in Appendix G.

Table 4.4 - Mean energy expenditure and substrate utilisation during each trial.*

Test drink	CHO (g)	CHO (g / kg BM)	Fat (g)	Fat (g / kg BM)	Energy (kJ)	Energy (kJ / kg BM)
M	166.5 (30.7)	1.95 (0.29)	28.8 (5.8)	0.34 (0.06)	3730 (663)	43.7 (6.4)
CE	148.5 (27.6)	1.74 (0.32)	28.8 (5.1)	0.33 (0.04)	3440 (560)	40.3 (6.0)
W	170.5 (39.7)	1.99 (0.37)	31.1 (8.6)	0.36 (0.09)	3877 (950)	45.2 (9.6)

*Fat and CHO oxidation calculated using Consolazio et al (1963) and modified by Peronnet and Massicotte (1991). Values are mean (SD)

4.6.3 Plasma osmolality and plasma volume

There was an increase in mean Posm from baseline to post exercise in all trials (M, CE and W). The change in Posm of 6.0 (3.5) mOsmol/kg (M), 5.0 (4.3) mOsmol/kg (CE) and 7.3 (2.4) mOsmol/kg (W) was not significantly different between trials ($p = 0.241$). The relative change in Posm was 2.1 (1.2) % (M), 1.7 (0.9) % (CE) and 2.6 (1.2) % (W) ($p = 0.225$).

Plasma volume changes were calculated relative to baseline. Within the sample population studied there was a high degree of heterogeneity between participants in the response to dehydration. The mean change in plasma volume post exercise for each trial was -5.1 (3.5) % (M), -3.7 (7.8) % (CE), -3.2 (4.5) % (W). There was no statistical difference identified between trials (p = 0.747).

4.6.4 Electrolytes

Post exercise plasma Na^+ was similar between trials (p = 0.909). The pooled mean at baseline was 140.4 (2.2) mmol/L and 141.1 (2.4) mmol/L post exercise (p = 0.540). There was an increase in K^+ in the M trial (3.9 (0.1) to 4.2 (0.1) mmol/L) (p = 0.004) and W (3.9 (0.3) to 4.2 (0.2) mmol/L) (p = 0.046) trial but not in the CE trial (4.0 (0.3) to 4.1 (0.4) mmol/L) (p = 0.094). Post exercise K^+ concentration was similar in each trial M, CE and W (p = 0.894).

4.6.5 Blood Glucose

There was no statistically significant change in blood glucose from baseline to post-exercise in M (p = 0.549), CE (p = 0.580), Wilcoxon signed rank test) and W trial (p = 0.068). Blood glucose concentration post exercise was similar between trials M (5.1 (0.5) mmol/L) CE (5.1 (0.7) mmol/L) and W 5.5 (0.7) mmol/L (p = 0.292).

4.7 Rehydration phase

4.7.1 Rehydration fluid volume

As mean body mass loss was similar in each trial (Section 4.6.1), the mean rehydration volume ingested was also similar. Rehydration volumes ingested were 2732 (426) (M), 2796 (556) (CE), 2764 (416) (W) (*p* = 0.844). Duration of the rehydration phase was between 2-3hours.

Table 4.5 - Mean hourly volume of test drink ingested during rehydration phase.

Volume (mL)	M	CE	W	P-value*
1h	1500	1500	1500	-
2h	907 (164)	904 (150)	961 (93)	0.411
3h	325 (290)	393 (445)	304 (370)	0.608
Total	2732 (426)	2796 (556)	2764 (416)	0.844
Total per kg BM	32 (2)	32 (3)	32 (3)	0.870

* One-way ANOVA. Mean values calculated from hourly intakes of each participant for Milk (M), carbohydrate-electrolyte (CE) and water (W) trials. Values are mean (SD)

4.7.2 Nutrition content of test drinks

The mean energy, macro-nutrients and electrolytes provided by each of the test drinks are summarised in Table 4.6 and 4.7. The CE drink contains 53% less energy per 100mL compared to M drink. Protein contributed 39% and fat 2% of the energy in the M drink. Water is not included in Table 4.6 as it does not provide any macro-nutrients. The K^+ content of the M drink is 93% greater than the CE drink. The CE drink contains 18% more Na^+ than the M drink (Table 4.7).

Table 4.6 - Macro-nutrient provision (total and g per kg BM) for Milk (M) and Carbohydrate-electrolyte drink (CE) during rehydration.

Nutrient	M Total	M (per kg BM)	CE Total	CE (per kg BM)	p-value*
Energy (kcals)	929 (145)	10.8 (0.7)	447 (89)	5.2 (0.5)	<0.01
Energy (kJ)	3962 (618)	46.2 (3.2)	1958 (389)	22.6 (2.2)	<0.01
Fat (g)	2.73 (0.4)	0.03 (0.0)	0	0	<0.01
CHO (g)	136.6 (21.3)	1.6 (0.1)	109.1 (21.6)	1.3 (0.1)	<0.01
Protein (g)	90.2 (14.1)	1.1 (0.1)	0	0	<0.01

* Paired sample t-test comparing mean total CHO provided by M (Milk) and CE (carbohydrate-electrolyte) drinks. Values are mean (SD) and represent the mean of total intakes calculated for each participant.

Table 4.7 - Total electrolytes and calcium ingested during each trial

Electrolyte	M	CE	W	p-value*
Na+ (mmol)	49 (8)	61 (12)	2 (0)	<0.01
K+ (mmol)	124 (19)	9 (2)	0.1 (0)	<0.01
Mg (mmol)	14 (2)	0.8 (0.2)	1 (0)	<0.01
Ca (mg)	3279 (193)	36 (3)	663 (38)	<0.01

*one way ANOVA, p-value indicates all test drinks were significantly different from each other with respect to electrolytes and calcium ingestion during each trial. Values represent the mean of total intakes calculated for each participant for each trial Milk (M), carbohydrate-electrolyte (CE) and water (W) trials. Values are mean (SD).

Using the data on CHO oxidised (Table 4.4) and CHO consumed (Table 4.6) the % replenishment was calculated as described in section 3.2. During the M trial 83 (10.5) % and the CE trial 74 (13.4) % of CHO was replenished in the rehydration phase ($p = 0.247$) (Figure 4.3). Milk provided 50g CHO in the first 30 minutes of rehydration which translates to 0.59 (0.08) g CHO/kg BM. CE provided less CHO 0.46 (0.06) g/kg BM).

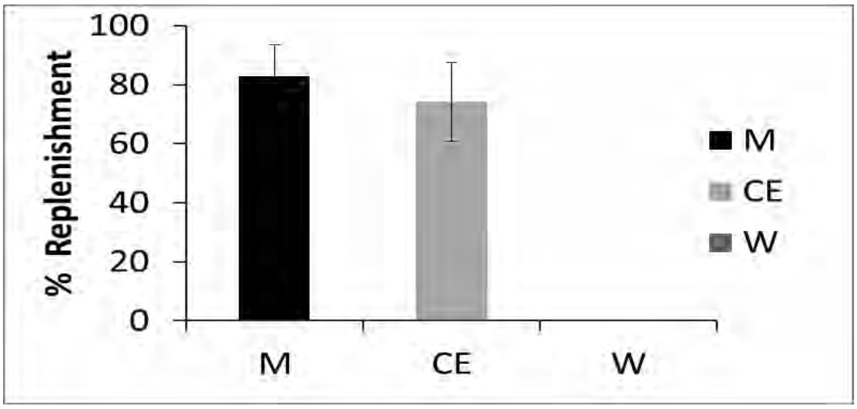


Figure 4.3 - CHO replenishment during the MILK (M) and carbohydrate-electrolyte (CE) trials. Values are mean (SD)

4.7.3 Plasma Osmolality

During all trials Posm decreased from post exercise onwards until the end of the rehydration phase from a mean of 293mOsmol/kg to 287mOsmol/kg ($p = < 0.001$, $\eta_p^2 = 0.857$, $1-\beta = 1.000$) (Figure 4.4). Posm AUC from post exercise in response to M ingestion was 724 (363) mOsmol/kg.5h^{-1} *vs*. -1659 (741) mOsmol/kg.5h^{-1} for CE *vs*. -2874 (594) mOsmol/kg.5h^{-1} for W with a main effect for test drink ($p = <0.01$, $\eta_p^2 = 0.770$, $1-\beta = 0.999$). There was a significant difference in Posm AUC between the M and W trial ($p = <0.01$) but not between M and CE trial ($p = 0.072$).

The first two hours of rehydration stimulated a greater decrease in Posm in the W and CE trials compared with the M trial. From post exercise to the end of the first hour of rehydration Posm in the M trial decreased by only -1 mOsmol/kg (from 292 (2) to 291(2) mOsmol/kg). This contrasts with a larger decrease of -8 mOsmol/kg (from 295 (2) to 287 (3) mOsmol/kg) in the W trial (p = 0.005) (Figure 4.4 (c)). The decrease of -4 mOsmol/kg (from 292 (3) to 288 (3) mOsmol/kg) in the CE was close to being significantly greater compared with the M trial (p = 0.050). This resulted in the M trial remaining 2.0 (1.4) % above baseline compared with CE and W trials which were 0.1 (1.4) % above and -0.3 (1.3) % below baseline, respectively.

At 2h of rehydration, Posm in the M trial (290 (3) mOsmol/kg) remained significantly higher than CE (285 (3) mOsmol/kg) (p = 0.037) and W (283 (3) mOsmol/kg) (p = 0.011). At the end of rehydration (5h) Posm had returned to within 2 mOsmol/kg of baseline in the M trial, was -2 mOsmol/kg of baseline in the W trial and had returned to baseline in the CE trial.

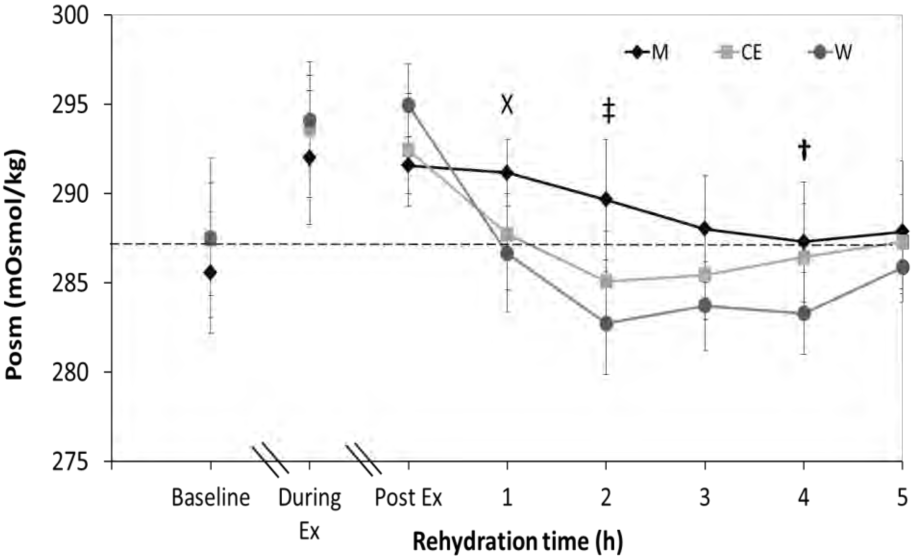


Figure 4.4 Posm during all three trials Milk (M), Carbohydrate-electrolyte (CE) and water (W). x = M significantly different from CE, ‡ = M significantly different from both CE and W, † = M significantly different from W. Values are mean with SD represented by error bars.

4.7.4 Plasma Volume

There was a main effect for test drink on plasma volume during rehydration (p = 0.004, η_p^2 = 0.358, $1-\beta$ = 0.951) (Figure 4.5). At 3h, plasma volume in the M trial (6.2 (5.1) %) and CE trial (6.7 (6.8) %) were both higher than the W trial (-1.1 (4.3) %) (p = 0.040). At the end of rehydration (5h), plasma volume was 2.9 (6.6) % for M trial, 3.1 (6.6) % for CE and 0.3 (5.3) % for the W trial, there was no difference between trials (p = 0.700). It is noted that there was individual heterogeneity in response across the three trials.

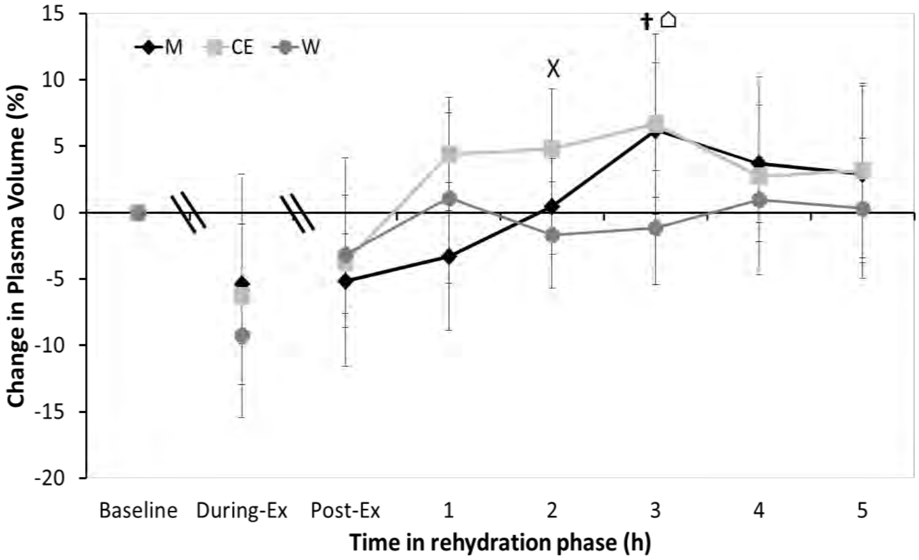


Figure 4.5 - Change in plasma volume relative to baseline during all trials. x = M significantly different from CE trial, † = M significantly different from W trial, ⌂ = CE significantly different from W trial. Values are mean with SD represented by error bars.

4.7.5 Electrolytes

There was no significant main effect of test drink on plasma Na^+ concentration levels (p = 0.062) or interaction effect of test-drink over time (p = 0.192) during rehydration. AUC relative to post-exercise plasma Na^+ was -287 (513) mmol/L.5h^{-1} (M), -257 (849) mmol/L.5h^{-1} (CE) and -861 (448) mmol/L.5h^{-1} (p = 0.218). Na^+ levels at 5h were not statistically significantly different from baseline for M (p = 0.356), CE (p = 0.386) or W (p = 0.413) (Figure 4.6).

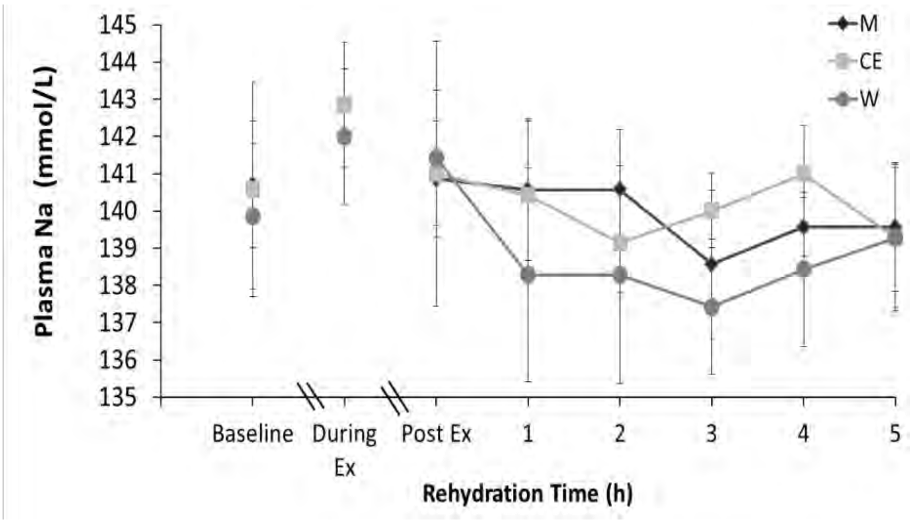


Figure 4.6 - Mean plasma Na$^+$ concentration during each trial. Values are mean with SD represented by error bars.

There was a main effect of test drink on K$^+$ concentration during rehydration (p = <0.01) and an interaction effect of test drink over time (p = <0.01). Plasma K$^+$ concentration AUC relative to post exercise was 99.0 (23.7) mmol/L 5h^{-1} (M) -69.0 (76.2) mmol/L.5h^{-1} (CE) and -34.3 (48.3) mmol/L.5h^{-1} (W). Plasma K$^+$ was significantly higher in the M trial during rehydration compared with CE (p = 0.008) and W (p = <0.01). At the end of rehydration (5h) K$^+$ concentration had returned to baseline levels in the CE (3.9 (0.4) mmol/L) and W (4.1 (0.2) mmol/L) trials but remained elevated above baseline in the M trial 4.3 (0.3) mmol/L) (p = 0.011) (Figure 4.7).

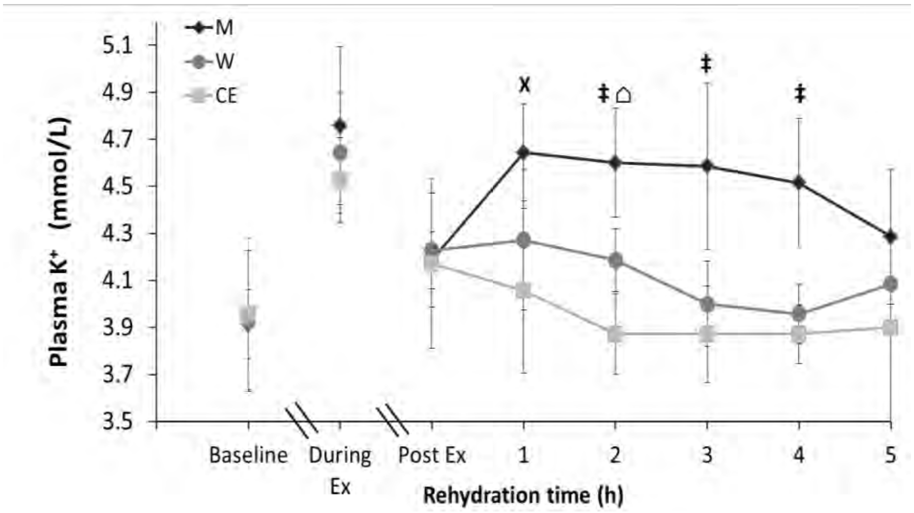


Figure 4.7 - Mean plasma K$^+$ concentration during each trial. x = M significantly different from CE, ‡ = M significantly different from W and CE,⌂ = CE significantly different from W. Values are mean with SD represented by error bars.

4.7.6 Blood Glucose

During rehydration there was an interaction effect of test drink over time (p = 0.000, η_p^2 = 0.412, 1-β = 0.996) (Figure 4.8). AUC for blood glucose concentration also showed a significant main effect of test drink, -108.0 (200.6) mmol/L.5h^{-1} (M), -29.8 (180.2) (CE), -281.4 (154.5) (W) (p = 0.043). At 1h of rehydration blood glucose in the CE trial was significantly higher than in the W trial (6.4 (1.3) vs. 4.5 (0.4) (p =0.042) but was not significantly different from M trial (4.7 (0.8) mmol/L (p = 0.118). In the M trial blood glucose decreased from 5.1 (0.5) mmol/L post-exercise to 4.4 (0.3) mmol/L at the end of rehydration which was lower than baseline (p = 0.023). During the CE trial there was an increase in blood glucose at the end of 1h of rehydration to 6.4 (1.3) mmol/L relative to baseline 4.8 (0.4) (p =0.031). This was followed by a decrease to 4.3 (0.4) mmol/L at 5h which was lower than baseline (p = 0.002). During the W trial blood glucose decreased from 5.5 (0.7) mmol/L post exercise to 4.5 (0.4) mmol/L at 5h which was lower than baseline (p = 0.030).

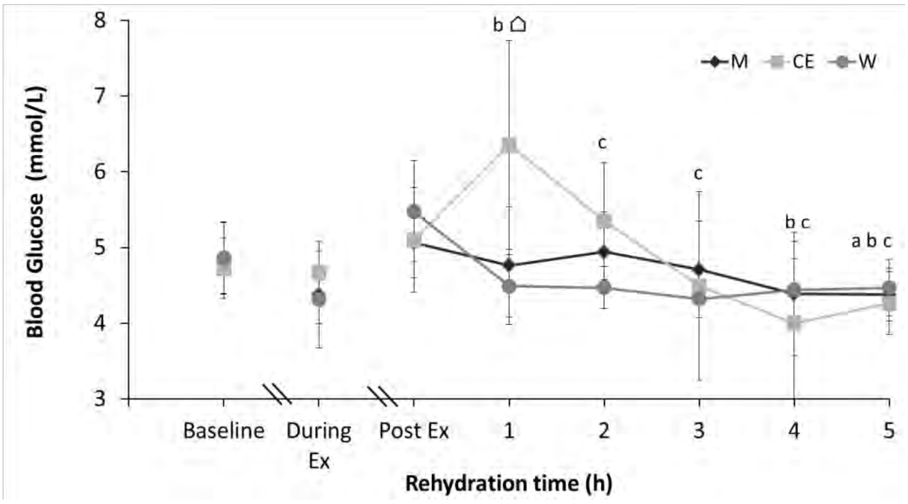


Figure 4.8 – Blood Glucose concentration during each trial Milk (M), Carbohydrate-electrolyte drink (CE) and Water (W). a = Trial M significantly different from baseline, b = Trial CE significantly different from baseline, c = Trial W significantly different from baseline, ⌂ = Trial CE significantly different from W trial. Values are mean with SD represented by error bars.

4.7.7 Urine Volume

Overall mean hourly urine volume was lower during the M trial (159 (75) mL/h) compared with W (286 (157) mL/h) (p = 0.018) but was not statistically significantly lower compared with CE (263 (144) mL/h) (p= 0.057) (Figure 4.9 (a)). At 2h urine volume was markedly lower in the M trial (91 (57) mL) compared with CE (314 (203) mL) (p = 0.017) and W (402 (213) mL) (p = 0.039). Peak urine output occurred at 3h for all test drinks, 254 (33) mL (M) compared with 466 (67) mL (CE (p = 0.052) and456 (35) mL (W) (p = 0.061). Overall total urine volume at the end of the rehydration phase was lower in the M trial (794 (92) mL) compared with W (1429 (345) mL) (p = 0.018) however this did not reach statistical significance compared to CE trial (1314 (434) mL) (p = 0.057).

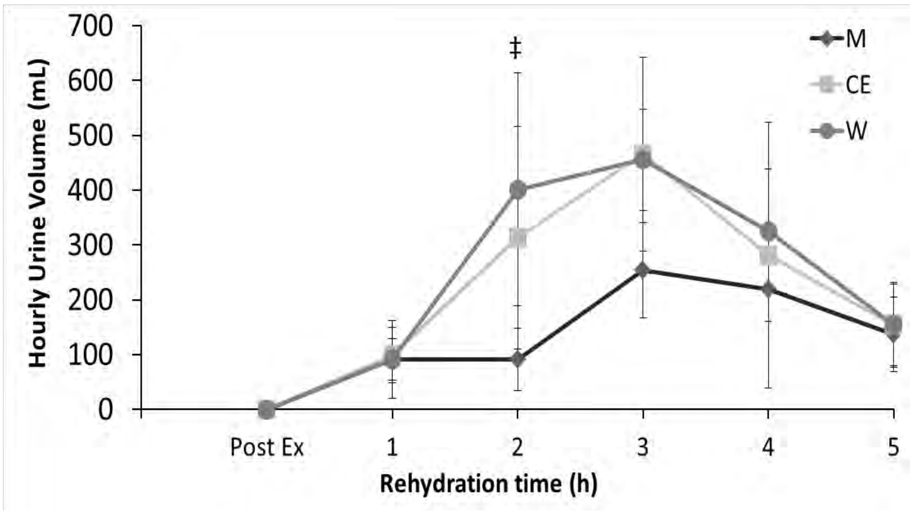


Figure 4.9 (a) - Mean Urine hourly urine volumes during each trial. ‡ = M significantly different from CE and W trial. Values are mean with SD represented by error bars.

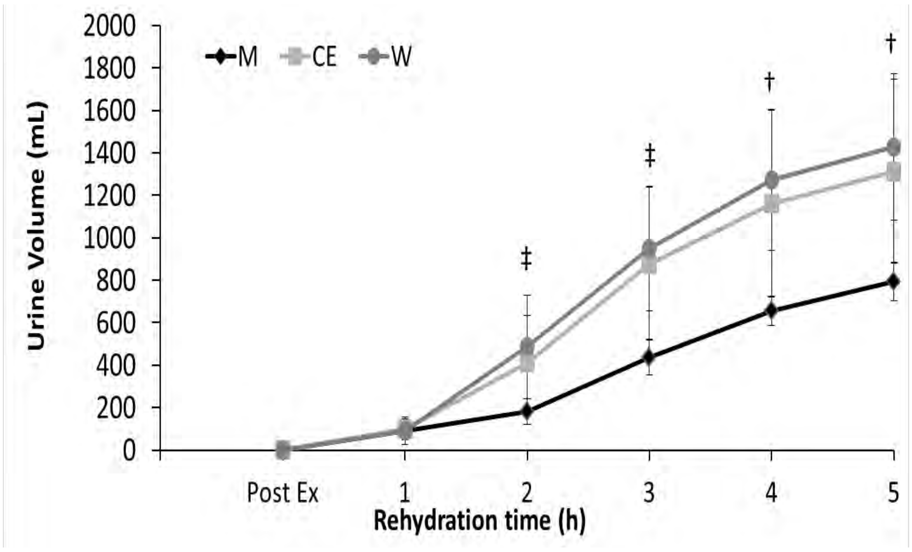


Figure 4.9 (b) - Cumulative urine volumes for each trial. ‡ = M trial significantly different from CE and W trials. † = M Significantly different from W trial. Values are mean with SD represented by error bars.

4.7.8 Urine Osmolality

Six of seven participants were included in the analysis as one subject was unable to provide urine at 1h of rehydration during the M trial. Mean urine osmolality during the M trial (558 (165) mOsmol/kg) was higher overall compared with CE (347 (260) mOsmol/kg) (p = 0.014) and W (308 (132) mOsmol/kg) (p = 0.010, η_p^2 =0.800, 1-β = 0.999) throughout the rehydration phase (Figure 4.10). The higher concentration of urine excreted in the M trial corresponds with the lower volume of urine (Figure 4.9 (b)).

At the end of the first hour of rehydration urine osmolality did not increase significantly from baseline during the M and W trial, however during the CE trial there was a significant increase from baseline 495 (272) mOsmol/kg to 749 (153) mOsmol/kg (p = 0.017). At the end of 2h when metered fluid ingestion was ongoing, urine osmolality remained higher in the M trial 761 (135) mOsmol/kg compared with the CE 169 (106) mOsmol/kg (p = 0.000) and W trial 193 (202) mOsmol/kg (p = 0.000) which both decreased markedly. During the CE and W trials the greatest decrease in Uosm occurred between 1h-2h with a reduction of 579 mOsmol/kg (77%) in the CE trial and 467mOsmol/kg (72%) in the W trial. This is in contrast to the M trial during which the greatest decrease occurred between 2h and 3h with a reduction of 375mOsmol/kg (49%). Urine osmolality returned to within baseline levels for all 3 trials, while it appears lower than baseline in the W trial at 5h ((519 (319) *vs* 369 (176) mOsmol/kg), this did not reach significance (p = 0.193).

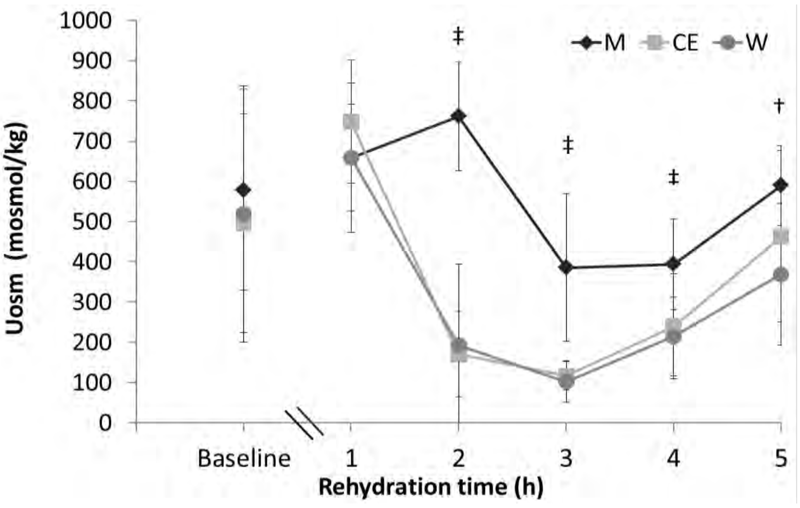


Figure 4.10 - Uosm during rehydration for each trial (n=6). ‡ = M Significantly different from CE and W trial, † = M significantly different from W trial. Values are mean with SD represented by error bars.

4.7.9 Plasma osmolality and Urine osmolality relationship

The linear relationship between Posm and Uosm during rehydration in each trial was analysed (Figure 4.11). During the M trial there was a non-significant weak positive correlation between Posm and Uosm (*r* = 0.018, p = 0.923). In the CE trial there was a significant medium positive correlation (*r* = 0.367, p = 0.046) and in the W trial there was a highly significant large positive correlation (*r* = 0.674, <0.01) (Figure 4.11). The relationship between Posm and Uosm is more defined in the case of W and CE trials. The response in the M trial is more gradual with small incremental hourly changes in both Posm (≤2mOsmol/kg/h) and Uosm (≤376mOsmol/kg/h), compared with the more pronounced dilutional changes that occurred in the CE trial (≤3mOsmol/kg/h, Posm and ≤587mOsmol/kg/h Uosm) and W trial (≤4mosmol/kg/h, Posm and ≤467mOsmol/kg/h) from 1-5h (Figure 4.4 and 4.10).

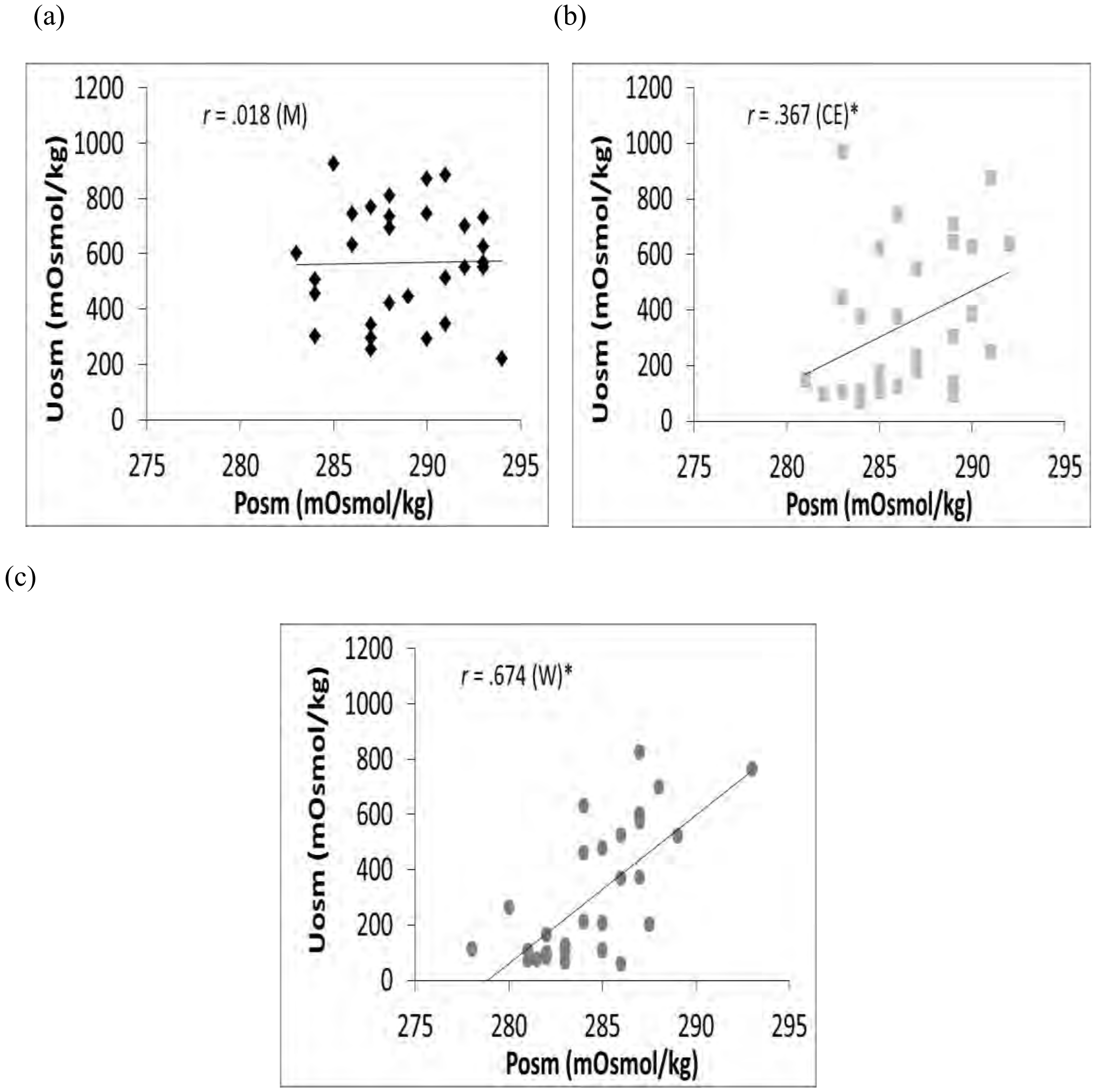


Figure 4.11 – Pearson product moment correlation of mean Posm and Uosm during rehydration (1-5h) for each trial (a) Milk (M), (b) Carbohydrate-electrolyte drink (CE) and (c) Water (W). * = significant correlation at p = 0.05 level.

4.7.10 Net Fluid Balance and % Fluid retention

There was a main effect for test drink on net fluid balance during rehydration ($p = 0.016$, $\eta_p^2 = 0.498$, $1-\beta = 0.779$). A higher overall net fluid balance was maintained in the M trial compared with the CE ($p = 0.028$) and W trials ($p = 0.019$) (Figure 4.12). After ingestion of 1500mL in the first hour of rehydration, no trial achieved positive balance as fluid intake was not sufficient to replace sweat losses. A positive fluid balance was attained for all drinks at 2h of rehydration, 404 (129) mL (M), 127 (254) mL (CE) and 112 (295) mL (W) ($p = 0.104$).

At 3h a positive balance was maintained in the M trial 474 (198) mL and to a lesser extent in the CE trial 54 (492) mL, (p = 0.069). The W trial returned to negative fluid balance of -51 (349) mL at 3h and was significantly different from the M trial (p = 0.019) but not the CE trial (p = 1.000). At 5h final fluid balance in the M trial was 117 (122) mL, compared with a negative net balance in the W trial -539 (390) mL (p = 0.011). Although mean final net fluid balance was negative in the CE trial -381 (460) mL this did not reach statistical significance compared with the M trial (p = 0.077).

The difference in NFB between M and CE was 499 (447) mL and between M and W was 656 (379) mL. The difference between CE and W was 157 (742) mL. This is equivalent to 0.6 (0.5) % BM between M and CE, 0.8 (0.4) % BM between M and W trials, and 0.2 (0.9) % BM between CE and W.

Relative net fluid balance was calculated to account for the inter-participant differences in BM loss and fluid ingested. Relative net fluid balance at the end of the rehydration phase was significantly higher in the M trial 5.9 (5.9) % compared with the CE -22.7 (23.3) % (p = 0.048) and W trial -30.9 (22.7) % (p = 0.019). There was no significant difference between the CE and W trials (p = 1.000).

Overall mean fluid retention was higher in the M trial 70.6 (3.9) % compared with 51.6 (15.5) % in the CE trial (p = 0.048) and W trial. 47.3 (14.5) % (p = 0.014). The CE and W trials were not significantly different (p = 1.000) (Figure 4.13).

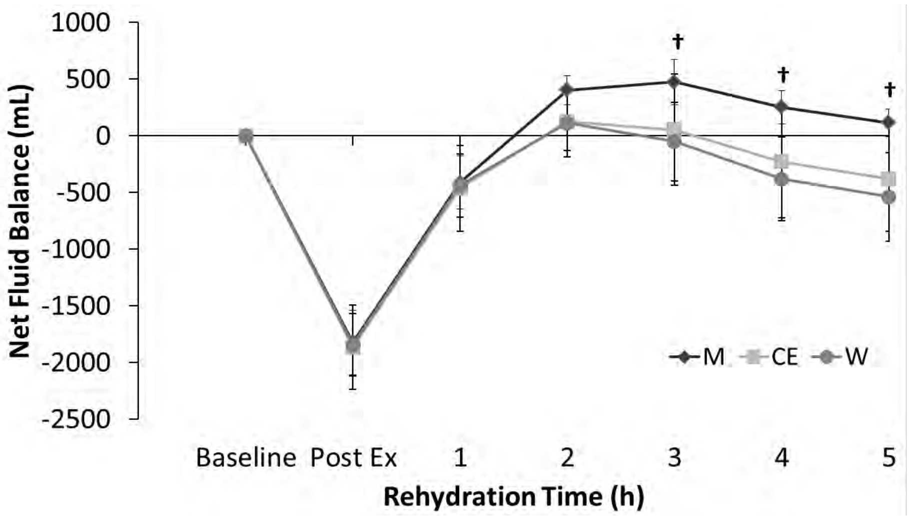


Figure 4.12 - Mean net fluid balance for each trial during rehydration Milk (M), Carbohydrate-electrolyte drink (M) and water (W). † = M significantly different from W. Values are mean with SD represented by error bars.

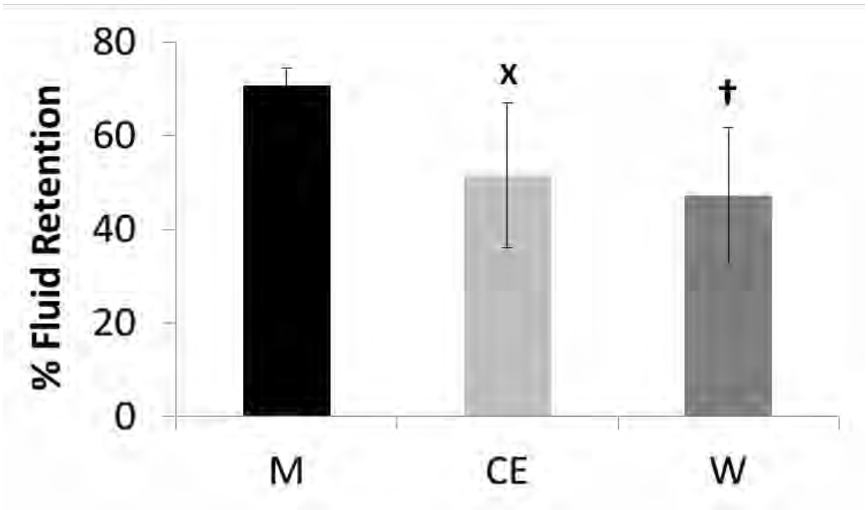


Figure 4.13 - Fluid retention at the end of the rehydration phase (5h) for each trial, Milk (M), Carbohydrate-electrolyte drink (CE) and Water (W). x = M significantly different from CE, † = M significantly different from W. Values are mean with SD represented by error bars.

4.7.11 Restoration of body mass and body water

Measurements of BM, TBW, ICW and ECW taken at baseline and at the end of rehydration are summarised in Table 4.8. There was a statistically significant difference between BM at baseline and at the end of the 5h rehydration phase for each trial (M, CE and W). The absolute difference in the M trial -0.24 (0.24) kg differed significantly from the CE -0.99 (0.15) kg ($p = 0.003$) and W -1.17 (0.25) kg trials ($p = 0.003$). In contrast there was no difference between the CE and W trials ($p = 0.498$). This represented a % difference relative to baseline of -0.29 (0.28) % (M), -1.17 (0.3) % (CE) and -1.38 (0.25) % (W).

The mean absolute difference in TBW from baseline to the end of the rehydration phase was 0.59 (0.91) kg (M), 0.33 (0.55) kg (CE) and 0.19 (0.64) kg (W). There was no statistically significant difference in the absolute changes in TBW between trials ($p = 0.559$) as was the case for ICW ($p = 0.721$) and ECW ($p = 0.195$) (Figure 4.14).

Table 4.8 - Absolute values of body mass, total body water, extracellular and intracellular body water at baseline and end of rehydration phase (5h).

Trial	BM	TBW	ECW	ICW
M Baseline	85.60 (11.51)	49.99 (4.47)	19.35 (1.49)	30.63 (3.11)
M 5h	85.36 (11.54)	50.58(4.25)	19.41 (1.52)	31.16 (2.82)
p-value	***0.039****	*0.138*	*0.272*	*0.132*
CE Baseline	86.16 (11.6)	49.77 (4.23)	19.42 (1.53)	30.36 (2.80)
CE 5h	85.17 (11.57)	49.98 (4.63)	19.38 (1.61)	30.59 (3.11)
p-value	***0.000****	*0.430*	*0.245*	*0.344*
W Baseline	85.71 (11.0)	49.75 (4.17)	19.37 (1.49)	30.40 (2.79)
W 5h	84.54 (10.95)	49.95 (4.20)	19.30 (1.48)	30.65 (2.78)
p-value	***0.000****	*0.456*	*0.245*	*0.230*

*Indicates significance using paired sample t-tests between baseline and measurement at 5h for; Total body water (TBW), extracellular water (ECW) and intracellular water (ICW) during the MILK (M), Carbohydrate-electrolyte drink (CE) and water (W) trials. Values are mean (SD).

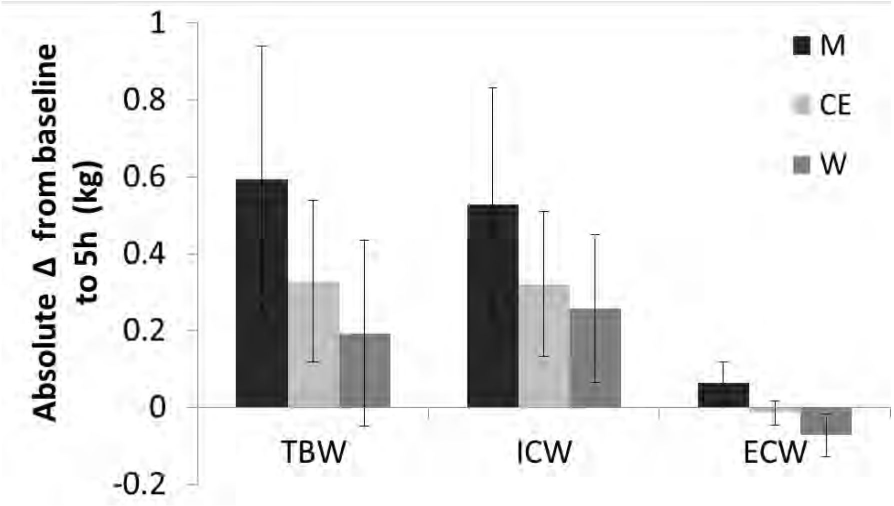


Figure 4.14 - Absolute difference in total body water (TBW), intracellular water (ICW) and extracellular water (ECW) relative to baseline at the end of rehydration (5h). Values are mean with SD represented by error bars.

4.8 Subjective Feelings of Hydration

The participant's responses to the associated feelings relating to hydration status are presented in figure 4.15. There was no difference between the trials on presentation at baseline for any of the measures except bloatedness (p = 0.003) which was higher in the M trial (22 (16) mm) compared with CE (6 (9) mm) (p = 0.029) but not W trial (10 (14) mm) (p = 0.117).

There was a marked increase in perceived thirst immediately post exercise for all trials. There was no significant difference in thirst scores between test drinks (p = 0.773). The ingestion of the test drinks reduced feelings of thirst from a pooled mean of 81 (6) mm to 45 (4) mm but this sensation gradually increased in the last two hours of the rehydration phase after fluid ingestion had ceased. There was a difference in perceived hunger during the rehydration phase between test drinks (p = 0.025). Overall participants felt more hungry in the W trial with a mean score of 79 (9) mm compared to 51 (9) mm from 1h-5h in the M trial (p=0.038). There was no difference in perceived levels of bloatedness between test drinks (p = 0.153) or significant changes over time (p = 0.280) during rehydration.

There was a difference in perceived mouth taste between test drinks during rehydration (p = <0.01). Participants scored mouth-taste lowest in the W trial (14 (4) mm) compared with CE (35 (13) mm) and M (44 (8) mm) trials. Immediately post-exercise perceived alertness was lower in the M trial (39 (18) mm) compared to W (63 (21) trials (p = 0.007) but similar to CE (57 (25) mm). However alertness level in the M trial increased to the same level as W and CE drinks at 1h and there was no difference in perceived alertness between test drinks during rehydration (p = 0.275). There was no difference in tiredness (p = 0.063), headache (p = 0.160) or refreshment (p = 0.064) between test-drinks.

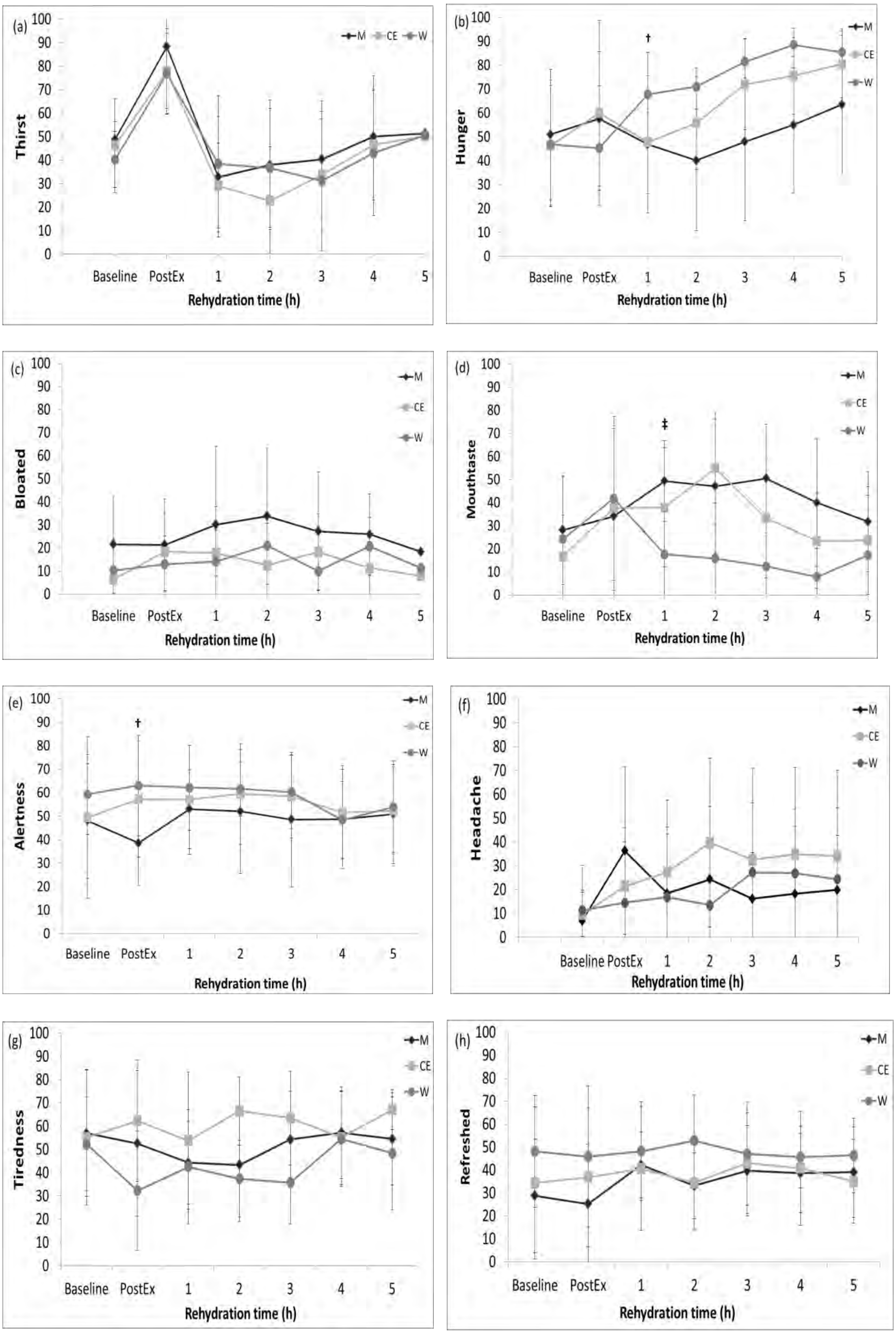


Figure 4.15 - Subjective feelings related to hydration status (a) Thirst, (b) Hunger (c) Bloated (d) Mouthtaste (e) Alertness (f) Headache (g) Tiredness (h) Refreshed. 100 mm Visual analogue scale. † = M significantly different from W trial. ‡ = M significantly different from CE and W trials. Values are mean with SD represented by error bars.

4.9 Subjective feelings related to drink acceptance

Subjective feelings over 1h and 2h of rehydration were analysed and are presented in Figure 4.16. In the M trial aftertaste scored higher than W (p =0.004) but there was no difference between M and CE trials (p = 0.907). There was no difference in perceived visual appeal (p = 0.317) or palatability (p = 0.755) between test drinks at any time-point. There was an overall higher perceived sweetness in the CE trial compared to M and W trials (p = <0.01 for both 1h and 2h). A mean score of 6.9 (2.8) mm was maintained in the CE trial compared to the M trial 3.5 (0.8) mm) and W 0.2 (0.1) trial, with no statistically significant change in sweetness within drinks from the 1h to the 2h. Perceived saltiness scored highest in the CE trial (2.9 mm), remaining unchanged over the 2h. The M trials also scored saltiness relatively low 1mm at 1h and 2mm at 2h, with no statistically significant change over time (p = 0.185). The difference in level of saltiness was only statistically significant between CE and W trials (p = 0.030).

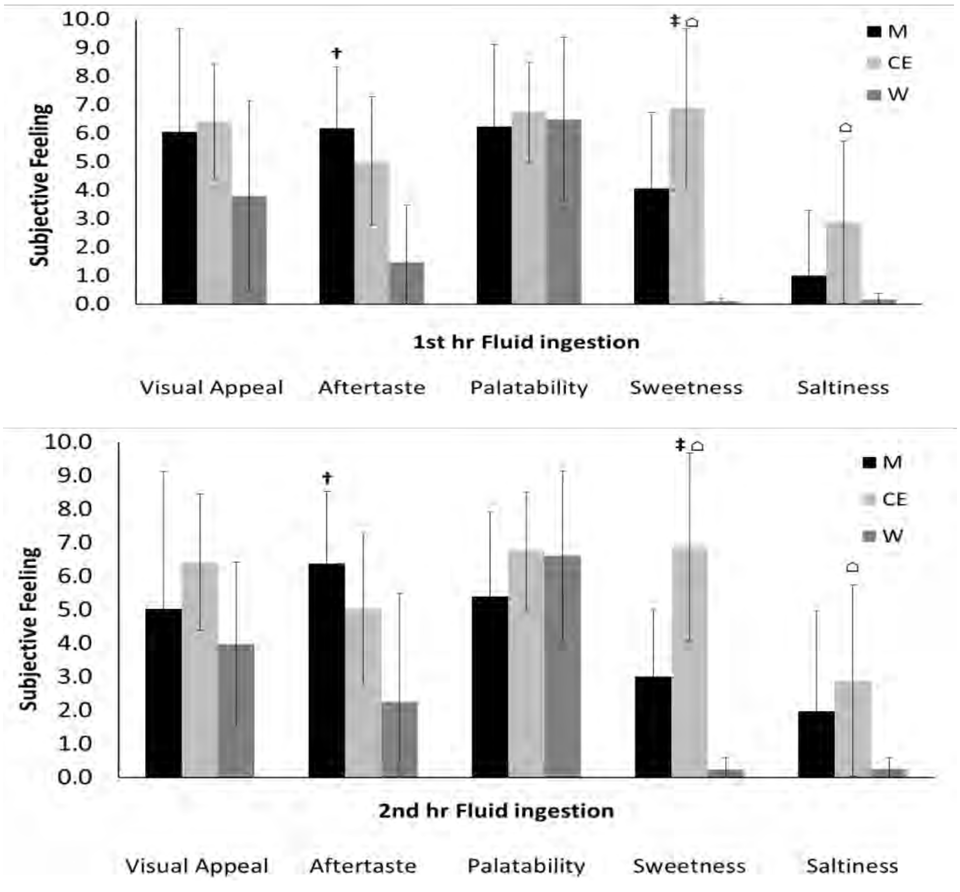


Figure 4.16 Subjective feelings related to drink taste and visual appeal in the 1h and 2h fluid ingestion period. † = M significantly different from W trial,‡ = M significantly different from CE and W trials. ⌂ = Trial CE significantly different from W trial. Values are mean with SD represented by error bars.

Chapter 5
Discussion

This study sought to investigate the effectiveness of 0.1% fat milk as a rehydration solution using a metered drinking protocol with 150% of BM loss replacement post exercise. Through measurement of Pvol, Posm and plasma electrolyte concentrations, the pattern of osmotic regulation during each trial is investigated in depth. The findings of this study correlate with findings from previous studies (Watson et al 2008, Shirreffs et al 2007b) indicating that milk is an effective rehydration solution. Milk maintained a positive net fluid balance and greater fluid retention at 5h post exercise, compared with water and a proprietary carbohydrate electrolyte solution. This study demonstrates that a metered approach to fluid ingestion enhances fluid retention when compared with rapid rehydration protocols and contributes to informing methodology design in future rehydration studies. This study, to the researcher's knowledge, is also the first of its kind undertaken in Ireland using Irish milk.

5.1 Plasma osmolality threshold for compensatory water retention

Posm is a key variable in regulating fluid balance. The variation in the osmotic response to exercise and heat induced dehydration is influenced by sweat rate and sweat Na^+ concentration which determines the level of plasma (extracellular) fluid losses from the body (Nose et al 1988a). Factors known to influence the sweat response and resulting Pvol change were controlled for in this study. Hydration status was controlled by prescribing a 500ml bolus 1h before arrival at the lab and a standardised breakfast 1h prior to exercise commencement. Hydration status influences erythrocyte size which determines packed cell volume (PCV) and haemoglobin concentration used to calculate the plasma volume change (Dill and Costill 1974). Posture changes during blood sampling can also effect Pvol measurement by up to ~8% (Shirreffs et al 1994) and this was controlled by ensuring that participants were at rest in a seated position prior to each blood sample.

Level of exercise intensity, air temperature and humidity were standardised during each trial. Physical adaptation to training in the heat was controlled for by randomising the test drinks assigned during each trial although it cannot be completely out-ruled as an influencing factor. All subjects were moderately active individuals at baseline, however cycling was not a sport in which all participants engaged in regularly.

The dehydration protocol used in this study achieved an atypical body water deficit equating to ~2% BM loss and resulted in Pvol decrease of -5.1 (3.5) % in the M trial, -3.7 (7.8)% (CE) and -3.2 (4.5) % in the W trial. In rehydration studies employing similar dehydration protocols using a similar population (adult males, BM ≈ 75kg) mean plasma volume changes of ~ -4% are observed from presented graphs within the study articles (Evans et al 2009, Watson et al 2008) and -5.5% (SD 4.7) reported by Shirreffs et al (1998). In this study, loss of plasma volume resulted in a mean increase in Posm of 6 mOsmol/kg (M), 5 mOsmol/kg (CE) and 7 mOsmol/kg (W) and was not significantly different between trials. These results are similar to findings in other studies with similar dehydration protocols (Evans et al 2009, Shirreffs et al 1998, Watson et al 2008). The increases are also in accordance with the statistical probability for dehydration model developed by Cheuvront et al (2011) in which it was calculated that an increase of ~5mOsmol/kg in Posm was likely to reflect dehydration. This increase in Posm also increased perceived feelings of thirst post exercise. Based on these findings it is likely that some degree of compensatory AVP response was stimulated although this cannot be stated definitively as plasma AVP levels were not measured in this study.

5.2 Osmoregulation of water balance during rehydration

By measuring Posm, Pvol, urine volume and Uosm, it was possible to track the effect of Posm on water retention during rehydration. In the first hour of rehydration Posm in the W trial decreased by an average of -8 mOsmol/kg which is similar to the changes found in other studies where ingestion of 500-mL water bolus induces −3 mOsmol/kg in Posm (Sollanek et al 2011, Robertson et al 1973). The decrease in Posm was less in the CE trial (-4 mOsmol/kg) and M trial (1 mOsmol/kg). In the case of the CE trial, the higher Na^+ content ingested was most likely responsible for maintaining a higher concentration gradient in the plasma while also increasing the plasma volume (extracellular compartment). This pattern of Pvol increase after ingestion of rehydration solutions containing moderate to high amounts of Na^+ has been demonstrated by Shirreffs et al (1996) and Nose et al (1988b). In the Shirreffs et al (1996) study a volume equivalent to 150% of BM loss containing 23mmol/L Na^+, induced a reduction in mean Posm from 295 mOsmol/kg to 288 mOsmol/kg at the end of fluid ingestion and an increase in Pvol of ~4% (from graph as value not reported in text of articles). When a similar volume solution containing 61mmol/L Na was consumed a greater increase in Pvol was observed. This led Shirreffs to the now widely accepted conclusion that when an adequate volume of fluid is ingested, Pvol increase is a function of the Na^+ content of the drink. In the M trial there was a minimal change in Posm (-1mOsmol/kg) at 1h rehydration and Pvol appeared to recover at a slower rate compared with the CE and W drinks. This is likely due to the delayed intestinal uptake of water due to the relatively slower rate of gastric emptying associated with milk. It may also be due to the high K^+ content, although studies relating to the influence of K^+ content on restoration of intracellular water and Pvol recovery are limited and inconclusive.

The difference in the magnitude of decrease in Posm in the first hour would have been expected to initiate a larger diuresis in the W trial compared with the CE and M trials. The reason for a similar diuretic response despite the difference in Posm decrease is possibly due to the metered rate of drinking employed which may have prevented a larger diuretic response. The effect that exercise can have on reducing renal blood flow and urine production may also have been a factor in the 1st h (Freund et al 1991). Secondly, although the decrease in Posm was large in the W trial, it was still within 1% of the baseline Posm and therefore the level of AVP in circulation was possibly greater than the level (~0.5pmol/L) at which maximal diuresis occurs (Baylis 1987) preventing over-stimulation of diuresis by the kidneys.

It is also of interest to note that similar to findings of the Popowski et al (2001) study which assessed blood and urinary measures of hydration status during progressive acute dehydration and rehydration, Uosm tended to lag behind Posm. This is noted particularly at the end of the 1st hour of rehydration during the CE and W trial, where despite a decrease in Posm, Usom did not decline. This can potentially be explained by the role of the kidney as the organ which responds to a change in ECW osmolality and volume. It is recognised that a lag time exists as Usom is a reflection of the kidneys filtration of plasma and subsequent response (water excretion or conservation) to Posm during periods of acute changes in body water turnover. This highlights the differences in the sensitivity of Usom compared to Posm as a marker of hydration during periods of rapid body water turnover (Kovacs et al 1999). The serial measurements of Uosm over 5h reflects the kidneys ability to retain each of the rehydration solutions, as opposed to hydration status.

Correlation of baseline Posm with Usom in this study (*r* = 0.584, p = <0.01) is also similar to the moderate but not statistically significant correlation (*r* = 0.43, p= >0.01) found between Uosm and Posm during acute changes in hydration in the Popowski et al (2001) study. The correlation between Posm and Uosm during rehydration in this study also indicates the differences in the osmotic regulation of fluid balance in response to the three different test drinks. In the case of the M trial there was no positive or negative linear relationship identified between the variables compared with CE and W which both demonstrated a positive significant linear relationship (Section 4.7.9, Figure 4.11). This is not surprising given the almost 'static' like response of Posm during the M trial with relatively small incremental hourly changes compared to the CE trial and W trial from 1-5h. This analysis further highlights the maintenance of an elevated plasma osmolality during rehydration in the M trial which resulted in greater water conservation.

At 2h of rehydration, Posm in the W trial dropped further (-5mOsmol/kg) below the mean baseline Posm to 283mOsmol/kg. In the CE trial a further reduction of -3mOsmol/kg to 285mOsmol/kg occurred. Findings from the studies of Robertson and Athar (1976) and Baylis (1987) indicate that at a Usom of 100mOsmol/kg, AVP level is low (0.4-0.5 pmol/L) and maximal diuresis occurs. From 2-4h in the W trial mean Uosm remained <202mOsmol/kg and in the CE trial was < 231msmol/kg with urine output at its peak at 2h and 3h. This compares with the M trial which had a Uosm approximately three times higher than both the CE and W trials and 45% less urine was produced compared with the CE trial and 77% less compared with the W trial at 2h. This indicates that AVP concentration was likely maintained at a higher level in the M trial particularly during 1h-3h. The elevated Posm in the M trial resulted in a lower total urine volume over the rehydration phase compared with W but was not significantly different compared with the CE trial . The reason for not achieving significance compared to the CE trial may be due to the effect of the metered ingestion preventing a greater diuresis.

5.3 Metered fluid ingestion

The rate at which the volume is ingested is a key regulator of the rate of gastric empting. Generally, the higher the volume of fluid ingested the more rapid the gastric emptying rate. The purpose of employing a metered rate of fluid ingestion in this study was to limit the formation of large volumes of urine associated with rapid fluid-consumption rates and promote greater fluid retention efficiency. In this study 1000mls of fluid was consumed in the first 30 minutes which equated to a pooled mean of 56% of the body mass loss and 37% of the total volume prescribed. In the next 30 minutes, a 500ml bolus was consumed providing a pooled mean replacement of 83% of the total body mass loss and 56% of the 150% volume prescribed in 60 minutes. For the remaining 1.5-2 h, a bolus of 500mL was ingested every 30minutes until the 150% volume was consumed.

Recent studies that have compared a set volume ingested at different rates have used volumes of 100% using water (Jones et al 2010) and 120% using a 7.6% Carbohydrate-electrolyte solution (Kovacs et al 2002). As the volumes are dissimilar to that ingested in this study, meaningful comparisons are not possible. Jones et al (2010) concluded that even with a metered rate of ingestion of water over 4h, replacement of 100% of fluid loss was not sufficient to achieve a positive fluid balance, indicating the importance of fluid composition. Fluid retention of milk by the metered approach used in this study was compared to studies using similar solutions (milk or milk protein constituents added to CE or water solutions) and total volume (150% replacement) but the rate of ingestion was 150% of BM loss ingested in 1h (Table 5.1).

Table 5.1 Comparison of % fluid retention in rehydration studies using milk or milk proteins with results from the present study.

Authors / Drink	Rehydration / Monitoring time	Urine Volume (mL)	Volume consumed	Osmolality (mOsmol/kg)	% Fluid retention
James et al (2011) CE+ Milk protein 1.5%	1h / 4h	1180 (330)	2320 (300)	265	49 (13)
James at al (2014) Whey Protein 2%	1h / 4h	1306	2074	14	37 (14)
Watson et al (2008) Milk 0.1% Fat	1h / 3h	525 (118)	2263 (241)	278	77 (6)
Shirreffs et al (2007) Milk 0.1% Fat	1h / 4h	611 (207)	1790 (420)	299	72 (4)
Present study Milk 0.1% Fat	2.5-3h / 5h 3h 4h	794 (99) 656 (75)	2732 (426)	280	71 (4) 83 (5) 76 (4)

When compared with the Shirreffs et al (2007b) study, the metered rate used in this study appears to have resulted in a 5% greater fluid retention of the milk consumed at 4h. The reason why a greater difference in retention was not observed may relate to the higher Na^+ content of the milk used in the Shirreffs et al (2007b) study (38.6mmol/l) compared with the present study (17.9mmol/L) which also resulted in a greater osmolality (299 *vs.*280 mOsmol/kg). Watson et al (2008) studied a milk drink of similar osmolality and Na^+ content but fluid balance was monitored for a shorter period (3h), however at 4h in the present study, there is only 1% difference in retention compared with the Watson at al 2008 study, indicating a greater degree of fluid retention using a metered approach, when accounting for difference in monitoring time.

A comparison is also made between % fluid retention of the CE (4% CHO) and W drinks in the present study to the Shirreffs et al (2007b) study which used a CE drink (6% CHO) and W (only 0.3mmol/L Na^+). The % fluid retention appears to have been greater in this study for the CE drink (52 *vs.* 38%) and also greater in the W trial (47 *vs.* 36%). This highlights the failure of hypotonic fluids to restore fluid losses even when using a metered approach.

5.4 Composition of rehydration solution and fluid retention

The nature of the study design utilised allowed analysis of a treatment effect of the composition of each test drink as all other factors influencing the response were controlled. The composition of the milk drink resulted in 20% greater fluid retention compared with the CE drink and 25% compared with the W drink. The maintenance of elevated K^+ throughout rehydration in the M trial is likely to have contributed to maintaining a higher Posm which stimulated the water conservation mechanism. Watson et al (2008) also found K^+ to remain elevated in the milk drink used (42mmol/L) compared with a 6% CE drink (1.6mmol/L) and found a similar pattern of elevated Posm and subsequent greater fluid retention. However, coconut water (5 mmol/L Na^+, 53 mmol/ K^+) which has a relatively high K^+ was found not to be more effective than water and a low Na^+ CE drink (19 mmol/L) in restoring fluid balance following exercise-induced dehydration of 2.8 %. (Saat et al 2002). Another study by Kalman et al (2012) compared coconut water from concentrate and a standard coconut water using a 125% replacement of BM loss protocol. This study only found a significant greater fluid retention in the coconut from concentrate drink compared with water (52% *vs.* 35%) at 2h post ingestion, there was no significant difference between the CE (5.6% CHO,) drink and either of the coconut waters. Similar findings were also found by Shirreffs et al (2007a) in her study comparing Apfelshorle with a 6.7% CHO solution and bottled mineral water. Coconut water is similar to milk in terms of CHO (5%) and K^+ content but it does not contain protein. This would lead to the conclusion that although K^+ may have some role in enhancing fluid retention it is likely to be in combination with other constituents particularly the protein content.

There are a number of mechanisms by which protein and milk proteins in particular are considered to have enhanced fluid retention properties which have been discussed previously. Studies that match test drinks for energy and electrolyte content allow more specific analysis of the effects of macronutrients on fluid retention. A recent study by James et al (2013) investigated whether the influence of increasing the protein content of a drink by > 2.5-3% enhances fluid retention further. A 2% protein

+ 4% CE drink was compared to a 4% protein + 2% CE drink with energy density and electrolyte content controlled. At 4 h both drinks were in negative fluid balance, -181 mL in the 2% drink and – 107mL in the 4% protein drink and there was no significant difference in fluid retention (58% *vs.* 64%). This suggests that the optimal effect of protein in a rehydration solution may lie between 2-3%. The M drink in the present study contained 3% protein and 5% CHO and attained a greater level of fluid retention at 5h (Table 5.1) This highlights that when the 150% replacement volume is ingested rapidly (1h) as was the case in both of these studies, even with greater protein content, reaching a positive fluid balance remains a challenge. The results of the present study and the results of the studies discussed here all suggest that protein in a rehydration solution enhances the retention of the fluid. The mechanisms by which this occurs are multi-factorial and relate both to gastric emptying and the osmotic tension created at the intestinal level during water absorption.

The overall energy density and protein content of milk has been estimated to slow gastric emptying time by approximately 14% compared with sports drinks (Shirreffs et al 2007b). A recent study by Clayton et al (2014) showed that delayed gastric emptying alters the relationship between plasma volume, urine volume and net fluid balance. A similar pattern of delayed Pvol recovery, elevated Posm and reduced urine output was observed for milk in this study. This indicates that a slower gastric emptying rate was most likely a key mediating factor influencing the osmotic-diuretic response and ultimately resulted in a greater overall fluid retention.

5.5 Restoration of BM and body water

Net fluid balance and % fluid retention appear to be the most common outcome measures used to report rehydration efficacy. Changes in BM calculated as the difference between baseline and post 5h rehydration under controlled conditions can also provide an indication of hydration status. Overall, the differences in BM restoration were statistically significantly different with Milk achieving a near complete restoration of BM (99.7 (0.1) %), compared to CE (98.8 (0.1 %) and water (98.6 (0.1) %). This is also reflected in the significantly greater % fluid retention achieved in the M compared with CE and W trials. The 1.4% deficit in BM in the W trial could imply greater susceptibility to impaired sports performance in subsequent bouts of exercise given further losses would be incurred during the next exercise session. One factor that may affect the interpretation of the BM results is faecal loss, which was not accounted for during this study. This limits the ability to draw definitive conclusions with regards to restoration of body mass. This is reflected in the fact that percentage fluid retention and net fluid balance are typically reported as the main outcome measures when assessing the effectiveness of rehydration solutions in the literature.

In this study additional measures of TBW, ICW, ECW were taken, by BIA at baseline and at the end of rehydration (5h) to assess if TBW, ICW and ECW had been restored. The lack of significant change in TBW, ICW and ECW from baseline to 5h implies a restoration of TBW, ICW, ECW during the rehydration phase in all trials. However, given that there were significant decreases in BM particularly in the CE and W trials at 5h, the accuracy of BIA under these conditions is questionable. The conditions under which the final measurement (5h) was taken were outside of the BIA manufacturer's guidelines which recommend a minimum of 3h after fluid or meal ingestion and avoidance of strenuous exercise for at least 12h. Skin temperature, composition of ingested fluids, changes in plasma osmolality and sodium concentration and fluid compartment shifts can all effect bioelectrical impedance measurements of fluid compartments (Kushner et al 1996, O'Brien et al 2002). The results of TBW, ICW and ECW are therefore difficult to interpret given the presence of these confounding factors and the known limitations of BIA measurement during acute dehydration and rehydration studies (Armstrong 2005).

5.6 Rehydration as part of total recovery strategy

Post exercise, the main considerations for an athlete are refueling of muscle and liver glycogen stores, replacement of fluid and electrolytes lost in sweat and regeneration and repair of muscle following catabolic stress and damage. Adequate fluid intake is essential as cellular hydration is important to optimise glycogen and protein synthesis due to the role of cell volume in cellular metabolism (Lang 2011). During exercise the body utilises carbohydrate as a source of energy which depletes glycogen stores. For athletes who are required to undertake multiple training sessions in a day the replenishment of glycogen stores is essential (Fournier et al 2004). If these stores are not replenished it may negatively affect performance in a subsequent bout of activity (Karp et al 2006).

In this study, the M drink provided 1.6g per kg BM and 1.3g per kg BM in the CE drink within 3h. There was a lower CHO content in the CE drink (3.9%) used in this study in comparison to standard carbohydrate solutions (6%) (Appendix H). Recommended CHO intakes of 1.0–1.5 g per kg BM to be consumed within the first 30mins post-exercise and at 2h intervals for up to 6h has been shown to result in higher levels of muscle glycogen replenishment than when ingestion is delayed for 2h (Jentjens and Jeukendrup 2003, Ivy et al 1988, Rodriguez et al 2009). The CHO provision in this study in the first hour was a total of 75g which provided 0.8-1.1g/kg BM in the M trial and 58.5g which provided 0.6-0.8g/kg BM in the CE trial. Although intake is less than the recommendation per hour, the co-ingestion of protein with CHO has been shown to enhance glycogen storage via the increased energy provision and through its stimulatory effect on insulin secretion, thereby enhancing the anabolic response (van Loon et al 2000). Therefore, despite the sub-optimal CHO provision from the M drink, the presence of protein should aid enhanced glycogen synthesis.

To optimise muscle protein synthesis post exercise, recommendations for protein intakes are 0.3 g/kg BM or a 20-25g protein portion, ideally from a high biological value / animal based source (Maughan 2012). In this study the protein content of the milk used was 3g per 100ml which provides a mean intake of 90g (approximately 1.1g per kg BM) which contributes to the recommended daily intake for an endurance athlete (1.2-1.4g/kg/day) and resistance training athlete (1.2-1.7g/kg/day) (Rodriguez et al 2009). Dairy protein provides all the essential amino acids including leucine which are integral to muscle metabolism. Studies of individual milk proteins, particularly whey which is high in leucine, also support beneficial effects on skeletal muscle amino acid uptake protein synthesis and muscle mass (Stark et al 2012). This has led to the development of a range commercially available protein powder supplements targeted at the athlete. Consumption of ~ 3–4 g of leucine after exercise is considered optimal to promote maximum protein synthesis and skimmed milk provides 2g of leucine in a 600ml serving (SDA 2014). Practically, therefore milk is an inexpensive and accessible product which can have a dual role in effective rehydration and muscle recovery and repair.

The type of CHO also affects glycogen synthesis, generally high glycaemic carbohydrate sources are recommended post exercise (Burke 1997). However, it has been shown that when iso-caloric amounts of carbohydrates or carbohydrates plus protein and fat are provided after endurance or resistance exercise, glycogen synthesis rates are similar (Burke et al 1995, Roy and Tarnopolsky 1998). Lactose is a low glycaemic index sugar, a disaccharide containing glucose and galactose. Galactose must be converted to glucose before being oxidised or stored as glycogen. In the present study a significant increase in blood glucose was observed in the CE drink at 1h of rehydration to 6.4mmol/L, followed by a more marked decline to 4.1mmol/L at 4h. This fluctuation did not occur in the M or W trials. The CE drink contained less CHO compared with the M drink (3.9% *vs.* 5%). The reason for the spike in blood glucose therefore likely relates to the rate at which it was absorbed into vascular circulation. The gastric emptying rate of milk likely slowed down the release of glucose into circulation compared with the high glycaemic sugars (glucose and fructose) contained within the CE drink. In the context of optimum nutrition for long-term health of an active person or an elite athlete, low glycaemic CHO should make up the greatest proportion of total CHO intake as they provide additional benefits in terms of vitamins, minerals and fibre which contribute to disease prevention and weight management (Rodriguez et al 2009).

5.7 Practical implications of research findings

The ultimate goal of rehydration as part of recovery is to optimise performance in the next bout of training or competition. Watson et al 2008 investigated exercise capacity during a second exercise session within a 24h period following rapid rehydration with either M or CE. A difference in net fluid balance of 326 ± 354 mL (0.4 ± 0.5% BM) entering the second exercise session did not result in greater exercise capacity and there were no differences found in Vo_2, perceived thermal stress, exertion, sweat rates or skin temperature between trials. This study found a marginally greater net fluid balance at 5h between M and CE 499 (447) mL (0.6 (0.5) % BM). This overall difference is relatively small in terms of overall total body water (~42L) and as previously described exercise capacity in warm conditions appears to be maintained up to losses of ~2% body mass. Using milk as a rehydration solution in a recovery programme is therefore likely to result in similar levels of exercise capacity compared with a proprietary sports drink.

In this study a metered approach to rehydration was employed in assessing the effectiveness of milk at restoring body mass loss post exercise. This approach was taken as it reflects more natural ad libitum drinking patterns among athletes, avoiding over distension of the stomach and any negative gastro-intestinal effects such as bloatedness. The results of the subjective feelings questionnaire regarding the level of bloatedness post-ingestion of milk in the first two hours of ingestion in this study scored relatively low, with a mean of 34 (13) mm compared with ~70mm rating in the Shirreffs et al (2007b) study. This would indicate improved gastro-intestinal tolerance using a metered approach.

All drinks were served at room temperature as this reflects the fact that rehydration drinks may not always be refrigerated when carried in the sports bag of an athlete on the go between training sessions. The palatability of a fluid plays a major role in volume of fluid consumed and the stimulus of drinking behaviour. The acceptability of milk as a post exercise drink appeared to be similar to the CE drink as there were no significant differences in palatability or visual appeal ratings, which is a positive finding given the drinks were not served at a cool temperature. Level of sweetness also affects palatability and results in greater ad libitum consumption of a drink (Passe et al 2004). Sweetness level scored higher in the CE drink which may indicate that, should *ad libitum* consumption be allowed, a higher volume of CE may have been consumed. Milk is already available in flavoured form with addition of chocolate and strawberry flavouring to increase sweetness. Assessing *ad libitum* consumption of flavoured versus non-flavoured milk as part of a rehydration strategy may be worthwhile.

Weight management is an important consideration for athletes as well as the general population. The satiety effects of dairy protein can have a positive influence on appetite control and reduced calorie intake (Dougkas et al 2011). A recent meta-analysis of randomised control trials found that inclusion of dairy products in calorie restricted diets led to a significantly greater reduction in body weight, waist circumference and fat mass, while maintaining lean body mass compared with low dairy weight loss diets (Abargouei et al 2012). While energy intake from the milk drink was higher overall, it provides a greater proportion per calorie of other essential nutrients such as protein and calcium compared with sports drinks. This may have benefits in terms of meeting higher protein and micro-nutrient requirements essential for an athlete while achieving reductions in fat mass if required. Optimising the skeletal health of athletes is of growing interest in the area of sports nutrition particularly among endurance and female athletes (Scofield and Hecht 2012). Calcium intakes are sub-optimal in adult females (IUNA 2011). The promotion of milk as an effective rehydration solution among female athletes may help to optimise calcium intakes in this population.

The average amount of lactose consumed during rehydration with milk was approximately 138g. Those with lactose intolerance generally can tolerate small doses of lactose 12g (approximately 200ml of milk) without showing overt symptoms (NCBI 2010). Large lactose loads may result in gastrointestinal disturbances such as bloating and diarrhoea after consumption, in those with undiagnosed lactase deficiency. Although this was not apparent in this study, it has been reported as an issue by Shirreffs et al 2007b. Post test day reporting of any gastrointestinal symptoms were not recorded as part of this study but no subject reported any extreme symptoms to the researcher throughout the time course of the trial. In the development of a rehydration strategy that includes milk, consideration of the presence of lactose intolerance is important.

Chapter 6
Conclusion

This study employed a metered approach to fluid ingestion in a rehydration study comparing (0.1% fat) milk with a proprietary carbohydrate-electrolyte sports drink (CE) and water (W) of the same volume. The metered rate of fluid ingestion was utilised in order to replicate *ad libitum* consumption pattern of sports persons post exercise. The key findings of this study were:

- Milk (0.1% fat) achieved and maintained a positive fluid balance at 2h of rehydration until the end of the 5h rehydration period with a final net fluid balance of 117 (46) ml.
- Both CE and W trials did not maintain a positive net fluid balance at 5h. The W trial was -539 (340) mL in negative balance which was significantly different from the M trial. The CE trial was -381 (460) mL in negative balance, although this did not achieve statistical significance compared with the M trial.
- Overall fluid retention was significantly higher after rehydration with Milk 71 (3.9) % compared with the proprietary carbohydrate-electrolyte drink 51.6 (15.5) % and water (W) 47.3 (14.5) %.
- The metered approach to fluid ingestion appears to have attenuated fluid retention for all three drinks when compared with studies using a rapid rate of fluid ingestion (<1h) of the same volume and similar rehydration solutions.
- The composition of milk (in particular the electrolyte profile, protein content and energy density) appears to have stimulated a greater compensatory water retention response probably by maintenance of a higher plasma osmolality during the rehydration phase compared with the water (W) and proprietary carbohydrate-electrolyte drink (CE).
- The metered rate of fluid ingestion appears to have minimised gastro-intestinal discomfort associated with rapid ingestion of large fluid volumes.

Based on these findings the null hypothesis (H_O) that following a reduction in body water loss equivalent to -2% of body mass achieved by exercise in a hot environment (30°C) and a metered rate of oral fluid replacement equivalent to 150% of the body mass loss, the retention of fluid replaced, measured over 5h, will not be affected by the composition of the rehydration solution (i.e. 0.1% fat Milk (M), Water (W), proprietary carbohydrate electrolyte solution (CE), can be rejected.

Some limitations to this study include the small sample size. However samples sizes tend to be small in these types of studies due to the nature of the intervention and the time commitment involved from the participants. Samples sizes generally range from 6-12 participants in the studies reviewed involving the testing of milk or its constituents as a rehydration solution. The statistical power attained in this study for the main effects reported in the results would suggest that the sample size was adequate for the principal outcomes.

It was not possible to measure with accuracy the restoration of body water, due to the limitations of BIA measurement. However, BM changes measured under controlled conditions can provide a more sensitive estimate of acute total body water changes than repeat measurements by dilution methods (Gudivaka et al 1999). One limitation with using BM loss in this study however, was the difficulty in ascertaining the contribution of faecal mass loss to the BM change as it was not recorded.

Participants with a Posm > 290msomol/kg and > 700mOsmol/kg Uosm at baseline could be considered to be hypo-hydrated based on current guidelines, however cut-offs for dehydration are debatable with a cut-off value of 301 (5) mOsmol/kg recently being proposed (Cheuvront et al 2010). In the design of this study consideration was given to prescribing fluid intakes 24h prior to the trial. However, it was felt that this may force the participants to engage in consumption that was not normal for them and alter their homeostatic Posm basal set-point and would not be reflective of their typical day to day hydration status. Considering that the participants in question presented in a similar state of hydration for each trial it is not thought to have impacted on the outcomes of the study.

The strengths of this study include the use of a comprehensive range of hydration assessment techniques and the frequency of sampling to track acute changes in hydration status during each trial. Based on the findings of the plasma osmolality data it may be possible that higher levels of AVP remained in circulation during rehydration in the M trial. In order to verify the stimulation and suppression of the osmotic regulation of AVP during the rehydration phase, measurement of AVP would have been useful. AVP is not commonly measured in rehydration studies of this nature. The technique used to measure AVP is a radio-immunoassay technique and an extraction process is usually required for accurate measurement and detection. It can be difficult to measure AVP in blood plasma because the peptide is small, is present in the blood in very small amounts (picograms) and due to a short half-life it degrades very quickly (Hew-Butler et al 2011). A review of the literature reveals that assays can detect levels of 0.5-2 pg/ml (Stachenfeld et al 2001). Plasma EDTA samples were collected during this study in order to undertake future analysis of AVP. Proceeding with the analysis will depend on the sensitivity of detection level of the radio-immunoassay available. This is currently being investigated.

To progress the evidence base for the application of milk as a rehydration solution post-exercise in a field-based study may be worthwhile. There are a number of challenges associated with measuring the intervention of a rehydration solution on hydration status in a non-laboratory setting. Research in the laboratory has the advantage of carefully controlled conditions to study mechanisms involved in fluid and electrolyte regulation. In this study conditions which affect sweat rate including air temperature, humidity and exercise intensity were all controlled. The laboratory setting also allows for frequent blood collection and the use of more complex equipment to prepare and analyse samples of more sensitive measures of hydration such as plasma osmolality. However, other simple measures of hydration status such as acute changes in body mass, urine specific gravity (which have been shown to correlate well with Uosm (Armstrong et al 1998)), urine colour and sensation of thirst, while all having their limitations can be justifiably used in a field setting. They are all recommended by international sporting authorities for hydration assessment of athletes during training and competition (Sawka et al 2007, Cheuvront and Sawka 2005).

An *ad libitum* protocol may be warranted in order to ascertain the volume of milk that would be tolerated voluntarily by sports people. Further research addressing factors that influence the choice of a rehydration solution and drinking practices among recreational and elite athletes post exercise are necessary, to address any perceived barriers to consuming milk as part of a rehydration strategy.

References

Abargouei, A. S., Janghorbani, M., Salehi-Marzijarani, M. and Esmaillzadeh, A. (2012) 'Effect of dairy consumption on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials', *Int J Obes (Lond),* 36(12), 1485-93.

Abbott Inc. Potassium/K and Sodium/ N. Abbott Point of care.com [onlne] available: http://www.abbottpointofcare.com/Customer-Info Center/ViewFile.aspx?id=98BD7D7A01DD47E2889C009A1D4D7EDC [Accessed 10th March 2014]

AIS (2014) 'Hydration', available: http://www.ausport.gov.au/ais/nutrition/faq/hydration [Accessed 10/7/2014].

Armstrong, L. E. (2005) 'Hydration Assessment Techniques', *Nutr Rev,* 63, S40-S54.

Armstrong, L. E. (2007) 'Assessing hydration status: the elusive gold standard', *J Am Coll Nutr,* 26(5 Suppl), 575S-584S.

Armstrong, L. E., Johnson, E. C., Munoz, C. X., Swokla, B., Le Bellego, L., Jimenez, L., Casa, D. J. and Maresh, C. M. (2012) 'Hydration biomarkers and dietary fluid consumption of women', *J Acad Nutr Diet,* 112(7), 1056-61.

Armstrong, L. E., Maresh, C. M., Castellani, J. W., Bergeron, M. F., Kenefick, R. W., LaGasse, K. E. and Riebe, D. (1994) 'Urinary indices of hydration status', *Int J Sport Nutr,* 4(3), 265-79.

Armstrong, L. E., Maughan, R. J., Senay, L. C. and Shirreffs, S. M. (2013) 'Limitations to the use of plasma osmolality as a hydration biomarker', *Am J Clin Nutr,* 98(2), 503-4.

Armstrong, L. E., Pumerantz, A. C., Fiala, K. A., Roti, M. W., Kavouras, S. A., Casa, D. J. and Maresh, C. M. (2010) 'Human hydration indices: acute and longitudinal reference values', *Int J Sport Nutr Exerc Metab,* 20(2), 145-53.

Armstrong, L. E., Soto, J. A., Hacker, F. T., Jr., Casa, D. J., Kavouras, S. A. and Maresh, C. M. (1998) 'Urinary indices during dehydration, exercise, and rehydration', *Int J Sport Nutr,* 8(4), 345-55.

Bain, B. J. (2006) *Blood Cells: a practical guide,* 4th Edition ed., United Kingdom: Wiley-Blackwell.

Baker, L. B., Dougherty, K. A., Chow, M. and Kenney, W. L. (2007) 'Progressive dehydration causes a progressive decline in basketball skill performance', *Med Sci Sports Exerc,* 39(7), 1114-23.

Baker, L. B. and Jeukendrup, A. E. (2014) 'Optimal composition of fluid-replacement beverages', *Compr Physiol,* 4(2), 575-620.

Baylis, P. H. (1987) 'Osmoregulation and control of vasopressin secretion in healthy humans', *Am J Physiol,* 253(5 Pt 2), R671-8.

Blann, D. A. (2007) *Routine Blood Results Explained,* 2nd ed., M&K Update Ltd.

BNF (2009) 'Lactose intolerance', available: http://www.nutrition.org.uk/nutritionscience/allergy/lactose-intolerance [accessed 5/9/2014].

Boirie, Y., Dangin, M., Gachon, P., Vasson, M. P., Maubois, J. L. and Beaufrere, B. (1997) 'Slow and fast dietary proteins differently modulate postprandial protein accretion', *Proc Natl Acad Sci U S A,* 94(26), 14930-5.

Broad, E. M., Burke, L. M., Cox, G. R., Heeley, P. and Riley, M. (1996) 'Body weight changes and voluntary fluid intakes during training and competition sessions in team sports', *Int J Sport Nutr,* 6(3), 307-20.

Burke, L. M. (1997) 'Nutrition for post-exercise recovery', *Aust J Sci Med Sport,* 29(1), 3-10.

Burke, L. M., Collier, G. R., Beasley, S. K., Davis, P. G., Fricker, P. A., Heeley, P., Walder, K. and Hargreaves, M. (1995) 'Effect of coingestion of fat and protein with carbohydrate feedings on muscle glycogen storage', *J Appl Physiol (1985),* 78(6), 2187-92.

Calbet, J. A. and MacLean, D. A. (1997) 'Role of caloric content on gastric emptying in humans', *J Physiol,* 498 (Pt 2), 553-9.

Casa, D. J., Armstrong, L. E., Hillman, S. K., Montain, S. J., Reiff, R. V., Rich, B. S., Roberts, W. O. and Stone, J. A. (2000) 'National athletic trainers' association position statement: fluid replacement for athletes', *J Athl Train,* 35(2), 212-24.

Casa D.J., Clarkson, P. M, Roberts, W. O. (2005) 'American College of Sports Medicine roundtable on hydration and physical activity: consensus statements' *Curr Sports Med Rep.* 2005 Jun;4(3):115-27..

Cheuvront, S. N., Carter, R., 3rd and Sawka, M. N. (2003) 'Fluid balance and endurance exercise performance', *Curr Sports Med Rep,* 2(4), 202-8.

Cheuvront, S. N., Ely, B. R., Kenefick, R. W. and Sawka, M. N. (2010) 'Biological variation and diagnostic accuracy of dehydration assessment markers', *Am J Clin Nutr,* 92(3), 565-73.

Cheuvront, S. N., Fraser, C. G., Kenefick, R. W., Ely, B. R. and Sawka, M. N. (2011) 'Reference change values for monitoring dehydration', *Clinical chemistry and laboratory medicine : CCLM / FESCC,* 49(6), 1033-7.

Cheuvront, S. N. and Kenefick, R. W. (2014) 'Dehydration: physiology, assessment, and performance effects', *Compr Physiol,* 4(1), 257-85.

Cheuvront, S. N., Kenefick, R. W., Charkoudian, N. and Sawka, M. N. (2013) 'Physiologic basis for understanding quantitative dehydration assessment', *Am J Clin Nutr,* 97(3), 455-62.

Cheuvront, S. N. and Sawka, M. N. (2005) 'Hydration Assessment of Athletes', 18(Sports Science Exchange 97)available: [accessed

Cockburn, E., Stevenson, E., Hayes, P. R., Robson-Ansley, P. and Howatson, G. (2010) 'Effect of milk-based carbohydrate-protein supplement timing on the attenuation of exercise-induced muscle damage', *Appl Physiol Nutr Metab,* 35(3), 270-7.

Costill, D. L. and Saltin, B. (1974) 'Factors limiting gastric emptying during rest and exercise', *J Appl Physiol,* 37(5), 679-83.

de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C and Schrezenmeir J (2001) Probiotics—compensation for lactase insufficiency. Am J Clin Nutr 73, 421S–429S.

Dill, D. B. and Costill, D. L. (1974) 'Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration', *J Appl Physiol,* 37(2), 247-8.

Dougkas, A., Reynolds, C. K., Givens, I. D., Elwood, P. C. and Minihane, A. M. (2011) 'Associations between dairy consumption and body weight: a review of the evidence and underlying mechanisms', *Nutr Res Rev,* 24(1), 72-95.

EFSA (2010) 'Scientific Opinion on Dietary Reference Values for water', *European Food Safety Authority,* 8((3)), 1459.

EFSA. (2011) 'Scientific Opinion on the substantiation of health claims related to carbohydrate-electrolyte solutions and reduction in rated perceived exertion/effort during exercise (ID 460, 466, 467, 468), enhancement of water absorption during exercise (ID 314, 315, 316, 317, 319, 322, 325, 332, 408, 465, 473, 1168, 1574, 1593, 1618, 4302, 4309), and maintenance of endurance performance (ID 466, 469) pursuant to Article 13(1) of Regulation (EC) No 1924/20061', *European Food Safety Authority,* 9(6):2211.

Elliot, T. A., Cree, M. G., Sanford, A. P., Wolfe, R. R. and Tipton, K. D. (2006) 'Milk ingestion stimulates net muscle protein synthesis following resistance exercise', *Med Sci Sports Exerc,* 38(4), 667-74.

Euromonitor (2014) 'Sports and Energy Drinks in Ireland', available: http://www.euromonitor.com/sports-and-energy-drinks-in-ireland/report [accessed

Evans, G. H., Shirreffs, S. M. and Maughan, R. J. (2009) 'Postexercise rehydration in man: the effects of carbohydrate content and osmolality of drinks ingested ad libitum', *Appl Physiol Nutr Metab,* 34(4), 785-93.

Evans, G. H., Shirreffs, S. M. and Maughan, R. J. (2009) 'Postexercise rehydration in man: The effects of osmolality and carbohydrate content of ingested drinks', *Nutrition,* 25(9), 905-913.

Evetovich, T. K., Boyd, J.C., Drake, S.M., Eschbach, L.C., Magal, M., Soukpup, J.T., Webster, M.J., Whitehead, M.T. and Weir, J.P. (2002) ‘Effect of moderate dehydration on torque, electromyography, and mechanomyography’, *Muscle and Nerve*, 26(2), 225–231.

Fallowfield, J. L., Williams, C. and Singh, R. (1995) 'The influence of ingesting a carbohydrate-electrolyte beverage during 4 hours of recovery on subsequent endurance capacity', *Int J Sport Nutr,* 5(4), 285-99.

Freund, B. J., Shizuru, E. M., Hashiro, G. M. and Claybaugh, J. R. (1991) 'Hormonal, electrolyte, and renal responses to exercise are intensity dependent', *J Appl Physiol (1985),* 70(2), 900-6.

Fukumoto, T., Tanaka, T., Fujioka, H., Yoshihara, S., Ochi, T. and Kuroiwa, A. (1988) 'Differences in composition of sweat induced by thermal exposure and by running exercise', *Clin Cardiol,* 11(10), 707-9.

Germann, W. J. (2005) *Principals of Human Physiology,* Second ed., Pearson.

Greenleaf, J. E. (1992) 'Problem: thirst, drinking behavior, and involuntary dehydration', *Med Sci Sports Exerc,* 24(6), 645-56.

Greiwe, J. S., Staffey, K.S., Melrose, D.R., Narve, M.D. and. Knowlton, R.G. (1998) 'Effects of dehydration on isometric muscular strength and endurance', *Medicine and Science in Sports and Exercise*, 30, 284–288.

Gudivaka R, Schoeller DA, Kushner RF, et al: Single- and multifrequency models for bioelectrical impedance analysis of body water compartments, *J Appl Physiol* 87:1087-1096, 1999.

Hall, W. L., Millward, D. J., Long, S. J. and Morgan, L. M. (2003) 'Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite', *The British journal of nutrition,* 89(2), 239-48.

Hew-Butler, T., Hoffman, M. D., Stuempfle, K. J., Rogers, I. R., Morgenthaler, N. G. and Verbalis, J. G. (2011) 'Changes in copeptin and bioactive vasopressin in runners with and without hyponatremia', *Clin J Sport Med,* 21(3), 211-7.

IOM (2005) 'Dietary reference intakes for Water, Potassium, Sodium, Chloride and Sulfate', *Institute of Medicine,*

IUNA (2011) *National Adult Nutrition Survey: Food and Nutrient intakes, Physical Measurements, Physical Activity Patterns and Food Choice Motives*, Irish University Nutrition Alliance.

Ivy, J. L., Lee, M. C., Brozinick, J. T., Jr. and Reed, M. J. (1988) 'Muscle glycogen storage after different amounts of carbohydrate ingestion', *J Appl Physiol (1985),* 65(5), 2018-23.

James, L. J., Clayton, D. and Evans, G. H. (2011) 'Effect of milk protein addition to a carbohydrate-electrolyte rehydration solution ingested after exercise in the heat', *The British journal of nutrition,* 105(3), 393-9.

James, L. J., Evans, G. H., Madin, J., Scott, D., Stepney, M., Harris, R., Stone, R. and Clayton, D. J. (2013) 'Effect of varying the concentrations of carbohydrate and milk protein in rehydration solutions ingested after exercise in the heat', *The British journal of nutrition,* 110(7), 1285-91.

James, L. J., Mattin, L., Aldiss, P., Adebishi, R. and Hobson, R. M. (2014) 'Effect of whey protein isolate on rehydration after exercise', *Amino Acids,* 46(5), 1217-24.

Jentjens, R. and Jeukendrup, A. (2003) 'Determinants of post-exercise glycogen synthesis during short-term recovery', *Sports Med,* 33(2), 117-44.

Jeukendrup AE. (2010) Carbohydrate and exercise performance: the role of multiple transportable carbohydrates. Curr Opin Clin Nutr Metab Care, 13: 452-457.

Jones, E. J., Bishop, P. A., Green, J. M. and Richardson, M. T. (2010) 'Effects of metered versus bolus water consumption on urine production and rehydration', *Int J Sport Nutr Exerc Metab,* 20(2), 139-44.

Judelson, D.A., Maresh, C.M., Anderson, J.M., Armstrong, L.E., Casa, D.J., Kraemer, W.J. and Volek, J.S. (2007) ‘Hydration and muscular performance: does fluid balance affect strength, power and high-intensity endurance?’, *Sports Medicine*, 37, 907-921.

Kalman, D. S., Feldman, S., Krieger, D. R. and Bloomer, R. J. (2012) 'Comparison of coconut water and a carbohydrate-electrolyte sport drink on measures of hydration and physical performance in exercise-trained men', *J Int Soc Sports Nutr,* 9(1), 1.

Karp, J. R., Johnston, J. D., Tecklenburg, S., Mickleborough, T. D., Fly, A. D. and Stager, J. M. (2006) 'Chocolate milk as a post-exercise recovery aid', *Int J Sport Nutr Exerc Metab,* 16(1), 78-91.

Karvonen, M. J., Kentala, E. and Mustala, O. (1957) 'The effects of training on heart rate; a longitudinal study', *Ann Med Exp Biol Fenn,* 35(3), 307-15.

Kenefick, R. W., Cheuvront, S. N., Elliott, L. D., Ely, B. R. and Sawka, M. N. (2012) 'Biological and analytical variation of the human sweating response: implications for study design and analysis', *Am J Physiol Regul Integr Comp Physiol,* 302(2), R252-8.

King, R. F., Cooke, C., Carroll, S. and O'Hara, J. (2008) 'Estimating changes in hydration status from changes in body mass: considerations regarding metabolic water and glycogen storage', *J Sports Sci,* 26(12), 1361-3.

Kovacs, E. M., Schmahl, R. M., Senden, J. M. and Brouns, F. (2002) 'Effect of high and low rates of fluid intake on post-exercise rehydration', *Int J Sport Nutr Exerc Metab,* 12(1), 14-23.

Kovacs, E. M., Senden, J. M. and Brouns, F. (1999) 'Urine color, osmolality and specific electrical conductance are not accurate measures of hydration status during postexercise rehydration', *J Sports Med Phys Fitness,* 39(1), 47-53.

Kushner, R. F., Gudivaka, R. and Schoeller, D. A. (1996) 'Clinical characteristics influencing bioelectrical impedance analysis measurements', *Am J Clin Nutr,* 64(3 Suppl), 423S-427S.

Lang, F. (2011) 'Effect of cell hydration on metabolism', *Nestle Nutr Inst Workshop Ser,* 69, 115-26; discussion 126-30.

Leahy, S., O'Neill, C., Sohun, R. and Jakeman, P. (2012) 'A comparison of dual energy X-ray absorptiometry and bioelectrical impedance analysis to measure total and segmental body composition in healthy young adults', *Eur J Appl Physiol,* 112(2), 589-95.

Liu K., B. G. a. R. L. (1983) 'Hypomagnasemia in a tennis player.', *Physiology of Sports Medicine,* 11, 79-80.

Martinez Gonzalvez, A. B., Nuno de la Rosa, Y. P. J. A., Sanchez Gascon, F., Villegas Garcia, J. A., Mulero Aniorte, F. and Contreras Gutierrez, J. C. (2005) '[Scintigraphic study of gastric emptying of rehydration drinks in athletes]', *Rev Esp Med Nucl,* 24(1), 19-26.

Maughan R., a. B. L. (2012) 'Nutrition for Athletes. A practical guide to eating for health and performance', *International Olympic Council*.

Maughan, R. J. (2003) 'Impact of mild dehydration on wellness and on exercise performance', *Eur J Clin Nutr,* 57 Suppl 2, S19-23.

Maughan, R. J. and Griffin, J. (2003) 'Caffeine ingestion and fluid balance: a review', *J Hum Nutr Diet,* 16(6), 411-20.

Maughan, R. J. and Leiper, J. B. (1995) 'Sodium intake and post-exercise rehydration in man', *Eur J Appl Physiol Occup Physiol,* 71(4), 311-9.

Maughan, R. J., Leiper, J. B. and Shirreffs, S. M. (1997) 'Factors influencing the restoration of fluid and electrolyte balance after exercise in the heat', *Br J Sports Med,* 31(3), 175-82.

Maughan, R. J., Leiper, J. B. and Vist, G. E. (2004) 'Gastric emptying and fluid availability after ingestion of glucose and soy protein hydrolysate solutions in man', *Exp Physiol,* 89(1), 101-8.

Maughan, R. J., Owen, J. H., Shirreffs, S. M. and Leiper, J. B. (1994) 'Post-exercise rehydration in man: effects of electrolyte addition to ingested fluids', *Eur J Appl Physiol Occup Physiol,* 69(3), 209-15.

Maughan, R. J. and Shirreffs, S. M. (1997) 'Recovery from prolonged exercise: restoration of water and electrolyte balance', *J Sports Sci,* 15(3), 297-303.

Maughan, R. J. and Shirreffs, S. M. (2010a) 'Dehydration and rehydration in competative sport', *Scand J Med Sci Sports,* 20 Suppl 3, 40-7.

Maughan, R. J. and Shirreffs, S. M. (2010b) 'Development of hydration strategies to optimize performance for athletes in high-intensity sports and in sports with repeated intense efforts', *Scand J Med Sci Sports,* 20 Suppl 2, 59-69.

Maughan, R. J., Shirreffs, S. M. and Leiper, J. B. (2007) 'Errors in the estimation of hydration status from changes in body mass', *J Sports Sci,* 25(7), 797-804.

Mitchell, J. W., Nadel, E. R. and Stolwijk, J. A. (1972) 'Respiratory weight losses during exercise', *J Appl Physiol,* 32(4), 474-6.

Mock T., Morrison D. and Yatscoff R. (1995) Evaluation of the i-STAT™ system: A portable chemistry analyzer for the measurement of sodium, potassium, chloride, urea, glucose, and hematocrit Clin Biochemistry 28 (2), 187-192.

Murray, R. (1987) 'The effects of consuming carbohydrate-electrolyte beverages on gastric emptying and fluid absorption during and following exercise', *Sports Med,* 4(5), 322-51.

NCBI (National Centre for Biotechnology Information (2010). Lactose Intolerance and Health. Evidence Reports/Technology assessments No. 192 [online], available: http://www.ncbi.nlm.nih.gov/books/NBK44616/ [accessed 7/9/214]

Nielsen, B., Sjogaard, G., Ugelvig, J., Knudsen, B. and Dohlmann, B. (1986) 'Fluid balance in exercise dehydration and rehydration with different glucose-electrolyte drinks', *Eur J Appl Physiol Occup Physiol,* 55(3), 318-25.

NIH (2013) 'Antidiuretic hormone blood test', [online], available: http://www.nlm.nih.gov/medlineplus/ency/article/003702.htm [accessed 10/9/2014]

Noakes, T. D., Adams, B. A., Myburgh, K. H., Greeff, C., Lotz, T. and Nathan, M. (1988) 'The danger of an inadequate water intake during prolonged exercise. A novel concept re-visited', *Eur J Appl Physiol Occup Physiol,* 57(2), 210-9.

Norton, L. E. and Layman, D. K. (2006) 'Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise', *J Nutr,* 136(2), 533S-537S.

Nose, H., Mack, G. W., Shi, X. R. and Nadel, E. R. (1988a) 'Role of osmolality and plasma volume during rehydration in humans', *J Appl Physiol (1985),* 65(1), 325-31.

Nose, H., Mack, G. W., Shi, X. R. and Nadel, E. R. (1988b) 'Shift in body fluid compartments after dehydration in humans', *J Appl Physiol (1985),* 65(1), 318-24.

O'Brien, C., Young, A. J. and Sawka, M. N. (2002) 'Bioelectrical impedance to estimate changes in hydration status', *Int J Sports Med,* 23(5), 361-6.

Osterberg, K. L., Pallardy, S. E., Johnson, R. J. and Horswill, C. A. (2010) 'Carbohydrate exerts a mild influence on fluid retention following exercise-induced dehydration', *J Appl Physiol (1985),* 108(2), 245-50.

Park, S., Onufrak, S., Blanck, H. M. and Sherry, B. 'Characteristics Associated with Consumption of Sports and Energy Drinks among US Adults: National Health Interview Survey, 2010', *J Acad Nutr Diet,* 113(1), 112-119.

Passe, D. H., Horn, M., Stofan, J. and Murray, R. (2004) 'Palatability and voluntary intake of sports beverages, diluted orange juice, and water during exercise', *Int J Sport Nutr Exerc Metab,* 14(3), 272-84.

Peronnet, F. and Massicotte, D. (1991) 'Table of nonprotein respiratory quotient: an update', *Can J Sport Sci,* 16(1), 23-9.

Pfeiffer, B., Stellingwerff, T., Hodgson, A. B., Randell, R., Pottgen, K., Res, P. and Jeukendrup, A. E. (2012) 'Nutritional intake and gastrointestinal problems during competitive endurance events', *Med Sci Sports Exerc,* 44(2), 344-51.

Popowski, L. A., Oppliger, R. A., Patrick Lambert, G., Johnson, R. F., Kim Johnson, A. and Gisolf, C. V. (2001) 'Blood and urinary measures of hydration status during progressive acute dehydration', *Med Sci Sports Exerc,* 33(5), 747-53.

Robertson, G. L. (1976) 'The regulation of vasopressin function in health and disease', *Recent Prog Horm Res,* 33, 333-85.

Robertson, G. L. and Athar, S. (1976) 'The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man', *J Clin Endocrinol Metab,* 42(4), 613-20.

Robertson, G. L., Mahr, E. A., Athar, S. and Sinha, T. (1973) 'Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma.', *J Clin Invest,* 52(8), 2340-2352.

Robertson, G. L., Shelton, R. L. and Athar, S. (1976) 'The osmoregulation of vasopressin', *Kidney Int,* 10(1), 25-37.

Rodriguez, N. R., DiMarco, N. M., Langley, S., American Dietetic, A., Dietitians of, C., American College of Sports Medicine, N. and Athletic, P. (2009) 'Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance', *J Am Diet Assoc,* 109(3), 509-27.

Roy, B. D. and Tarnopolsky, M. A. (1998) 'Influence of differing macronutrient intakes on muscle glycogen resynthesis after resistance exercise', *J Appl Physiol (1985),* 84(3), 890-6.

Saat, M., Singh, R., Sirisinghe, R. G. and Nawawi, M. (2002) 'Rehydration after exercise with fresh young coconut water, carbohydrate-electrolyte beverage and plain water', *J Physiol Anthropol Appl Human Sci,* 21(2), 93-104.

Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J. and Stachenfeld, N. S. (2007) 'American College of Sports Medicine position stand. Exercise and fluid replacement', *Med Sci Sports Exerc,* 39(2), 377-90.

Sawka, M. N., Cheuvront, S. N. and Carter, R., 3rd (2005) 'Human water needs', *Nutr Rev,* 63(6 Pt 2), S30-9.

Sawka, M. N. and Coyle, E. F. (1999) 'Influence of body water and blood volume on thermoregulation and exercise performance in the heat', *Exerc Sport Sci Rev,* 27, 167-218.

Sawka, M. N. and Montain, S. J. (2000) 'Fluid and electrolyte supplementation for exercise heat stress', *Am J Clin Nutr,* 72(2 Suppl), 564S-72S.

Sawka, M. N., Young, A. J., Francesconi, R. P., Muza, S. R. and Pandolf, K. B. (1985) 'Thermoregulatory and blood responses during exercise at graded hypohydration levels', *J Appl Physiol (1985),* 59(5), 1394-401.

Scofield, K. L. and Hecht, S. (2012) 'Bone health in endurance athletes: runners, cyclists, and swimmers', *Curr Sports Med Rep,* 11(6), 328-34.

SDA (2014) 'Protein and amino acid supplementation Fact sheet', available: http://www.sportsdietitians.com.au/resources/upload/110701%20Protein%20and%20Amino%20Acid%20Supp%20for%20Athletes.pdf [Accessed 12/9/14].

Seifarth, C. C., Miertschischk, J., Hahn, E. G. and Hensen, J. (2004) 'Measurement of serum and plasma osmolality in healthy young humans--influence of time and storage conditions', *Clinical chemistry and laboratory medicine : CCLM / FESCC,* 42(8), 927-32.

Seifert, J., Harmon, J. and DeClercq, P. (2006) 'Protein added to a sports drink improves fluid retention', *Int J Sport Nutr Exerc Metab,* 16(4), 420-9.

Sherman, W. M., Plyley, M. J., Sharp, R. L., Van Handel, P. J., McAllister, R. M., Fink, W. J. and Costill, D. L. (1982) 'Muscle glycogen storage and its relationship with water', *Int J Sports Med,* 3(1), 22-4.

Shirreffs, S. M., Aragon-Vargas, L. F., Keil, M., Love, T. D. and Phillips, S. (2007a) 'Rehydration after exercise in the heat: a comparison of 4 commonly used drinks', *Int J Sport Nutr Exerc Metab,* 17(3), 244-58.

Shirreffs, S. M. and Maughan, R. J. (1994) 'The effect of posture change on blood volume, serum potassium and whole body electrical impedance', *Eur J Appl Physiol Occup Physiol,* 69(5), 461-463.

Shirreffs, S. M. and Maughan, R. J. (1998) 'Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses', *Am J Physiol,* 274(5 Pt 2), F868-75.

Shirreffs, S. M. and Sawka, M. N. (2011) 'Fluid and electrolyte needs for training, competition, and recovery', *J Sports Sci,* 29 Suppl 1, S39-46.

Shirreffs, S. M., Sawka, M. N. and Stone, M. (2006) 'Water and electrolyte needs for football training and match-play', *J Sports Sci,* 24(7), 699-707.

Shirreffs, S. M., Taylor, A. J., Leiper, J. B. and Maughan, R. J. (1996) 'Post-exercise rehydration in man: effects of volume consumed and drink sodium content', *Med Sci Sports Exerc,* 28(10), 1260-71.

Shirreffs, S. M., Watson, P. and Maughan, R. J. (2007b) 'Milk as an effective post-exercise rehydration drink', *The British journal of nutrition,* 98(1), 173-80.

Shirreffs, S. M., Watson, P., Maughan, R. J., Karp, J. R., Johnston, J. D., Tecklenburg, S., Mickleborough, T. D., Fly, A. D. and Stager, J. M. (2007c) 'Milk as an effective post-exercise rehydration drink

Sollanek, K. J., Kenefick, R. W., Cheuvront, S. N. and Axtell, R. S. (2011) 'Potential impact of a 500-mL water bolus and body mass on plasma osmolality dilution', *Eur J Appl Physiol,* 111(9), 1999-2004.

Sparks, S. A. and Close, G. L. (2013) 'Validity of a portable urine refractometer: the effects of sample freezing', *J Sports Sci,* 31(7), 745-9.

Stachenfeld, N. S. (2014) 'The interrelationship of research in the laboratory and the field to assess hydration status and determine mechanisms involved in water regulation during physical activity', *Sports Med,* 44 Suppl 1, S97-104.

Stachenfeld, N. S., Splenser, A. E., Calzone, W. L., Taylor, M. P. and Keefe, D. L. (2001) 'Sex differences in osmotic regulation of AVP and renal sodium handling', *J Appl Physiol (1985),* 91(4), 1893-901.

Stark, M., Lukaszuk, J., Prawitz, A. and Salacinski, A. (2012) 'Protein timing and its effects on muscular hypertrophy and strength in individuals engaged in weight-training', *J Int Soc Sports Nutr,* 9(1), 54.

Tanita (2005) *Multi-Frequency Body Composition Analyzer MC180MA Instruction Manual,* UK: Tanita UK.

Taylor and Machado-Moreira. (2013) Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans, *Extreme Physiology & Medicine 2:4.* Available: http://www.extremephysiolmed.com/content/2/1/4 1[Accessed: 16th January 2015]

Telgi, R. L., Yadav, V., Telgi, C. R. and Boppana, N. (2013) 'In vivo dental plaque pH after consumption of dairy products', *Gen Dent,* 61(3), 56-9.

Thirup, P. (2003) 'Haematocrit: within-subject and seasonal variation', *Sports Med,* 33(3), 231-43.

van Loon, L. J., Saris, W. H., Kruijshoop, M. and Wagenmakers, A. J. (2000) 'Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures', *Am J Clin Nutr,* 72(1), 106-11.

Vist, G. E. and Maughan, R. J. (1995) 'The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquids in man', *J Physiol,* 486 (Pt 2), 523-31.

Wang, Z., Deurenberg, P., Wang, W., Pietrobelli, A., Baumgartner, R. N. and Heymsfield, S. B. (1999) 'Hydration of fat-free body mass: review and critique of a classic body-composition constant', *Am J Clin Nutr,* 69(5), 833-41.

Wapnir, R. A., Wingertzahn, M. A. and Teichberg, S. (1997) 'L-arginine in low concentration improves rat intestinal water and sodium absorption from oral rehydration solutions', *Gut,* 40(5), 602-7.

Watson, P., Love, T. D., Maughan, R. J. and Shirreffs, S. M. (2008) 'A comparison of the effects of milk and a carbohydrate-electrolyte drink on the restoration of fluid balance and exercise capacity in a hot, humid environment', *Eur J Appl Physiol,* 104(4), 633-42.

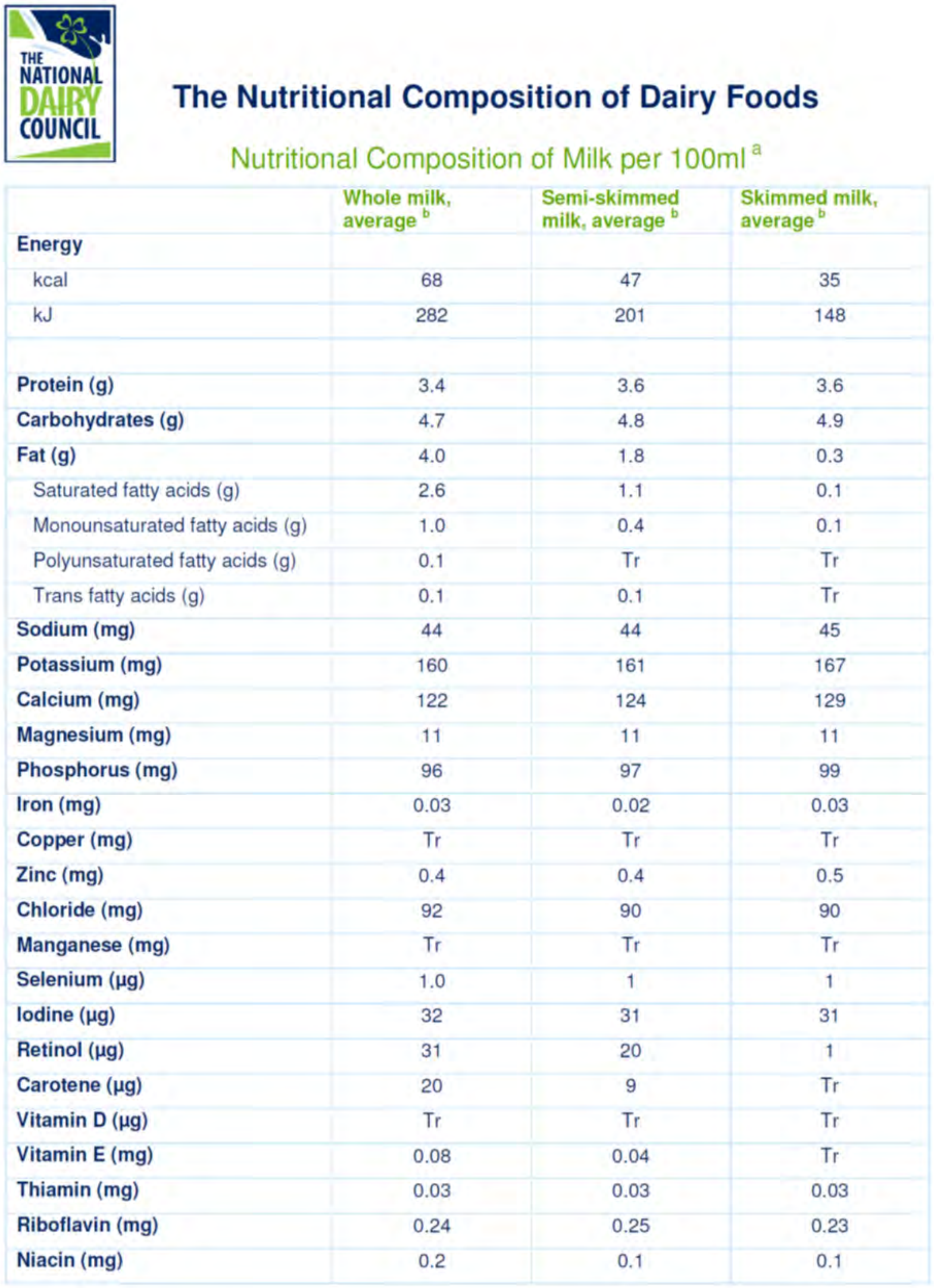
Wemple, R. D., Morocco, T. S. and Mack, G. W. (1997) 'Influence of sodium replacement on fluid ingestion following exercise-induced dehydration', *Int J Sport Nutr,* 7(2), 104-16.

Wong, S. H., Williams, C. and Adams, N. (2000) 'Effects of ingesting a large volume of carbohydrate-electrolyte solution on rehydration during recovery and subsequent exercise capacity', *Int J Sport Nutr Exerc Metab,* 10(4), 375-93.

Zerbe, R. L., Miller, J. Z. and Robertson, G. L. (1991) 'The reproducibility and heritability of individual differences in osmoregulatory function in normal human subjects', *J Lab Clin Med,* 117(1), 51-9.

Appendices

Appendix A: Nutritional composition of milk products



THE NATIONAL DAIRY COUNCIL

The Nutritional Composition of Dairy Foods

Nutritional Composition of Milk per 100ml [a]

	Whole milk, average [b]	Semi-skimmed milk, average [b]	Skimmed milk, average [b]
Energy			
kcal	68	47	35
kJ	282	201	148
Protein (g)	3.4	3.6	3.6
Carbohydrates (g)	4.7	4.8	4.9
Fat (g)	4.0	1.8	0.3
Saturated fatty acids (g)	2.6	1.1	0.1
Monounsaturated fatty acids (g)	1.0	0.4	0.1
Polyunsaturated fatty acids (g)	0.1	Tr	Tr
Trans fatty acids (g)	0.1	0.1	Tr
Sodium (mg)	44	44	45
Potassium (mg)	160	161	167
Calcium (mg)	122	124	129
Magnesium (mg)	11	11	11
Phosphorus (mg)	96	97	99
Iron (mg)	0.03	0.02	0.03
Copper (mg)	Tr	Tr	Tr
Zinc (mg)	0.4	0.4	0.5
Chloride (mg)	92	90	90
Manganese (mg)	Tr	Tr	Tr
Selenium (μg)	1.0	1	1
Iodine (μg)	32	31	31
Retinol (μg)	31	20	1
Carotene (μg)	20	9	Tr
Vitamin D (μg)	Tr	Tr	Tr
Vitamin E (mg)	0.08	0.04	Tr
Thiamin (mg)	0.03	0.03	0.03
Riboflavin (mg)	0.24	0.25	0.23
Niacin (mg)	0.2	0.1	0.1

Appendix B: Participant Information Sheet

EHSREC 2014_02_14

Thank you for participating in this trial. In order for the test days to run smoothly and ensure that the results we get are as accurate as possible, there is some preparation required on your part. We thank you for your co-operation.

Short Test days 1 and 2

Before you arrive at the Body Composition Lab PG054, Physical Education and Sports Science Building, for this experiment:

- Refrain from any form of organised training or strenuous exercise of greater than 20mins for a period of 24 hours
- **Avoid alcohol for 24 hours** before the trial
- Avoid excessive caffeine
- You should arrive having **fasted from midnight**
- Avoid taking the sports supplements e.g. protein, creatine for 24 hours.
- ******Drink 500mls of water 1 hour before arrival at the lab*****
- **Complete a 24 hr food and fluid diary before 1 of the short days – will be agreed with researcher in advance.**

What to expect on test day 1 and 2:

The session will last maximum 30-60mins.

The following tests will be completed:

- Weight checked.
- 1 x blood and urine sample.
- Your heart rate will be measured.
- Bio-electrical impedance analysis and DXA (DXA will be performed on 1 of the short days)
- Perform cycling exercise for approximately 10-15 minutes.
- ***Before* Test days 3,4 and 5 (long test days)**

- Refrain from any form of organised training or strenuous exercise of greater that 20mins for a period of 24 hours
- **Avoid alcohol for 24 hours** before the trial
- You should arrive having **fasted from midnight**
- ******Drink 500mls of water 1 hour before arrival at the lab*****

- **Maintain the same diet and fluid intake in the 48 hours prior to each test day as recorded in the previous Dietary record.**
- **Physical activity diary** – please bring completed diaries with you on the day
- **Clothing:** Bring 2-3 sets of clothing, wear a t-shirt as a base layer. Bring a few changes as you won't want to put on your sweaty clothes again, if you need to exercise further.
    - Please bring with you toiletries for showering after exercise and a change of clothes.
    - Bring some warm comfortable clothing to wear after exercise is complete as you may feel cold during the monitoring period after exercise.

If you are unable to attend for any reason please contact Suzanne on: 087 6115355 at any time.

What to expect when you arrive:

- Weight checked.
- Blood and urine samples will be obtained for every hour during the course of the test day (7 samples)
- Provided with Breakfast
- Perform cycling exercise for approximately 60-90mins
- Drink a rehydration solution in a volume calculated based on your body mass loss through sweating.
- Complete Visual analogue scale questionnaires every hour during the test day
- Abstain from eating or drinking anything other than what is provided to you.
- Every time you wish to urinate after exercise you will be asked to urinate into a container and your urine volume will be measured.
- Remain in the laboratory for the full monitoring period after exercise (5 hours).

Appendix C: Physical Activity Readiness Questionnaire

UNIVERSITY of LIMERICK
OLLSCOIL LUIMNIGH

Department of Physical Education & Sport Sciences

PRE-TEST QUESTIONNAIRE

NAME …………………………………. Ref. No.
………………...

Date of Birth …………………………… Age:
…………………….

Test procedure ………………………….

As you are to be a subject in this laboratory/project, would you please complete the following questionnaire. Your cooperation in this is greatly appreciated. Any information contained herein will be treated as confidential

Please tick appropriate box

		YES	**NO**
	Has the test procedure been fully explained to you?	☐	☐
1.	Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?	☐	☐
2.	Do you feel pain in your chest when you do physical activity?	☐	☐
3.	In the past month, have you had chest pain when you were not doing physical activity?	☐	☐
4.	Do you lose your balance because of dizziness or do you ever lose consciousness?	☐	☐
5.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?	☐	☐

6. Is your doctor currently prescribing drugs for your blood pressure or heart condition? ☐ ☐

7. Do you know of any other reasons why you should not undergo physical activity? This might include severe asthma, diabetes, a recent sports injury, or serious illness. ☐ ☐

8. Have you any blood disorders or infectious diseases that may prevent you from providing blood for experimental procedures? ☐ ☐

9. Are you lactose intolerant? ☐ ☐

- If you have answered **NO** to <u>all</u> questions then you can be reasonably sure that you can take part in the physical activity requirement of the test procedure

I ………………………………. declare that the above information is correct at the time of completing this questionnaire Date ……/……/…….

Please Note: If your health changes so that you can then answer YES to any of the above questions, tell the experimenter/laboratory supervisor. Consult with your doctor regarding the level of physical activity you can conduct.

- If you have answered **YES** to one or more questions:
  Talk with your doctor in person discussing with him/her those questions you answered yes.
  Ask your doctor if you are able to conduct the physical activity requirements.

Doctor's signature ……………………………….……… Date ……/……/…….

Signature of Experimenter……………………………….. Date ……/……/…….

Appendix D: Nutritional content of controlled breakfast

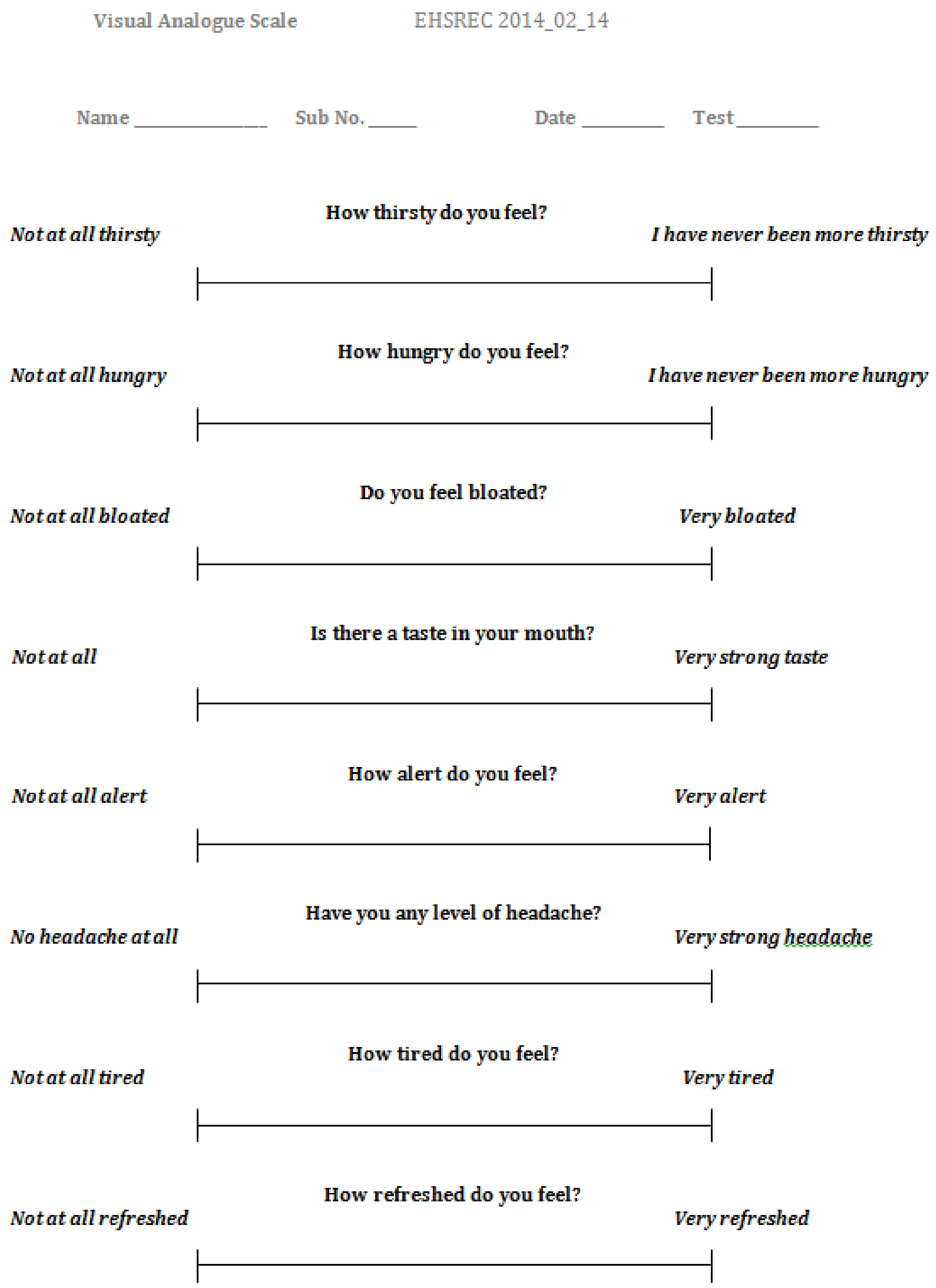
	Cornflakes (40g)	**Full fat Milk (200mls)**	**Nutrigrain Bar (40g)**	**Total**
Energy (kJ)	1064	564	777	2405
Energy (kcals)	151.2	136	185	472.2
Fat	0.36	8	7	15.36
CHO	33.6	9.4	26	69
Sugars	3.2	9.2	11	23.4
Protein	2.8	6.8	3	12.6
Salt	0.5	0	0.34	0.84
Sodium	0.20	0.088	0.14	0.42

A 100mls of water also provided as fluid to help promote gastric emptying and digestion.

Products used in preparation of controlled breakfast:

- Kellogg’s Nutrigrain Breakfast Granola Slices (Honey)
- Kelloggs Cornflakes
- Golden Vale Full Fat Milk

Appendix E: Visual Analogue Scale



Visual Analogue Scale EHSREC 2014_02_14

Name _____________ Sub No. _____ Date ________ Test ________

How thirsty do you feel?

Not at all thirsty — *I have never been more thirsty*

How hungry do you feel?

Not at all hungry — *I have never been more hungry*

Do you feel bloated?

Not at all bloated — *Very bloated*

Is there a taste in your mouth?

Not at all — *Very strong taste*

How alert do you feel?

Not at all alert — *Very alert*

Have you any level of headache?

No headache at all — *Very strong headache*

How tired do you feel?

Not at all tired — *Very tired*

How refreshed do you feel?

Not at all refreshed — *Very refreshed*

Appendix E: Visual Analogue Scale

Visual Analogue Scale EHSREC 2014_02_14

Name ________________ Sub No. ______ Date __________ Test __________

Visual appeal

Not at all appealing **Very appealing**

Aftertaste

No aftertaste **Strong aftertaste**

Palatability

Not palatable at all **Very Palatable**

Sweetness

Not at all sweet **Very sweet**

Saltiness

Not at all salty **Very salty**

Appendix F: Food diary

Food & Fluid Diary Instructions & Guidelines

In order to measure your nutritional intake it over the 2 day period it is required that you fill out a *Food & Fluid Diary* as accurately as possible to give the most detailed description of your dietary intake. The following pages will provide information & guidelines on how to fill out the *Food & Fluid Diary* correctly.

Important points to note

- In order to obtain an accurate representation of your dietary intake it is important that you do not alter or change your diet in any way from what you consider is your normal diet for the duration of the 2 day period.

- Do not write down what you think the researchers would want to see. All information is confidential and no person's diet will be "judged" by the researchers.

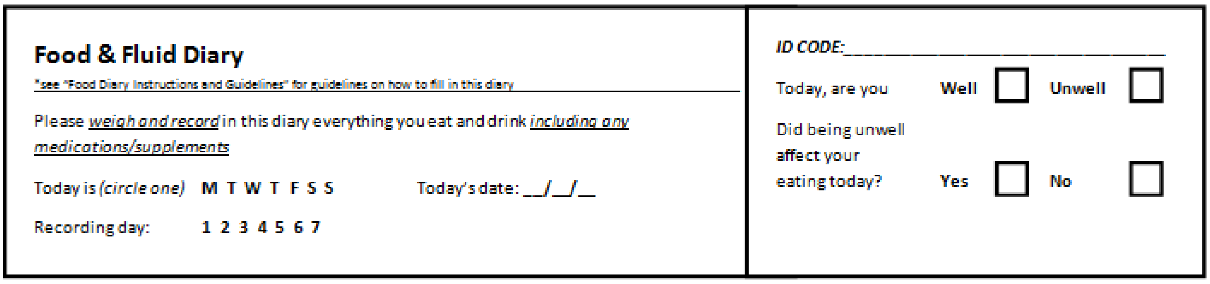
- Be as accurate and honest as possible in your recording of food & fluid intake over the 2 day period (*see guidelines following)*.

- All food & fluid intake should be recorded on the food & fluid diary.
- All medications/ vitamin & mineral supplements should be recorded on the *Food & Fluid Diary.*
- **Avoid any form of protein / sports supplements** in the **24 hours** before the test day.

The Food & Fluid Diary

Recording in your *Food & Fluid Diary:*

Record your ID Code, the date, the day, the recording day, whether you are well or unwell on this day and whether this affected your eating habits for the day at the top of each set of pages for that particular day.



Food & Fluid Diary

*see "Food Diary Instructions and Guidelines" for guidelines on how to fill in this diary

Please *weigh and record* in this diary everything you eat and drink *including any medications/supplements*

Today is *(circle one)* **M T W T F S S** Today's date: __/__/__

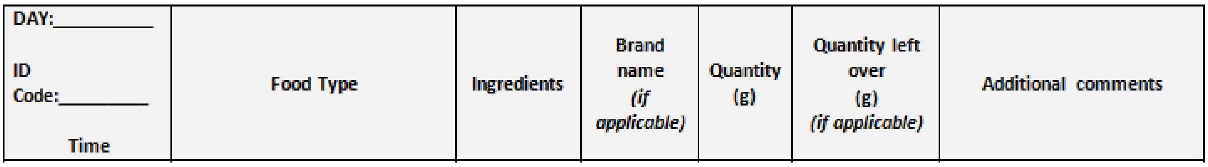
Recording day: **1 2 3 4 5 6 7**

*ID CODE:*______________________________

Today, are you **Well** ☐ **Unwell** ☐

Did being unwell affect your eating today? **Yes** ☐ **No** ☐

If a second sheet or subsequent sheets are required record the day and your ID number above the column marked "Time" in the spaces provided.



DAY:_________ ID Code:________ Time	Food Type	Ingredients	Brand name *(if applicable)*	Quantity (g)	Quantity left over (g) *(if applicable)*	Additional comments

Explanation of column terms

Time:

Record the time each meal/snack was eaten e.g. 07:00, 12:30, 13:27, 19:15. If recording using a 12 hour clock method please state the time followed by am/pm.

Food type:

Provide a description of the food e.g. a bowl of porridge, 2 slices of wholegrain/white/wholemeal toast, 1 yoghurt, 1 sandwich, Chicken stir-fry etc. If a food states whether it is low fat/light/fortified with vitamins or minerals/ no added sugar/ whole/ semi-skimmed/skimmed it is important to state this also (e.g. low fat fortified milk instead of just milk or even low fat milk).

If a meal consists of more than one ingredient (see example chicken stir fry), list in this column just the words “chicken stir fry” and list the individual constituents of the meal in the *ingredients* section.

Any multivitamin/ mineral supplements must be recorded and their name and brand name also be recorded as well as if they have been taken with something else (e.g. 1 sachet Iron supplement in 200ml orange juice).

Ingredients:
In this column you should list the ingredients of a particular meal.

E.g. 1: Bowl of porridge: the ingredients that should be listed here are porridge oats, milk (type) and anything added e.g. honey/ blueberries/ seeds etc *(see sample food & fluid diary).*

E.g. 2: Chicken stir fry example: the ingredients that should be listed individually here are e.g. chicken fillet, red pepper, onion, mange tout, leek, carrots, noodles and olive oil.

If recording a sports supplement the ingredients would be for example protein powder and milk (type)/water.

Brand Name:
Record the brand name of products where applicable e.g. for the Iron supplement mentioned previously in this column you should record a brand name such as “Spatone” or “Holland & Barrett” etc.

In the case of milk you should record a brand name such as “Avonmore” or “Golden Vale”

Quantity

You will be provided with electronic weighing scales for the 2 day period. Individual ingredients of a meal should be weighed where possible and their weight recorded in grams (g) or millilitres (ml). For example if making a sandwich the bread, meat or filling, and vegetables should be weighed separately and recorded separately.

Wrappers of less well known food items should be kept and placed in the folder in the correct day. If unsure about any item please keep the packaging/ wrappers.

If cooking and eating a full pizza it is sufficient to record the weight of the pizza stated on the box.

The quantity of milk added to porridge or breakfast cereals should also be measured independent of the cereal. This can be done by:

1. Place a bowl on the weighing scales
2. Press the “tare” button to bring it to 0
3. Place the cereal in the bowl
4. Record the weight of the dry cereal in your diary
5. Press the “tare” button once again to bring it back to 0
6. Pour in the milk
7. Record the weight of the milk in your diary

Similarly for butter/spread on a slice of bread the bread should be weighed, the scales “tared”, the bread buttered & re-weighed and the difference recorded as the weight of the butter/spread.

FLUIDS: The quantity of water or other fluids consumed should also be recorded in the food & fluid diary.

Quantity left over:

If you do not eat all of a particular meal/snack the quantity remaining should be weighed and recorded in this column.

Additional Comments:

Use this section to add any important notes/ comments that you feel may be important. Cooking & preparation methods are very important and should be listed here e.g. fried/ boiled/ poached/ microwaved/ oven cooked/ toasted / ADDED salt etc.

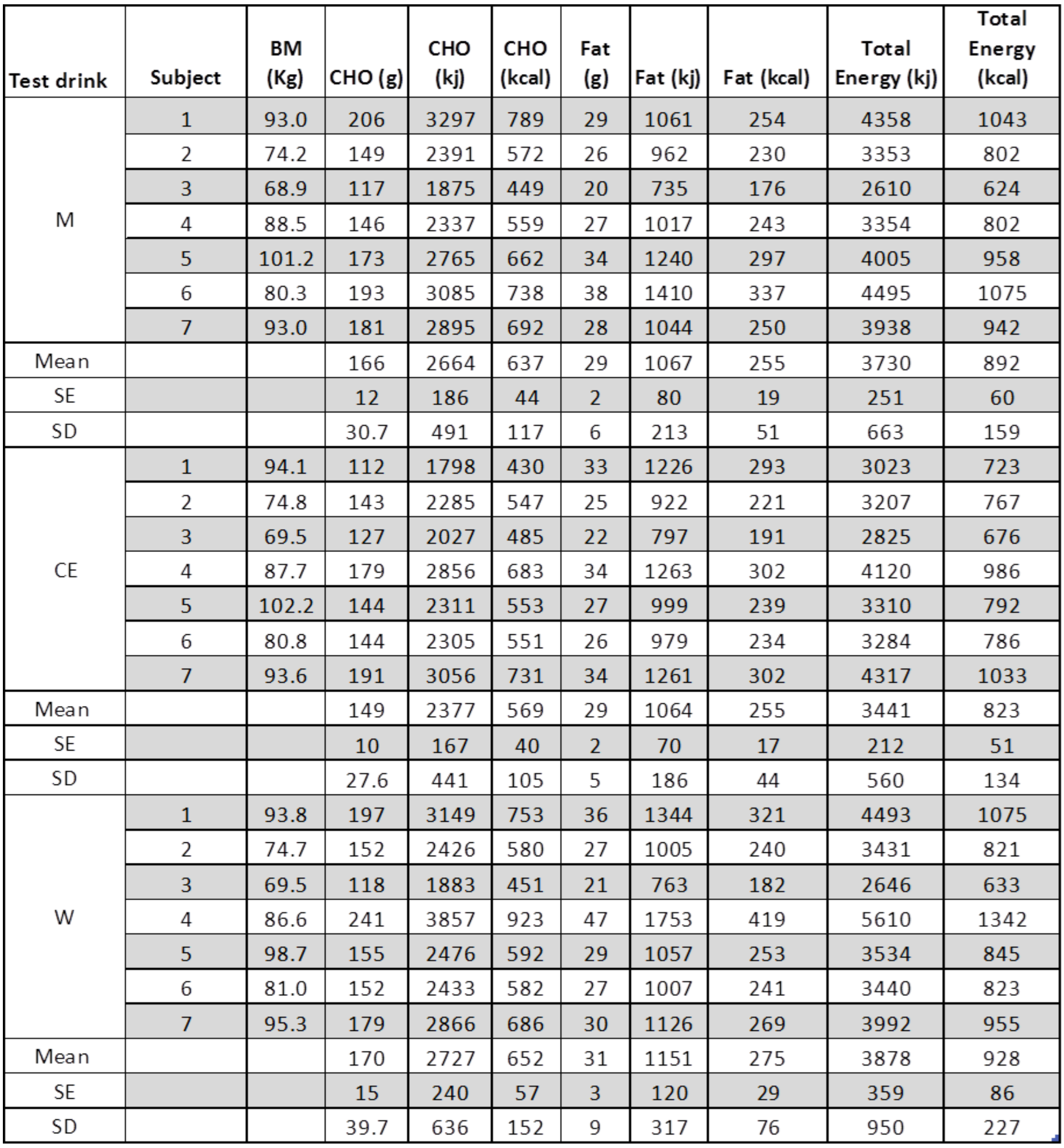
Appendix G: Pre-trial Dietary intake and Substrate utilisation during each trial

Fluid, electrolyte, carbohydrate, protein and energy intakes prior to each trial Milk (M), Carbohydrate-electrolyte drink (CE) and water (W)

	M	CE	W	P value*
Fluid (mL)	3401 (519)	2607 (1025)	3078 (1229)	0.156
Sodium (g)	3.0 (2.3)	3.1 (2.2)	3.0 (2.4)	1.000
Potassium (g)	3.6 (1.3)	3.7 (1.7)	3.3 (0.7)	0.867
CHO (g)	258 (148)	248 (203)	308 (87)	0.867
Protein (g)	114.8 (8.5)	110.8 (88.6)	125.7 (37.3)	0.651
Energy (MJ)	10.1 (2.6)	10.1 (2.1)	10.3 (2.0)	0.651

*Friedman test for non-parametric data, values stated are median and inter-quartile range (IQR)

Substrate Utilisation for each participant during each trial (Milk (M), Carbohydrate-electrolyte drink (CE) and water (W)



Test drink	Subject	BM (Kg)	CHO (g)	CHO (kj)	CHO (kcal)	Fat (g)	Fat (kj)	Fat (kcal)	Total Energy (kj)	Total Energy (kcal)
M	1	93.0	206	3297	789	29	1061	254	4358	1043
	2	74.2	149	2391	572	26	962	230	3353	802
	3	68.9	117	1875	449	20	735	176	2610	624
	4	88.5	146	2337	559	27	1017	243	3354	802
	5	101.2	173	2765	662	34	1240	297	4005	958
	6	80.3	193	3085	738	38	1410	337	4495	1075
	7	93.0	181	2895	692	28	1044	250	3938	942
Mean			166	2664	637	29	1067	255	3730	892
SE			12	186	44	2	80	19	251	60
SD			30.7	491	117	6	213	51	663	159
CE	1	94.1	112	1798	430	33	1226	293	3023	723
	2	74.8	143	2285	547	25	922	221	3207	767
	3	69.5	127	2027	485	22	797	191	2825	676
	4	87.7	179	2856	683	34	1263	302	4120	986
	5	102.2	144	2311	553	27	999	239	3310	792
	6	80.8	144	2305	551	26	979	234	3284	786
	7	93.6	191	3056	731	34	1261	302	4317	1033
Mean			149	2377	569	29	1064	255	3441	823
SE			10	167	40	2	70	17	212	51
SD			27.6	441	105	5	186	44	560	134
W	1	93.8	197	3149	753	36	1344	321	4493	1075
	2	74.7	152	2426	580	27	1005	240	3431	821
	3	69.5	118	1883	451	21	763	182	2646	633
	4	86.6	241	3857	923	47	1753	419	5610	1342
	5	98.7	155	2476	592	29	1057	253	3534	845
	6	81.0	152	2433	582	27	1007	241	3440	823
	7	95.3	179	2866	686	30	1126	269	3992	955
Mean			170	2727	652	31	1151	275	3878	928
SE			15	240	57	3	120	29	359	86
SD			39.7	636	152	9	317	76	950	227

Appendix H: Composition of commercial rehydration beverages

Nutrient Per 100mL	Spar Isotonic Sports Drink	Spar Energy Drink	Centra Isotonic Sports Drink	Powerade Ion 4	Luco zade Sport	Coconut Water (Vita Coco)
CHO (g)	5.76	17.7	6.7	3.9	6.4	5
Fat (g)	0	0	0	0	0	0
Protein (g)	0.02	0	0	0	0	0
Energy density (kcal)	25.4	72	27	16	28	18
Energy density (kJ)	106.2	305	114	70	112	72
Sodium (mg)	300	400	250	500	250	0.01
Potassium (mg)	29	28	29	38	45	195