

# **Hydration and fluid balance:**

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**Studies on body composition, drink formulation and ageing**

*By*

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**A thesis submitted to the University of Stirling in partial  
fulfilment for the degree of**

**Doctor of Philosophy**

**Physiology, Exercise and Nutrition Research Group**

**Faculty of Health Sciences and Sport**

**November 2016**

*"Above all, don't fear difficult moments. The best comes from them."*

**Rita Levi-Montalcini**

## Declaration

I declare that this thesis was composed by myself and that all the data were collected and analysed by myself except part of the data presented in Chapters 4 (Part A) and 5 that were part of a multicentre project where Philip AA Cordery under the supervision of Phillip Watson and Ronald J Maughan from Loughborough University and Alberto Dolci and Samuel J Oliver under the supervision of Neil P Walsh from Bangor University performed the data collection and sample analysis in their respective locations. Neither the thesis nor the original work has been submitted to this or any other institution for a higher degree.

A handwritten signature in black ink, appearing to be 'Nidia Rodriguez Sanchez', written in a cursive style.

Nidia Rodriguez Sanchez (10/11/16)

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## THANK YOU...

First of all, thank you to Dr Stuart Galloway who, since I first showed my interest in coming to Stirling, was very kind and helped to make my arrival and first few days at Stirling easier. Thanks for being really supportive during the entire Ph.D. journey, and always being there in the early morning trials, teaching me in the lab and going through all the writing process with me. It has been such a privilege to work alongside you. I have learned so much from you. Thank you for giving me the opportunity to pursue my Ph.D. under your supervision.

Thank you very much to all the members of Physiology, Exercise & Nutrition Research Group, Kevin, for all your support since we met in Mexico in October 2012, for all your support when I arrived at Stirling and during my Ph.D. Ian W, Olly, Naomi, Iain G, thank you for your help during my trials.

Gill and Chris, thank you very much for all your help in the lab and in the panic moments. Thank you for sharing lots of cups of tea and cake! Gill, thank you for all those ELISA, iLab and freezer moments of fun. Chris, thank you for all your patience and support in the lab.

Sophie, Lindsay, Chris M, Mike, Lewis, Tom, Adam, Andy, thank you very much for helping me too when I was running my trials, getting some pilot data or practising some of the skills that I acquired during my Ph.D. (a.k.a. Blood club!). Colin and Jordan, thanks for making the 3A57 a great place to be!

Barbara, Gill, Lindsay, Roslyn, Naomi and Sophie, thank you for your friendship, for all the teas, coffees, cakes, pancakes, dinners and all the lovely moments we have shared.

Thank you to my friends back in Mexico, Ingrid, Marthita, Adri and Sam, thanks for always being there for me regardless of the time or distance.

Gracias Papá por siempre apoyarme en mis decisiones y ayudarme a realizar mis sueños. Gracias Mamá por tu apoyo y por siempre estar, por escucharme y por las palabras de aliento. Gracias por ser mis papás, los quiero mucho.

Thank you to Alistair for your continued love, patience and belief in me. You have made this journey a much happier one for me. I love you so much.

Thank you to all my participants, without you this thesis would not be possible. Thank you for all your time, your enthusiasm and your altruistic willingness to help to develop science. I will always be indebted to you.

**To my family and all my friends in Scotland and in Mexico,  
thanks for all your love and support and for believing in me!**

## Abstract

The thesis reports on 6 studies (2 of which were part of a multi-centre trial) examining hydration and fluid balance.

The first study described in this thesis investigated the impact of hydration status on Dual energy x-ray absorptiometry (DXA) and other methods that are popular tools to determine body composition in athletes. We observed that it is important to ensure a euhydration when assessing body composition, particularly when considering changes associated with nutritional or exercise interventions.

The second and third studies reported identified beverages that promote longer term fluid retention and maintenance of fluid balance in adults. We investigated the effects of 13 different commonly consumed drinks on urine output and fluid balance when ingested in a euhydrated state, with a view to establishing a beverage hydration index (BHI), i.e., the volume of urine produced after drinking expressed relative to a standard treatment (still water) for each beverage. The beverages with the highest BHI were oral rehydration solution, full fat milk and skimmed milk. BHI may be a useful measure to identify the short term hydration potential of different beverages when ingested in a euhydrated state.

The fourth study aimed to systematically examine the influence of carbohydrate, sodium and caffeine content of beverages on the BHI. The BHI was greater in beverages with higher carbohydrate or higher sodium content, but not influenced by caffeine. The carbohydrate content of beverages has no effect on BHI at concentration up to 10% carbohydrate. Sodium content of beverages in concentrations of 27mmol/L and higher can improve the hydration potential of beverages. Caffeine doses in beverages up to 400mg/L do not have an impact upon diuresis when ingested in a euhydrated state.

The fifth study compared net fluid balance (NFB) responses to the ingestion of commonly consumed drinks in young and older men. We observed that in young adults milk helps to maintain positive net fluid balance for longer than other drinks. In older adults this effect of milk is not observed despite similar net electrolyte balance responses. Future work should more fully explore these potential differences in fluid balance responses to drink ingestion between young and older adults.

The final study investigated the hydration habits of Scottish young and older adults (+50 years old), identifying their fluid choices, volume, and preferences in relation to time of day. The results showed that 26.1% of the young females, 30.3% of the young males, 25.8% of the older females and 50.4% of the older males did not meet the EFSA fluid recommendations. We also observed that the difference between those who met and those who did not meet the EFSA adequate intake could be attributed to differences in water ingestion, mainly during the mid-morning (after breakfast until 11 am) and during the early-afternoon (after lunch time up to 5 pm). It was concluded that these moments might be key when implementing interventions to improve hydration status especially in the older population.

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## List of publications

### PUBLISHED

Maughan RJ, Watson P, Cordery PAA, Walsh NP, Oliver SJ, Dolci A, **Rodriguez Sanchez N**, and Galloway SDR. A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. *The American Journal of Clinical Nutrition*, 103.3 (2015): 717-23. doi:10.3945/ajcn.115.114769

**Rodriguez-Sanchez N** and Galloway SDR. Errors in dual energy x-ray absorptiometry estimation of body composition induced by hypohydration. *Int.J.Sport Nutr.Exerc.Metab* 25.1 (2015): 60-68.

### IN PREPARATION

Maughan RJ, Watson P, Cordery PAA, Walsh NP, Oliver SJ, Dolci A, **Rodriguez-Sanchez N**, and Galloway SDR. Carbohydrate and sodium, but not caffeine, content of beverages influences the beverage hydration index in humans. *In preparation for the British Journal of Nutrition*.

**Rodriguez-Sanchez N** and Galloway SDR, Effect of age and different composition of drinks on body fluid balance. *In preparation for the European Journal of Nutrition*.

**Rodriguez-Sanchez N** and Galloway SDR, Hydration habits in Scottish adults. *In preparation for the European Journal of Nutrition*.

**Rodriguez-Sanchez N** and Galloway SDR, Hydration potential of commonly consumed drinks in an office-working environment. *In preparation for the European Journal of Applied Physiology*.

**ABSTRACTS/CONFERENCE PRESENTATIONS-POSTERS**

**Rodriguez-Sanchez N** and Galloway SDR, Errors in the estimation of body composition induced by hypohydration, Mini-oral presentation at the European College of Sport Science Annual Congress in Amsterdam, The Netherlands, July 2014.

Galloway SDR and **Rodriguez-Sanchez N**, Hydration potential of commonly consumed drinks in an office-working environment, Poster presentation at the American College of Sports Medicine Annual Meeting in Boston, United States, June 2016.

**Rodriguez-Sanchez N** and Galloway SDR, How do different drinks affect body fluid balance: does age make a difference?, Poster presentation at the American College of Sports Medicine Annual Meeting in Boston, United States, June 2016.

**Rodriguez-Sanchez N**, Maughan RJ, Watson P, Cordery PAA, Walsh NP, Oliver SJ, Dolci A and Galloway SDR, Systematic evaluation of carbohydrate, sodium, and caffeine content of drinks on the beverage hydration index, Presentation at the International Sports Exercise and Nutrition Conference in Newcastle, United Kingdom, December 2016.

## List of abbreviations

BV	blood volume
CV	coefficient of variation
CV	cell volume
d	day (s)
DXA	dual energy X-ray absorptiometry
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
g	gram
G	gauge
h	hour (s)
Hb	haemoglobin
Hct	haematocrit
HDPE	High-density polyethylene
BHI	beverage hydration index
IFB	initial fluid balance
K	Potassium
kg	Kilogram
L	Litres
mL	millilitres
μL	microliter
mOsm	milliosmole
Na	sodium
nm	nanometres
°C	degrees Celsius
PV	plasma volume



## CHAPTER 1. LITERATURE REVIEW

### 1.1 Introduction

The maintenance of fluid balance is an important concern for regular exercisers, athletes, older adults and clinicians. Whilst the balance between fluid intake and fluid loss, and thus total body water, is normally tightly controlled in healthy adults, there are many situations where effective fluid replacement is required: 1) to help maintain performance during endurance exercise; 2) to replace fluid and salt losses following exercise; 3) to help maintain hydration status and cognitive function during daily activities; and 4) to improve clinical outcomes in patients. Many studies have been conducted in these areas in an attempt to identify fluid provision guidelines and optimal fluid replacement beverage formulation. These studies have identified variables such as energy and macronutrient content of drinks, as well as electrolyte/mineral composition as key factors influencing fluid replacement, and the restoration of total body water.

#### 1.1.1 What controls hydration/fluid balance?

Homeostasis to maintain a constant water and mineral balance requires the coordination of many inputs including neural pathways and integrative centres in the brain as well as peripheral effects. These centres are sensitive to neuro-hormones produced for the adjustment of diuresis, natriuresis and blood pressure regulation (angiotensin, mineralocorticoids, vasopressin, and atrial natriuretic factor). These neuro-hormones alter the function of different organs to regulate body water and inorganic ion balance (kidney, sweat and salivary glands). These mechanisms are activated with deficits or excesses of body water, and in particular to changes in circulating blood volume. At rest, a water deficit increases the ionic concentration of the extracellular compartment (increased osmolality, reduced plasma volume) and this draws water from the intracellular compartment, making the cells shrink. This osmotic stimulus is also detected by two sensors in the brain, one which controls drinking behaviour (thirst) and the other which controls renal function (excretion of urine). A fluid deficit will lead not only to a decrease in glomerular filtration rate and a subsequent renin angiotensin aldosterone system response to reduce sodium excretion, but will at the same time increase the release of arginine vasopressin from the posterior pituitary to alter renal tubular water reabsorption. These actions counter the reduced effective

circulating volume, and when combined with the thirst response will increase fluid intake to restore body water balance. If there is an excess of water, the lower ionic concentration of body fluids (reduced osmolality, increased plasma volume) will result in the opposite actions.

Thus, the role of thirst, sodium balance and renal function are integrated to help regulate body water volume and maintain circulating blood volume and cardiovascular function. Under normal conditions this physiological regulation ensures that plasma volume and osmolality are maintained within normal limits by initiating physiological and behavioural modifications. However, certain situations can increase sweat losses and insensible water losses, such as exercise, and others may increase faecal water losses, such as diarrhoea. In addition, changes in drinking behaviour, renal function or salt appetite throughout the lifespan can impact upon body water regulation (such as in older adults).

#### *1.1.1.1 Thirst*

Thirst is a key component of the regulatory mechanisms. Thirst is a subjective perception that delivers the urge to drink in humans and animals. Key physiological signals for thirst are plasma hyperosmolality with consequential cellular dehydration and hypovolemia (Thompson, Bland et al. 1986). The need to drink can be driven by habitual, cultural and psychogenic drives and also by the regulatory response to reductions in the fluid content of the water body compartments, hypertonicity of the extracellular fluid, or increases in the circulating concentration of some dipsogenic hormones (such as angiotensin and aldosterone) and neural signals from low and high pressure baroreceptors (Johnson and Thunhorst 1997). The multifactorial nature of factors driving drinking behaviour makes thirst a complex area to study. Indeed, the reported wide variation in fluid intake of adults in UK and US populations supports the role for many non-physiological inputs into drinking behaviour (Agostoni, Bresson et al. 2010).

#### *1.1.1.2 Renal function*

Arginine vasopressin, angiotensin II, natriuretic peptides, vasoactive intestinal peptide, urotensin II, insulin and non-genomic actions of corticosteroids are all involved in acute alterations in renal ion and water transport (McCormick and Bradshaw 2006).

The kidneys play a central role in regulating inorganic ion composition and fluid volume in the internal environment. Having low molecular weights and not being bound to protein, sodium and water freely filter from the glomerular capillaries into Bowman's space and they undergo considerable reabsorption (normally more than 99%). Most renal energy utilization goes to accomplish this reabsorptive task (Prowle and Bellomo 2010). The bulk of sodium and water reabsorption (about 2/3rds) occurs in the proximal tube, but the major hormonal control of reabsorption are exerted in the collecting ducts. Sodium reabsorption is an active process happening in all tubular segments except the descending limb of the loop of Henle and water reabsorption occurs by diffusion and is dependent upon sodium reabsorption. Water movement across the tubular epithelium can happen, however, only if the epithelium is permeable to water. Water permeability fluctuates from tubular segment to segment and depends mainly on the presence of water channels called aquaporins in the plasma membranes (Agre, Sasaki et al. 1993). Water permeability of the proximal tubule is always very high, so the water molecules are reabsorbed by this segment almost as fast as sodium ions; therefore, the proximal tubule always reabsorbs sodium and water in the same proportions. The water permeability of the last portions of the tubules (cortical and medullary collecting ducts) can change depending on physiological inputs. The principal determinant of this controlled permeability and consequently of water reabsorption in the collecting tubes is the action of arginine vasopressin (Hew-Butler 2010), that stimulates the insertion into the luminal membrane by exocytosis of a group of aquaporin water channels produced by the collecting duct cells. When vasopressin plasma concentration is high the water permeability of the collecting tubes is greater. Water reabsorption is maximal and the final urine volume is small (<1% of the filtered water). Without vasopressin the water permeability of collecting tubes is very low and very little water is reabsorbed causing a larger volume of water that remains in the tubule to be excreted in the urine. The increased urine excretion resulting from low vasopressin is termed water diuresis. A decreased extracellular volume triggers increased vasopressin secretion and this increases the water permeability on the collecting ducts, more water is reabsorbed and less is excreted. Vasopressin secretion by the posterior pituitary is controlled by cardiovascular baroreceptors with a low extracellular volume stimulating vasopressin secretion and a high extracellular volume, inhibiting it. Vasopressin is also influenced by osmoreceptors in the hypothalamus with a high body fluid osmolality stimulating vasopressin secretion and a low osmolality inhibiting it (Stockand 2010, Thornton 2010, Perrier, Rondeau et al. 2013). Aldosterone, produced by the adrenal cortex, stimulates sodium reabsorption by the cortical collecting

ducts. Angiotensin II is a component of the renin-angiotensin aldosterone system. Angiotensin II exerts many effects but the most important are its stimulation of the secretion of aldosterone and its constriction of arterioles. Plasma angiotensin II is high during salt depletion and low when the individual is sodium replete, and this change in angiotensin II brings about the changes in aldosterone secretion.

During exercise there is a stimulus for the release of vasopressin caused by an increase in osmolality and a decrease in plasma volume. A study by Castenfors (1977) demonstrated that renal blood flow decreases during severe exercise to allow a maximal redistribution of cardiac output to the exercising muscles. Despite this reduced renal blood flow the basal urinary water excretion is relatively little influenced during prolonged exercise, however urinary sodium excretion decreases due to increased aldosterone secretion.

### *1.1.1.3 Gastric emptying and intestinal absorption*

The rate of fluid delivery to the circulation is influenced by the combined processes of gastric emptying and intestinal water transport. Hunt and Pathak (1960) performed early experiments to examine the osmotic effects of some molecules and ions on gastric emptying. In their studies they administered a known volume of different test meals to their participants. The influence on gastric emptying of several solutes in test meals was determined by plotting the volume of test meal remaining in the stomach after a fixed interval against the osmolar concentrations of the solute in the original meal. They observed that: 1) at low concentrations the addition of sodium chloride to test meals increased the rate of gastric emptying; and 2) increasing the concentration of glucose in test meals containing sodium chloride progressively reduced the action of sodium chloride on gastric emptying (i.e. higher glucose delayed gastric emptying). The authors believed the findings could be explained by the presence of an osmoreceptor mechanism in the intestine which feeds back to inhibit gastric emptying. Later, Fordtran and Saltin (1967) studied the effect of exercise on gastric emptying and intestinal absorption. They observed that intestinal absorption is not significantly affected by exercise and that gastric emptying rate is only slightly decreased (at moderate intensity). They also observed that at least 50g of glucose can be emptied from the stomach during 1 h of heavy exercise; an amount that might be helpful to replenish the carbohydrate used during exercise. Other gastric emptying studies observed that the rate of emptying was dependent on the type of solute ingested and on its

concentration. Isotonic saline solutions typically empty faster than water; however, the addition of small amounts of glucose (5%) to water causes a marked inhibition of gastric emptying rate (Hunt and Pathak 1960, Hunt 1961). Given that gastric emptying rate is only slightly inhibited by heavy exercise, it seems likely that gastric emptying and intestinal absorption of saline solutions would be rapid enough to replace all the losses by sweat caused during heavy exercise, even in hot environments.

Costill and Saltin (1974) examined the effects of solute volume, temperature, osmolality and glucose concentration on gastric emptying rates during exercise and at rest. They observed that at rest the addition of small amounts of glucose induced a diminution in the gastric emptying rate. Other findings were that the volume of fluid remaining in the stomach 15 min after ingestion was somewhat greater when the solution was delivered at 35°C compared with colder fluids. They also demonstrated that the maximal rate of gastric emptying could be achieved with a bolus volume of 600 ml. Costill and Saltin (1974) also concluded that exercise only affected gastric emptying rate at intensities above 70%  $VO_{2max}$ , and that the composition of glucose and water solutions administered during prolonged exercise should be determined on the basis of individual requirements.

Before moving on it is important to briefly review two methodological factors that must be considered when evaluating the outcomes of studies. These factors include the way in which hydration status is measured in studies and identifying why and when a focus on hydration monitoring might be important.

### 1.1.2 Assessment of hydration status

Body water is contained within the numerous organs and tissues and circulation and is regulated through the complex interaction of many physiological and psychological factors. Total body water (TBW) can be most simply subdivided into extra cellular fluid (ECF) and intra cellular fluid (ICF) compartments. ECF is composed of three major compartments: plasma, interstitial and connective tissue water. The largest component is ICF which accounts for around 23 litres (55% of TBW) or 33% of total body mass in an average male (**Table 1-1**).

**Table 1-1 Body fluid compartments in an adult 70 kg male**

Extracellular fluid (ECF) and intracellular fluid (ICF) compartments shown. ISF = Interstitial fluid, CT = Connective tissue.

	% of Body Mass	% of Total Body Water	Approx. Volume (Litres)
ECF	27	45	19
Plasma	4.5	7.5	3.2
ISF	12.0	20.0	8.4
Dense CT water	4.5	7.5	3.2
Bone water	4.5	7.5	3.2
Transcellular	1.5	2.5	1.0
ICF	33	55	23
TBW	60%	100%	42 litres

Due to the importance of water balance it is essential to consider different ways in which body water and thus, hydration status, can be assessed. There are many hydration assessment techniques; however it is not possible to establish a single gold standard method. The techniques available include dilution techniques, neutron activation, changes in body mass, haematological and urine indices, tear or saliva analysis, bioelectrical impedance analysis, and dual energy x-ray absorptiometry (**Table 1-2**).

### *1.1.2.1 Dilution techniques*

These techniques are based on the dilution principle stating that a dose of tracer introduced in a volume dilutes uniformly within it. The measurement of the concentration of the tracer once equilibrium has been reached allows the calculation of the volume. The most commonly used tracers are  $^2\text{H}$ ,  $^3\text{H}$ ,  $^{18}\text{O}$ , deuterated water and  $^{18}\text{O}$  enriched water. Measurement of the concentrations requires either mass spectrometry ( $^{18}\text{O}$  and  $^2\text{H}$ ) or infrared spectrometry ( $^2\text{H}$ ). The main inconvenience for these dilution techniques is the cost of analysis and length of time until data are generated.

### *1.1.2.2 In vivo neutron activation analysis*

Total body neutron activation analysis originally was applied to the study of calcium and nitrogen metabolism. Subsequently, analyses of total body chloride, potassium, and sodium were used to calculate extra cellular volume (ECV), intra cellular volume (ICV), and total exchangeable extracellular sodium, respectively (Yasumura, Cohn et al. 1983, Xiong, Borovnicar et al. 1998) Total body water was calculated as the sum of extracellular fluid plus intracellular fluid. One whole-body scan thus provided a non-invasive measurement of fluid compartment sizes, with the assurance of small error and elemental equilibrium. However, this method is now rarely used for human body water analysis.

**Table 1-2: Summary of methods used for assessment of hydration status**

Methods have been evaluated by two independent researchers and ratings for each measure. The ratings are indicated as \* = small / low; \*\* = moderate; \*\*\* = great / high.

Method	Cost	Speed of measurement	Ease of use	Validity	Reliability	Expertise required	Portability
Dilution techniques	***	***	***	***	***	***	*
In vivo neutron activation analysis (Xiong, Borovnicar et al. 1998)	***	***	***	***	***	***	*
Blood and plasma volume	**	**	**	***	***	**	*
Serum/plasma osmolality	**	**	**	***	***	**	**
Sodium concentration	**	**	**	**	**	**	*
Hormone determination	***	***	***	***	***	***	*
Urine osmolality	**	**	**	***	***	**	**
Urine specific gravity	*	*	***	**	**	**	***
Urine colour	*	*	*	**	*	*	***
Tear osmolality	***	**	**	*	**	**	**
Salivary parameters	**	**	**	*	*	**	*
Bioelectrical impedance	**	**	**	*	**	**	***
Dual energy X Ray Absorptiometry	***	**	**	**	**	**	*



### *1.1.2.3 Body mass changes*

Tracking changes in body mass is a method widely used to determine acute changes in hydration status and particularly when assessing dehydration caused by heat stress and/or exercise over a short period of time. For this method, the main losses of water are from sweating or from urination. With this simple method it is assumed that the specific gravity of urine and of sweat is around 1.0 so it can be concluded that 1 gram change in body mass corresponds to 1 millilitre of urine and sweat output. However, corrections can be applied for respiratory water loss, loss due to substrate exchange, and release of metabolic water in exercise (Maughan, Shirreffs et al. 2007, King, Cooke et al. 2008) to improve upon the estimates of water loss. The major disadvantage of this method is that it can be affected by gastrointestinal function and food and fluid intake. It is, therefore, recommended to use this method in combination with other markers.

### *1.1.2.4 Haematological indices.*

#### *Blood volume and plasma volume*

Blood parameters are widely used to evaluate hydration status. Changes in haemoglobin concentration and in haematocrit can be used as indicators of changes in blood volume and plasma volume to assess change in hydration status. However, these changes represent changes in intravascular volume alone and not in TBW. Haemoglobin and haematocrit can be valid as intravascular hydration change markers as long as the baseline blood sample is obtained in a stable rested/hydrated condition, and blood is drawn without stasis with participants maintaining the same posture for all sample collections.

#### *Serum/plasma osmolality, sodium concentration and hormones*

Serum osmolality and plasma sodium markers are known as hydration status markers and they can be measured and analysed quickly and easily. When the subject is dehydrated, both parameters have been shown to be significantly increased (Mack, Weseman et al. 1994). It has been shown that serum/plasma osmolality can be elevated by a dehydration of 1% body mass loss (Popowski, Oppliger et al. 2001). However in some studies, where participants have lost more than 3% of their body mass mainly through sweat losses, there was no change in serum osmolality (Francesconi, Hubbard et al. 1987, Armstrong, Maresh et al. 1994, Armstrong and Maresh 1998). Serum/plasma sodium and serum/plasma osmolality (S<sub>osm</sub> and P<sub>osm</sub>, respectively) are the most potent triggers for stimulating plasma arginine vasopressin. The measurement of hormone responses such as arginine vasopressin, renin,

aldosterone and atrial natriuretic hormone response is useful for determining the impact of fluids upon hydration status when combined with other measures (Schrier, Berl et al. 1979, Stachenfeld, Gleim et al. 1996).

### *Other blood markers*

Some studies have used serum uric acid and it has been considered as a criterion for the diagnosis of dehydration (Lind and Ljunghall 1992, Yamamoto, Moriwaki et al. 2004, Yoshihara, Hirotoomi et al. 2007).

#### *1.1.2.5 Urinary indices*

Urine osmolality (Uosm) and urine specific gravity are widely used as they are very easy to measure. Urine colour and the urine colour scale (Armstrong, Maresh et al. 1994) also appear to be good indicators of hydration, and in most circumstances they reflect hydration status. However, urine markers can be influenced by diet composition, dietary supplements, drugs and illness (Baron, Courbebaisse et al. 2015, Cheuvront, Kenefick et al. 2015). Urine specific gravity is highly associated ( $r^2 = 0.96$ , (Armstrong and Maresh 1998)) with urine osmolality while being a more affordable and easy to use technique. Urine colour is also a suitable tool when urine specific gravity is not available but should probably not be used in isolation (Armstrong, Pumerantz et al. 2010). Overall, urinary markers seem to be reliable hydration status assessment methods, but can be altered independently of the level of hydration status by factors such as severe dehydration, alcohol intake (Shirreffs and Maughan 1997), muscle mass (Hamouti, Del Coso et al. 2010), rapid rehydration (Figaro and Mack 1997), caffeine consumption (Grandjean, Reimers et al. 2000) and illness (Fletcher, Slaymaker et al. 1999). In addition, Uosm:Posm ratio has also been used as a hydration status biomarker (Armstrong, Johnson et al. 2013).

#### *1.1.2.6 Tear osmolality*

Tear fluid is isotonic with plasma, and plasma osmolality is widely accepted as a marker of intravascular fluid balance. Fortes et al (2011) used a portable device (TearLab®) that may allow assessment of hydration status. They examined the relation between plasma osmolality and urine specific gravity with tear osmolality while they dehydrated their participants. The volume required to use this device was 50 nL. They concluded that tear osmolality increased with dehydration and tracked alterations in plasma osmolality with comparable utility to urine specific gravity. Other studies have shown there are more ocular measurements for non-invasive hydration assessment such as tear break up time and intraocular pressure,

however they concluded that further investigations are needed to determine the degree of reliability of these as hydration status markers (Sollanek, Kenefick et al. 2012, Munoz, Johnson et al. 2013).

#### *1.1.2.7 Salivary parameters*

Walsh et al (Walsh, Laing et al. 2004) studied saliva flow rate, total protein concentration and osmolality to identify if they were sensitive non-invasive markers of whole body hydration status. They observed that some changes in saliva total protein concentration and osmolality, and to a lesser extent flow rate, were related with changes in body mass during progressive acute dehydration. However, it has been demonstrated that saliva indices have large inter-individual variability (Oliver, Laing et al. 2008). Another study analysed an elderly population and the researchers correlated the salivary spinability (elasticity) with serum markers of chronic dehydration, including serum uric acid (Yoshihara, Hirotoomi et al. 2007).

#### *1.1.2.8 Bioelectrical impedance*

Bioelectrical impedance has been used as a technique to assess hydration status (Piccoli 2010). These authors conclude that it is a non-invasive, rapid and accurate method to assess total body water in healthy subjects. However, there are multiple factors that might affect the results such as skin temperature, food or fluid intake, and body posture before the measurement (Shirreffs and Maughan 1994). All of these factors must be controlled to ensure that reliable estimates of changes in body water can be obtained.

#### *1.1.2.9 Dual X Ray absorptiometry (DXA)*

The DXA method is mainly used to measure bone mineral content, fat mass and fat free soft tissue mass. The device uses two X-Ray sources producing photo beams with two discrete energies. DXA is becoming a very popular methodology for body composition but few have considered the impact of hydration status on body composition estimates. Despite it being a quick and easy measure, it is not widely accessible due to the cost of buying and maintaining the equipment.

#### *1.1.2.10 Other technology*

Embedded piezoresistive microcantilever sensors fabricated using proprietary hydrogel formulations produce sensor elements capable of tracking osmolality changes in both saliva mimic solutions and in actual human saliva during preliminary dehydration testing. These sensors may be used in small, inexpensive and portable hydration monitoring instruments

(Gunter, Delinger et al. 2005, Stewart, Reed et al. 2007). Other recent investigations suggest the use of smart phone technologies to track sweat pH (and thus estimate sweat Na content) using a colorimetric method (Oncescu, O'Dell et al. 2013). The information available is limited to date but this type of technology may be useful in the future.

In summary, there are a variety of methods available with many being simple easy to measure parameters. It seems that a combination of measures is most likely optimal for assessing hydration status and change in hydration over time. Indeed, recent reviews of these methods by Armstrong (Armstrong 2007, Armstrong, Johnson et al. 2013) and Chevront (Chevront, Kenefick et al. 2013) highlight that no single method is valid for all situations. As such, within this literature review we will only consider studies that have used more than one hydration assessment method for tracking the outcomes.

### 1.1.3 Importance of hydration

At the most basic level maintaining body water is essential for human survival. The challenge to maintaining body water is to balance fluid intake with fluid losses. An individual's fluid intake is driven by thirst and food intake and is thus episodic, yet our fluid losses through evaporative losses, urine output, faecal water losses and breathing (insensible losses) are mainly continuous. Due to the intermittent nature of fluid ingestion our hydration status fluctuates throughout the day and in certain groups such as older adults and children, or sports participants a chronic mild dehydration may be evident. Daily fluid requirements from ingested drinks in the UK and Europe are suggested to be 1.6 L per day for women and 2.0L per day for men (Agostoni, Bresson et al. 2010). Other fluid requirements will be delivered through food with around 400ml/day from food expected in women, and 500ml/day from food expected in men. However, in the USA fluid intake recommendations (from ingested drinks) are higher at 3.0 L per day for men and 2.2 L per day for women (IOM 2005). The Nutrition Reference Values for Australia and New Zealand have established an adequate daily fluid intake of 2.6 L for male adults and of 2.1 L for female adults coming from plain water, milk and other drinks (NHMRC 2006). The World Health Organization recommends 2.9 L per day for male adults and 2.2 L per day for female adults as part of their guidelines for water intake (WHO 2005). Most of these recommendations are based on the results of nutritional surveys and they do not specify any special recommendation for the older adults. The Recommended Dietary Allowances that are considered as the dietary standards for the

United States population is based in the Food and Nutrition Board (FNB). The FNB water recommendation is 1 ml water/kcal of energy expenditure since 1945 (FNB 1945). However the FNB increased the amount of water required to 1.5 ml water/kcal of energy expenditure to cover variations in activity level, sweating and solute load (**Table 1-3**). These discrepancies between recommendations and guidelines might confuse the general population on the actual amount of daily required fluids.

**Table 1-3 Recommended water intake by different institutions and worldwide organisations.**

	EFSA (2010)	IOM (2005)	WHO (2005)	NHMRC (Australia and New Zealand) (2006)
Males	2.5 l/day	3.7 l/day	2.9 l/day	3.4 l/day
Females	2.0 l/day	2.7 l/day	2.2 l/day	2.8 l/day
<i>These values include the water included in the food. The Organisations recognised that ~80% of this recommended water intake should come from beverages.</i>				

The volume and composition of fluid intake throughout the day is likely to be important in maintaining fluid balance. It is known that factors such as energy content and osmolality of drinks will influence the rate of gastric emptying (Vist and Maughan 1995) and that glucose and electrolyte composition and osmolality will impact upon intestinal water transport (Schedl, Maughan et al. 1994, Gisolfi, Summers et al. 1995, Shi, Summers et al. 1995). Furthermore, the electrolyte content of drinks is likely to also impact upon the retention of fluid within the extracellular or intracellular compartments and thus could influence the required frequency of further fluid ingestion. Other components within drinks ingested, such as caffeine and alcohol, can also impact upon fluid losses if taken in sufficient quantities. Therefore, the volume and composition of the range of beverages typically ingested during a day will have a significant bearing upon drinking behaviour and maintenance of hydration status.

Maintaining an adequate hydration status may be important for prevention of chronic diseases (Manz and Wentz 2005), with some evidence that poor hydration is linked to an

increased risk of several conditions such as urolithiasis, urinary tract infections, constipation, hypertension and dental caries. However, it should be acknowledged that it is difficult to directly link dehydration to health (Armstrong 2012). While strong evidence of a causal effect of dehydration on these conditions is lacking there is stronger evidence that mild hypohydration in the region of 2% body mass loss will adversely affect physical and mental performance (Sawka and Noakes 2007, Cheuvront, Kenefick et al. 2010, Masento, Golightly et al. 2014). At rest, mild-moderate dehydration (1-2% of body mass loss) has been shown to impair cognitive function including memory performance and psychomotor processing speed (Gopinathan, Pichan et al. 1988, Cian, Barraud et al. 2001, Patel, Mihalik et al. 2007, Suhr, Patterson et al. 2010) whilst increasing the perceived effort of a given task (Szinnai, Schachinger et al. 2005). Dehydration also increases self-reported mood disturbance by increasing fatigue, confusion, anger and depression whilst reducing alertness (Cian, Barraud et al. 2001, Szinnai, Schachinger et al. 2005, Ely, Sollanek et al. 2013).

Dehydration is most common in an athletic setting. Body water losses increase as respiration and sweat rates are elevated, particularly when the opportunity to replace fluids is limited. Dehydration has been shown to impair endurance, resistance (Kraft, Green et al. 2012) and maximal aerobic power performance (Sawka and Noakes 2007), although tasks with a larger aerobic component are influenced to a greater extent (Maughan 2003). During exercise, the goal is to prevent fluid deficits >2% body mass loss, which appears the critical water deficit that is able to impair performance in many individuals. Similar to at rest, both mild and severe dehydration increase cardiovascular strain and core temperature during exercise (Armstrong, Maresh et al. 1997, Kenefick, Cheuvront et al. 2010, Lopez, Casa et al. 2011). A plasma volume decrease will result in increased heart rate and decreased stroke volume. With severe dehydration, decreased cardiac output can also occur and eventually lead to circulatory collapse (Sawka, Young et al. 1985, Hamilton, Gonzalez-Alonso et al. 1991, Montain and Coyle 1992, McConell, Burge et al. 1997, Sawka, Montain et al. 2001). In addition to reduced peripheral blood flow as a means for maintaining central venous pressure, skeletal muscle blood flow may also decrease during exercise in a dehydrated state (González-Alonso, Calbet et al. 1998, González-Alonso, Calbet et al. 1999). Lower perfusion pressure and systemic blood flow, rather than vasoconstriction, were deemed responsible for reduced muscle blood flow. It should be noted that greater fluid losses (3-4% body mass) under heat-stress conditions (30-35 °C) will exacerbate the physiological strain. Mild dehydration has also been shown to hinder cognitive performance during exercise, a vital

factor in many sports where concentration, reaction time, decision making and execution of skilled tasks are important (Ganio, Armstrong et al. 2011, Lopez, Casa et al. 2011, Armstrong, Ganio et al. 2012). In addition, dehydration during exercise has been demonstrated to impair postural balance (Derave, Clercq et al. 1998), rates of gastric emptying (Neufer, Young et al. 1989, Rehrer, Beckers et al. 1990, Van Nieuwenhoven, Vriens et al. 2000) and alter metabolic function (increase glycogen utilisation (Hargreaves, Dillo et al. 1996, González-Alonso, Calbet et al. 1999, Logan-Sprenger, Heigenhauser et al. 2012)). Impaired postural balance (Derave, Clercq et al. 1998) and altered rates of gastric emptying have only been shown to occur during dehydration in exercise rather than at rest (Rehrer, Beckers et al. 1989), further emphasising the need for separate reporting of hydration evaluation under resting and exercising conditions in this review.

Furthermore, a recent meta-analysis of 17 studies ((Watson 2015), personal communication) revealed that hypohydration results in an increased rating of perceived exertion (RPE) during exercise. This effect was most evident with >2% hypohydration induced prior to or during exercise, in hot conditions, and particularly later in exercise (beyond 60 minutes). While the authors acknowledge that the data gathered from laboratory trials may not directly reflect outdoor air flow conditions, they do state that the data are valid for a large number of people who exercise in gyms using treadmills and cycle ergometers with relatively little air flow. The relationship between hypohydration and RPE therefore suggests that fluid ingestion to maintain euhydration (within 1% body mass loss) is likely to reduce perception of effort during exercise, and/or could enable a higher work rate to be sustained for the same RPE.

Evidence suggests that thirst sensation is not stimulated until fluid losses reach 1-3% of BM in man (Fitzsimons 1972), yet exercise capacity / performance can be hindered from fluid deficits of just 1-2% of body mass in some cases. To reduce the chance of dehydration during exercise, ACSM guidelines state that 5-7 ml/kg/body mass of fluid should be consumed slowly during the 4h period prior to exercise enabling athletes to begin exercise in a euhydrated state (American College of Sports, Sawka et al. 2007). Considerable variability in individual sweat rate and concentration make it difficult to produce definitive guidelines for fluid ingestion during exercise; athletes are therefore advised to adopt individual fluid replacement programmes to prevent fluid losses >2% of body mass. Following exercise, it is recommended to replace 150% of fluid losses by consumption of a carbohydrate-electrolyte drink. Fluid, in excess of the volume lost during exercise, will compensate for elevated post-exercise metabolic/respiratory rates and ongoing sweat losses (Shirreffs, Taylor et al. 1996).

It seems apparent that failure to replace sodium losses will delay the return to a euhydrated state (Maughan and Leiper 1995), and therefore rehydration beverages are recommended to contain sodium and small amounts of potassium to replace additional electrolyte losses, stimulate thirst, increase fluid retention and reduce excessive urine production. However, the optimal combination of sodium, potassium and energy source for effective rehydration has not yet been fully elucidated.

Factors including age, gender and environmental conditions can exacerbate the detrimental effects of dehydration. Age-related impairments in renal function, thirst sensation and increased laxative or diuretic use put older adults (70 years+) at greater risk of involuntary dehydration, thus further exacerbating age-related declines in postural balance and cognitive function (Suhr, Hall et al. 2004). Typically, men have higher sweat rates and electrolyte losses compared to women (Avellini, Kamon et al. 1979, Shapiro, Pandolf et al. 1980). Additionally, women have higher circulating oestrogen concentration which stimulates greater arginine vasopressin (AVP) release and increases renal water and electrolyte retention in comparison to men (Stachenfeld, Splenser et al.). Although only a subtle difference, the influence of gender on fluid losses and retention puts males at greater risk of dehydration. During heat exposure, in particular when combined with exercise, heat production increases and the need to dissipate heat to prevent rising core temperature is of greater importance. In hot environments, the physical transfer of heat is not possible, so evaporation of sweat becomes the body's main pathway to dissipate excess heat. The severity of the implications caused by dehydration in temperate environments (18-30°C) is increased during heat exposure at rest and during exercise; cardiovascular strain (Kenefick, Chevront et al. 2010), impaired gastric emptying (Neufer, Young et al. 1989) and metabolic alterations (González-Alonso, Calbet et al. 1999) are all exacerbated. Furthermore, the risk of heat strain (Sawka, Young et al. 1992) and heat stroke (Carter III, Chevront et al. 2005) is increased. Awareness of hydration status is also important in exposure to cold-air environments during which thirst sensation can be blunted (O'Brien, Freund et al. 1996, O'Brien, Freund et al. 2005, Lewis, Fraser et al. 2013). Thus, multiple factors must be considered when evaluating what constitutes an effective hydration drink in humans.



### 1.3.1 Energy/macronutrient content of drinks and hydration

#### 1.3.1.1 Daily living

Logan-Sprenger et al (2013) are the only authors to have published a study examining the influence of macronutrient content of drinks on fluid delivery / retention at rest without prior exercise. In four experimental trials they examined urine specific gravity (USG) and urine volume responses to ingestion of 600ml of fluid (water, salt water, 3% carbohydrate electrolyte beverage, or 6% carbohydrate electrolyte beverage) taken over half an hour in participants who had abstained from fluid overnight. There was no difference in USG or urine volume between trials but in all cases USG declined and urine volume increased over the 1 hour study period. Their study indicates that over a short follow-up period of only 1 h fluid can be effectively delivered and stimulate a diuresis regardless of the macronutrient content of the fluid (3% vs 6% carbohydrate). No differences were noted for proportion of fluid volume retained between trials, however, longer term follow-up of urine output (>1h) could have provided further insight into fluid retention. Additionally, the electrolyte content of the drinks was slightly different (40mM Na<sup>+</sup> for salt water vs 20mM Na<sup>+</sup> for both carbohydrate-electrolyte beverages). The impact of an electrolyte difference in this range is unlikely to significantly influence fluid retention (see electrolyte/mineral section below).

Further work is required to explore energy/macronutrient composition effects on hydration and fluid balance during normal daily living.

#### 1.3.1.2 During exercise

During exercise the impact of energy/macronutrient content of drinks has largely focused on performance outcomes with relatively few studies focused on hydration/fluid balance. Of those monitoring fluid balance responses to drink ingestion the focus has almost exclusively been on carbohydrate content of drinks ingested during endurance exercise (**Table 1-5**). Strategies for effective hydration during exercise largely aim to provide fuel for muscle contraction, and/or fluid to restore blood/plasma volume. Restoration of the fluid losses incurred through sweating in the heat are a particular focus, and effective hydration aims to ensure that cardiovascular and thermoregulatory function are maintained to prolong exercise capacity or improve exercise performance.

Koulmann et al (1997) examined fluid delivery to the circulation during exercise in a hypohydrated state. Their study used deuterium tracers to track the appearance of the tracer into the plasma during exercise. The appearance of deuterium should reflect the rate of gastric emptying and intestinal absorption of fluid and particularly of water in drinks. Interestingly, their study observed no difference in the rate of deuterium accumulation between drinks containing no carbohydrate (mineral water) and drinks composed of a 6% glucose-electrolyte solution, a 6% maltodextrin solution, or a 6% maltodextrin-electrolyte solution. However, plasma volume restoration was greater with the glucose-electrolyte drink suggesting better extracellular fluid replacement. The lack of difference in deuterium accumulation could be explained by unaccounted for differences in rates of tracer efflux from the circulation.

**Table 1-4: Studies examining energy/macronutrient content of drinks on hydration during exercise**

BM - body mass; CV - Cardiovascular; FI -fluid intake; GI - Gastrointestinal; HR - Heart rate; Posm - plasma osmolality; PV - plasma volume; Sosm - Serum osmolality; Uosm - Urine osmolality; UV - Urine volume; VO<sub>2</sub>max - maximal oxygen uptake.

Authors	Year	Age	Gender	Sample size	Study design	Hydration assessment	Duration of follow-up	Outcome
Koulmann et al	1997	27	Male	6	5 trials examining rate of water absorption from drinks following a period of passive heating to induce 2% BM loss. Trials involved 60 min of treadmill exercise in the heat (35°C) with fluid (no fluid, mineral water, 6% glucose-electrolyte drink, 6% maltodextrin solution or 6% maltodextrin-electrolyte solution) equating to 50% of prior fluid loss ingested before exercise.	Deuterium accumulation in plasma, PV, Posm, sweat loss, UV.	Throughout exercise.	No differences were observed between any drinks for deuterium accumulation in plasma throughout exercise. However, PV restoration was greater with the glucose-electrolyte beverage compared to all other drinks.
Galloway et al	1998	26	Male	6	3 trials (cross over design): Trials were no fluid, 15% carbohydrate-electrolyte or 2% carbohydrate electrolyte solutions. Subjects cycled to exhaustion at 70% of VO <sub>2</sub> max in the cold (10°C), ingesting 7.14 ml.kg of fluid immediately prior to exercise and then consumed 3.57ml/kg (of water or 2% CHO-E) or 1.79ml/kg (15% CHO-E) of fluid every 10 min during exercise.	BM, PV, Uosm	Throughout exercise.	The 2% carbohydrate drink was most effective for fluid replacement during exercise in the cold, although there were no differences in thermoregulatory or cardioresp response between trials. Exercise capacity was not different between trials.
Galloway et al	2000	27	Male	6	3 trials (cross over design): subjects cycled to exhaustion at 60% of VO <sub>2</sub> max in the heat (30°C), ingesting 7.14 ml.kg of fluid immediately prior to exercise and then consumed 3.57ml/kg of fluid every 10 min during exercise. Trials were no fluid, 15% carbohydrate-electrolyte or 2% carbohydrate electrolyte solutions.	BM, PV, Uosm.	Throughout exercise.	The 2% carbohydrate drink was most effective for fluid replacement during exercise in the heat, although there were no differences in thermoregulatory or cardioresp response between trials. Exercise capacity was also greater in the 2% carbohydrate trial compared to other trials however the mechanisms for improve capacity are unclear.
Lee et al	2008	24	Male	8	4 trials exercising at 70% peak oxygen consumption with ingestion of 1.5ml/kg of water, CHO-E solution, low fat milk, or low fat milk plus glucose. Fluid ingested before and every 10 minutes during exercise.	Sosm, Uosm, serum electrolytes, PV, BM.	Throughout exercise.	No differences between trials in any cardiovascular responses (PV or HR). Suggesting that low fat milk or low fat milk plus glucose were equally effective as CHO-E drink ingestion or water during exercise. However, GI discomfort rating were higher for milk and milk plus CHO than the other two trials.
Peacock et al	2012	26	Male	12	3 trials (cross over design); three 20 min intervals of CV exercise at 75% of max HR, 20 min resistance exercise and 20 min recovery where access to fluid was <i>ad libitum</i> . The three trials were water (W), carbohydrate-electrolyte (CES) or no fluid (NF).	BM (urine output, weight of drinks bottle, sweat loss), Uosm	At end of recovery period.	Voluntary intake of fluid is greater with CES than W. CES resulted in more adequate hydration and better maintenance of affective states (pleasure ratings) than W or NF in gym based exercise.
Peacock et al	2013	25	Male	10	2 trials (cross over): subjects dehydrated by 1.2% of body mass through fluid restriction. Subjects then completed 2x 2min CV exercise, 20 min resistance exercise and 20 min cycling at self selected speed with <i>ad libitum</i> fluid intake; water or carbohydrate-electrolyte.	Uosm, FI and BM.	30 min after the final cycling exercise.	CES increased <i>ad libitum</i> fluid intake compared to water and resulted in more adequate hydration status. CES was also associated with increase in self selected exercise intensity.
Kingsley et al	2014	24	Male	14	3 trials (cross over design): subjects completed a simulated soccer match (90min). A 60g gel was ingested 20 min pre exercise and 10 min into the second half of exercise. 5.25ml/kg/BM were ingested at 15, 30, 60 and 75 min into the match; 9.6% carbohydrate-caffeine- electrolyte solution with carbohydrate-electrolyte gels (HCHO), 5.6% carbohydrate-electrolyte solution with electrolyte gels (CHO) and electrolyte solutions with electrolyte gels (PL)	PV, Posm, BM.	At the end of exercise.	Although sprint performance was improved with HCHO solution (142g/h), hydration status was negatively influenced compared to the CHO (54g/h) and PL solutions (0g/h).

Galloway et al (Galloway and Maughan 1998, Galloway and Maughan 2000) examined plasma volume restoration during exercise in hot and cold environments. The aim of these studies was to investigate the potential value of fluid delivery (using a large volume of a 2% carbohydrate-electrolyte (CHO-E) solution) vs substrate delivery (using a moderate volume of a 15% CHO-E solution) on cardiovascular function, thermoregulation and subsequent exercise capacity. Under hot conditions fluid replacement was hypothesized to be key focus (to offset sweat losses) while in cold conditions substrate provision could be considered the main focus. Interestingly, in both of these studies the 2% beverage produced the greatest restoration of plasma volume during the endurance exercise task, and in the heat this also equated to an improved endurance exercise capacity. These studies primarily emphasize the fact that ingestion of high carbohydrate hypertonic solutions will delay gastric emptying and intestinal absorption of fluid, while ingestion of dilute hypotonic solutions will lead to earlier and greater fluid transport into the circulation (regardless of the environmental conditions). Thus, for fast fluid replacement during exercise the use of dilute hypotonic CHO-E solutions would be recommended. Even with the high fluid ingestion rate used in these studies there was no evidence of hyponatraemia due to the high sodium content of all the ingested drinks (65mM Na<sup>+</sup>).

Lee et al(2008) examined cardiovascular markers of fluid delivery (plasma volume, plasma osmolality, heart rate response) with the ingestion of a carbohydrate-electrolyte solution, or two different carbohydrate-protein-electrolyte solutions (low fat milk, or low fat milk plus glucose) compared to water during endurance exercise. They observed that there were no differences in any cardiovascular responses to exercise between trials. They suggested that this meant no adverse effects of milk ingestion. They also highlighted that the responses to the ingestion of combined macronutrient drinks such as milk were no different from carbohydrate-electrolyte solutions during exercise. However, some additional gastrointestinal distress symptoms were reported in the milk and milk plus glucose trials.

Peacock et al (Peacock, Thompson et al. 2012, Peacock, Thompson et al. 2013) conducted two studies investigating fluid balance and voluntary fluid intake during a typical gym exercise session. Their participants were provided with fluid ad libitum (water or a CHO-E drink). In both studies voluntary fluid intake was greater with the CHO-E beverage, leading to a less negative net fluid balance at the end of each session. This larger voluntary intake was also associated with greater feelings of pleasure and higher work intensities sustained during the different work bouts. These observations not only corroborate earlier findings of greater

fluid intake with flavoured sweetened beverages, but also introduce the concept of greater self-selected work intensity and greater enjoyment of a gym session when carbohydrate is ingested. However, the positive observations must be countered by the potential negative effect of additional energy intake in gym sessions when ingesting a CHO-E drink. The additional carbohydrate would also alter the metabolic response to exercise away from fat oxidation towards a reliance on oxidation of the ingested substrate.

Kingsley et al (2014) using a mixture of gels and carbohydrate electrolyte solutions, to replace sweat losses, examined the impact of a high rate of carbohydrate feeding (Costill and Saltin 1974) vs. moderate rate of feeding (Munoz, Johnson et al. 2013) or placebo (0 g/h) on hydration during simulated soccer matches. Sprint ability during the matches was fastest on the high carbohydrate ingestion trial but was significantly different from placebo only. Based on plasma osmolality measures alone they reported that hydration status was negatively influenced by the highest rate of carbohydrate ingestion. There was a 2.3% increase in plasma osmolality on the high carbohydrate trial with no change in moderate carbohydrate and placebo trials. However, plasma volume changes between trials were not different. The authors suggest that these observations indicate that when the priority is hydration, then high rates of CHO ingestion are not effective compared to lower ingestion rates. It would seem that the authors have slightly over interpreted their data from a single parameter in this study. However, it should also be noted that caffeine was an additional potentially confounding variable as it was only ingested in the high carbohydrate trial.

In summary, from the limited studies that have actually examined changes in hydration markers during exercise with feeding of drinks containing different energy/macronutrient content or composition, it seems that effective hydration can be maintained when sufficient volume of moderate carbohydrate content (2-6% CHO solution) or a low fat protein-carbohydrate solution is ingested during exercise. Particularly in hot conditions, where sweat losses may be large, it would seem that a more dilute hypotonic drink would be most effective for fluid delivery / replacement. These observations are largely in keeping with the gastric emptying and intestinal absorption literature. However, due to the relative low volume of studies in this area the evidence base must only be considered as moderate.

### 1.3.1.3 Recovery from exercise

The largest number of studies focused around energy/macronutrient content of drinks and effective hydration has been on post-exercise fluid replacement. The range of studies in this section mainly cover the impact of carbohydrate content of solutions on rehydration, as well as the role of protein or carbohydrate-protein combinations (**Table 1-6**). The evidence base in this section is strong but is based on methodological procedures that do not necessarily reflect all real life situations.

The impact of carbohydrate content on rehydration has been considered in several studies with the dual rationale that slower emptying and absorption might delay a subsequent diuresis, but also would elevate blood glucose and insulin to help promote body water retention in tissues through enhanced glycogen synthesis. In some of the studies examining the impact of carbohydrate content on effective post-exercise rehydration, those of Evans et al (Evans, Shirreffs et al. 2009, Evans, Shirreffs et al. 2009) and Clayton et al (2013) have focused on carbohydrate content by administering drinks containing 0%, 2% and 10% glucose, while Osterberg et al (2010) examined 0%, 3%, 6% and 12% sucrose solutions. The focus of these studies has been on subsequent fluid delivery and retention. In all of these studies an exercise heat stress period was used to induce a hypohydration in the region of 2-3% of body mass loss prior to rehydration.

In the two studies by Evans et al they examined the rehydration response with either administration of fluid *ad libitum* over 2 hours (Evans, Shirreffs et al. 2009) or in a fixed volume of 150% of body mass loss over 1 hour (Evans, Shirreffs et al. 2009). In both of these studies all of the carbohydrate drinks contained an equal amount of sodium (25-30mM). Over the subsequent 5.5h recovery period no further fluid was ingested. In the *ad libitum* fluid intake study there was no difference between the volumes of fluid ingested in each trial, no difference in urine outputs, and no subsequent difference in the net fluid balance response during the recovery period. In the prescribed drinking study (Evans, Shirreffs et al. 2009) participants ingested the drinks over a shorter time period (1h vs. 2h) but in a similar volume to the freely chosen *ad libitum* intake study (2L compared to 2.3L). In the 2009b study urine output was less in the first hour with the 10% glucose trial compared to 2% and 0%. The lower early urine output led to a higher fraction of the drink being retained on the 10% drink trial (46% of drink retained) vs the 0% trial (27% retained). Whether the drinks were emptied and absorbed was not monitored in either study but it is likely that a significant portion of the fluid administered on the 10% trial was still in the stomach or

gastrointestinal tract 1-2 h into the recovery period (where the largest differences in urine output are likely to be observed). The authors concluded that with prescribed drinking there is a potentially beneficial effect of hypertonic high carbohydrate solutions on hydration and fluid balance. However, with an extended drinking period and *ad libitum* drinking this same effect was not observed. The extended drinking period led to no difference in urine output in the early part of the recovery period, and therefore no difference in net fluid balance at the end of the study period. These observations highlight the important role that time course of fluid intake can have in monitoring effective rehydration.

The potential impact of carbohydrate content was further highlighted by Osterberg et al (2010) who replaced 100% of sweat losses with 0%, 3%, 6% or 12% sucrose-electrolyte solutions (all 18mM Na<sup>+</sup>) delivered over a 90 minute drinking period. The only difference between trials was a significantly greater fluid retention (82% retained) on the 12% trial compared to the 0% trial (~72% retained). Retention on the other carbohydrate trials was similar (75% and 75%, for 3% and 6% sucrose solutions, respectively). The outcomes from this study considered alongside those of Evans et al (Evans, Shirreffs et al. 2009, Evans, Shirreffs et al. 2009) are interesting. They suggest that the expected delayed emptying and absorption of higher carbohydrate content solutions (up to 12%) is not having a large impact on fluid retention/urine output, when the volume ingested is less than or equal to prior body mass losses, or when the volume is consumed over a longer time period.

Clayton et al (2013) investigated this further and measured gastric emptying and net fluid balance to determine what proportion of fluid was likely still sitting in the gastrointestinal tract at 1 hour following ingestion of low (2%, hypotonic) and high (10%, hypertonic) carbohydrate solutions. They followed up the Evans et al (2009) model by investigating the effect of replacing 150% of mass loss over 1 hour post exercise, to determine a corrected fluid balance (by accounting for fluid remaining in the stomach, but not fluid in the intestine). Their data replicated the earlier finding from the same group (Evans, Shirreffs et al. 2009) by demonstrating a greater retention of fluid in the 10% trial. They also observed the same urine output response and difference in net fluid balance (a larger fluid deficit at 2, 3 and 4 hours in 2% trial compared to 10% trial). When adjusting for the volume of fluid remaining in the stomach 1 hour after the end of the fluid ingestion period the picture was reversed. The corrected net fluid balance demonstrated a significant deficit from pre-exercise on the 10% trial of around 900ml compared to no significant deficit from pre-exercise on the 2% trial. The volume of test drink remaining in the stomach 1 hour after cessation of ingestion was

7ml for the 2% trial and 893 ml in the 10% trial. This equates to <0.5% of the total volume for the 2% drink but amounts to 42% of the total volume for the 10% drink. The absence of intestinal perfusion does not allow for fluid flux in the intestine to be estimated but it is likely that considerable fluid flux into the intestine would occur on the 10% trial. These emptying and absorption characteristics of a 10% solution lead to delayed fluid delivery as evidenced by the plasma volume data. The 2% hypotonic drink effectively restored/expanded plasma volume throughout the recovery period, while plasma volume changes did not become positive until the end of the recovery period (3 hours post-ingestion) on the 10% trial. These data highlight the differences between effective fluid delivery and effective fluid retention. Collectively, all of these studies indicate that the carbohydrate content of drinks only exerts a small positive effect on net fluid balance when urine output alone is considered. However, if fast fluid delivery is the focus then a concentrated carbohydrate drink is unlikely to deliver this.



**Table 1-5: Studies included in the analysis of energy/macronutrient content of drinks on hydration post-exercise.**

Abbreviations are as for previous Tables.

Authors	Year	Age	Gender	Sample size	Study design	Hydration assessment	Duration of follow-up	Outcome
Seifert et al	2006	20 - 28	Male / female	8 male / 5 female	3 trials cross over design: subjects dehydrated by 2.5% through exercise then ingested fluid equal to body mass loss; CHO + PROT (6g CHO, 1.5g PROT, 153 mg Na, 18 mg K), CHO (6g CHO, 46mg Na, 12.5g K) or placebo (water).	Blood analysis (PV), Sosm, Uosm, BM.	3h post exercise	Fluid retention in the CP trial was 15% greater than the C trial and 40% greater than the W trial. Water ingestion led to dilution of serum.
Shirreffs et al	2007	24	Male / female	5 male / 6 female	4 trials (cross over design): all dehydrated to 1.8% of BM by exercise-heat stress. Subjects consumed 150% of BM loss of water (W), carbohydrate electrolyte (CE), milk (M) or milk + sodium (M+NA). Hydration status monitored for 5 h post beverage consumption.	Urine excretion, urine electrolyte excretion and Uosm, fluid balance and subjective feelings of drink taste.	5 h post exercise	Urine excretion was increased with CE and W drink vs milk drinks. Subjects were in positive fluid balance or euhydrated 1h into the recovery period with milk drinks vs W and CES. Milk is suggested effective recovery drink.
Watson et al	2008	23	Male	7	2 trials (cross over design): subjects dehydrated by 2% BM loss through exercise heat stress then consumed fluid in volume 150% of fluid losses 30 min post ex; carbohydrate electrolyte (137g CHO) or milk (113g CHO/75g PROTEIN). Drinks not matched for energy density.	BM, urine output, Uosm and Sosm.	3 h post ingestion (30 min post exercise)	Milk was a more effective rehydration drink, improving retention. There was no difference between exercise capacity in either trial, 4h post the initial exercise bout.
Okazaki et al	2009	21 & 68	Male	16 (8 young, 8 old)	2 trials (cross-over): control and protein-carbohydrate feeding immediately after exercise (72 min of intermittent exercise at 19°C) in young and old. ~200ml of fluid ingested containing ~12g PROT and 35g CHO or 0g PROT and 7g CHO. 5 h recovery at 28°C.	PV (Evans blue method), BM, Hct, Hb, Albumin, UV, Posm and plasma electrolytes.	5 h recovery period and 23h of recovery	Albumin content recovered following exercise but to a greater extent with CHO-PROT vs control, and to a greater extent in young vs. old. Protein and carbohydrate combination helps to improve the response in old.
Evans et al (a)	2009		Male / female	6 male / 3 female	3 trials cross over design: subjects dehydrated by 2% of BM through exercise heat stress. 30 min post exercise subjects consumed fluid <i>ad libitum</i> for 120 min; 31mmol/L Na and 0, 2 or 10% glucose concentration.	Blood analysis, BM.	6 h post ingestion	<i>Ad lib</i> FI was not different between trials and no differences in net fluid balance or fluid retention were observed. The authors suggest that <i>ad lib</i> intake of a hypertonic solution is as effective as a hypotonic solutions at restoring whole body fluid balance post-exercise.
Evans et al (b)	2009	26	Male	6	3 trials cross over design: subjects dehydrated by 1.9% of BM through exercise heat stress. 30 min post exercise subjects consumed fluid volumes 150% of BM losses; 25mmol/L Na and 0, 2 or 10% glucose concentration.	Blood analysis, BM.	6 h post ingestion	Hypertonic glucose solutions may be more effective at restoring and maintaining hydration status after sweat loss compared to more dilute solutions when sodium content is comparable.
Osterberg et al	2010	34 (19 - 46)	Male	15	5 trials (cross over design): dehydrated by 2-3% BM through exercise heat stress. Following a 30min equilibration period they consumed fluid equal to 100% of BM loss; flavoured placebo with no electrolytes (P), flavoured placebo with electrolytes (PE), 3, 6 and 12% carbohydrate electrolyte drinks. Drinks were consumed over a 40 min period.	Blood analysis, Uosm and BM.	4 h post exercise	Fluid retention was greater in the carbohydrate trials compared to the P. However fluid retention in PE was not significantly different from the 3 and 6 % CHO drinks but was less than 12% CHO drink. There was no difference in fluid retention between the three CHO drinks. Carbohydrate at the given content exerts a mild influence on fluid retention in post-exercise recovery.
James et al	2011	21	Male	8	2 trials (cross over): dehydrated by 1.9% BM by intermittent exercise in the heat. Rehydrated with 150% of fluid losses with 65g/l carbohydrate solution or 40g/l carbohydrate with 25g/l of milk protein (CP) 15 min post exercise, every 15min over an hour period.	BM, Uosm.	4h post exercise	When matched for energy density and fat content, a CP drink is more effective for rehydration than a Calone. CP augments fluid retention more than C.
James et al	2012	21	Male	12	2 trials (cross over): subjects were dehydrated by 1.86% through exercise heat stress and rehydrated 15 min post exercise over a 1 h rehydration period. Subjects consumed fluid equal to 1.5x BM losses; 65g/l carbohydrate electrolyte (C), 50g/l carbohydrate + 15g/l of whey protein (CP). Drinks were matched for energy and electrolyte content.	Uosm, BM, drink retention (drink volume - urine output)	4h post exercise	When matched for energy and electrolyte content, there was no difference in post exercise fluid balance and rehydration status between C and CP.
James et al	2013	22	Male	8	3 trials (cross over): subjects were dehydrated by 1.8% BM through exercise heat stress and rehydrated 15 min post exercise over a 1 h rehydration period. Subjects consumed fluid equal to 1.5x body mass losses; 60g/l carbohydrate electrolyte (C), 40g/l carbohydrate + 20g/l of milk protein solution (CP20) or 20g/l carbohydrate + 40g/l of milk protein solution (CP40). Drinks were matched for energy and electrolyte content (21mm Na, 5 mM K).	Uosm, BM, drink retention (drink volume - urine output)	4h post exercise	Carbohydrate-milk protein solution was better than carbohydrate alone at retention of fluid volume and resulting in a better maintenance of fluid balance over 4 h.
Clayton et al	2014	24	Male	8	2 trials (cross-over design): intermittent exercise in the heat (35°C) induced 1.8% BM loss. Rehydration with either 2% hypotonic drink or 10% hypertonic carbohydrate drinks (with 29mM Na and 0.4 mM K) in a volume equivalent to 150% BM loss. Drinks were ingested in 4 aliquots over 1 h.	Gastric emptying, UV, Uosm, PV, Sosm.	4h post-exercise	Net fluid balance was greater on 10% trial from 2 h to the end of the 4 h period. Urine output was greater on 2% trial but at 2h total fluid volume was emptied from the stomach on 2% whereas 42% still remained in the stomach on the 10% trial. When net fluid balance corrected for fluid in stomach the 2% trial achieved a greater net fluid balance.
Desbrow et al	2014	24.9	Male	15	4 trials(cross over design): dehydrated to 2% BM loss through exercise. 30 min equilibration period before ingesting fluid equal to 150% of body mass loss over a 1h period; cows milk (286KJ), soy milk (273 KJ), milk based liquid meal suppl (417 KJ) and sports drink (129 KJ).	USG, blood analysis, BM.	4 h post 1h ingestion period	Milk based drinks were more effective rehydration drinks than traditional sports drinks. Additional energy, protein and sodium in milk based meal replacements facilitated superior fluid recovery following exercise. (NB: PV and electrolyte content were unaffected by trial), only net BM and retention.
James et al	2014	22	Male / female	7 male / 3 female	2 trials (cross over): subjects were dehydrated by 2% BM through exercise heat stress and rehydrated 15 min post exercise over a 1 h rehydration period. Subjects consumed fluid equal to 1.5x BM losses; mineral water (W); or mineral water plus 20g/L whey protein isolate (W/P). Drinks were essentially electrolyte free.	Uosm, BM, drink retention (drink volume - urine output)	4h post exercise	Additional of whey protein isolate to mineral water does not influence fluid retention or net fluid balance over 4 hours. Net fluid balance was negative at 4 hours in both trials.
Tai et al	2014	27	Male / female	10 male / 10 female	3 trials (cross over): subjects were dehydrated by ~2.5% BM through exercise heat stress and rehydrated through fluid ingestion every 15 min post exercise over a 1 h rehydration period. Subjects consumed fluid equal to 1.5x BM losses; branched chain amino acid electrolyte drink (AE); carbohydrate electrolyte drink (Gatorade, GE), or flavoured water (Crystal light). Drinks were not matched for energy or electrolyte content.	UV, fluid retention, USG,	4h post-exercise	No differences were noted for UV or fluid retention. USG returned to baseline during the amino acid electrolyte trial but was significantly higher than both carb-electrolyte or flavoured water trials at 4h post-exercise. The authors suggest this could reflect greater cellular rehydration.
Hobson et al	2015	24	Male / female	13 male / 3 female	2 trials cross over design: dehydrated to 1.9% BM loss through exercise heat stress. Consumed fluid 150% of BM losses; Carbohydrate @ 60g/l CHO or a carbohydrate + whey protein isolate (CP, 60g/l CHO + 20g/l whey protein isolate). Both drinks contained 7 mmol/l of sodium and 4-5 mmol/l of potassium.	Blood analysis , Uosm and BM.	4 h post ingestion	Addition of WP to CHO drinks did not enhance or inhibit rehydration.

The focus of an effective hydration drink should most probably be on ways to enhance retention of a fluid that is rapidly delivered to the circulation, rather than focusing on fluids that are emptied and absorbed slowly. However, the influence of other macronutrients, particularly protein, on post-exercise rehydration has been of recent interest to researchers.

The potential impact of protein on oncotic pressure for regulation of extracellular fluid volume has been presented as a key rationale for many of these investigations. Of these studies the early observations of Seifert et al (2006) led the way. They observed that addition of a small amount of protein (1.5%) to a carbohydrate-electrolyte sport drink reduced urine output, and led to a 15% greater fluid retention than a standard carbohydrate-electrolyte drink. Drinks were consumed over 20 minutes at the beginning of a 3 hour rehydration period in a volume equal to body mass loss. Shirreffs et al (2007) and Watson et al (2008) examined this further by exploring the impact of low-fat milk on restoration of fluid balance in comparison to a carbohydrate-electrolyte solution. In both studies, participants lost approximately 2% of body mass through exercise-heat stress and ingested a volume equivalent to 150% of body mass loss over 60 minutes, beginning 20-30 minutes post-exercise. Similar to the observations of Seifert et al (2006) there was a significant reduction in urine output on the milk ingestion trials (amounting to a 40-50% reduction in total urine volume over 4 hours). In both studies a net positive fluid balance was achieved at the end of the rehydration period on the milk trials. However, a negative fluid balance was evident on carbohydrate-electrolyte drink or water ingestion trials. From these studies it cannot be determined whether the more effective fluid retention was due to delayed emptying and absorption of the milk/protein drinks, but this does seem to be the likely mechanism. Shirreffs et al (2007) provide some support for this notion as they observed no influence of additional sodium provision to a milk drink on fluid balance. Furthermore, the difference in content and combination of sodium and potassium in the milk drinks (40-60mM Na<sup>+</sup> and 40-50mM K<sup>+</sup>) compared to the carbohydrate-electrolyte drink (20-25mM Na<sup>+</sup> and 2mM K<sup>+</sup>) is unlikely to have contributed to such large alterations in fluid retention in these studies (see electrolyte/mineral section below).

In 2009, Okazaki et al (2009) provided a further interesting insight into the potential oncotic effects of protein addition to a carbohydrate-electrolyte solution for rehydration post-exercise. They observed that a small volume (~200ml) of a protein-carbohydrate combination taken post-exercise promoted an increase in plasma volume and plasma albumin content. These observations suggest that the additional protein led to increased albumin synthesis

post-exercise, and that an oncotic effect to maintain albumin concentration occurred to expand plasma volume. However, interpretation of the study data is complicated by the potential impact of meal and snack ingestion prior to and following exercise in their design.

James et al (James, Clayton et al. 2011, James, Gingell et al. 2012, James, Evans et al. 2013, James, Mattin et al. 2014) and Hobson et al (2015) conducted a series of studies investigating the impact of protein addition on post-exercise rehydration. All of the studies were focused on carbohydrate plus protein (powdered milk protein, or whey) and involved ingestion of a volume equivalent to 150% of body mass loss ingested over a 1 hour period. The dehydration protocol induced mass loss of ~2% in all studies and rehydration was monitored over the subsequent 4 hours. James et al (2011) examined rehydration with 65g/L carbohydrate-electrolyte solution compared to a 40g/L carbohydrate plus 25g/L milk protein solution. They observed that the carbohydrate-protein combination was more effective than carbohydrate alone for fluid retention over the 4 hour recovery period. As predicted, this was due to differences in urine output between trials in the first 1-2 hours following ingestion. James et al (2012) went on to examine the addition of whey protein to carbohydrate-electrolyte solution, presumably in an attempt to determine the influence of whey vs predominantly casein protein composition in their earlier study. Interestingly, they observed no effect on fluid retention or net fluid balance with whey addition (15g/L whey protein). This finding suggests that the casein protein rather than whey likely contributes to better fluid retention through known effects on delaying gastric emptying (Burn-Murdoch, Fisher et al. 1978).

The observation of possible casein vs. whey protein differences also prompts the question about whether additional protein really acts on plasma volume through oncotic pressure effects over a period as short as 4 hours post-exercise. James et al (2013) went on to examine the effects of varying the amount of milk protein ingested in rehydration drinks. They examined a carbohydrate-electrolyte solution (6% carbohydrate, 21mM Na<sup>+</sup>) and compared the fluid retention with two carbohydrate-protein combinations (4% carbohydrate, 2% milk protein, 20mM Na<sup>+</sup> and 2% carbohydrate, 4% milk protein, 21mM Na<sup>+</sup>). They replicated their earlier findings of greater fluid retention with a carbohydrate-protein solution compared to carbohydrate alone, but did not observe any difference between the higher or lower protein contents. James et al (2014) and Hobson et al (2015) revisited the question about the role of whey protein in restoration of fluid balance and fluid retention following exercise induced dehydration. James et al (2014) examined the impact of addition of whey protein isolate (2%) to mineral water on fluid retention vs. a mineral water

control. Both drinks were essentially electrolyte free, were administered in a volume equivalent to 150% of body mass loss over a 1 h ingestion period, and both had low osmolality (water 2 mOsmol/kg and water plus protein 14 mOsmol/kg). The data revealed no difference in urine volume or fluid retention over the rehydration recovery period. Both drinks left participants in a net negative fluid balance situation from 2 hours after fluid ingestion ended, but there was evidence of significant plasma volume expansion relative to water on the water plus protein trial. These data suggest that whey protein isolate neither enhances nor impairs hydration status, but they add some evidence that whey protein may act to promote an oncotic effect on plasma volume. The low osmolality and electrolyte free nature of the drinks also likely compromised the ability of the fluids to be retained. Hobson et al (Hobson and James 2015) went back to comparing carbohydrate vs. carbohydrate plus whey protein isolate (20g/L of whey isolate), in a very similar study to that of James et al (2012). They replicated the findings of that study with no impact of protein addition to a 6.2% carbohydrate-electrolyte beverage on urine output or fluid retention. Net fluid balance was negative on both trials by the end of the recovery period, and they did not observe any differential effects of the drinks on the plasma volume response. However, they did observe a possible interaction effect demonstrating a higher plasma albumin content on the carbohydrate-protein trial between pre-exercise and 2, 3 and 4 hours post drink ingestion. This time effect was not observed on the carbohydrate trial. In all of these studies it seems that the protein type influences fluid retention more so than protein dose, and that a combination of protein with carbohydrate and electrolytes seems to result in the best chance of fluid retention after a 4-5 hour recovery period.

Two other recent post-exercise rehydration studies have also contributed something to this field. Desbrow et al (2014) examined a range of beverages and compared drinks containing carbohydrate-electrolytes (Powerade, 7.3% carbohydrate), cow's milk (4.9% carbohydrate, 3.6% protein, 3.8% fat), soy milk (5.1% carbohydrate, 3.2% protein, 3.8% fat) and a liquid meal replacement product (Sustagen Sport, 17.6% carbohydrate, 6.5% protein, and 0.2% fat). Clearly the energy content of these different drinks was not matched, but the study still provides an insight into the drivers for fluid retention in a post-exercise recovery period. The data revealed that fluid retention was the best on the Sustagen sport meal replacement trial (65% retention) over the 4 hour follow-up period. The next best drinks were soy milk (47% retention) and cow's milk (40% retention), and then the carbohydrate-electrolyte drink (17% retention). The difference in total fluid retention between drinks was large. We know from

the previous studies covered in this section that only a small effect of carbohydrate content was observed in the range of 2-12%. It is possible that at almost 18% there may be a larger effect on fluid retention (delayed fluid delivery) induced by the meal replacement product. Furthermore, the meal replacement contained more protein than tested in the studies by James and co-workers which would also induce a greater delay in gastric emptying and intestinal absorption. The authors caution that the high total energy content of the meal replacement, when ingested in large volumes, would often be impractical for effective fluid replacement. Tai et al (2014) compared a carbohydrate-electrolyte drink (6.7% carbohydrate) with a branched-chain amino acid electrolyte drink (0.8% BCAA solution, with 2.6% carbohydrate) and a flavoured water drink. The hydration and fluid balance data indicate no difference between trials with around 40-44% of all fluid being retained. A particular problem with this study is the different electrolyte and mineral content of the drinks which could have influenced the outcome (see electrolyte section). There was also no control of carbohydrate or protein content between trials. As such, we can neither recommend nor dismiss a potential role of branched-chain amino acids on fluid retention.

In summary, from these post-exercise studies it seems that if fluid retention is the goal (i.e. reducing urine output and reducing requirement for additional fluid intake over a 4-5 hour recovery period) then delaying fluid delivery should be targeted. A delay in fluid delivery can be easily achieved through slowing gastric emptying rate with higher carbohydrate content (>10% carbohydrate) or carbohydrate (6% carbohydrate) plus additional protein (casein most appropriate). However, where fast fluid delivery is required then fast emptying and absorption is essential. Fast emptying and absorption can be achieved through ingestion of low carbohydrate contents (2-4% carbohydrate) and could include some protein (2% whey). However, where fast fluid delivery and high fluid retention is required, it seems that the fast delivery formulation would need to have additional electrolytes added (see electrolyte / mineral section below).

### **1.3.2 Electrolyte content of drinks and effective hydration**

#### ***1.3.2.1 Daily living***

In inactive adults in temperate climates hydration throughout the day is mostly influenced by fluid ingestion volume, as insensible losses are not high. Due to this fact, and probably the perception that hydration in daily living is not an essential area of study, there have been very few studies specifically focused on understanding the role of electrolytes/minerals on

hydration throughout daily life. A recent descriptive study of the UK population (n=1724 adults aged 19-64 y, based on the National Diet and Nutrition Survey) described the fluid intake habits and choice of drinks for ingestion (Gibson and Shirreffs 2013). This analysis has revealed that total fluid intake ranges widely (500-5000ml per day) in both sexes. This wide range of fluid intake is interesting from a behavioural as well as physiological standpoint, and the analysis highlights that in the UK at least, hot drinks contribute the highest proportion to daily beverage intake.

Of the studies examining the role of electrolytes on hydration during daily living activities the quality of work is generally low (**Table 1-7**). For example, Miller et al (Miller, Mack et al. 2009) examined the impact of a very low volume of fluid ingestion (1ml/kg body mass) on hydration markers (plasma and urine osmolality, urine volume and urine specific gravity) over a 1 h post ingestion period in 9 euhydrated males. Their intervention examined whether a high electrolyte solution (pickle juice: 415mM Na<sup>+</sup>, 27mM K<sup>+</sup>, 48mM Ca<sup>2+</sup>, 17mM Mg<sup>2+</sup>) helped to retain fluid, compared with a carbohydrate-electrolyte solution (18mM Na<sup>+</sup> and 3mM K<sup>+</sup>) or normal tap water (16mM Na<sup>+</sup>). Unsurprisingly, there were no differences in the key hydration outcome measures between trials. The low volume of ingested fluid likely made it very difficult to detect differences between drinks over a short follow-up period.

**Table 1-6. Studies included in the analysis of electrolyte composition of drinks on hydration during daily living activities.**

Abbreviations are as for previous Tables.

Authors	Year	Age	Gender	Sample size	Study design	Hydration assessment	Duration of follow-up	Outcome
Greenleaf et al	1998	23 - 46	Male	5	6 trials cross over design: following 24h moderate dehydration (by ingestion of only dry food)- water, 19.6 Na, 157 Na, 19.6 Na +glucose, Performance drink (19.6 Na) and Power drink (23.7 Na) in vol of 12ml/kg/bw. Then rested for 70 min.	Posm, Uosm, BM, blood analysis (change in Hb-Hct transformation equation)	70 min post ingestion	Sodium content of the drink was more important than total osmotic content (gluc +Na) for increasing plasma volume in resting conditions. Also, K (5mM) plus sodium (19.6mM) in a drink containing 10% carbohydrate was the next most effective beverage at increasing plasma volume at rest.
Zorbias et al	2002	22-26	Male	30	3 trials (randomly assigned to one): unsupplemented ambulatory controls, 30 day bed rest regime with no fluid + salt supplements, 30 day bed rest with fluid + salt supplements (FSS).	Posm, PV, Uosm and BM	the duration of the 30 days.	FSS may be used to maintain body hydration status and electrolyte values during bed rest.
Roberts	2006	23 (3)	Male / female	33 male / 18 female n=51	3 groups of tree-planters given access to electrolyte drink (E), CHO-E drink or Water daily over 30 days.	BM, PV, FI and BP	1 month of daily fluid ingestion	Approx. 4.5L of electrolyte beverage ingested daily - no diffs in BM, PV, FI or BP between trials after 1 month.
Miller et al	2009	25 (2)	Male	9	cross over design: only 1ml/kg of body mass (86.5ml total volume) of pickle juice, CHO-E or tap water consumed.	USG, Uosm, UV, Posm, PV	up to 60 min post ingestion	No differences in plasma sodium, magnesium, calcium, osmolality and UV 60 min post ingestion of fluids. Water ingestion decreased plasma potassium conc 60 min post ingestion. The high Na content of pickle juice (415mM) led to a short lived elevation in plasma sodium in the initial 30 min post-ingestion.
James and Shirreffs	2014	24 (4)	male / female	6 male / 6 female n=12	3 trials of a 2h rehydration period ingesting 1.25xBM loss induced by 24 h fluid and energy restriction (2.1% BM loss). Drinks were flavoured water (P), P plus 50mM Na, and P + 30mM K and were administered in 6 aliquots (every 20 min).	UV, BM, Uosm, urine electrolytes	4 h post-drinking	Ingestion of the higher Na drink was best for fluid balance restoration (Na drink better than water). K drink was no different to water or Na drink.

Another daily living study examined fluid ingestion during prolonged low intensity activity in a warm environment (19-24°C) over a period of 30 days in 33 males and 18 females (Roberts 2006). This study compared three groups of workers consuming either an electrolyte beverage (18mM Na<sup>+</sup> and 3mM K<sup>+</sup>), water, or a carbohydrate–electrolyte beverage (18mM Na<sup>+</sup> and 3mM K<sup>+</sup>). The authors placed an emphasis on the potential negative effects of sodium ingestion on blood pressure. Participants consumed on average 4.5 L/day of the assigned fluid over the 30 day study period. Outcome measures were change in plasma volume on work days pre and post, body mass, and blood pressure. No differences were noted in outcomes between groups, highlighting that during manual labour the ingestion of large amount of electrolyte containing beverages does not impact upon body mass, plasma volume or blood pressure over a 30 day period. The study is generally weak and has only been included in this review as it was felt that it could contribute to an understanding of daily fluid needs under manual working conditions in a temperate environment.

The more interesting studies examining electrolyte content of fluids and hydration during daily living are those of Zorbas et al (2002) and Greenleaf et al (1998). Zorbas et al (2002) conducted a bed rest study in 30 male volunteers to examine the role of electrolyte supplementation on maintenance of hydration status during a prolonged period of bed rest. They had 3 groups: normal ambulatory controls unsupplemented, bed rest unsupplemented, and bed rest prescribed to fluid intake (30ml/kg per day water) with an electrolyte supplement (0.1 g NaCl per kg body mass per day, mean of 7.5g per day). Participant's plasma and urine osmolality, plasma volume, and body mass were tracked over a 30 day period. The study observed that prescribed fluid and salt helped to prevent a decline in some markers of hydration status (plasma volume) and prevented a decline in body mass that was evident in the unsupplemented bed rest group. The fluid and salt supplementation helped to maintain normal haematocrit and normal plasma sodium, potassium, chloride and magnesium concentrations compared to unsupplemented bed rest in which the variables all increased markedly. These data suggest that fluid and salt supplementation helped sustain an expansion of the extracellular fluid compartment and maintained fluid and electrolyte balance over a 30 day bed rest period. Greenleaf et al (Greenleaf, Jackson et al. 1998) examined acute hydration responses (over 70 minutes sitting at rest) to a range of beverages ingested at rest in a small (n=5) group of males. In their study a 24 h period of only dry food ingestion was applied to induce a moderate hypohydration prior to each of the 6 main trial days. The 6 trial days included a baseline monitoring period followed by ingestion of 12ml/kg



body mass of water, a 19.6mM Na<sup>+</sup> solution, a 157mM Na<sup>+</sup> solution, a 19.6mM Na<sup>+</sup> plus glucose 10%) solution, a performance drink (19.6mM Na<sup>+</sup> and 5mM K<sup>+</sup> with 10% mixed carbohydrate) and a power drink (23.5mM Na<sup>+</sup> and 2.5mM K<sup>+</sup> with 10% mixed carbohydrate). All fluid (mean 936ml) was ingested within a 5 minute period (mean ingestion time was 4.8 min) and key outcome variables were plasma and urine osmolality, urine excretion rate, body mass, and blood analysis (change in plasma volume). The data indicated that the sodium content of the drink was more important than total osmotic content (e.g. glucose plus sodium) for increasing plasma volume under resting conditions. The most effective drink at elevating plasma volume was the high sodium solution. Also of interest was that the drink containing potassium (5mM) with low sodium (19.6mM) and containing 10% carbohydrate was the next most effective beverage at increasing plasma volume by the end of the 70 minute rest period.

The most recent study in this category is that of James and Shirreffs (2015). Their study examined rehydration over 2 hours following a 24 hour period of fluid and energy restriction that resulted in a loss of 2.1% of initial body mass. Fluids were ingested in a volume that equated to 125% of body mass loss in six equal volumes ingested every 20 minutes over a 2 hour rehydration period. Analysis of urine parameters (volume, osmolality, and electrolytes) was conducted over the subsequent 4 hour period. The drinks ingested were a flavoured water (5mM Na<sup>+</sup>, 1mM K<sup>+</sup>), flavoured water plus 50mM sodium (57mM Na<sup>+</sup>, 1mM K<sup>+</sup>), and flavoured water plus 30mM potassium (5mM Na<sup>+</sup>, 32mM K<sup>+</sup>). Fluid balance restoration was significantly better than flavoured water with the high sodium drink, due to a lower cumulative urine output. The potassium containing drink had an intermediate effect but was not different to water or to the sodium containing drink. However, the authors point out that fluid retention of the volume administered was relatively low (32%) compared with retention of a similar beverage in a post-exercise rehydration situation (69%). The authors attribute the difference in fluid retention to the isosmotic state likely to be present after fluid and energy restriction compared to the hyperosmotic state likely after exercise induced dehydration. Unfortunately, no blood parameters were collected to provide evidence for their assertions, but the concept is certainly of interest when considering the effectiveness of hydration drinks ingested during daily living where an isosmotic state of hypohydration is most likely to exist. They also highlight that the ingestion of a sodium beverage did not result in increased sodium losses in urine whilst ingestion of a potassium beverage did increase urinary potassium excretion. These observations may explain why potassium alone, or potassium addition to a

sodium containing drink, does not have any further measureable impact upon hydration status.

In summary, since there are few studies in this area of electrolyte content it is difficult to recommend an optimal drink composition for effective hydration during typical daily activities such as resting in bed, light or moderate manual work and following fluid and food restriction. It seems that sodium content of drinks is important and it is also likely that the fluid volume and frequency of ingestion could be key factors, as previously highlighted by Jones et al (Jones, Bishop et al. 2010). However, the concept of additional potassium in a drink to promote intracellular hydration requires further study and the potential effectiveness of this approach could be influenced mostly by tight regulation of potassium balance.

### *1.3.2.2 During exercise*

Strategies for fluid ingestion during exercise are primarily driven at maintaining body mass loss within 1-2% of a baseline euhydrated state, and maintaining cardiovascular function and thermoregulation through effective restoration of blood and plasma volume. Many studies have examined the impact of a combination of carbohydrate and electrolytes on fluid delivery, and cardiovascular and thermoregulatory responses to exercise. However, few have specifically investigated the impact of electrolytes/mineral composition on the effectiveness of drinks aimed at promoting hydration during exercise. Of the studies examining electrolyte/mineral content of drinks during exercise the general strength of evidence, for manipulation of electrolyte/mineral content to promote effective hydration, is again weak (**Table 1-8**).

One of the earliest studies examining electrolyte composition of drinks on hydration during exercise was conducted by Vrijens and Rehrer (1999). Their study in 10 male volunteers was really focused on understanding hyponatraemia. The design included 2 experimental trials with ingestion of either water, or an 18mM sodium and carbohydrate solution during 2.5-3 hours of exercise in the heat (34°C). Urine volume, plasma volume and body mass changes were noted. The key outcome observed was that replacing a volume of water similar to that lost as sweat (approx. 1.2-1.3L/h) resulted in a decline in plasma sodium concentration. Replacing the same volume of fluid with a drink containing carbohydrate and 18mM sodium did not result in a plasma sodium decline. The authors conclude that even a low

concentration of sodium in a drink can have a significant effect on plasma sodium when consuming volumes approximately equivalent to large sweat losses.

Another early study by Greenleaf et al (1998) examined acute hydration responses (over 70 minutes of supine exercise) to a range of beverages ingested at rest in a small (n=4) group of males. In their study a 24 h period of only dry food ingestion was applied to induce a moderate hypohydration prior to each of the 6 main trial days. The 6 trial days included a baseline monitoring period followed by ingestion of 12ml/kg body mass of water, a 19.6mM Na<sup>+</sup> solution, a 157mM Na<sup>+</sup> solution, a 19.6mM Na<sup>+</sup> plus glucose (10%) solution, a performance drink (19.6mM Na<sup>+</sup> and 5mM K<sup>+</sup> with 10% mixed carbohydrate) and a power drink (23.5mM Na<sup>+</sup> and 2.5mM K<sup>+</sup> with 10% mixed carbohydrate). All fluid (mean 898ml) was ingested within a 5 minute period (mean ingestion time was 3.2 min) and key outcome variables were plasma and urine osmolality, urine excretion rate, body mass, and blood analysis (change in plasma volume). The data revealed no significant differences between drinks in the PV change noted with exercise. These data suggest that during exercise the restoration of plasma volume is likely more reflective of the volume of fluid ingested and intensity of exercise being undertaken, rather than absolute composition of the ingested drink (albeit under supine exercise situations). Anastasiou et al (2009) have provided some support for this observation. In their study they conducted 4 trials of a 3 h variable format exercise task under hot conditions (30°C). The trials included consumption of two carbohydrate-electrolyte drinks varying only in their sodium content (36mM Na<sup>+</sup> vs. 20mM Na<sup>+</sup>) compared with water, or flavoured/coloured water (placebo). All drinks were ingested in a volume to match sweat loss. The low sodium and higher sodium drinks were equally effective at maintaining plasma volume and plasma osmolality during 3 h of exercise in the heat. However, they did observe a small significant decline in plasma volume over the exercise period in placebo and water trials (~2% decline). It appears from the statistical methods used that no interaction effect (treatment x time) was observed and therefore this reported difference between responses in trials is purely a time effect with no interaction. Therefore, the data as a whole seem to support the notion that during exercise the volume of fluid ingested and intensity of exercise is more important in maintenance of blood or plasma volume.

Table 1-7. Studies included in the analysis of electrolyte composition of drinks on hydration during exercise

Abbreviations are as for previous Tables.

Authors	Year	Age	Gender	Sample size	Study design	Hydration assessment	Duration of follow-up	Outcome
Greenleaf et al	1998	23-46	Male	4	6 trials cross over design: following 24h moderate dehydration (by ingestion of only dry food)- water, 19.6 Na, 157 Na, 19.6 Na +glucose, Performance drink (19.6 Na) and Power drink (23.7 Na) in vol of 12ml/kg/bw. Then exercised for 70 min on cycle ergometer.	Posm, Uosm , BM, blood analysis (change in Hb-Hct transformation equation)	Termination of exercise	Stabilisation of PV between 15 - 70 min was not related to sodium or osmotic content although water was the least effective. It seems that during exercise, fluid with greater hypervolemic action is not as effective as at rest.
Vrijens and Rehrer	1999	24.8 (2.8)	Male	10	2 trials (cross over): exercised for 3h at 55% of VO2 max on cycle ergometer in hot conditions (34C, 65% RH) consuming water (w), or a carbohydrate electrolyte drink (G) equal to fluid losses.	UV, blood analysis (change in PV) and BM (compared change in BM with urinary losses and FI to estimate sweat loss)	Termination of exercise (exhaustion or 3h)	Decreased plasma sodium concentration occurs with ingestion of plain water during exercise, when sweat losses are large.
Cuddy et al	2008	23(2)	Male / female	male / 4 female	2 groups: 1 water (n=8) and 1 water with electrolytes (n=8) containing Mg, Na, K, & sulphate (Elete product). 15h work shift in hot conditions (wildfire suppression). FI <i>ad libitum</i> .	FI, change in nude BM and USG. Thermoreg parameters recorded (core, skin and ambient temperature each hour) and work activity.	15 h	The group using the electrolyte additive (5.4mM Na, 3.3mM K, 2mM Mg) had a lower FI over the shift without any negative effects on thermoregulation, activity or urine specific gravity / body mass loss. Suggests greater fluid retention and requirement to carry large fluid volumes is less.
Anastasiou et al	2009	24.2 (2)	Male	13	4 trials of 3h variable format exercise in the heat (30C): consuming HNa CE drink (36.2mM Na), LNa CE drink (19.9mM Na), water, or flavoured/coloured water, to match mass loss.	Serum Na, Posm, PV change	Termination of exercise (3h)	LNa and HNa equally effective in maintaining PV and plasma Osm during 3h exercise in heat. When ingesting fluid to match sweat loss electrolyte free drinks resulted in lowering of serum Na.
O'Neal et al	2012	24 (4) range 19-35	Female	27	5 trials of <i>ad libitum</i> intake of water (W) acidified water (AW), AW plus electrolytes (AWE), AW plus flavouring (AWF) and AWF plus electrolytes (AWFE) during 60 min of walking (26°C WGBT) and 1 hour of recovery.	BM, USG	2h	Lower intake of AW and AWE (321 and 366ml respectively) than AWF (661ml) and AWFE (688ml) during and post-exercise. Water intake was 512ml. Sweat loss was 485g AW, 539g AWE, 529g AWF, 530g AWFE, & 547g W. No diff in USG pre to post walk between trials or in total urine voided over 2h (range 167 to 275ml). AW and AWE were rated as salty compared to AWF and AWFE. AWE and AWFE same electrolyte composition.

Cuddy et al (2008) examined the impact of adding small amounts of additional electrolytes to water during long duration submaximal exercise in the heat (15 h, mean temperature  $\sim 30^{\circ}\text{C}$ ) in wildfire fighters. Two groups of participants were studied with one group ( $n=8$ ) provided with water alone, and the other group ( $n=8$ ) provided with water plus electrolyte additive. The electrolyte additive was a commercial product (Elete) that only provided  $5.4\text{mM Na}^+$ ,  $3.3\text{mM K}^+$  and  $2.0\text{mM Mg}^{2+}$ . Despite the low electrolyte provision in the water plus electrolyte group a lower fluid intake was reported than in the water alone group (3.3 L less over 15 hours). The authors interpreted this outcome to mean that fluid retention was better and that the requirement to carry fluid could be reduced if an electrolyte additive was used during prolonged activity under hot conditions. A key limitation of the study is the use of two independent groups. Despite monitoring activity and frequency of fluid ingestion and reporting no differences between groups the difference in total fluid intake could reflect a palatability issue, large differences in urine output, or large differences in sweat rates between groups. Since urine output was not recorded the differences in body mass over 15 h do not readily reflect fluid retention over the whole period. In fact, body mass loss was 0.5% in the water trial group vs. 1.3% in the water plus electrolyte group. It is difficult to support the author's views on fluid retention differences with such low amounts of electrolyte addition especially when cumulative urine losses and sweat losses are not measured in the two independent groups.

O'Neal et al (2012) also investigated effects of electrolytes and/or flavouring/acidification of water on fluid intake, body mass losses, and urine specific gravity in female walkers. The study incorporated 5 trials with ad libitum intake of water, acidified water, acidified water [plus electrolytes,  $9.3\text{mM Na}^+$ ,  $3.4\text{mM K}^+$ ], acidified water plus flavouring, or acidified water with flavouring and electrolytes. Trials were conducted during walking over 1 hour duration at  $26^{\circ}\text{C}$  and monitoring continued into 1 hour of recovery from exercise. There was a lower intake of fluid when no flavouring was present but also no difference between any trials in urine specific gravity or total urine voided over the 2 h monitoring period. Perceived saltiness of the drinks was higher in drinks without flavouring despite no differences in electrolyte content. This study really only demonstrates that addition of flavouring can influence palatability and fluid intake. The impact of electrolyte addition was minimal, likely due to the small amount of electrolytes added.

In summary, during exercise the impact of electrolyte addition appears to be under evaluated. Those studies demonstrating a benefit of electrolyte addition have added only small amounts of electrolytes, or show no impact of adding additional electrolytes. However, a lack of any electrolytes (particularly sodium) appears to influence retention of plasma sodium concentration especially when sweat volume loss and fluid intake volumes are high. As such, during exercise a modest amount of sodium in a drink would appear warranted.

### *1.3.2.3 Recovery from exercise*

The impact of electrolyte composition of fluids for restoration of fluid balance after exercise has been a much bigger focus of research (**Table 1-9**). Clearly, post-exercise fluid replacement can have important implications for recovery from exercise and particularly so when a subsequent exercise period will occur within a few hours (such as when training twice a day).

Table 1-8. Studies included in the analysis of electrolyte composition of drinks on hydration post-exercise

Abbreviations are as for previous Tables.

Authors	Year	Age	Gender	Sample size	Study design	Hydration assessment	Duration of follow-up	Outcome
Nielsen et al	1986	24	Male	6	4 trials: given in 9x 300ml portions at 15min intervals post exercise in 3% dehydrated state; control (C), high potassium (K), high sodium (Na), high sugar (S). All drinks had 43mM Na and 2.5% glucose except for high Na which had 128mM Na, and high sugar which had 5% glucose and 4.4% sucrose and low Na and K content.	Blood analysis (Hb/Hct and PV, plasma electrolytes) and urine electrolytes and Uosm muscle water and electrolyte content.	135 min post-exercise	PV restoration was greatest with high sodium concentration. Lowest with potassium drink (K plus Na) . No diff in muscle water content assessed post rehydration between trials. Effects of K added to a low Na drink (<43mM) not evaluated here so true effect of potassium addition possibly not revealed.
Nose et al	1988	28	Male	6	4 h of recovery from 90-110 min of a heat (36 °C, less than 30% RH) and exercise (40% maximal aerobic power) exposure, which caused body weight to decrease by 2.3%. 1st h post-ex no fluid, then <i>ad libitum</i> fluid over next 3 h. Fluids were water with placebo capsules or water with NaCl capsules (0.45g per 100ml water).	Posm, UV, BM.	3 h	Greater retention of fluid (71% vs 51%) in NaCl ingestion trial due to lower urine output. Posm remained elevated and PV was expanded in the NaCl trial compared to baseline.
Maughan et al	1994	22	Male	8	4 trials: Dehydrated by 2% BM through exercise then ingested glucose, NaCl, KCl or a drink containing all three in volumes 100% of fluid losses, over a 30 min period post exercise.	Blood analysis (Sosm) and Uosm.	6h post exercise	Volume of fluid excreted was greater with the glucose (no electrolyte) beverage compared to the other drinks. Recovery of plasma volume was slowed with potassium beverage - possible beneficial effect on intracellular rehydration.
Maughan & Leiper	1995	31 (4)	Male	6	4 trials (cross over): subjects dehydrated by 1.9% through exercise heat stress. For a 30 min period starting 30 min post exercise subjects ingested fluid volumes 1.5 times their BM loss; 2, 26,52, 100 mmol.l of sodium.	Blood and urine measures.	5.5h post ingestion	1.5 h post ingestion, the effect of the different drinks on rehydration was significant, with the volume of urine excreted being inversely related to the sodium content of the drink. With lower sodium concentrations (2 and 26mmol/l) subjects were in negative fluid balance 5.5h post ingestion, but euhydrated with drinks of 52 and 100 mmol/l.
Shirreffs et al	1996	L group (27(3)) H group (33(4))	Male	12 (6 in each group)	2 groups: Low sodium (LNa, 23mM) and High sodium (HNa, 61mM) provided in volumes of 50%, 100%, 150% and 200% of BM loss following dehydration of 2.1% BM loss.	Urine output, urine composition and electrolyte balance, fluid balance, blood and serum measurements.	6h post exercise	Fluid volume seems to interact with sodium content such that there is no difference in the restoration of fluid balance at 6 h post following ingestion of 150% of fluid loss regardless of Na conc (23 or 61mM).
Wemple et al	1997	20.7 (19-21)	Not specified	6	3 trials (cross over): dehydrated by 3% of BM loss through exercise heat stress. Rehydrated <i>ad libitum</i> with artificially sweetened water (H2O), flavoured sucrose drink with 25 mmol/l of NaCl (LNa) or 50mmol/l NaCl (HNa).	Blood analysis (Hb/Hct, osm), urine output and BM.	3 h post exercise	FI with a small amount of sodium (LNa) increases voluntary fluid compared to flavoured H2O and HNa resulting in significantly improved rehydration after 3h. But ongoing evaporative losses during seated rest at 27°C not recorded.
Shirreffs et al	1998	28	Male / female	4 male / 2 female	4 trials (cross over): subjects dehydrated by 1.89% of BM by exercise-heat stress. 40 min after exercise subjects consumed a 0, 25, 50 or 100 mmol/l sodium beverage (25mM NaCl, balance NaAc) equivalent to 150% of mass loss over 60 min.	Urine output, urine composition and electrolyte balance, fluid balance, blood and serum measurements.	6h post exercise	Greater sodium content, reduced urine excretion volume. Subjects were in sodium balance on trial 50 but only in positive sodium balance and fluid balance in trial 100. Urinary loss of potassium was greater in trial 100. Sodium intake is important for repletion of sodium and fluid balance. However 100mmol/l may have consequences for potassium loss.

Saat et al	2002	22 (3)	Male	8	3 trials, water, coconut water (53mM K, 5mM Na) and CE drink (4mM K, 19mM Na) administered in a volume of 120% of fluid loss over 1 h post exercise (50% at 0, 40% at 30 min and 30% at 60 min post ex) following 90 minutes of exercise-heat stress (31C) that resulted in 2.8% BM loss.	Uosm, BV, UV, BM, serum electrolytes, Sosm.	2h post-exercise (1 h post end of drinking)	% Rehydration was highest with CE drink (80% restoration) vs. 75% for coconut water and 73% for water but these diffs were not significant. UV, BM, Uosm, Sosm all similar on all trials. Serum K higher on CW trial.
Ismail et al	2007	21 (1)	Male	10	4 trials. Coconut water (CW), CW with added sodium (SCW), sports drink (CE) and water (W) administered following exercise-heat stress (32°C for 90 min) to induce 3.1% BM loss. 120% of fluid loss was replaced over 1 h post exercise (50% at 0, 40% at 30 min and 30% at 60 min post ex) then followed up for a further 1 h.	Uosm, BV, UV, BM, serum electrolytes, Sosm.	2h post-exercise (1 h post end of drinking)	At end of period hypohydration was greatest with W compared to all other trials. W restored 59%, CE 68%, CW 65% and SCW 69%. UV was lower with sodium containing drinks (CE and SCW) than W. BM, Uosm, Sosm all similar on all trials but serum K higher on CW and SCW trials.
Shirreffs et al (a)	2007	24	Male / female	5 male / 6 female	4 trials (cross over design): all dehydrated to 1.8% of BM by exercise-heat stress. Subjects consumed 150% of BM loss of water (W), carbohydrate electrolyte (CE), milk (M) or milk + sodium (M+NA). Hydration status monitored for 5 h post beverage consumption.	Urine excretion, urine electrolyte excretion and osmolality, fluid balance and subjective feelings of drink taste.	5 h post exercise	Urine excretion was increased with CE and W drink vs milk drinks. Subjects were in positive fluid balance or euhydrated from 1h into the recovery period until the end with both milk drinks but not with W and CES. Milk without added sodium is likely an effective recovery drink (but both milks had higher Na than CES or W).
Shirreffs et al (b)	2007	23 (2)	Male/female	4 male / 4 female	4 trials (cross-over design): all dehydrated to 1.9% of BM by exercise-heat stress. Subjects consumed 150% of BM loss of carbohydrate electrolyte (CE), water/apple juice mix (AP), mineral water as Evian (E) or mineral water as San Benedetto (S) over a 1h rehydration period. Hydration status monitored for 4 h post beverage consumption.	Blood analyses (Hb/Hct, Sosm), urine indices (volume / composition).	5 h post exercise	Net fluid balance was best with CE (23mM Na). The apple juice drink (8mM Na with 30mM K) caused a delay in restoration of PV. Both water drinks resulted in the greatest net negative fluid balance after 5 h.
Merson et al	2008	25 (5)	Male	8	4 trials following exercise in the heat (35C, 2% BM loss): rehydration by consuming 1.5x mass loss of drinks containing either 1mM, 31mM, 40mM or 50mM of NaCl in 4 equal volumes over 1 h. All drinks contained 2% CHO.	BM, Sosm, Hb/Hct for PV, serum electrolytes, UV.	4 h post-drinking.	Rehydration was directly related to sodium content of the drinks (greatest fluid retention with 40 and 50mM compared to 1mM Na drinks).
Kalman et al	2012	26.6(5.7)	Male	12	4 trials following 60 min treadmill exercise in the heat (36C, 2% BM loss): rehydration by consuming 1.3x mass loss of water (W), bottled coconut water (CW), coconut water from concentrate (CWC) or CE drink. No information given on composition of the drinks.	BM, Posm, USG.	2 h post rehydration	Fluid retention greater at 2hs post with CWC than bottled water. Change in Posm and BM (0.6kg vs 0.9kg) supposedly less at 2h post compared to pre-exercise with CWC compared to water (but stats are weak - multiple t-tests). CWC resulted in 1 exclusion due to GI issue and higher rating of stomach upset for both coconut drinks compared with other drinks.
Perez-Idarraga and Aragon-Vargas	2014	24 (3)	Male / female	10 male / 2 female	4 trials following exercise in the heat (32C, 2.1% BM loss); rehydration consuming 1.2x mass loss of fluid ingested over 1h. Water, CE sport drink (18mM Na, 4 mM K), coconut water (5mM Na, 71mM K) or a special formula (18mM Na, 50mM K). All drinks contained 4% CHO.	BM, UV.	3 h post-drinking	CE sport drink had greater fluid retention than water but was not different from coconut water or special formula drinks (for net fluid balance).



The studies in this section provide a stronger evidence base founded on systematic evaluation of the impact of volume and electrolyte composition on net fluid balance. The studies have used periods of 2-6 hours following rehydration to examine fluid retention / urine losses. In all cases a hypohydration of 2-3% of initial body mass was induced during a period of exercise-heat stress.

Nose et al (1988) examined the role of sodium chloride (NaCl) capsule ingestion alongside *ad libitum* water intake on restoration of fluid balance following exercise in the heat. They observed that ingestion of NaCl promoted an expansion of plasma volume, retention of elevated plasma osmolality, and enhanced fluid retention (reduced urine output) when compared to *ad libitum* ingestion of water alone. These observations provided an early indication that sodium content of beverages would be important in restoration of extracellular fluid volume and fluid retention in the post-exercise period.

Nielsen et al (1986) and Maughan et al (1994) both examined the role not only of Na<sup>+</sup> but also of K<sup>+</sup> on restoration of fluid balance in the post-exercise period. They hypothesized that since K<sup>+</sup> was the major intracellular ion that intracellular fluid compartments may be preferentially restored with a K<sup>+</sup> containing beverage and that the most effective rehydration may likely occur with a combined Na<sup>+</sup> and K<sup>+</sup> solution. Nielsen et al (1986) used a control solution (2.5% glucose, 43mM Na<sup>+</sup>) and compared to the same solution with added potassium (2.5% glucose, 43mM Na<sup>+</sup>, 51mM K<sup>+</sup>), added sodium (2.5% glucose, 128mM Na<sup>+</sup>), or a sugar solution of glucose plus sucrose (5% glucose, 4.4% sucrose, 13 mM Na<sup>+</sup> and 6mM K<sup>+</sup>). They observed that plasma volume restoration, after exercise induced dehydration (3% body mass) was greatest when the high sodium solution was ingested, but it was also expanded to a similar extent with the control drink (containing moderate sodium content). The plasma volume restoration was least with the potassium containing drink but was not different to the sugar solution. These data suggest that restoration of plasma volume is likely influenced by delayed gastric emptying and intestinal absorption of fluid in the sugar solution trial. The delayed response in restoration of plasma volume with the potassium containing drink could be interpreted as a preferential restoration of intracellular fluid compartments. However, the muscle water content data did not support this concept (Maughan, Owen et al. 1994). A similar study design examined the impact of a low carbohydrate solution (1.6% glucose), a potassium solution (25mM K<sup>+</sup>), a sodium solution (60 mM Na<sup>+</sup>) or a solution containing all three, on restoration of fluid balance after exercise in the heat (2% body mass loss). The urinary losses of fluid were greatest with the glucose solution alone and were

lower on all other trials (with no differences between these other trials). These data suggest that the addition of sodium or potassium to a solution promotes greater fluid retention but the effects are not additive. However, plasma volume restoration was slower with the potassium solution which the authors interpreted as being indicative of a preferential restoration of intracellular fluid volume rather than a delayed fluid delivery to the circulation. However, urine potassium losses were not evaluated. The combined data of Nielsen et al and Maughan et al therefore suggest that additional potassium in rehydration solutions is not warranted when adequate amounts of sodium are present.

A series of studies by Maughan and Leiper (1995), Shirreffs et al (Shirreffs, Taylor et al. 1996, Shirreffs and Maughan 1998) and Merson et al (2008) combined with that of Wemple et al (1997) all subsequently confirmed the important role for sodium in restoration of fluid balance and palatability of ingested fluids following exercise-heat stress induced dehydration. These studies also provided evidence that when fluid volume is increased, the amount of sodium required to restore fluid balance over a rehydration period can be reduced. The balance point appears to be a sodium content of between 25-50mM for optimal fluid consumption and retention, when volumes ingested equate to 150% of body mass loss.

Shirreffs et al went on to examine the impact of a broader range of drinks in two studies published in 2007 (Shirreffs, Aragon-Vargas et al. 2007, Shirreffs, Watson et al. 2007). These studies examined restoration of fluid balance (ingestion of 150% of body mass losses) with commonly consumed beverages such as mineral water, sparkling mineral water, apple juice (8 mM Na<sup>+</sup>, 30mM K<sup>+</sup>), carbohydrate-electrolyte sports drink (23mM Na<sup>+</sup>, 2mM K<sup>+</sup>), skimmed milk (39mM Na<sup>+</sup>, 45mM K<sup>+</sup>), and skimmed milk plus sodium (58mM Na<sup>+</sup>, 47mM K<sup>+</sup>) drinks. These studies support a role for higher sodium content of drinks to promote fluid retention over the rehydration period. The data obtained also provide striking evidence for reduced urine output and enhanced fluid retention from skimmed milk compared to other drinks. The higher electrolyte content of the milk drinks may be responsible but also the likely delayed fluid delivery (due to casein) and potential oncotic impact of protein on fluid retention (albumin synthesis, see macronutrient section) are probably also contributing factors.

The final group of four studies in this section placed a focus on coconut 'milk' in rehydration after exercise (Saat, Singh et al. 2002, Ismail, Singh et al. 2007, Kalman, Feldman et al. 2012, Pérez-Idárraga and Aragón-Vargas 2014). The emphasis on coconut water comes from the

traditional use of coconut 'milk' as a drink, and the reported lower sodium content (5-10mM Na<sup>+</sup>) and higher potassium content (40-60mM K<sup>+</sup>) than most drinks aimed at rehydration (Kuberski, Roberts et al. 1979). Given the lack of positive findings for additional potassium in drinks containing an adequate sodium content (Nielsen, Sjøgaard et al. 1986, Maughan, Owen et al. 1994) it might not be surprising to suspect that coconut water would be of similar effectiveness as a drink containing a higher sodium content (25-50mM Na<sup>+</sup>). It could also be hypothesized that additional small amounts of sodium in coconut water drinks would have no additive effect on net fluid balance following a rehydration period. In fact, these are almost exactly the observations from the four coconut water studies. While most of the studies report that coconut water was acceptable to participants, one study reports a higher rating of stomach upset and loss of one participant due to a gastrointestinal issue (Kalman, Feldman et al. 2012). The carbohydrate content of coconut water is reported to be anywhere from 2-15% dependent upon the product. The higher end of carbohydrate content may be responsible for gastrointestinal problems if large volumes are ingested. Likewise the sodium content appears to vary between 20-50mM and potassium content from 35-70mM in commercial products available in the UK making it difficult to establish exactly the composition of 'coconut water'. These differences in composition appear to reflect whether the drink is from concentrate, from young or mature coconuts, and other processing such as addition of water/preservatives. Certainly the composition in commercially available products is not entirely in keeping with the composition reported for fresh coconuts by Kuberski et al (Kuberski, Roberts et al. 1979).

In summary, it appears that the electrolyte content of the drink has been well studied for post-exercise rehydration. Effective hydration comes from ingestion of a sufficient volume (150% of body mass loss) of a moderate sodium content drink (25-50mM). Where no sodium is in the drink effective hydration can come from potassium in the drink, but the addition of potassium to sodium does not appear to have an additive effect.

## 1.4 Summary

It seems from the small volume of relevant literature published that the macronutrient content, particularly carbohydrate in the range 2-12%, does not have a significant impact on fluid retention at rest when euhydrated or mildly dehydrated. However, protein does seem to play a role with milk resulting in greater fluid retention post-exercise, which most likely

reflects delayed fluid delivery (Maughan 2003). Furthermore, the electrolyte composition, particularly sodium content of drinks, seems to have the biggest influence on fluid retention.

Importantly, the lack of a significant number of studies in this area suggests a large knowledge gap in the research around fluid intake and hydration throughout daily living. It is understandable that fluid intake requirements and effective restoration of fluid balance are not considered of critical importance in normal daily living activities. However, with recent evidence reporting the wide range of fluid intake in the population, and a considerable number with low total fluid intake, provision of guidelines for fluid composition to promote effective hydration may be warranted. To do this, studies examining responses of electrolyte addition to drinks ingested in habitual low and high fluid ingestion groups may be a way of generating valuable data. Indeed, Perrier et al (Perrier, Vergne et al. 2013) examined markers of hydration status in two groups representing the extreme ends of fluid intake behaviour (low fluid drinkers, <1.2L/day; and high fluid drinkers, 2-4L/day) in 71 adults. They observed that the low fluid intake group had marked differences in urine hydration markers (lower urine volume and higher urine specific gravity and osmolality) and plasma concentration of fluid regulating hormones (higher vasopressin). Despite these differences plasma osmolality was similar in both groups suggesting that adaptation to low / high fluid intake has enabled maintenance of normal plasma osmolality. Given the preliminary evidence for a link between poor hydration status and development of chronic disease this seems like an interesting area open for future research.

### 1.4.2 During exercise

The literature on effective hydration during exercise suggests that volume is the most important variable but consideration should be given to both electrolyte and macronutrient content. Fast fluid delivery is likely to be a priority during exercise as fluid remaining in the stomach or intestinal lumen may lead to discomfort. Delivering fluid to the circulation quickly will be particularly of interest during exercise under warm/hot environmental conditions. For fast fluid delivery to the circulation low carbohydrate contents are required (range 2-6%). With fast fluid delivery and potentially a moderately large volume consumption (up to an amount matching sweat losses) then consideration should also be given to the sodium content of beverages to ensure retention of serum sodium concentration. Clearly, the volume requirement and composition of a drink will be related to the task at hand (<1 h vs 3-

4h) and the environmental conditions (Kenefick and Cheuvront 2012). Since the data supporting these recommendations is surprisingly sparse, further work is required to investigate the optimal formulation of beverages for effective maintenance of fluid balance during exercise. A key issue in this section is that many studies were not specifically designed to examine macronutrient and/or electrolyte composition effects on hydration status. This leaves a distinct lack of strong evidence about the role of macronutrients and electrolytes in drinks for promoting the maintenance of hydration status during exercise. Previous calls for more work on practical aspects of drinking during exercise (Garth and Burke 2013) are certainly echoed by the evidence presented in this current review.

### 1.4.3 Recovery from exercise

An interesting aspect to note in this section is that effective hydration is typically measured as the proportion of fluid retained after a period of 3-5h. This is a gross measurement that does not necessarily reflect the rate of fluid delivery to the circulation and the subsequent retention of that fluid. Solutions aimed at promoting fast fluid replacement are likely to be hypotonic with low carbohydrate content (<4%). In contrast, the presence of a high carbohydrate content (>12%) and/or milk protein (casein in particular) will delay gastric emptying. Furthermore, strongly hypertonic solutions in the small intestine will result in a net secretion of water into the intestinal lumen thus delaying any subsequent diuresis. While a reduction in urine output may be a key goal, these features of concentrated solutions can induce measurable decreases in plasma volume during or after exercise. In effect, there is the potential for concentrated solutions to result in a transient reduction in extracellular fluid volume. As such, in many studies the reported greater fluid retention with higher macronutrient content drinks may merely reflect slower fluid delivery. In situations where fast fluid delivery is important then it seems that increasing energy/macronutrient content of a drink (using carbohydrate and/or protein) would not be the most effective strategy. Additionally, the negative impact of fast fluid delivery is generally fast fluid excretion, and therefore a high electrolyte concentration (>50 mM sodium) would be necessary to ensure the retention of fluid that is delivered quickly. Furthermore, with adequate sodium content in drinks there appears to be no additional beneficial effect of additional potassium. Thus, for optimal fluid replacement/retention post-exercise studies support the use of a large volume (150% of body mass losses) of a carbohydrate/protein (up to 10% CHO) solution containing moderate amounts of sodium.

#### 1.4.4 Other considerations

##### Hydration in the older adults

In some population groups such as females or older adults there may be negative impacts of promoting hydration such as hypo or hypernatremia associated with different hormonal responses, or impaired ability to maintain homeostasis through the many systems involved in fluid regulation (Miller 1997). These ageing/gender induced differences can be further influenced by disease or medication induced alterations in renal function or diuresis. As such, careful consideration is needed on an individual basis before recommendations for females or older adults can be made.

Age related changes in fluid balance regulation make older adults susceptible to water imbalance, and many of them do not reach their recommended daily intake of oral fluids (Agostoni, Bresson et al. 2010). Some of the key changes in the older people are the reduction in voluntary fluid intake, reduced skeletal muscle mass, and reduction in daily activities. Furthermore, the kidneys also demonstrate functional changes leading to more dilute urine production and thus increased water losses, while changes in the gastrointestinal (GI) tract or prescription medications may lead to altered faecal water loss in comparison to young adults.

It is well known that the worldwide population is ageing, and predictions in the majority of developed countries demonstrate a growing number and proportion of older adults. This demographic transition presents new challenges to health care providers for older adults in these countries, and it would be interesting to investigate how to maintain optimal fluid balance in older adults. This knowledge can be vital for the development of future hydration guidelines and hydration monitoring indices for healthy aging. Indeed, older adults have been shown to have a higher risk of developing dehydration than younger people, and many clinicians recognize that acute hypohydration is a precipitating factor in a number of acute medical conditions in older adults (Ferry 2005).

##### *Demographic information*

In recent years there has been a great deal of interest in the demographic transition, characterised by lower birth rates and greater life expectancy (Bongaarts 2009). The number of people aged 65 years or more worldwide was 420 million in 2000, by 2030 the expected number for this group is 550-973 million. By that date, older adults will represent 20%, 24.8%

and 33% of the global population in the United States, China and Europe respectively. This number exceeds the number of children below 15 years (CDC 2006). Currently, ten million people in the United Kingdom are over 65 years old. The latest projections are for 5.5 million more old adults in 20 years and the number will have nearly doubled to around 19 million by 2050. By now, one in six of the UK population is 65 years old and over, by 2050 one on four will be (Cracknell 2010).

### *Hydration and ageing*

Maintaining adequate fluid balance is an essential component of health at every stage of life. Age related changes, as decreases in muscle mass, renal function and thirst sensation, in addition to limited access to fluids and laxative or diuretic use, make older adults more vulnerable to shifts in water balance that can result in overhydration or, more frequently, dehydration (Mentes 2006). Older adults have been shown to have a higher risk of developing dehydration than younger people (Ferry 2005). Many clinicians also recognize that acute hypohydration is a precipitating factor in a number of acute medical conditions in older adults (Ferry 2005). Fluid intake of older adults has been shown to vary between the towns of Europe and between men and women (Mentes 2006). A high percentage of the female population had a fluid intake below the recommended cut off value of 1700 g. In most towns about 70 percent of daily fluid intake came from the food groups “milk products”, “alcoholic drinks”, “juices” and “other non-alcoholic drinks”. Women were found to be at higher risk of dehydration because of much lower fluid intakes than men (Haveman-Nies, de Groot et al. 1997).

Dehydration is a common cause of morbidity and mortality in older adults. It causes the hospitalization of many patients and its outcome may be fatal. Dehydration is a common cause of fluid and electrolyte disturbance in older adults, occurring in approximately 1% of community hospital admissions (Snyder, Feigal et al. 1987). Dehydration is often related with infection (Lavizzo-Mourey 1987). In one study, 82 % of dehydrated older adults had an infection (Mahowald and Himmelstein 1981). The risk of infection in older adults has been linked with dehydration and the mortality rate can be as high as 50% in the absence of early diagnosis (Ferry 2005). Some analysis of the cause of death in older adults care home in England and Wales from the Office of National Statistics under the Freedom of Information Act revealed that 1158 care home residents suffered dehydration-related deaths between 2003 and 2012 (AgeUK 2013). This statistic cannot identify exactly if these individuals died because of dehydration or if dehydration was just a contributing factor. However, providing

better guidelines and monitoring for health care workers could reduce the numbers admitted to hospital due to dehydration, or help prevent deaths in care home settings. It has been reported that mortality associated with disturbances in water balance in the older adults may be as high as 40% to 70% (Warren, Bacon et al. 1994). There is also evidence that older adults are the most vulnerable to periods of extreme heat. Dehydration as a result of abnormal weather conditions may, therefore, have some important implications for those responsible for forward planning in the healthcare facilities (Maughan 2012). Improvement in detection of disorders of water balance in the older population may yield more timely interventions and targeted approaches to management of fluid balance in older adults (Weinberg, Pals et al. 1994).

### *Older adult care*

Maintenance of water balance is indispensable to normal physiologic function and healthy ageing. Adequate information must be given regularly not only to older people, but also to their families, caregivers, and healthcare professionals. Dehydration is a common problem in nursing homes and in the community, due often to failures in detection and appropriate management. In many cases, the cause is iatrogenic due to diuretics or drugs that impair the intake of food and fluid (Allison and Lobo 2004). Better training in the detection, prevention and management of fluid and electrolyte imbalance is needed to reduce common and serious morbidity associated with this problem to which the older adults are especially prone, owing to their diminished physiological reserves and increased comorbidity (Allison and Lobo 2004)

### *Body composition in older people*

As we age the proportion of fluid in our bodies reduces, from over 70% of our weight as newborn babies, to 60% in childhood and about 50% in older adults (Altman 1961). Total body water (TBW) generally declines with increasing age (Baumgartner, Stauber et al. 1995). This suggests that ICF is decreasing with increasing age (Mazariegos, Wang et al. 1994). Most ICF is confined in the non-fat compartment of the body, called fat free mass which includes organs, extra cellular fluids and bone. Skeletal muscle mass comprises the largest part of the fat free mass, about 53-54% (Wang, Visser et al. 1996, Lee, Wang et al. 2001). As body water falls with the age the buffering capacity against dehydration is decreased, possibly with serious consequences to health. Fat free mass, and particularly muscle, store large amounts of fluid as around 70% of their composition is water (adipose tissue is 10-40% water) but as muscle mass decreases with ageing, the fluid reserve is reduced (Martin, Daniel et al. 1994).



Muscle mass decreases in older adults mainly because there is an important reduction in the intra-cellular water compartment. Total body water is decreased and this can have an important impact for the prescription of water-soluble drugs (Morley JE 1998). The reduction of skeletal muscle mass causes the diminution of glycogen storages (Evans 1995), and this is also related with the total body water decrease.

### *Thermoregulation in older adults*

Ageing is associated with a progressive decrease in thermal perception. Intrinsic structural changes in ageing skin may induce physiological changes that affect the skin's ability to function as an interface between the internal and external environments (Farage, Miller et al. 2008). Ageing is associated with reductions of up to 50–60% in the principal functions of the skin, including thermoregulation and sensory perception (Cerimele, Celleno et al. 1990). Age related changes in the density of sensory epidermal nerve fibres may be responsible for the apparent decreases in thermal sensitivity (Goransson, Mellgren et al. 2004) as well as the decline in transmission properties of the peripheral nervous system (Guergova and Dufour 2011). These reductions in sensation would reflect neuronal loss as well as changes at the cellular and molecular levels, which alter sensory neuron responses (Wang and Albers 2009). It has been demonstrated that there is an impairment of thermoregulatory function due to diminished or absent sweating and it is thought to be one of the factors responsible for increased mortality in older adults during heat waves (Foster, Ellis et al. 1976).

### *Thirst regulation and ageing*

Key physiological signals for thirst are plasma hyperosmolality with consequential cellular dehydration and hypovolemia due to low blood volume and low arterial pressure (Thompson, Bland et al. 1986). Older people are more susceptible to dehydration and electrolyte abnormalities than younger people. This is partly because they are less sensitive to thirst and to changes in water and sodium balance that naturally occur as people age (Schols, De Groot et al. 2009). The thirst regulation mechanisms can break down somewhat as people age; this is in part because the fluid reserve is smaller so dehydration can happen quicker with water depletion (Olde Rikkert, Deurenberg et al. 1997). As a result of changing thirst perception older adults trend to drink less fluid compared with younger people (Mack, Weseman et al. 1994, Waldreus, Sjostrand et al. 2011) and also it has been shown there is a physiological hypodipsia when older adults are under stressful conditions (Rolls and Phillips 1990).

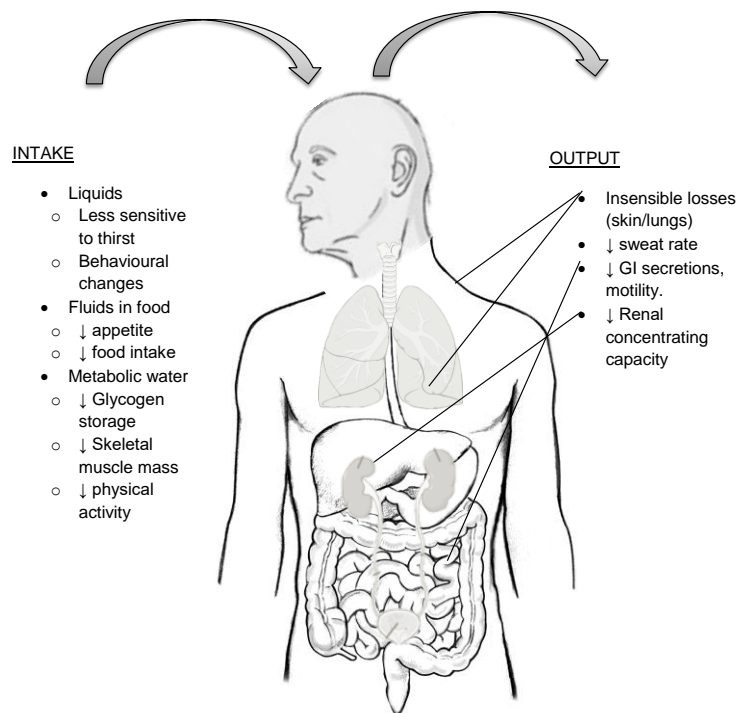
Normal physiological changes of ageing increase the likelihood of fluid-electrolyte disorders in the older adults (Luckey and Parsa 2003). The most important of these changes are:

- Decrease in total body water
- Decrease in glomerular filtration rate
- Decrease in urinary concentrating ability
- Increase in antidiuretic hormone
- Increase in atrial natriuretic peptide
- Decrease in aldosterone
- Decrease in thirst mechanism
- Decrease in free water clearance

### *Functional and anatomical changes in the ageing kidney*

The ageing process results in remarkable changes in the kidney. These changes are both anatomical and functional and have been considered the cause of the increases propensity of the older adults to acute or chronic failure. (Esposito and Dal Canton 2010) The kidney undergoes functional and structural changes during the ageing process. The renal functional reserve is diminished in the older adults predisposing these individuals to the development of acute kidney injury when the balance of vasoconstrictive and vasodilatory hormones are altered, particularly with medications (Rodriguez-Castro and Cordova 2011). There is a progressive decrease in the baseline function of the kidney after young adulthood. In most individuals between 30 and 85 years, there is a 20 to 25% loss of renal mass, most of which is cortex (Beck 2000). The ageing kidney also shows hyalinization of blood vessel walls and decrease in the number of glomeruli. This process progresses to hyalinising arteriosclerosis and scattered arteriolar obliteration with a resultant loss of nephrons secondary to ischemia (Lindeman, Romero et al. 2001). The kidneys also present functional changes; they exhibit an impaired concentrating capacity over time and a 10% decline in renal blood flow per decade after young adulthood. Functionally, the most studied change in the aged kidney is the decline in the glomerular filtration rate (Luckey and Parsa 2003). In a healthy cohort examined for 20 to 30 years, most individuals showed a fall in the glomerular filtration rate of about 10 mL/min per decade (Lindeman, Tobin et al. 1985). As kidney function is reduced with age, the loss of fluids may not be as well controlled in older adults because the ability to concentrate urine and so retain fluids falls (Bolignano, Mattace-Raso et al. 2014). Normal ageing is also associated with changes in the hormonal regulatory systems involved in the

maintenance of water and sodium balance (Miller 1997). Therefore the changes caused with ageing involve several interrelated physiological mechanisms, and behavioural changes, that induce an impaired ability to regulate body fluid balance in older adults (**Figure 1-1**).



**Figure 1-1. Changes in factors affecting fluid balance with ageing**

### *Gastrointestinal (GI) tract and ageing*

There is an increase in GI disorders on function and motility with ageing. However, even though an increased prevalence of several GI motor disorders (i.e., dysphagia, dyspepsia, anorexia and constipation) occurs in older people, ageing per se appears to have only a minor direct effect on most GI functions, in large part because of the functional reserve capacity of the GI tract (Salles 2007). It is commonly assumed that complaints of chronic constipation or alterations in colonic functioning are natural consequences of the ageing process, but, motor function of the colon is overall well preserved in the healthy older adults (Talley, O'Keefe et al. 1992). Meal-related increases in sigmoid and rectal motility are unchanged. Delayed colonic transit can be induced in active healthy older adults who are made inactive (Liu, Kondo et al. 1993). In relation with motility, the majority of studies indicate that small intestinal motility does not change with normal ageing (Broghna, Ferrara et al. 1999). In

subjects over 80, the transit of faecal material through the colon is slowed as a result of the reduced number of neurons in the plexus, especially the myenteric plexus (Madsen and Graff 2004). When gastric secretions have been studied it has been found that baseline and stimulated production of HCl are both reduced with ageing. However this changes does not appear to occur when the gastric mucosa is intact. Pepsin secretion seems to be within normal limits in older persons who are healthy and reduced in those with *Helicobacter pylori* infections (Feldman, Cryer et al. 1996).

The GI tract represents an organ system that is characterized by rapid proliferation; ageing GI tissues illustrate markedly different phenomena from aged post mitotic cells. A state of hyper proliferation occurs in the epithelial cells of the stomach, the small intestine, and the large intestine of stable fed aged rodents when compared to young mature rodents (Xiao, Moragoda et al. 2001). The number of gastric and colonic mucosal cell undergoing apoptosis was found to be lower in older animals (Atillasoy and Holt 1993). Nutritional modulation of mucosal cell proliferation is affected by ageing. Age-associated changes in GI mucosal cell proliferation could also be secondary to alterations in hormonal influences, especially in the gastric mucosa. The gastric mucosa is responsive to different peptides, i.e., gastrin, bombesin, epidermal growth factor, changes at different stages of live (Majumdar 2003). These changes in motility and in gastric secretion will impact also the water losses through faeces. In older patients with constipation, the addition of fibre accelerates colonic transit. When delayed colon transit has been identified, this seems to be localized to the distal colon and rectum. In healthy older adults, colonic transit does not seem to differ from earlier years. Overall, the predominant physiologic changes contributing to constipation in the older adults have less to do with altered colonic transit and more likely relate to changes in anorectal function (Merkel, Locher et al. 1993).

Another aspect that alters the intake of fluids through food is the anorexia of ageing. It has been recognized that there are changes in hunger, poor intake of food may result from social isolation, physical handicaps to meal preparation, dental problems, dry mouth, changes in taste and smell, or cognitive dysfunction. In addition, taste sensation has also been shown to decrease with age (Morley 1997, Donini, Savina et al. 2003) which may lead to reductions in water intake in meals and fluids.

### *Evaluation of hydration status in older adults*

Change in body mass is the simplest indicator of changes in total body water. However, it is essential to know the initial stable body mass, which is not always possible particularly in older adults. The clinical signs of dehydration are very weak in older adults and are often simply functional modifications (blood pressure decreases, orthostatic hypotension, declines in diuresis, increased concentration of urine, etc.). Another sign is muscle cramps and fatigue, with declines in performance caused by decreases in the muscular intracellular volume. Asthenia and concentrated urine are two other warning signs (Ferry 2005). It is important to develop and validate a simple and easy method to assess the hydration status in the older adults, considering all the characteristics and conditions that they can present, including the diuretics and drugs they are prescribed and also considering the difficulties that older adults may have to move or scroll. Having a simple and practical method to evaluate hydration status could help in maintaining euhydration and could be useful for the older adults, their families and their caregivers, preventing dehydration consequences.

### *Hydration and cognitive function*

There is some evidence of impairments of cognitive function at moderate levels of hypohydration. Even short periods of fluid restriction (1-2% loss of body mass) lead to reductions in the subjective perception of alertness and ability to concentrate as well as increases in self-reported tiredness and headache (Shirreffs, Merson et al. 2004). It has been also demonstrated that particular cognitive abilities and mood states are positively influenced by water consumption. The impact of dehydration on cognition and mood is particularly relevant for those with poor fluid regulation, such as older adults (Masento, Golightly et al. 2014). Being dehydrated by just 2% impairs performance in tasks that require attention, psychomotor and immediate memory skills, as well as assessment of the subjective state. The performance of long term and working memory tasks and executive functions is more preserved, especially if the cause of dehydration is moderate physical exercise (Adan 2012). Dehydration has also been suggested as a contributing factor to cause of falls in older adults (Baraff, Della Penna et al. 1997).

Ageing is associated with impaired physiological reserve and a reduced ability to maintain homeostatic control over a variety of body functions, including maintenance of body water balance. These changes include reduced cardiac and renal reserve, making older adults more vulnerable to changes in water and electrolyte gain or loss, with a resulting increase in

dehydration-related morbidity and mortality. It is important to develop more research aimed at understand how older adults respond to fluid intake, how to evaluate their hydration status in a practical and non-invasive way, and how this group respond when ingesting different kinds of drinks in comparison to the responses in young adults.

### Hydration in the female adults

Gender differences in body composition and total body water are fairly well documented with lower blood volume/plasma volume and lean mass, and greater fat mass in an average female compared to an average male. There is also evidence from animal and human studies that in female's increases in available oestrogen may promote fluid and sodium retention and thus contribute to body mass gain prior to ovulation, as well as retention of fluid/sodium in the luteal phase (Witten and Bradbury 1951, Preedy and Aitken 1956). However, other reports demonstrate little effect of menstrual phase hormonal changes in fluid balance during dehydration/rehydration experiments (Stachenfeld, DiPietro et al. 1999). Oestrogen is thought to have a possible fluid retention action through either an alteration in posterior pituitary secretion of vasopressin, or through a direct effect on the kidneys, via specific renal oestrogen receptors, that may interact with vasopressin action to reduce urine output (Dignam, Voskian et al. 1956, Stachenfeld and Keefe 2002). In the most recent review on this topic Curtis et al (Curtis 2009) highlighted that an interaction between oestrogen and vasopressin may lead to fluid being retained despite lower plasma sodium concentration and elevated blood volume. Other studies have also indicated that oestrogen could act on the renin-angiotensin-aldosterone system to influence sodium retention through a stimulation of aldosterone secretion. Interestingly, progesterone seems to inhibit the action of aldosterone such that fluid / sodium retention would only occur under conditions of high oestrogen and low progesterone (Fanestil and Park 1981). Although, this association has been clearly demonstrated in oestrogen treated rats (Barron, Schreiber et al. 1986) the same outcomes have not been studied in depth in humans. However, there are reports of more cases of hyponatraemia in female athletes than in males (Wagner, Knechtle et al. 2012) that lends some support to this notion and which may be further exacerbated by lower sweat rates in females. The combination of these observations suggests that some consideration of the individual hormonal status of females may be warranted when recommending fluid replacement strategies.

## 1.5 Conclusions

This literature review highlights the need for more work particularly on furthering our understanding of effective hydration drinks in daily living, and a need to focus on effective fluid delivery aimed at promoting maintenance/restoration of fluid balance during exercise. While the review indicates that our knowledge of effective strategies for post-exercise rehydration is most advanced it is clear that the conclusions from this work cannot be uniformly applied to other situations. Furthermore, it is clear that special consideration should be given to future work in female and/or older adult groups of participants. The potential variation in fluid requirements/composition for effective fluid replacement throughout the menstrual cycle as well as in older adults would be interesting areas for future study. Research focused on these areas would help to provide guidance on effective fluid replacement in specific target groups.

## CHAPTER 2. GENERAL METHODS

### 2.1 Participants and Ethical approval

Volunteers for all studies were recruited through adverts posted around Campus and the community of Bridge of Allan (shops, churches, cafes, etc.) or via the University of Stirling's website. Local Sports clubs were also contacted and requested to distribute the invitation through their mailing lists.

The participants for the study reported in Chapter 3 were male and female athletes aged 17-40 years currently training or competing in their sports, they were recruited mainly from local sports clubs. Volunteers for this study were involved in a range of sports. This study was approved by the University of Stirling School of Sport Research Ethics Committee and the NHS East of Scotland Research Ethics Committee (Reference # 12/ES/0040)

For the experimental trials of studies reported in Chapters 4a and 5, the inclusion criteria were healthy recreationally active males aged 18-35 years old and with weekly alcohol intake no higher than 20 alcohol units. Ethical approval was granted by the School of Sport Research Ethics Committee for both studies (References # 591 and # 693 respectively). Both studies were done in collaboration with two other laboratories: Bangor and Loughborough.

In the field-based trial reported in Chapter 4b, the data collection was undertaken in a call centre. The participants were invited to take part in the experiment during a working day and gave consent for their participation. The volunteers were males and females aged 20-57 years.

The volunteers for the experimental part of the study reported in Chapter 6 were healthy active males aged 18-35 years or >50 years with a weekly alcohol intake no higher than 20 units. Participants under any medication that might affect the body fluid balance (such as diuretics) were excluded. The study was approved by the School of Sport Research Ethics Committee (Reference #753). Chapter 7 includes the results and analysis of a Hydration Habits Audit that was distributed through a website and through the circulation of the hardcopy questionnaire of the Audit to older adults with the support of The Food Train and local sports clubs. The participants included were males and females and the overall age range was 18-93 years.



## 2.2 Pre-screening procedures

All the volunteers for all the experimental studies were asked to attend for a pre-screening consultation where the study was explained in detail and if they had any question, it was resolved. The participants signed a copy of the Participant Information Sheet, a consent form where they were also informed of their right to withdraw at any time without any explanation. Each participant gave his or her written consent to take part in the study.

During the pre-screening consultation for the study reported in Chapter 3 the participants were provided with a set of scales and they were asked to record their body mass first thing in the morning for 7 days prior the first trial in the laboratory.

For all the experimental studies the volunteers were asked to record their food and fluid intake and also any exercise they performed 48 hours before their first visit to the laboratory. They were provided with a food/fluid/exercise format to complete this task. Participants were also asked to replicate their food and fluid intake and any activity in the 48 hours prior to each subsequent trial day.

## 2.3 Initial preparation and urine collection procedure

Participants were asked to refrain from any strenuous physical activity and alcohol ingestion for the 24 hours prior to each experimental trial. After an overnight fast of at least 10 hours, the participants emptied their bladder upon waking and collected an aliquot in a sterile urine collection tube. In the study reported in Chapter 3, on each trial day, the participants were asked to drink 500 mL of water in their own homes 2 hours before coming to the laboratory. In the studies reported in Chapters 4a, 4b, 5 and 6, the volunteers were asked to drink a bottle of 500 mL of still water (Highland Spring<sup>®</sup>) 1 hour before arriving at the laboratory. Water was provided during the pre-screening consultation and these guidelines were implemented in an attempt to ensure participants were euhydrated when the trials were initiated.

In the study reported in Chapter 3, when the participants arrived to the laboratory they were asked to empty their bowels and their bladder and a sample of urine was stored from this void. This sample was used to determine initial hydration status based on osmolality and also in the case of the female participants, the urine sample was used to perform a pregnancy

test. A pregnancy test was required in this study due to exposure to a low dose of ionizing radiation during whole body dual energy x-ray absorptiometry scanning.

In the case of the study reported in Chapter 4a, on arrival to the laboratory the participants were asked to remain seated in a comfortable environment for 10 min. A single 5-mL blood sample was collected via venepuncture from an antecubital vein by a trained and experienced phlebotomist, using a BD collection set and blood was dispensed into a serum tube. The sample was maintained for at least 1 hour at room temperature to allow clotting before being spun in a centrifuge (at 4000 rpm for 15 minutes).

For the studies reported in Chapters 5 and 6 on arrival to the laboratory, the participants were asked to lie on a plinth and a trained and experienced phlebotomist positioned a cannula (BD Nexiva Closed IV Catheter System) in an antecubital vein. After being sure the participant was comfortable with the procedure, the participant was asked to move to a seated position for 15 minutes. After this period, an 8 mL blood sample was obtained using a 10 mL dry syringe and it was dispensed into three tubes: 2 mL into an EDTA tube that was kept in an ice bath, 2 mL into another EDTA tube and 4 mL into a serum tube which were maintained at room temperature for the duration of the trial. These blood volumes were collected at each time point in both studies. Before each blood sample was taken, the participants were seated for at least 10 minutes to avoid any postural changes in blood and plasma volume, however, participants largely remained seated throughout the trials with the exception of the requirement to stand to empty their bladder each hour. In the study reported in Chapter 5, the blood EDTA samples that were kept in the ice bath were used for hormone analysis (aldosterone and vasopressin), from the EDTA samples that were kept at room temperature duplicate aliquots (100  $\mu$ l) were used for an immediate deproteinization in 1 mL of ice cold 0.3 N perchloric acid (PCA) that was centrifuged later and the supernatant was used to determine glucose (Maughan 1982). The rest of the blood in the EDTA tube was used for the determination of haematocrit and haemoglobin to calculate changes in blood, plasma and cell volumes (Dill and Costill 1974) and the serum samples were used to determine osmolality and electrolytes. For the study described in Chapter 6, the serum samples were used to determine osmolality, electrolytes, and creatinine.

In the studies reported in Chapters 4a, 5 and 6, after collecting the blood samples (via venepuncture or through cannulation), the participants were asked to completely empty their bladder and collect the entire urination volume in a 2 L wide neck bottle. The urine

voided mass was measured using a set of electronic scales (to the nearest 1 g) with the mass of the empty plastic bottle subtracted to enable the estimation of the urine volume. The urine mass was obtained making the assumption that 1g of urine equals 1mL. A small and variable error is introduced by the fact that the specific gravity of urine is not equal to 1.0, but is usually about 1.001 – 1.030 (Lentner, Lentner et al. 1981). The small error introduced would not be meaningful. For example, if the urine specific gravity of 300 g of a sample was 1.020, the urine volume would be 294.11 mL, representing a 1.96% difference if it was considered as 300 mL. An aliquot of 5 mL from each urine sample collection was transferred for further analysis into a plain screw-capped tube. Near nude body mass (only underwear) of participants was obtained to the nearest 10g behind a screen. After the volunteers ingested the tested drink, they were asked again to void the bladder in the container and the process of obtaining the mass and collecting an aliquot was repeated. The procedures were repeated again at the end of each hour of the study period. If the participants needed to urinate before the sample time point (end of each hour), this urine volume was retained and it was mixed thoroughly with the urine collected at the end of the hour. Urine samples were stored for a maximum of 7 days at 4°C. Determination of urine osmolality was performed in the next 48 hours and if the study required it, the determination of sodium and potassium concentrations were done in a maximum period of 7 days after obtaining the sample. When these analyses were complete, the samples were frozen and stored at -80°C for any subsequent analysis.

## 2.4 Body mass

In the study reported in Chapter 3, nude body mass was recorded before and after each exercise bout from a set of scales (Seca, Birmingham, UK). Participants were asked to dry themselves to remove any residual sweat left on their bodies behind a screen before their body mass was obtained.

In the studies reported in Chapters 4a, 5 and 6, near-nude body mass (underwear only) was measured to the nearest 10 g (Jadever IP68 Waterproof Floor Scale) behind a screen.

## 2.5 Drinks and drink preparation

In the studies reported in Chapters 4a, 5 and 6 each participant ingested still water (Highland Spring<sup>®</sup>) as control and three other drinks in a randomized, counter-balanced order. In the study reported in Chapter 4a, the participants ingested 3 drinks from a group of 12 commonly used beverages (sparkling water, cola, diet cola, sports drink, oral rehydration solution, orange juice, lager beer, hot black coffee, hot black tea, cold black tea, full fat milk and skimmed milk). The hot drinks were brewed with freshly boiled still water and served at 60 °C with the temperature maintained in a hot water bath. The hot drinks were ingested with no sugar/no milk added. In the study reported in Chapter 5, the drinks with different carbohydrate concentrations were prepared using sucrose, still water (Highland Spring<sup>®</sup>) and a lemon no sugar added concentrated squash. In the study reported in Chapter 6, the participants ingested apart from the control drink (water), orange-mango juice, a sports drink and skimmed milk. All cold drinks were stored at a standard refrigerated temperature (4-6 °C) until serving. All drinks were tested for osmolality, sodium and potassium after preparation within 48 h and 5 d after collection respectively.

## 2.6 Osmolality determination

The osmolality determination was based on the principle that increased concentration of a solute in a solution causes lowering of its freezing point and thus increases osmolality. This method is known as freezing point depression osmometry. For all the studies, a Löser osmometer from Camlab was used. The osmometer was calibrated using 3 known controls: zero (deionized water), a 300 mOsm/kg and a 900 mOsm/kg standard solutions. Two 100 µl aliquots of sample (urine or serum) were transferred into a 1.5 mL microtube. Each microtube was positioned in the measuring head. The freezing point was determined and the osmolality was shown on the digital display. All the samples were measured in duplicate.

## 2.7 Blood sampling

In studies reported in Chapters 4a, 5 and 6, blood was collected through venepuncture (BD Vacutainer blood collection set 21G x .75") or using a cannula (BD Nexiva 20G x 1.25"). The general procedure for blood sampling was as follows: The most common site for

venepuncture was at the antecubital fossa, located at the anterior and medial aspect of the elbow. The median cubital, the cephalic and the basilica veins lie close to the surface of the skin at this point, and this makes them visible or palpable, and consequently they are easily accessed. It has also been observed that the use of these veins can minimise discomfort with venepuncture/cannulation (Yamada, Yamada et al. 2008).

In the studies reported in Chapters 5 and 6, the blood samples were obtained through cannulation and drawn into a sterile plastic syringe before being dispensed in the required blood collection tubes for each experiment. Before each blood sample was drawn, the participants were seated for at least 10 minutes to avoid any impact of postural changes on estimates of changes in blood and plasma volume.

## 2.8 Electrolytes determination

The method used to determine electrolytes concentration in studies described in Chapters 4a, 5 and 6 was flame photometry. The principle of this method relies upon the fact that compounds of the alkali and alkaline earth metals can be thermally dissociated in a flame and that some of the electrons will be further excited to a higher energy level and when they return to the ground state they emit light that falls in the visible region of the spectrum. Each element has a specific wavelength. For example, the element emission wavelength for sodium (Na) is 589 nm, producing a yellow flame and for potassium (K) the wavelength is 766 producing a violet flame colour. At certain ranges of concentration, the intensity of the emission is directly proportional to the number of atoms returning to the ground state. The flame photometer has a photo detector that measures the intensity of the light emitted by the flame based on the principle that the light emitted is proportional to the sample concentration.

Samples and Standard solutions were diluted using a Hamilton Microlab 500 Dispenser using a flame photometer diluent solution in a 1:200 ratio (dilution factor of the diluent solution was 1ml of flame photometer diluent concentrate in 1L of DI water). Samples and standard solutions were dispensed in duplicate into borosilicate glass tubes. A calibration curve was obtained using different standard solutions and the flame photometer was blanked with the "0" solution (deionized water). Using the highest concentration in the standard solutions (standard solutions for sodium: 0 mmol/L, 25mmol/L, 50 mmol/L and 100 mmol/L; for

potassium: 0 mmol/L, 2.5 mmol/L, 5 mmol/L and 10 mmol/L), the flame photometer was set up, the intermediate concentration solutions were measured to develop the different points of the calibration curve (Calibration curves mean  $r^2=0.996$ ). Two serum controls with known electrolyte concentrations were used to verify the standard curve. For sodium concentration analysis, the serum samples were pre-diluted 1:2 and when the urine samples overpassed the highest concentration standard solution (100 mmol/L) they were also diluted in 1:2 ratio. For potassium concentration, the urine samples they were pre-diluted 1:12 to fall in the range of the calibration curve. The sample tubes were read on the flame photometer and every 10 samples, the blank and the highest concentration standard solution were read again to verify the readings were still correct.

## 2.9 Hormone analysis

In the study described in Chapter 5, aldosterone and vasopressin were determined. The samples used for hormone analysis were whole blood in EDTA tubes that were immediately kept in an ice bath, then they were centrifuged at 4000 rpm for 15 minutes. The plasma was transferred to Eppendorf tubes and stored at  $-80^{\circ}\text{C}$ .

### 2.9.1 Aldosterone

In the study reported in Chapter 5, plasma aldosterone concentration was determined using the Enzo Life Sciences Aldosterone Enzyme Immunoassay (ELISA) kit.

When the assay was performed, the samples were thawed leaving them at room temperature for 60 minutes and then they were mixed using a vortex for 15 seconds each. When the samples were ready, they were diluted 1:8 before starting the assay procedure. The assay procedure as indicated in the manual was followed.

### 2.9.2 Vasopressin

In the studies reported in Chapters 5 and 6, plasma aldosterone concentration was determined using the Enzo Life Sciences Arg<sup>8</sup>-Vasopressin ELISA kit. To perform the assay, it was required to do an extraction on the samples. 500 $\mu\text{L}$  of plasma were pipetted into 2mL Eppendorf tube. Then 1 mL of ice cold acetone was added and the sample was vortexed, after this, the samples were centrifuged at 12,000g for 20 min. 300 $\mu\text{L}$  of supernatant were transferred to a new microcentrifuge tube. 1.5 mL of ice cold petroleum ether was added to

each of the samples. They were vortexed and centrifuged again. The top ether layer was discarded and 200µL of the remaining aqueous layer was transferred to another tube for each sample. The samples were then dried down in a centrifugal evaporator at room temperature for 24h. After this process, the samples were reconstituted with 200µL of assay buffer. After the extraction was completed, the procedure indicated in the kit manual was followed.

## 2.10 Haematocrit

In the studies described in the Chapters 5 and 6, haematocrit was determined. The principle of this method is anticoagulated whole blood centrifugation with the volume occupied by the red cells expressed as a percentage of the total volume. Haematocrit was determined with whole blood anticoagulated with EDTA and it was analysed within six hours of collection as it was stored at room temperature. Samples were prepared in triplicate. After mixing thoroughly on a mixer roller for at least 30 minutes capillary tubes were filled 2/3 to 3/4 full. Capillary tubes were sealed placing them into the sealant (Critoseal TM) at a 90-degree angle. Tubes were then placed in the micro haematocrit centrifuge with the sealed end toward the periphery. Centrifuge should be well balanced before starting the spinning period. The samples were centrifuged for five minutes. Haematocrit was determined using a micro haematocrit reading device. Results were recorded to the nearest 0.5 unit (%) whole number.

## 2.11 Haemoglobin

To determine haemoglobin concentration, in the studies reported in Chapters 5 and 6, Haemoglobin reagent set was used (BioSupply UK). This set is based on the cyanmethemoglobin method that is the most widely accepted (Eilers 1967). The principle of this method is as follows: erythrocytes are lysed by a stromatolytic agent in the presence of a surfactant and release their haemoglobin into solution. Haemoglobin is oxidized to methaemoglobin by ferricyanide, and the methaemoglobin is converted in to the stable cyanmethemoglobin by addition of KCN (Potassium cyanide). The absorbance of cyanmethemoglobin is measured at 540 nm and colour intensity is proportional to haemoglobin concentration.

The samples that were analysed were whole blood with EDTA as anticoagulant and they were mixed on the roll mixer for at least 30 minutes at room temperature prior to assay. Using the Hamilton Microlab 500 Dispenser 0.01 mL (10 µl) of each sample was dispensed with 2.0 mL of haemoglobin reagent into borosilicate tubes in duplicate. There were also duplicate blank tubes where just haemoglobin reagent was dispensed, and duplicate standard tubes (15 mg/dL). Likewise, there were control solutions dispensed alongside the sample dispensing procedure to ensure that the standard curve was correct. All tubes were allowed to stand for up to 30 minutes at room temperature. The spectrophotometer (Hitachi U-2001) was set to 540 nm (previous warm up of the lamp). The spectrophotometer was zeroed with the reagent blank and the range was calibrated with the 15 mg/dL standard solution before each sample was read in duplicate.

In studies reported in Chapters 5 and 6, haematocrit and haemoglobin concentrations were used to estimate changes in blood, plasma and red cell volume relative to the baseline blood sample based on Dill and Costill method (1974).

## 2.12 Calculations performed

### 2.12.1 Net fluid balance

Net fluid balance describes the equilibrium of the input and the output of fluids in the body to allow metabolic processes to function correctly (Welch 2010). For the studies reported in Chapters 5, 6 and 7 net fluid balance was calculated based on the urine output mass per time point. All the participants started at zero and then achieved a positive net fluid balance of 1000 (g) based on the fixed volume of the fluid they were asked to drink (1 L). To calculate the fluid balance at any time point given, the total mass of urine (cumulative urine mass) that had been collected following the drinking period was subtracted from the initial fluid balance.

$$\text{Initial Fluid Balance} = \text{IFB} = 1000 \text{ g}$$

$$U_n = \text{urine mass at } n \text{ time point}$$

$$\text{Net fluid balance after 4 hours} = \text{IFB} - (U_1 + U_2 + U_3 + U_4)$$



### 2.12.2 Blood volume, plasma volume and cell volume changes.

In the studies reported in Chapters 5 and 6, the changes in blood volume, plasma volume and cell volume relative to the baseline blood samples were estimated using Dill & Costill method (1974).

### 2.12.3 Beverage Hydration index

In the studies reported in Chapters 4, 5 and 6 a beverage hydration index (BHI) was calculated for different drinks and different populations. The hydration index was obtained by dividing the total urine output over a period of time when the control drink was ingested (still water – Highland Spring<sup>®</sup>) by the total urine output for the same period of time with the intake of another beverage.

$$BHI = \frac{\text{Total urine output when control was ingested}}{\text{Total urine output when a test drink was ingested}}$$

The water content of the drinks used in the studies reported in Chapter 5, 6 and 7 varied from 100% to 87%, and consequently the amount of water ingested varied between drinks. It was deemed appropriate to do a correction to the HI taking into account the different volumes of water actually ingested in the different trials. The HI was normalised accordingly to the water content of the drinks to reflect the effect of the drink itself, excluding the differences in water content.

### 2.13 Coefficient of variation for analytical procedures.

The coefficient of variation (CV) was calculated as the standard deviation of the difference between duplicates and expressed as a percentage of the mean value obtained for samples produced in the studies reported in this thesis (Hopkins, 2000). To obtain the CV for each procedure, analysis of replicates was used, except for the DXA estimations that data were obtained actually from scans analysed in the study.

The following table (**Table 2-1**) shows the CV for analytical procedures performed in the studies reported in this thesis.

**Table 2-1. Coefficient of variations for the analytical procedures performed in the studies described in this thesis.**

Assay/Procedure	CV (%)	Range
Difference between trials DXA estimated total mass (before exercise) (g)	1.17	-2007 to 1714
Difference between trials DXA estimated whole body fat-free soft tissue mass (before exercise) (g)	1.24	-1503 to 1466
Difference between trials DXA estimated whole body fat (before exercise) (g)	3.92	-1201 to 1309
Beverage hydration index	18.00	0.89-1.54
Serum osmolality (mOsm/kg)	0.23	289 to 292
Serum sodium concentration (mmol/L)	0.97	137 to 142
Serum potassium concentration (mmol/L)	2.74	3.0 to 3.3
Urine osmolality (mOsm/kg)	0.23	677 to 681
Urine sodium concentration (mmol/L)	1.96	86 to 92
Urine potassium concentration (mmol/L)	3.09	41 to 45
Blood glucose concentration (mmol/L)	2.71	4.125 to 4.367
Insulin ( $\mu$ U/mL)	6.52	9.873 to 10.828
Plasma aldosterone concentration (pg/mL)	3.23	152.3 to 159.4
Plasma vasopressin concentration (pg/mL)	12.83	2.48 to 4.46
Creatinine (Serum) ( $\mu$ mol/L)	4.55	68-79
Creatinine (Urine) ( $\mu$ mol/L)	0.80	9437-9720

## CHAPTER 3. ERRORS IN DUAL ENERGY X-RAY ABSORPTIOMETRY ESTIMATION OF BODY COMPOSITION INDUCED BY HYPOHYDRATION

### ABSTRACT

This study examined the impact of hydration status on Dual energy x-ray absorptiometry (DXA) and other methods that are popular tools to determine body composition (BC) in athletes. DXA is often used for analysis of fat-free soft tissue mass (FFST) or fat mass (FM) gain/loss in response to exercise or nutritional interventions. The aim of this study was to assess the effect of exercise-heat stress induced hypohydration (HYP, >2% of body mass (BM) loss) vs. maintenance of euhydration (EUH) on DXA estimates of BC, sum of skinfolds (SF), and impedance (IMP) measurements in athletes. Competitive athletes (23 males and 15 females) recorded morning nude BM for 7 days before the first main trial. Measurements on the first trial day were conducted in a EUH condition, and again after exercise-heat stress induced HYP. On the second trial day, fluid and electrolyte losses were replaced during exercise using a sports drink. A reduction in total BM (mean  $\pm$  SD) of  $1.6 \pm 0.4$  kg;  $2.3 \pm 0.4\%$  HYP, and total FFST ( $1.3 \pm 0.4$  kg), mainly from trunk ( $1.1 \pm 0.5$  kg), was observed using DXA when participants were HYP, reflecting the sweat fluid losses. Estimated fat percent increased ( $0.3 \pm 0.3\%$ ), however, total FM did not change ( $0.1 \pm 0.2$  kg). SF and IMP declined with HYP (losses of  $1.5 \pm 2.9\%$  and  $1.6 \pm 3\%$  respectively) suggesting FM loss. When EUH was maintained there were no significant changes in BM, DXA estimates, or SF values pre to post exercise, but IMP still declined. We conclude that use of DXA for FFST assessment in athletes must ensure a EUH state, particularly when considering changes associated with nutritional or exercise interventions.

### 3.1 Introduction

Body composition assessment is an important part of athlete monitoring and is widely used to assess changes following exercise or nutritional interventions. Athletes competing in gravitational, weight class and aesthetic sports often reduce their body mass / fat mass, or maintain it as low as possible to gain a competitive advantage. In extreme cases, athletes could develop severe medical problems sometimes with fatal consequences (American College of Sports, Sawka et al. 2007). Considering these practices, the International Olympic Committee Medical and Scientific Commission set up a Working Group on Body Composition Health and Performance to determine whether optimum body composition and/or minimum values for body fat content and body water content could be established (Sundgot-Borgen, Meyer et al. 2013). Publications arising from the IOC working group highlight that greater understanding of factors influencing all aspects of body composition estimation is important (Ackland, Lohman et al. 2012, Meyer, Sundgot-Borgen et al. 2013, Sundgot-Borgen, Meyer et al. 2013).

Dual energy X-ray absorptiometry (DXA) was originally designed to measure specific bone regions (bone mineral density of hip and spine) of older adults, has been used for over two decades, and is considered the gold standard technique for these assessments (Blake and Fogelman 2009). More recently DXA has become a popular and accessible tool to determine fat and lean tissue composition. Many factors affect body composition estimation by DXA, one of which is soft tissue hydration. DXA scanning assumes soft tissues are normally hydrated for accurate partitioning into fat and lean fractions (Plank 2005) and that there is a constant hydration status of fat-free soft tissue mass (73%) ((Pietrobelli, Wang et al. 1998). However, hydration of fat-free soft tissue mass can range from 67-85% (Moore and Boyden 1963). Acute changes in hydration status can therefore alter fat-free soft tissue mass DXA estimates (Lohman, Harris et al. 2000). Several clinical studies suggest DXA is able to detect small individual changes in total mass, soft and lean tissue mass in healthy adults and patients (Going, Massett et al. 1993, Kohrt 1995, Kohrt 1998, Pietrobelli, Wang et al. 1998). To date, the effect of hypohydration followed by rehydration on DXA estimates of fat-free soft tissue mass and fat mass has only been analysed in a non-athletic group using a 24h fluid restriction protocol (Going, Massett et al. 1993) and has not used the most recent scanning technology. Therefore, analysing and understanding the effects of hypohydration on body composition estimation in an athlete population could be very important for sports nutrition practitioners and researchers. This is particularly true when assessing minimum body fat

criteria and/or fat-free soft tissue mass changes in response to nutritional or exercise interventions.

Studies on the influence of daily activities, meal ingestion, and acute exercise on body composition estimates have already been performed in healthy controls (Horber, Thomi et al. 1992) and more recently on athletes (Nana, Slater et al. 2011, Nana, Slater et al. 2013). Furthermore, previous work has not compared DXA estimates with skinfolds/impedance analysis outcomes following an acute fluid deficit. We hypothesize that DXA estimates of fat-free soft tissue mass will track fluid balance deficits incurred during exercise-heat stress and that control of hydration status is a crucial part in assessment of body composition in athletes.

## 3.2 Methods

We recruited 38 participants (23 males, 15 females) from different athletic clubs representing the range of physiques found among athletic populations. The study was approved by the University of Stirling Research Ethics Committee and the NHS East of Scotland Research Ethics Committee. Participants were excluded from the study if they were older than 40 years (older than typical athletic population on whom our research is focused) or not currently training / competing in their sport. Participants were involved in a range of sports (running, cycling, rowing, rugby, boxing, football, gymnastics, triathlon, martial arts, rock climbing, and tennis). Subject characteristics were: age  $28.1 \pm 5.5$  years, height  $172.6 \pm 9.3$  cm, stable baseline body mass  $69.5 \pm 10.6$  kg. Females were asked to complete all laboratory visits during the same menstrual phase to avoid potential changes in body fluid and body mass. To achieve this we obtained menstrual cycle phase history information from them prior to, and during participation in the study.

### 3.2.1 Early morning body mass measurement

Participants were provided with a set of scales (Seca Quadra 808, Birmingham, UK) to record body mass for 7 days before the first test in their own homes. The scales were individually calibrated against known mass (range: 0-90 kg) prior to use. Calibration correlation coefficients were 0.99-1.00. Morning, fasted, nude body mass was recorded after emptying

bladder and bowels to establish stability of body mass over the period before starting the trials. To reduce potential variance all participants used the same set of scales throughout the entire study period. No correction was applied to mass recordings to account for the slight differences between sets of scales.

### 3.2.2 Study design overview

Participants attended the laboratory on three occasions. The first visit was for pre-screening, signing consent and issuing of scales for daily body mass recording. The second visit was 1 week later and was the first main trial day (Day 1) involving anthropometric measurements, impedance analysis and then DXA scanning. These measurements were conducted on entering the laboratory in a euhydrated condition, and again after a period of exercise-heat stress aimed at producing a fluid deficit of  $\geq 2\%$  of the initial body mass. A further week later participants attended for a final visit (Day 2) in which we repeated all of the measurements and the same exercise-heat stress work period as Day 1, but fluid losses and estimated energy/glycogen usage were replaced using a carbohydrate-electrolyte sports drink (Gatorade<sup>®</sup>) to maintain body mass (**Figure 3-1A**).

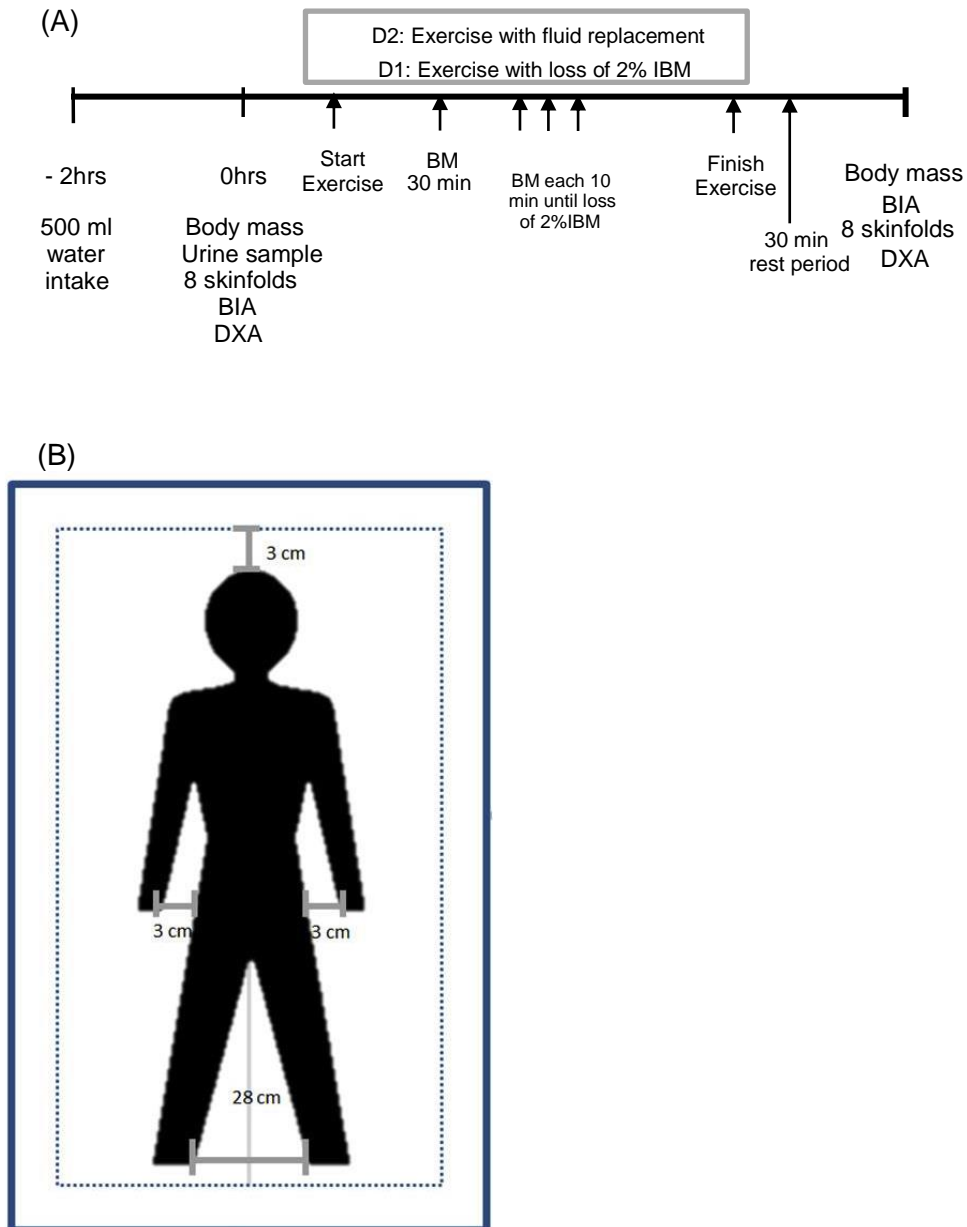
### 3.2.3 Standardized baseline conditions

For both days of testing, participants were asked to attend to the laboratory in the morning after fasting for at least eight hours, without doing strenuous exercise or ingesting alcoholic beverages the previous day. Participants were instructed to drink 500ml of water 2h before entering the laboratory to ensure euhydration. On arrival at the laboratory, participants emptied their bladder and bowels and provided a urine sample for initial hydration status assessment. Urine samples were measured for osmolality using a freezing point depression osmometer (Roebbling, Camlab, UK). Initial nude body mass and anthropometric measures including stature and 8 skinfolds (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh and medial calf) using a Harpenden caliper were recorded. Land marking and measurements were done by the same International Society for the Advancement of Kinanthropometry (I.S.A.K.) level 3 trained anthropometrist following the International Standards for Anthropometric Assessment (Stewart 2011). Impedance was then estimated using a single frequency (50 kHz) bioelectrical impedance analysis device

(Bodystat 1500) with participants in a supine position. All readings were obtained within 1 minute of adopting the supine posture. This procedure was to avoid erroneous impedance readings through fluid shifts that occur with prolonged periods in this position (Shirreffs and Maughan 1994). Following these initial measurements participants were carefully positioned for one whole body DXA scan.

#### 3.2.4 DXA scan

Body composition was measured from a whole body scan using a narrowed fan-beam DXA (iDXA GE Healthcare) with analysis performed using GE Encore 13.40.038 Software (GE Healthcare). All scans were performed and analysed by the same trained technician. iDXA was calibrated with phantoms as per manufacturer guidelines each day before measurement. All scans were undertaken using the standard thickness mode; automatically chosen by the software. Subjects wore minimal clothing (underwear) and removed jewellery and metallic objects for scans. We established a protocol for undertaking whole body scans which emphasized consistency in the positioning of subjects on the scanning area of the DXA instrument (**Figure 3-1B**).



**Figure 3-1 Study design (A). The exercise time varied depending on each subject and positioning protocol (B) for dual energy X ray absorptiometry (DXA).**

Subjects were centrally aligned in the scanning area of the DXA instrument, we measured 3 cm from the distance of the top line drawn on the surface of the bed to the vertex of the head of the participants, the hands were in a prone position and we ensured that the distance between the thumbs and the legs was 3 cm, we placed a foam block between their feet which was transparent under the DXA scan to maintain a constant distance between the feet of 28 cm in each scan. All the distances were measured with a metric ruler in each scan. The scans were analysed automatically by the software, with regions of interest subsequently confirmed by the technician prior to data analysis. IBM= initial body mass.



### 3.2.5 Exercise protocol

Following scanning, participants undertook an exercise-heat stress protocol. On Day 1, exercise was performed without any food/fluid ingestion. Exercise was conducted on a stationary cycle ergometer (Monark 874E) in a warm environment ( $26.4\pm 0.9^{\circ}\text{C}$ ) with participants wearing a plastic bin bag and warm clothing to enhance heat stress. The protocol consisted of 30 minutes of cycling at a predetermined fixed load ( $160\pm 25\text{W}$  (males),  $94\pm 16\text{W}$  (females)) and pedal cadence (70 rpm) followed by subsequent 10 minute bouts. Between exercise bouts participants were asked to dry themselves off before undertaking a nude body mass measurement. Nude body mass measurement was required to ensure sweat in clothing did not influence mass loss assessment, and all measures were made with participants behind a privacy screen. Repeated bouts of cycling were performed until a 2% body mass loss was achieved. Activity duration, heart rate, power and pedal cadence were recorded during exercise. Following a 30 minute rest period to cool down, shower, and empty bladder we obtained final nude body mass, skinfold, impedance, and DXA measures.

On Day 2 participants replicated the exact intensity and duration of exercise performed on Day 1, with ingestion of a known volume of sports drink (Gatorade®) to replace the fluid losses experienced on Day 1. On Day 2, the aim was to maintain initial body mass and euhydration status. The study was not counter-balanced because sweat losses were determined on day 1 and on day 2, the sweat losses were replenished to ensure euhydration.

### 3.2.6 Statistical analysis

Statistical analyses were conducted using Minitab, version 16.1.0. For tabulated and graphical data, mean and standard deviation (SD) values were used. Differences related to pre- or post-exercise scanning, gender or hydration status were tested using repeated measures analysis of variance and general linear model. P values  $< 0.05$  were considered significant. Reliability measures for pre- and post-exercise anthropometric measurements, impedance and DXA scans also were conducted. Paired t-tests were used to assess whether absolute differences existed between pre-exercise measurements or measurements pre- and post-exercise on Days 1 and 2 also to analyse pre exercise characteristics on both days to analyse if there was any difference in baseline conditions.

We also compared the results from repeat DXA scans and calculated the coefficient of variation (CV); defined by the SD of difference in duplicate measurements expressed as a percentage of the overall mean data (Hopkins 2000).

### 3.3 Results

Analysis of menstrual cycle phase history (female participants) revealed 40% (n=6) were in follicular phase, 53% (n=8) in luteal phase and 7% (n=1) presented amenorrhea confirmed by a sports medicine physician.

#### 3.3.1 Reliability of baseline measures and conditions prior to and during each trial day

Participant body mass was not different across the 7 days preceding the trials and on trial days (**Figure 3-2A**). Urine osmolality (Day 1, 268 mOsm/kg (min: 98, max: 1203); Day 2, 290 mOsm/kg (min: 94, max: 1196)) demonstrated that most participants were generally well hydrated based on ACSM euhydration criteria (American College of Sports, Sawka et al. 2007). However, urine osmolality values were sometimes variable within individuals between trials (**Figure 3- 2B**) despite following the pre-trial water ingestion criteria.

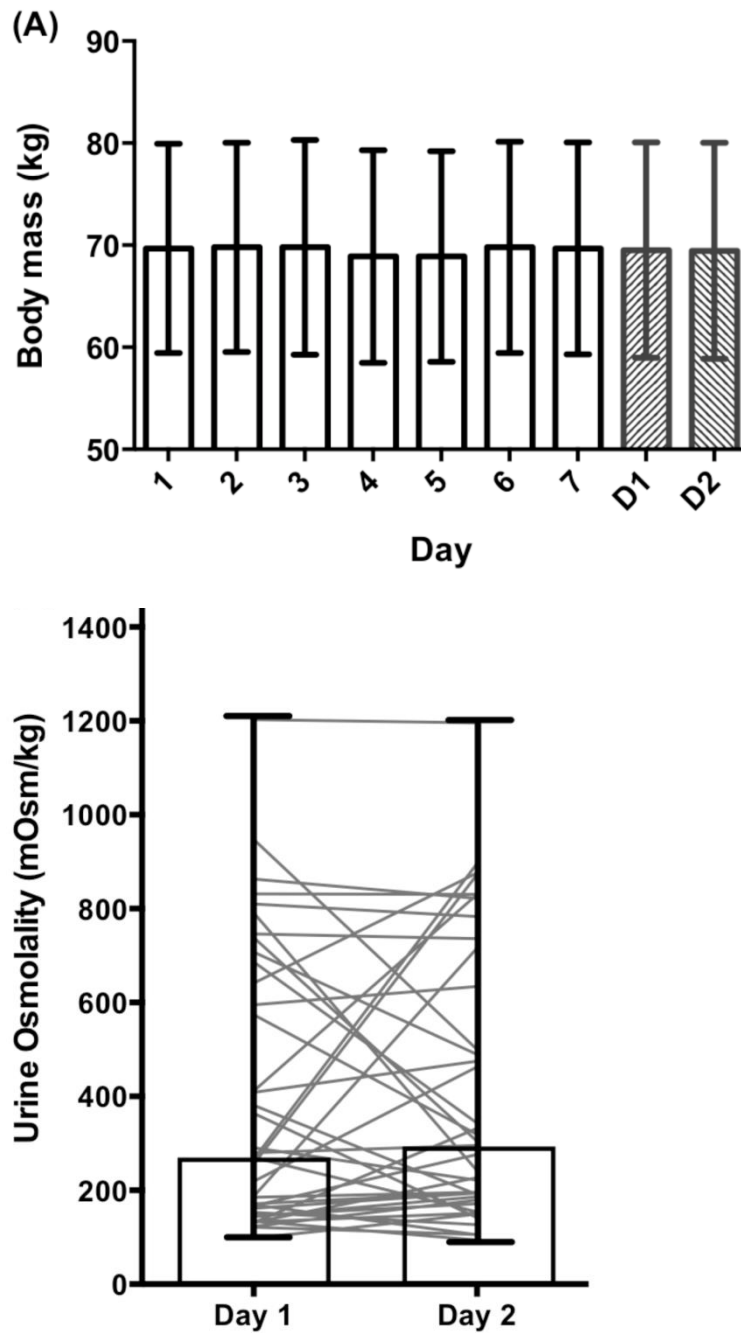


Figure 3-2 Mean (SD) nude body mass (A) of the participants over 7 days prior to beginning the trials and also the body mass before exercise on both trial days (D-1 and D-2) and urine osmolality (B) determined on attending the laboratory on Day 1 and Day 2 for initial hydration status assessment.

Values are shown as the median (range) for n=38. Individual data are also plotted in the figure.

All other pre-exercise data, including DXA measurements, were consistent between trial days. When analysing CV for the DXA body composition estimates between the pre-exercise scans on Day 1 and on Day 2, data were considered reliable. We found trivial CV of bone mineral density, fat expressed as a percentage, whole body tissue mass, whole body fat mass, whole body fat-free soft tissue mass and estimated body mass demonstrating minimal variability between Day 1 and Day 2 (**Table 3-1**). Room temperature (Day 1; 26.4±0.9°C, Day 2; 26.7±0.9°C) and relative humidity (Day 1; 37±5%, Day 2; 38±6%) were similar between trials. Average exercise duration for 2% body mass reduction was 60.9±12.1 min (males: 55.7±11.2 min; females: 69.0±8.5 min). Average exercising heart rate was 155±13 beats per minute (Day 1) and 150±14 beats per minute (Day 2) representing (81±7 and 78±8% of age predicted maximum heart rate, respectively (**Table 3- 2**).

**Table 3-1 Baseline conditions prior to exercise for each trial day showing percentage change (%Δ), and coefficient of variation (CV %) between the Day 1 and Day 2 pre-intervention values.**

	Pre-Day 1	Pre-Day 2	%Δ	CV (%)
<b>Body Mass (kg)</b>	69.5 (10.6)	69.5 (10.6)	-0.09	0.99
<b>Sum of skinfolds (mm)</b>	89.6 (35.4)	88.4 (34)	-1.08	6.97
<b>Impedance (Ω)</b>	504 (66)	507 (69)	0.68	21.08
<b>Bone mineral density (g/cm<sup>2</sup>)</b>	1.243 (0.142)	1.239 (0.139)	-0.30	1.05
<b>Whole body tissue (% Fat)</b>	20.9 (7.1)	20.8 (7.1)	-0.08	3.30
<b>Whole body tissue (kg)</b>	67.1(10.2)	67.0 (10.3)	-0.11	1.17
<b>Whole body fat (kg)</b>	13.8 (4.6)	13.8 (4.5)	-0.40	3.92
<b>Whole body fat-free soft tissue mass (kg)</b>	53.3(10.4)	53.3 (10.4)	0.01	1.24
<b>Bone mineral content (kg)</b>	2.9 (0.6)	2.9 (0.6)	0.05	1.24
<b>DXA estimated total mass (kg)</b>	70.0 (10.6)	69.9 (10.7)	-0.09	1.17

Table 3-2 Exercise and environmental characteristics recorded on each trial day.

	Day	All (n=38)	Males (n=23)	Females (n=15)
<b>Heart rate during exercise (bpm)</b>	1	155 (13)	156 (14)	154 (11)
	2	150 (14)	152 (14)	146 (13)
<b>% age predicted max heart rate</b>	1	81 (7)	82 (8)	80 (6)
	2	78 (8)	80 (8)	76 (7)
<b>Power (W)</b>	1	134 (39)	160 (25)	94 (16)
	2	135 (39)	160 (26)	96 (14)
<b>Room temperature (°C)</b>	1	26.4 (0.9)	26.4 (1.0)	26.4 (0.6)
	2	26.7 (0.9)	26.6 (1.0)	26.9 (0.7)
<b>Relative humidity (%)</b>	1	37 (5)	37 (5)	35.6 (2.9)
	2	38 (6)	38 (5)	38.3 (7.0)
<b>Exercise duration (min)</b>	1 & 2	60.9 (12.1)	55.7 (11.2)	69.0 (8.5)

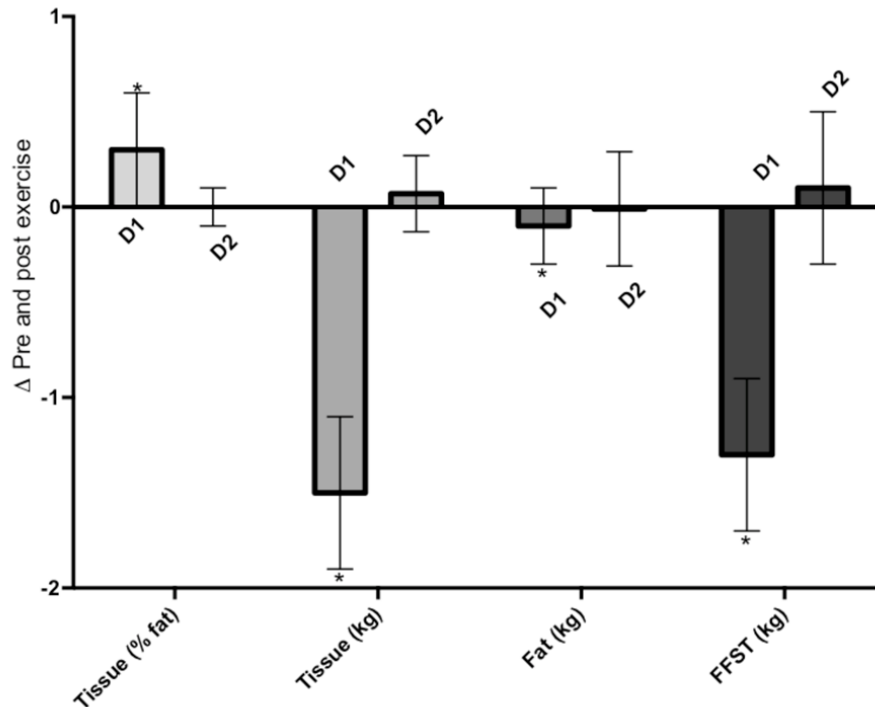
Day 1 refers to the hypohydration trial; Day 2 refers to the euhydration trial.

### 3.3.2 Effects of exercise induced hypohydration on body mass and estimates of body composition

Exercise on Day 1 led to a mean body mass reduction of  $1.6 \pm 0.4$  kg ( $2.3 \pm 0.4\%$  hypohydration). Sex differences in losses were  $1.8 \pm 0.3$  kg (males) and  $1.3 \pm 0.3$  kg (females); representing  $2.4 \pm 0.4\%$  and  $2.2 \pm 0.3\%$  hypohydration, respectively. On Day 2 the mean fluid intake to match sweat losses was  $1.5 \pm 0.4$  L ( $1.6 \pm 0.3$  L (males) and  $1.2 \pm 0.3$  L (females)) and body mass was maintained during the exercise period.

DXA body composition values (including bone mineral density (BMD) and fat, lean and total mass), sum of skinfolds, and impedance are summarized in Table 3. On Day 1 there was a statistically significant reduction of  $1.5 \pm 0.4$  kg (2.2%) in total tissue mass and  $1.3 \pm 0.4$  kg (2.5%) in fat-free soft tissue mass from pre- to post-exercise (**Figure 3-3**). However, significant increases in fat mass percentage were observed following hypohydration ( $0.3 \pm 0.3\%$ ); no

change in absolute fat mass (kg). With fluid replacement on Day 2 there were no significant changes in any DXA estimates.



**Figure 3-3 Absolute changes from pre to post exercise induced hypohydration on Day 1 and on Day 2:**

Change in fat percentage (tissue %fat), change in tissue mass (tissue, kg) and change in fat mass (Fat, kg) and fat-free soft tissue mass (FFST, kg) estimated by DXA.

(\*indicates significant difference)

Differences in body segment composition (arms, legs and trunk) were also assessed pre- and post-exercise using DXA data (**Figure 3-4**). On Day 1, trunk (tissue fat percentage increased ( $0.5 \pm 0.7\%$ ) while total tissue mass ( $1.2 \pm 0.5\text{kg}$ ) and lean tissue mass ( $1.1 \pm 0.6\text{kg}$ ) decreased, with no changes in segment composition pre- post-exercise noted on Day 2. Sum of skinfolds was significantly lower on Day 1 (**Table 3-3**) following hypohydration ( $1.5 \pm 2.9\%$ ; all participants). Analysed by gender the reduction in males was  $1.4 \pm 3.5\%$  and in females  $1.6 \pm 2.0\%$ . On Day 2, sum of skinfolds decreased by  $0.3 \pm 2.5\%$  (full group) and  $0.1 \pm 2.7\%$  and  $0.8 \pm 2.1\%$ , respectively for males and females, but there was no significant interaction effect. Impedance was significantly reduced by hypohydration on Day 1 (**Table 3-3**) from  $504 \pm 66 \Omega$  to  $495 \pm 64 \Omega$ . On Day 2 there was also a significant reduction in impedance from  $507 \pm 69 \Omega$  to  $498 \pm 61 \Omega$ .

**Table 3-3 Body composition data analysed on each trial day.**

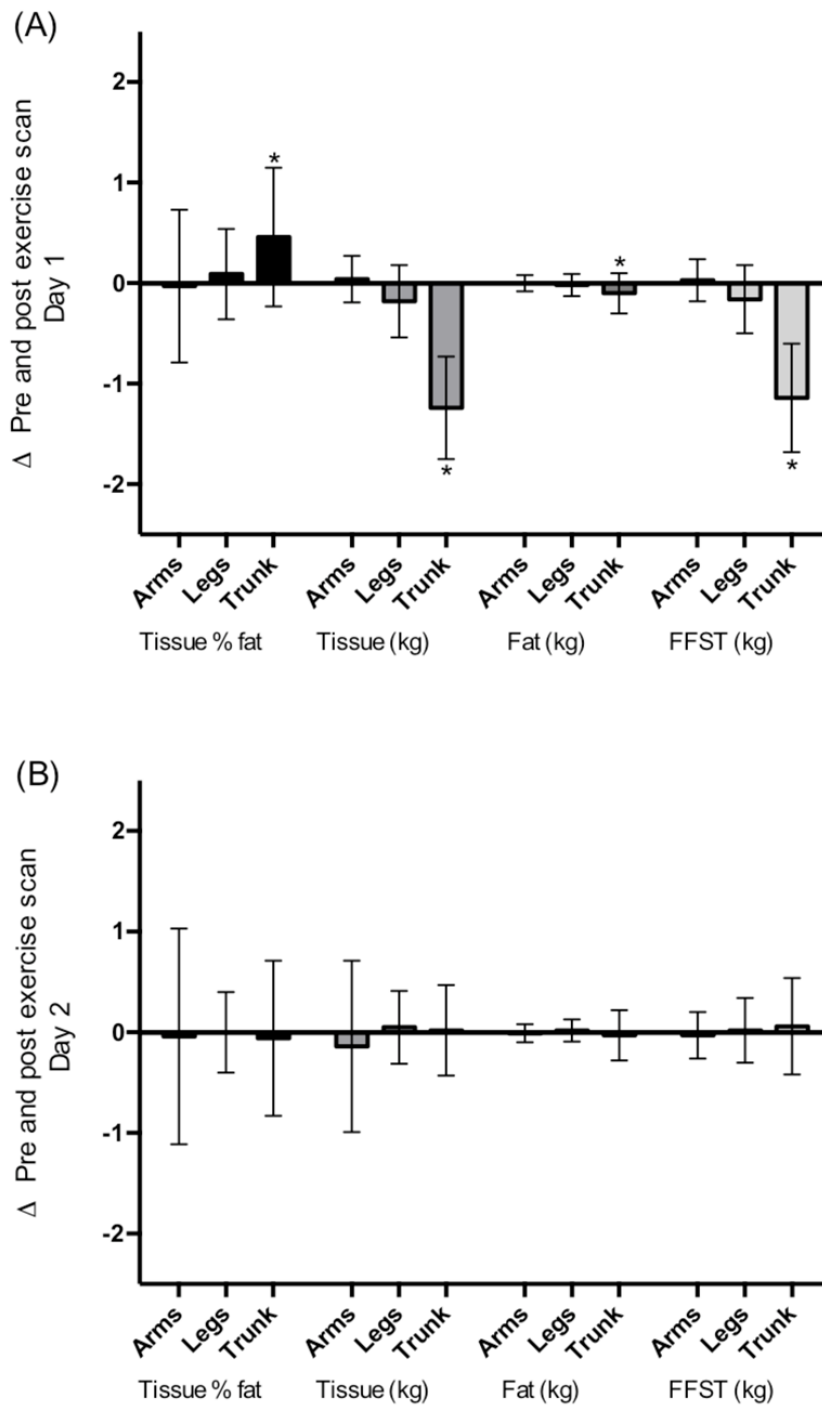
Pre and post exercise (Pre-ex, Post-ex) values, percentage change

pre to post exercise (%Δ), and mean difference (95% confidence interval) between pre and post-exercise values are shown. Day 1

refers to the hypohydration trial; Day 2 refers to the euhydration trial.

	Day	Pre-exercise	Post-exercise	%Δ	Mean diff (95% CI)
<b>Body Mass (kg)</b>	1	69.5 (10.6)	67.9 (10.3)*	-2.28	-1.6 (1.5, 1.7)
	2	69.5 (10.6)	69.5 (10.6)	0.05	0.0 (-0.1, 0.0)
<b>Sum of skinfolds (mm)</b>	1	89.6 (35.4)	88.2 (34.6)*	-1.48	-1.4 (0.6, 2.2)
	2	88.4 (34)	88 (33.2)	-0.25	-0.4 (-0.4, 1.1)
<b>Impedance (Ω)</b>	1	504 (66)	495 (64)*	-1.60	-8 (-4, -12)
	2	507 (69)	498 (61)*	-1.63	-9 (-3, -15)
<b>BMD (g/cm<sup>2</sup>)</b>	1	1.243 (0.142)	1.241 (0.100)	-0.19	-0.002 (-0.002, 0.006)
	2	1.239 (0.139)	1.239 (0.100)	-0.01	0.032 (-0.060, 0.003)
<b>Tissue (% fat)</b>	1	20.9 (7.1)	21.2 (7.2)*	0.28	-0.3 (-0.4, -0.2)
	2	20.8 (7.1)	20.8 (7.1)	-0.39	-0.0 (-0.1, 0.00)
<b>Tissue (kg)</b>	1	67.1 (10.2)	65.6 (10.0)*	-2.19	-1.5 (-1.4, -1.6)
	2	67.0 (10.3)	67.1 (10.3)	0.11	-0.1 (-0.1, -0.1)
<b>Fat (kg)</b>	1	13.8 (4.6)	13.7 (4.5)	-0.92	-0.1 (-0.1, -0.2)
	2	13.8 (4.5)	13.7 (4.5)	-0.10	-0.1 (-0.1, 0.1)
<b>Fat-free soft tissue mass (kg)</b>	1	53.3 (10.4)	51.9 (10.2)*	-2.54	-1.3 (-1.2, -1.5)
	2	53.3 (10.4)	53.4 (10.5)	0.15	-0.1 (-0.2, 0.0)
<b>BMC (kg)</b>	1	2.9 (0.5)	2.9 (0.5)	-0.33	-0.0 (-0.0, -0.0)
	2	2.9 (0.5)	2.9 (0.5)	-0.21	-0.0 (-0.0, -0.0)
<b>Estimated mass (kg)</b>	1	70.0 (10.6)	68.5 (10.4)*	-2.12	-1.5 (1.4, 1.6)
	2	69.9 (10.7)	70.0 (10.7)	0.10	0.1 (0.1, 0.0)

\* indicates significant difference from pre-exercise.



**Figure 3-4 Changes in DXA body composition in different body segments.**

Part A shows absolute changes between pre and post exercise on Day 1. Part B presents the differences between pre and post exercise on Day 2. (\*indicates significant difference)



### 3.4 Discussion

This is the first study in a trained athlete population to examine the effects of combined exercise-heat stress and accompanying hypohydration on DXA estimates of whole and regional body composition. This type of intervention related to hydration status (hypo- and hyper-hydration) has only been analysed previously in non-athletic groups using 24h fluid restriction or dialysis (Horber, Thomi et al. 1992, Going, Massett et al. 1993). In the present study, baseline measures (initial body mass, initial hydration status, sum of skinfolds, impedance and body composition), and environmental conditions were all consistent and demonstrated that under these experimentally controlled conditions estimates of body composition are reliable. In our study, exercise-induced hypohydration reduced total mass, total tissue mass and fat-free soft tissue mass estimates from DXA. With maintenance of euhydration we did not observe any significant differences in estimations and measurements from pre- to post-exercise.

A recent study investigated the effects of exercise and *ad libitum* meal/fluid intake on DXA estimates of body composition in cyclists (Nana, Slater et al. 2013). The authors observed these factors are associated with changes in mean estimates of total and regional body composition that range from trivial to small but substantial (Nana, Slater et al. 2013). The loss of body mass examined in the present study represents a common level of hypohydration that could be presented as a non-optimal hydration strategy or as part of an intentional dehydration to “make the weight” in category classified sports (Sundgot-Borgen and Garthe 2011, Ackland, Lohman et al. 2012, Klungland Torstveit and Sundgot-Borgen 2012). With hypohydration we observed a significant reduction in total tissue and fat-free soft tissue mass determined by DXA from pre- to post-exercise that was not evident when euhydration was maintained. Sum of skinfolds and impedance data demonstrated reductions from pre- to post-exercise on the hypohydration trial suggesting a loss in fat mass that was not evidenced in DXA scan data. This no agreement between DXA and skinfold and impedance results can be explained as follows. Skinfold results correspond with previous studies which have shown hydration affects elasticity and compressibility of tissues modifying the measurement of skinfolds (Ward, Rempel et al. 1999). The output from impedance matches with previous findings which demonstrated small fluid changes (gains or losses) could be misinterpreted as changes in fat content of an athlete (Saunders, Blevins et al. 1998).

DXA changes in body composition by region in the pre- to post-exercise scans on the hypohydration trial revealed changes were mainly localised to the trunk region. The localisation of effects to the trunk could be explained by losses in specific body fluid compartments, particularly blood volume. A reduction in blood volume would lead to reduced central blood volume when lying in a supine position for scanning, as the flow from splanchnic and renal circulations is redistributed (Rowland 2001, Rowland and Roti 2004, Kenney 2008). Although previous studies have found an effect of sex (Buehring, Krueger et al. 2013), the current research observed the significant differences with hypohydration were consistent between genders. When euhydration was maintained there was no significant change in body mass, whole body or regional DXA scan indices, or sum of skinfolds from pre- to post-exercise. A previous study in non-athletes observed, following intake of 0.8–2.4 L of water, that the determination of bone mineral content and of fat mass by DXA were not affected, while estimation of fat-free soft tissue mass in the trunk region was considerably increased (Horber, Thomi et al. 1992). The present work adds to this literature by demonstrating the magnitude of change in fat-free soft tissue mass estimates with a 2% hypohydration in an athlete population.

Participants were asked to carefully control baseline conditions prior to arrival in the laboratory to achieve reliable measurements for body composition from DXA, skinfolds, and impedance analysis. The values obtained were clearly consistent between pre-exercise assessments on the two trial days. This suggests our attempts to avoid variation in baseline body mass and composition, by controlling hydration status, dietary intake, and prior exercise, were effective. In female participants we ensured they completed the trials during the same phase of their menstrual cycle as body mass may fluctuate throughout the menstrual cycle. Findings from a previous study on 41 females demonstrated average body mass increased by 0.3% between follicular and luteal phases (Pliner and Fleming 1983). Research suggests an increase in body mass during luteal phase is not attributable to fluid retention; but rather an alteration in energy intake (Pliner and Fleming 1983, Chihal 1990, Tomazo-Ravnik T 2006). By considering menstrual cycle phase we could track the unique differences between trials with changes in hydration status from pre- to post-exercise alone.

In conclusion, this research provides additional guidance for future use of DXA in athletes, such as ensuring athletes are euhydrated before scanning. Assessment of hydration status should be considered optimal practice for test-retest scans, and consideration should be given to menstrual cycle phase in females. Thus, by controlling hydration status prior to scanning practitioners can more accurately evaluate fat-free soft tissue mass changes in athletes as part of nutritional or exercise interventions.

## CHAPTER 4. DEVELOPMENT OF A HYDRATION INDEX.

### ABSTRACT

The second and third studies reported in this chapter focused in the identification of beverages that promote longer term fluid retention and maintenance of fluid balance in adults. Maintenance of fluid balance has real clinical and practical benefit in situations in which free access to fluids is limited or when frequent breaks for urination are not desirable. The post ingestion diuretic response is likely to be influenced by several beverage characteristics, including the volume ingested, energy density, electrolyte content, and the presence of diuretic agents. The study reported in **Part A** investigated the effects of 13 different commonly consumed drinks on urine output and fluid balance when ingested in a euhydrated state, with a view to establishing a beverage hydration index (BHI), i.e., the volume of urine produced after drinking expressed relative to a standard treatment (still water) for each beverage. Each subject ( $n = 72$ , euhydrated and fasted male subjects) ingested 1 L still water or 1 of 3 other commercially available beverages over a period of 30 min. Urine output was then collected for the subsequent 4 h. The BHI was corrected for the water content of drinks and was calculated as the amount of water retained at 2 h after ingestion relative to that observed after the ingestion of still water. Total urine masses (mean  $\pm$  SD) over 4 h were smaller than the still-water control ( $1337 \pm 330$  g) after an oral rehydration solution (ORS) ( $1038 \pm 333$  g,  $P$ , 0.001), full-fat milk ( $1052 \pm 267$  g,  $P$ , 0.001), and skimmed milk ( $1049 \pm 334$  g,  $P$ , 0.001). Cumulative urine output at 4 h after ingestion of cola, diet cola, hot tea, iced tea, coffee, lager, orange juice, sparkling water, and a sports drink were not different from the response to water ingestion. The mean BHI at 2 h was  $1.54 \pm 0.74$  for the ORS,  $1.50 \pm 0.58$  for full fat milk, and  $1.58 \pm 0.60$  for skimmed milk. BHI may be a useful measure to identify the short term hydration potential of different beverages when ingested in a euhydrated state. The study in **Part B** of this chapter aimed to investigate the effect of 4 different commonly consumed drinks on urine output and net fluid balance over 3 hours in a field trial of office-workers. Twenty-three participants (euhydrated, males ( $n=7$ ) and females ( $n=16$ ), age: (mean  $\pm$  SD) males  $31.3 \pm 10.4$  y; females  $33.1 \pm 9.8$  y, BMI: males  $29.9 \pm 4.4$ ; females  $27.4 \pm 3.7$ , arrived at work in a euhydrated state. After emptying their bladder and recording body mass they ingested 1 L of fluid over the following hour as either water, coffee, orange juice or semi-skimmed milk. Energy content of the drinks was 0 kcal/L (water), 4 kcal/L (coffee), 470 kcal/L (orange juice) and 500 kcal/L (milk). Urine output was

collected immediately, and each hour for 2 hours, following fluid ingestion for volume and electrolyte analysis. On completion a final body mass was obtained. Mean  $\pm$  SD total urine mass loss over 2 hours for still water was  $1007 \pm 108$  g and was significantly different to milk  $797 \pm 181$  g ( $P < 0.05$ ). Urine losses with orange juice ( $953 \pm 246$  g) and coffee ( $1067 \pm 164$  g) were not different to water, but coffee was also different to milk ( $P < 0.05$ ). Net fluid balance was positive at 2 h after milk ingestion ( $+203 \pm 181$  ml) and was significantly different ( $P < 0.05$ ) from water ( $-7 \pm 108$  ml) and coffee ( $-67 \pm 164$  ml) but not different from orange juice ( $+48 \pm 246$  ml). Net Na<sup>+</sup> balance was significantly different from water ( $-495 \pm 207$  mg) after ingestion of orange juice ( $-973 \pm 298$  mg) and milk ( $-295 \pm 253$  mg). Net K<sup>+</sup> balance was significantly different from water ( $-315 \pm 64$  mg), after ingestion of orange juice ( $+576 \pm 171$  mg) and milk ( $+901 \pm 118$  mg). It was concluded that a variety of drinks can be ingested during normal daily living / working to help maintain fluid balance. Ingestion of milk led to a reduced urine output compared with the other drinks, most likely due to its electrolyte content and casein protein content. The retention of fluid volume following milk ingestion may be important in situations where frequent work breaks need to be avoided.

## PART A: A RANDOMIZED TRIAL TO ASSESS THE POTENTIAL OF DIFFERENT BEVERAGES TO AFFECT HYDRATION STATUS

### 4a.1 Introduction

Loss of water from the body is a continuous process but intake is episodic and hydration status will therefore fluctuate throughout the day. Maintaining an adequate hydration status is important in preventing the adverse outcomes that result from acute and chronic hypo- or hyperhydration (Manz and Wentz 2005, Maughan 2012). There is good evidence for a link between poor hydration and an increased risk of kidney stones, constipation, coronary heart disease and stroke, and there is some evidence for a link between poor hydration and renal disease, bladder and colorectal cancer, dental diseases and broncho-pulmonary disorders (Manz and Wentz 2005). While there is good evidence that hypohydration, if sufficiently severe, will adversely affect physical performance (American College of Sports, Sawka et al. 2007), there is also growing evidence of an effect of hydration status on cognitive performance and mood (Shirreffs, Merson et al. 2004, Masento, Golightly et al. 2014).

An adequate daily water intake is defined in the US by the Institute of Medicine (IOM 2005) at 3.7 L for men and 3.0 L for women and in Europe by the European Food Safety Authority (Agostoni, Bresson et al. 2010) as 2.5 L for men and 2.0 L for women. The distribution of fluids over the course of the day and their composition may, however, also be important in determining how well an individual is able to maintain an adequate hydration status. The volume and composition of ingested drinks has a strong influence on the rates at which they empty from the stomach and are absorbed in the small intestine, thus affecting their entry into the body water pool (Maughan 1998). Beverage components are also metabolised and excreted on different time scales. These various factors are likely to result in different hydration status profiles in the first few hours after ingestion of different beverages. Increasing osmolality and energy density will slow gastric emptying, limiting the rate of entry of nutrients (and water) into the small intestine. Osmolality, carbohydrate type and concentration and electrolyte (primarily sodium) content will all affect the rate of intestinal solute and water absorption (Schedl, Maughan et al. 1994). Oral rehydration solutions (ORS) intended for use in the treatment of diarrhoea disease are formulated to maximise the rate of absorption and retention of fluid. They are typically hypotonic and contain low concentrations (4-5%) of glucose or other carbohydrates and relatively high concentrations (50-80 mmol/L) of sodium (Schedl, Maughan et al. 1994). In contrast, the presence of

strongly hypertonic solutions in the small intestine will result in a net secretion of water into the intestinal lumen, inducing a measurable decrease in plasma volume and, in effect, a transient reduction in body water content (Evans, Shirreffs et al. 2009). Many commonly consumed beverages also contain variable amounts of caffeine or alcohol, both of which will, in sufficient doses, have diuretic actions that might compromise the maintenance of an adequate hydration status (Shirreffs and Maughan 1997, Maughan and Griffin 2003).

These various factors may result in different hydration status profiles in the first few hours after ingestion of a fixed volume of different beverages. Rapid absorption will tend to promote a diuretic response while the presence of high concentrations of electrolytes, and perhaps also other solutes, will tend to delay urinary water losses. It should therefore be possible to develop a Hydration Index (HI) that will define the hydration response to any particular drink, in much the same way as the Glycemic Index (GI) defines the blood glucose response to ingestion of foods (Jenkins, Wolever et al. 1981). In the case of a HI, the cumulative volume of urine passed over a fixed period of time is in effect the area under the curve for renal water excretion. The urine volume passed relative to a standard treatment (still water) can therefore be calculated as the HI of a beverage.

Therefore, the aim of the present study was to assess fluid balance responses to the ingestion of a fixed volume of commonly-consumed beverages ingested when in a euhydrated state, with a view to establishing the feasibility of a HI. We hypothesized that drinks containing a high electrolyte content or high energy content would have greater fluid retention and thus a higher HI compared to plain water. Conversely, drinks containing nutrients with known diuretic actions, such as alcohol and caffeine, would have lower Hydration Index values.

## **4a.2 Methods**

### **4a.2.1 General Study Design**

Three separate laboratories (Loughborough, Bangor and Stirling) collaborated to test 72 recreationally active, healthy males. Ethics approval for the study was obtained separately from the Ethics Committees of the three Institutions involved.

A randomization table was generated based on each participant undertaking a maximum of four experimental trials, which included water plus three other test drinks administered in a randomized fashion, and was based upon each experimental site assessing all available test drinks ([www.randomization.com](http://www.randomization.com)). Rehydration study data (Shirreffs and Maughan 1997, Evans, Shirreffs et al. 2009) informed the sample size estimates and indicated a minimum sample size for each test drink of  $n=12$ . Although not a cluster randomized trial we factored in an additional sample size weighting to account for possible increased variance due to data collection across three different sites. The final sample size estimate based on 80% power with mean total urine output of 900 ml, pooled SD of 300 ml, and a mean difference detectable of 220 ml required a total of  $n=15$  observations per drink. We therefore aimed to recruit  $n=30$  at each site and allowing for loss to follow-up this ensured completion of  $n=24$  at each site, giving  $n=17$  observations on any given test drink.

#### 4a.2.2 Pre-Trial Standardization/Exclusion criteria

At each site; 24 healthy, physically active men between 18 and 35 years old were recruited. For the total sample of  $n=72$  the mean (SD) characteristics were: age 24(4) y; height 178 (6) cm; body mass 77.3 (9.9) kg; water intake 2.0 (0.8) L/d: **Table 4-1**). Those with a history of cardiovascular, renal, muscle-skeletal or metabolic diseases, as determined from a pre-participation health screen questionnaire, were excluded. As body mass was used as an index of euhydration, those currently undertaking an energy-restricted diet and/or exercise plan were also excluded. Participants were asked to record their diet including their fluid intake (household measures technique) as well as any exercise performed, in a diary over the 2 days before the first trial and asked to replicate this before their subsequent visits. Participants were also asked not to perform any strenuous exercise or consume alcoholic beverages in the 24 h preceding all trials.



**Table 4-1 Participant physical characteristics and daily water intake. Data are mean (SD).**

	Bangor (n = 24)	Loughborough (n = 24)	Stirling (n = 24)	All (n = 72)	ANOVA <i>P</i> - value
Age (y)	24 (4)	26 (3)	25 (5)	25 (4)	<i>P</i> = 0.05
Height (cm)	177 (7)	180 (6)	179 (7)	178 (6)	<i>P</i> = 0.48
Mass (kg)	76.2 (12.3)	77.4 (7.3)	78.3 (9.8)	77.3 (9.9)	<i>P</i> = 0.77
BMI (kg/m <sup>2</sup> )	24.2 (3.3)	24.0 (1.6)	24.5 (2.6)	24.2 (2.6)	<i>P</i> = 0.77
Water intake (L/d)	2.0 (0.9)	2.0 (0.6)	2.1 (0.8)	2.0 (0.8)	<i>P</i> = 0.75

#### 4a.2.3 Experimental Procedures

Following an overnight fast, of at least 8 h, participants emptied their bladder upon waking; retaining an aliquot in a sterile collection tube. One hour before arriving at the laboratory, volunteers were instructed to consume 500 ml of still water (Highland Spring™, Perthshire, UK) over the course of 15 minutes. Upon arrival in the laboratory, volunteers remained seated in a comfortable environment for 10 minutes. A single 5 ml blood sample was collected via venepuncture from an antecubital vein and blood was dispensed into a serum tube. Participants were then asked to void their bowels and bladder before measurement of near-nude body mass (underwear only) to the nearest 50 g behind a screen. Approximately 30 minutes after arrival at the laboratory participants then ingested 1 L of the assigned test drink over a period of 30 minutes (4 equal volumes administered 7.5 min apart). It was not possible to conceal the drink allocation from participants on the day of trials due to using commercially available products, and since a range of hot, cold, sparkling and still drinks were administered. A fixed volume, rather than a volume relative to body mass, was chosen as

most drinks are served and ingested in containers of a standard volume. Participants were asked to empty their bladder at the end of the drinking period and again at the end of each hour of the study period. If a participant requested to pass urine before the hour was complete, this was collected and then added to any further urine produced at the end of the corresponding hour. After the final urine sample was collected, near-nude body mass was recorded once again.

#### 4a.2.4 Drinks and drink preparation

Each participant consumed still water (Highland Spring™, Perthshire, UK) and three of the following drinks in a randomized, counter-balanced order: sparkling water (Highland Spring™, Perthshire, UK), cola (Coca-Cola®, Uxbridge, UK), diet cola (Diet Coke®, Uxbridge, UK), sports drink (Powerade®, Coca-Cola®, Uxbridge, UK), oral rehydration solution (ORS: Dioralyte™, Sanofi. One, Surrey, UK), orange juice (Tesco Everyday Value, Hertfordshire, UK), Lager beer (Carling®, Staffordshire, UK), hot black coffee (Nescafe® Original, York, UK), hot black tea (PG tips®, Unilever, London, UK), cold black tea (PG tips®, Unilever, London, UK), full fat milk (3.6% fat; Tesco, Hertfordshire, UK) or skimmed milk (0.1% fat; Tesco, Hertfordshire, UK). The nutrient composition of the test drinks is presented in **Table 4-2**.

All cold drinks were stored at standard refrigerated temperature (4-6°C) until serving. Tea, coffee and ORS were prepared according to manufacturer's instructions, being prepared with still water (Highland Spring™ still water). Hot black coffee and black tea were brewed with freshly boiled still water (Highland Spring™ still water) and served at 60°C, with the temperature being maintained in a hot water bath. Cold black tea was brewed in the same manner, then stored and served at standard refrigerated temperature (4-6°C). A 5 ml sample of each drink preparation was aliquoted into plain tubes. All drinks were tested for osmolality and Na/K after preparation within 48 h and 5 days after collection, respectively.

**Table 4-2 Drink composition. Drink water and macronutrient content was obtained from drink labels, whereas osmolality, sodium (Na), potassium (K) and caffeine content were determined by in-house analysis.**

Drink	Water Content (%)	Energy (kcal/L)	CHO (g/100 ml)	Fat (g/100 ml)	Protein (g/100 ml)	Osmolality (mmol/kg)	Na (mmol/L)	K (mmol/L)	Caffeine mg/L
Still water	100	0	0	0	0	2	0	0	0
Sparkling water	100	0	0	0	0	7	1	0	0
Cola	89	420	10.6	0	0	432	2	0	96
Diet Cola	100	4	0	0	0	23	2	0	127
Sports drink	96	160	3.9	0	0	297	21	4	0
Oral rehydration solution	97	80	1.8	0.1	0	229	55	20	0
Orange juice	89	470	10.5	0.1	0.5	570	1	33	0
Lager	94	330	2.2	0	0.4	774	1	6	0
Coffee	99	4	0.1	0	0	34	1	7	212
Tea	100	0	0	0	0	16	1	4	179
Cold tea	100	0	0	0	0	18	1	5	179
Full fat milk	88	640	4.7	3.6	3.2	286	18	41	0
Skimmed milk	91	350	5.0	0.1	3.4	282	19	40	0

#### 4a.2.5 Urine and Serum Analysis

All urine collected during the study was passed into a 1 L plastic container. The volume of each urine pass was determined by measuring the mass on an electronic balance (to the nearest 0.1 g), with the mass of the empty plastic container subtracted to enable the estimation of urine volume. From each urine sample a 5 ml aliquot was dispensed into a plain screw-capped tube. This was stored at 4°C for the analysis of urine osmolality and sodium/potassium concentrations. Urine and serum osmolality was measured in duplicate using freezing-point depression method (either Gonotec Osmomat, Berlin, Germany or Advanced Instruments, MA, USA) within 48 h of collection. Urine Na/K concentrations were measured in duplicate using flame photometry (Corning Flame Photometer, Cambridge, UK) within 5 days of collection. Collection, handling and storage of urine and serum were in accordance with the Human Tissues Act. Stored samples were discarded once satisfied analysis was completed.

Whole blood in the serum tube was allowed to stand for 1 h at room temperature to clot before centrifugation (10 min, 4°C, 2000-3000 g). Serum was then dispensed into an appropriate storage tube (e.g. Eppendorf) and stored at 4°C for measurement of osmolality.

To help ensure consistency in the data analysed across sites, seven independently prepared quality control solutions were also analysed in replicates of ten by each research group. These contained undisclosed concentrations of Na/K and a measured osmolality. Two-way random effects intra class correlation coefficient analysis (ICC) suggested good agreement between the different institutions for osmolality, Na/K analysis where ICC were all 0.999 or greater. In addition, Bland-Altman limits of agreement analysis indicated that bias between any two institutions was less than 2% for osmolality, less than 1% for Na and less than 2% for K.

#### 4a.2.6 Data and statistical analysis

Participant characteristics, pre-trial participant preparation and urine responses to the still water trial from each institution were initially compared using a one-way ANOVA. To confirm that hydration status was similar before each trial, serum and urine osmolality were compared between drinks by ANOVA.

The main outcome measure was cumulative urine mass after ingestion of each drink. This was also expressed as a hydration index (HI) by dividing each individual's cumulative urine

mass after still water with cumulative urine mass for each other test drink consumed. Individual hour cumulative urine mass and HI of each drink was compared by ANOVA and Dunnett's Post Hoc comparison test to determine which drinks differed to still water.

To assess the practical meaning of the HI differences observed between still water and each of the test drinks, the difference was compared to the normal variation determined from a separate repeatability analysis. For this purpose twelve participants ingested the same drink on two occasions. The drinks used for this repeatability analysis were the same as those used in the present study. The repeatability of the HI was equal to a coefficient of variation of 18% (~180 ml).

Even though a fixed volume of each of the test drinks was consumed, the presence of other components in some of these drinks means the water content of drinks varied from 88% to 100% (Table 2). It might therefore be argued that the HI should be corrected for the differences in water intake. If, however, the aim was to estimate the effects of the different drinks on body water content, then the uncorrected values would be more appropriate. For clarity the data have been expressed both ways.

All other secondary outcome measures (net fluid balance, HI corrected for water content, cumulative urine electrolyte loss) were analysed by ANOVA with Dunnett's Post Hoc comparison test. In addition, the meaningfulness of group differences was also calculated using Cohen's *d* effect size (Cohen 1988) and 95% CI of differences between means.

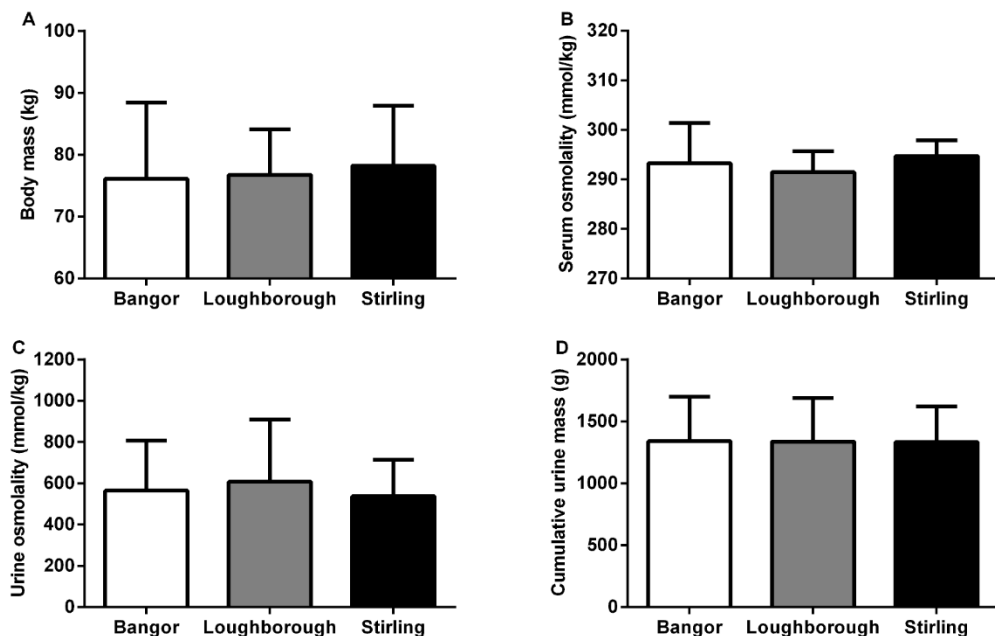
All statistical analyses were completed using a computerized statistical software package (GraphPad Prism version 6 for Windows, GraphPad Software, La Jolla California USA). Statistical significance was accepted at  $P < 0.05$ . Data are presented as mean (SD).

### 4a.3 Results

The study was conducted between February and August 2014. The study was completed when the target number of participants ( $n=72$ ) had finished the study, providing  $n=17$  observations on each test drink in total across the three sites, with  $n=72$  observations on water. In total  $n=86$  participants were recruited and randomized but loss to follow-up occurred due to vomiting following ingestion of the tea ( $n=6$ ) and ORS ( $n=1$ ), screened out ( $n=1$ ) or voluntary withdrawal from the study due to external factors ( $n=6$ ).

### 4a.3.1 Institutional comparison of pre-trial standardization and urine output response to a standard drink

Before ingestion of drinks on the still water trial body mass (**Figure 1A**,  $P = 0.76$ ), serum osmolality (**Figure 1B**,  $P = 0.14$ ) and urine osmolality (**Figure 1C**,  $P = 0.62$ ) were not different suggesting that participants' preparation before trials was similar at each institution. We also confirmed that cumulative urine mass after the still water drink trial was similar at each institution, which further suggests that the participants in the three institutions had similar fluid regulation (**Figure 1D**,  $P = 0.99$ ). It was therefore deemed reasonable to combine the data from the three institutions for the main study.



**Figure 4-1 Institutional comparison of pre-trial standardization and urine response to a standard drink on the still water trial.**

Body mass (A), serum osmolality (B), urine osmolality (C) immediately before still water ingestion at the three institutions. Four-hour cumulative urine mass to still water drink trial at the three institutions (D). No differences were observed between institutions for body mass, ( $P = 0.76$ ), serum osmolality ( $P = 0.14$ ), urine osmolality ( $P = 0.62$ ), or cumulative urine mass ( $P = 0.99$ ) suggesting at each institution that participants' preparation for trials was similar and that participants in the three institutions had similar fluid regulation.

#### 4a.3.2 Pre-drink ingestion hydration status

Serum osmolality (293(6) mmol/kg,  $P = 0.22$ ) and urine osmolality (582(265) mmol/kg,  $P = 0.47$ ) was similar immediately before drinks were ingested on each trial.

#### 4a.3.3 Urine output and fluid balance

Urine mass did not differ between trials immediately ( $P = 0.72$ ) or 1 h after the ingestion of the drinks ( $P = 0.16$ ). Two hours after drink ingestion cumulative urine mass was lower than the still water drink after the ingestion of full fat milk ( $P < 0.01$ ), skimmed milk ( $P < 0.01$ ), ORS ( $P = 0.02$ ) and orange juice ( $P = 0.05$ , **Figure 4-2A**). Consequently, net fluid balance was positive for full fat milk, skimmed milk, ORS and orange juice 2 h after drinks were consumed but negative for all other drinks consumed (**Figure 4-2B**). In addition, full fat milk, skimmed milk and ORS cumulative urine mass was lower, and net fluid balance was higher, than still water at 3 and 4 h after drinks were ingested. The effect sizes at 4 h in comparison to still water were 0.95 for full fat milk, 0.87 for skimmed milk, and 0.90 for ORS (all large effects) with an effect size of 0.60 for orange juice (a medium sized effect). The mean differences (95% CI) in cumulative urine output were 294 ml (165 - 423) for full fat milk, 340 ml (202 - 478) for skim milk, and 362 ml (232 - 492) for ORS.

- Still water   ○ Sparkling water   ▲ Cola   ▼ Diet cola   ◆ Sports drink
- Oral rehydration solution   ▽ Orange juice   ■ Lager
- ▲ Coffee   ◇ Tea   \* Cold tea   △ Full fat milk   □ Skimmed milk

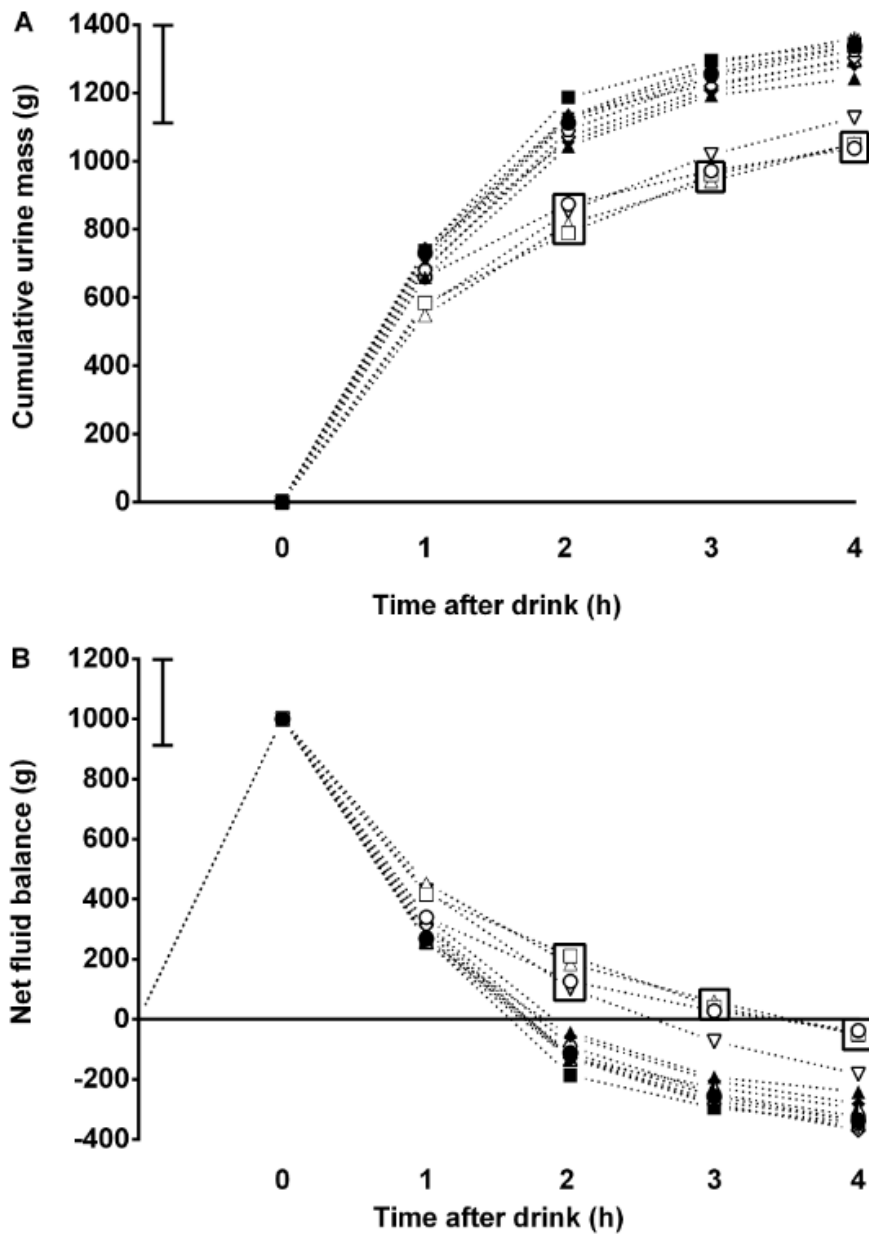


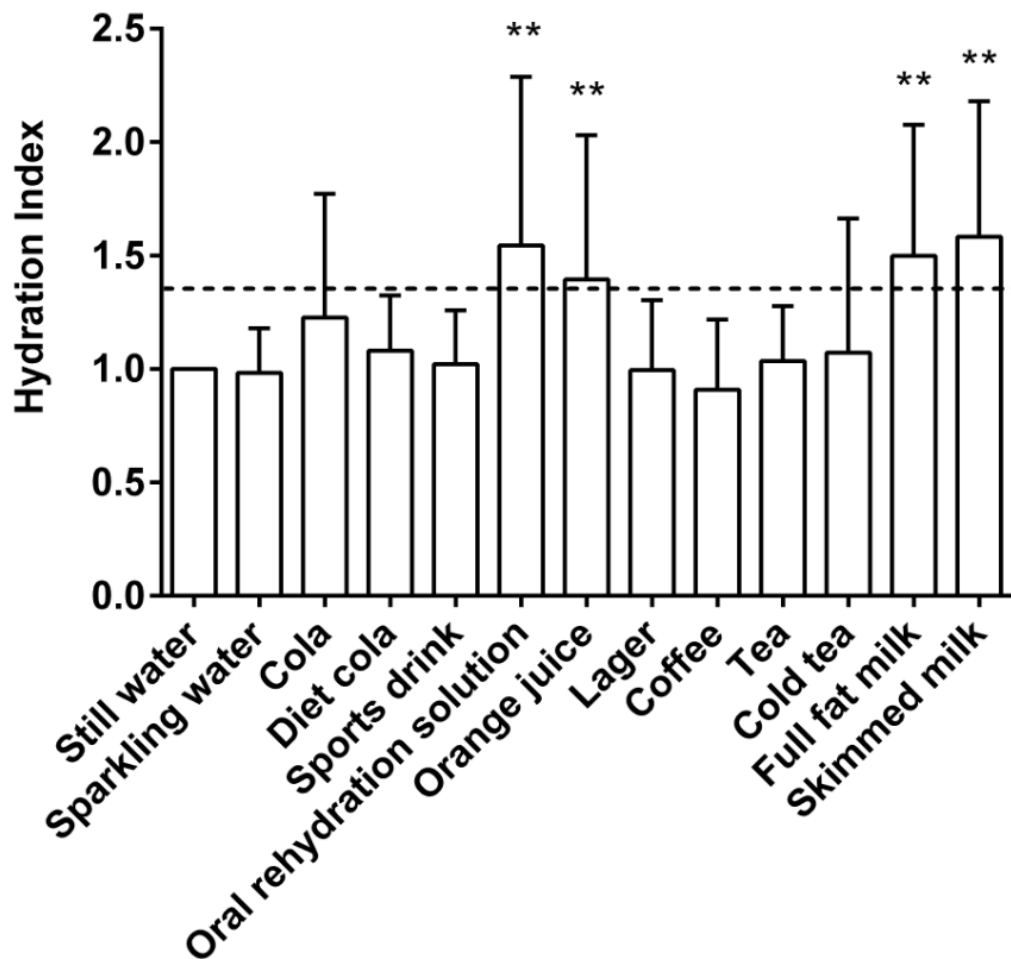
Figure 4-2 Urine output and fluid balance after 1 L of a various commonly-consumed and commercially available drinks.

Cumulative urine mass (A) and net fluid balance (B). Drinks with different responses to still water are presented within rectangular boxes. The vertical error bar in top left corner represents the overall mean SD for all drinks during the 4-hour collection.



#### 4a.3.4 Hydration index

After 2 h full fat milk, skimmed milk, ORS and orange juice had a higher HI than still water (All differences  $P < 0.01$ , **Figure 4- 3**). The effect sizes at 2 h were 1.22 for full fat milk, 1.37 for skim milk, 1.03 for ORS, and 0.86 for orange juice (all large to very large effects). The higher HI between still water and full fat milk, skimmed milk, ORS and orange juice also exceeded twice the CV of the HI measure. Mean differences (95% CI) for 2 h HI values were 0.50 (0.23 – 0.77) for full fat milk, 0.58 (0.30 – 0.86) for skimmed milk, 0.54 (0.19 – 0.89) for ORS and 0.39 (0.08 – 0.70) for orange juice. Additionally, full fat milk, skimmed milk, and ORS hydration indexes were greater than still water at 3 and 4 h after drink consumption.



**Figure 4-3 Hydration index for 13 commonly-consumed and commercially available drinks.**

Differences from still water highlighted by asterisks \* equals  $P < 0.05$ , \*\* equals  $P < 0.01$ . The dashed line represents twice the CV of the HI measure.

#### 4a.3.4 Hydration index corrected for water content

The water content of the drinks used in this study varied from 100% to 88% (Table 2), and consequently the amount of water ingested varied between drinks. It might be appropriate therefore to recalculate the HI to take account of the different volumes of water ingested on the different trials. The HI values presented in **Figure 4-4** have been normalized by the drinks' water content to reflect the effect of the drink itself on hydration status excluding the differences in water content. As was the case without the correction for drink water content, the corrected HI for full fat milk ( $P = 0.02$ ), skimmed milk ( $P < 0.01$ ) and ORS ( $P < 0.01$ ) were higher than that for still water. The effect sizes for corrected HI data at 2 h were 0.89 for full fat milk, 1.15 for skimmed milk, and 1.01 for ORS (all large effects). The mean differences (95% CI) for corrected 2 h HI were 0.32 (0.08 – 0.56) for full fat milk, 0.44 (0.18 – 0.70) for skimmed milk, and 0.50 (0.16 – 0.84) for ORS. The HI for orange juice was, however, no longer different than still water ( $P = 0.16$ ) with an effect size of 0.58 (a medium effect) and a mean difference (95% CI) of 0.24 (-0.03 – 0.51).

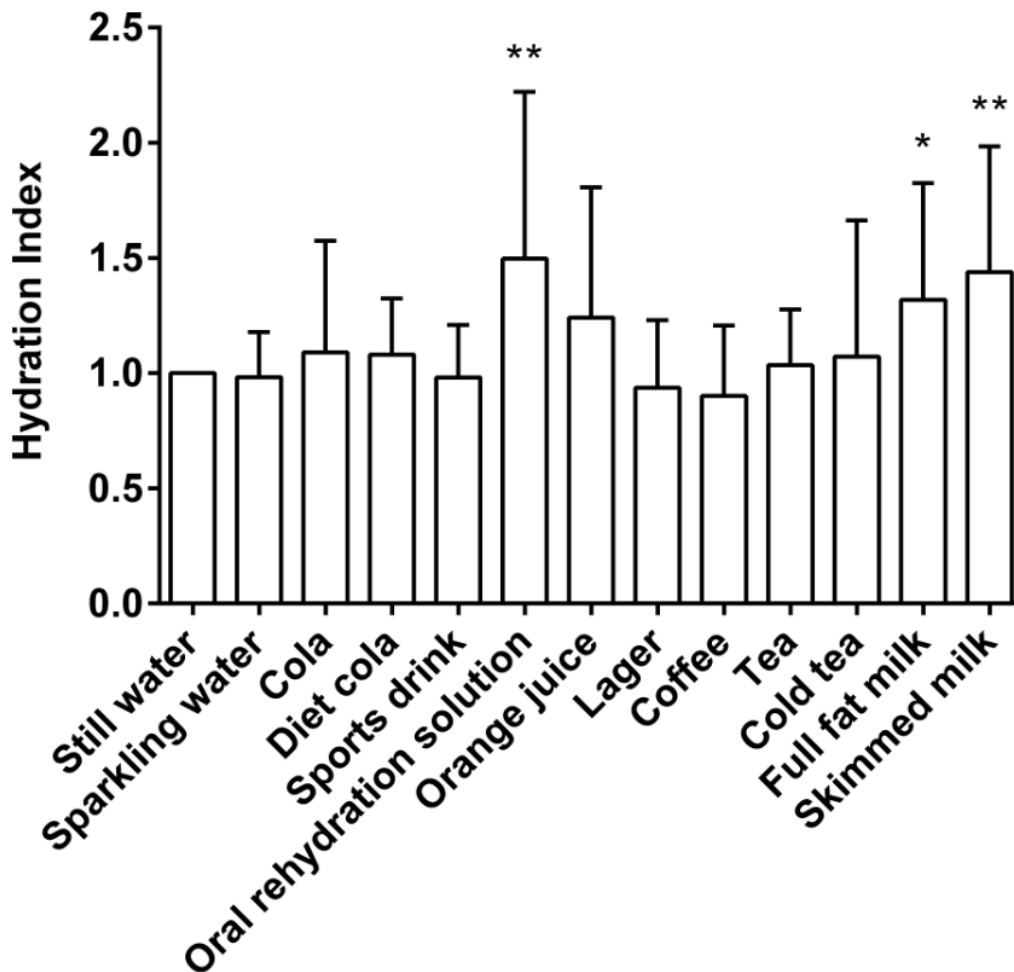


Figure 4-4 Hydration index for 13 commonly-consumed and commercially available drinks after correction for water content of drink ingested.

Differences from still water highlighted by asterisks \* equals  $P < 0.05$ , \*\* equals  $P < 0.01$ .

#### 4a.3.5 Urinary electrolyte excretion and balance

Several drinks had greater sodium or potassium balances than still water 2 h after drinks were consumed (**Figure 4-5**). Drinks with positive sodium or potassium balances were typically those with the highest HI. That is, ORS had a positive sodium balance (**Figure 4-5A**), whilst orange juice, full fat and skimmed milk had positive potassium balances (**Figure 4-5B**).

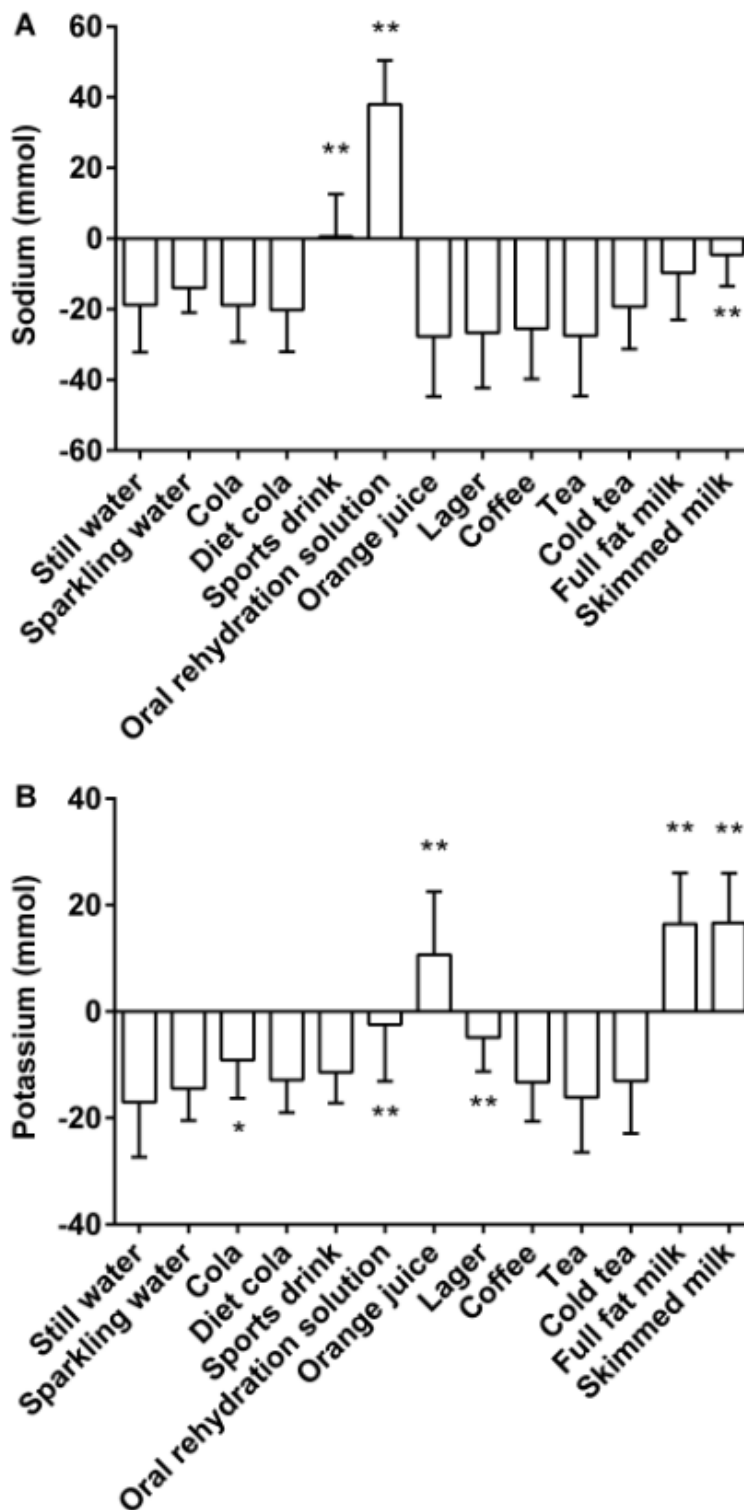


Figure 4-5 Sodium (A) and potassium (B) net balances 2 hours after 1 L of a various commonly-consumed and commercially available drinks.

Differences to still water highlighted by asterisk, \* equals  $P < 0.05$ , \*\* equals  $P < 0.01$ .

#### 4a.4 Discussion

In this study, a group of volunteers consumed a standard volume (1 L) of a range of different beverages over a period of one hour and urine output was measured over the subsequent four hours. The calculated HI revealed that drinks containing the highest macronutrient and electrolyte contents were the most effective at maintaining fluid balance. In the present work we chose to examine the response to a fixed volume of fluid, rather than a quantity relative to body mass, as this more closely mimics real-world behaviour (e.g. individuals will typically purchase and consume a bottle of cola, or a cup of coffee, in a single sitting). A 1 L bolus was chosen as it would sufficiently challenge fluid balance and would initiate a marked diuresis in the hours following ingestion; a similar objective is achieved during an oral glucose tolerance test, where 75 g of glucose is ingested rapidly to challenge glucose clearance.

The differences noted in the urine volume and calculated HI during the monitoring period might be attributed in part to differences in the water content of the different drinks. Stahl et al. (Stahl, Kroke et al. 2007) recognized that the amount of water present in a fixed volume of beverage varies due to the presence of other nutrients, meaning the amount of water available to influence hydration status can markedly differ; an observation these authors termed the Post-absorptive Hydration Index (PAI). The water content of the test beverages in the present study ranged from 100% for still water to 88% for full fat milk. Correction of the urine output to account for differences in the volume of water ingested made little difference to the relative HI responses (Figures 4 & 5), suggesting that such a correction may not be required when considering drinks with characteristics similar to those used in the present study.

In addition to variations in the water content of a beverage, the present HI model recognizes that the presence of additional nutrients in a beverage will also significantly influence the retention of fluid, meaning that beverages with similar water contents may display markedly different effects on long term hydration status. There are several elements of a beverage that might affect fluid balance in the hours following ingestion: the macronutrient content, the electrolyte (primarily sodium and potassium) content, and the presence of diuretic agents (primarily caffeine and alcohol). Ingested drinks with a high-energy content, whether in the form of carbohydrate, fat, protein or alcohol will empty from the stomach more slowly than energy-free drinks and will thus potentially reduce or delay the diuresis that follows compared with the ingestion of a bolus of still water (Shirreffs, Watson et al. 2007, Evans,

Shirreffs et al. 2009). This effect has the potential to contribute to the retention of ingested fluids within the body water space. The drinks in the present study with the highest energy density were full-fat milk 640 kcal/L; orange juice 470 kcal/L; lager 330 kcal/L; cola 420 kcal/L; skimmed milk 350 kcal/L. A high-energy content was generally associated with a high HI, but a comparison of the responses to cola, lager and orange juice suggest that other factors also play a significant role (e.g. electrolytes, alcohol).

In the present study, no water or salt deficit was induced before the beginning of the study. Indeed, steps were taken to ensure that individuals were well hydrated at the start of each trial. Acute administration of a bolus of water plus sodium chloride or other sodium salts results in a transient increase in total body water: this hyperhydration is prolonged relative to that observed after the intake of still water (Sims, van VLIET et al. 2007). In the present study, the ORS and milk drinks contained relatively high concentrations of sodium and potassium, the orange juice contained a moderate amount of potassium, while the remaining drinks contained relatively trivial concentrations of these electrolytes. It is notable that the drinks with the highest electrolyte content tended to have the highest HI.

The known diuretic effects of caffeine and alcohol, because of their action in inhibiting the release of ADH (Eggleton 1942, Fredholm 1983) would influence the response to ingested drinks that contain caffeine or alcohol. A review of the literature suggests that an acute dose of less than about 250-300 mg of caffeine is unlikely to have a measurable effect on urine output, though such an effect is likely to be seen when the dose exceeds about 300 mg (Maughan and Griffin 2003). Indeed, a recent report by Killer et al (Killer, Blannin et al. 2014) observed no difference in hydration status of a group of 50 young males whether they drank 4 x 200 ml of coffee per day (containing ~75 mg caffeine in each bolus) or whether the coffee was replaced by water. Similarly, we did not observe an impact of moderate caffeine intake on net fluid balance in the present study. Furthermore, the alcohol content of the lager did not increase diuresis over other drinks, but the alcohol may have countered the hypothesized impact of energy density on the HI. Shirreffs and Maughan (Shirreffs and Maughan 1997) studied the administration of beer containing 0-4% alcohol on restoration of fluid balance after exercise-induced dehydration. With acute administration of a large volume (average of 2212 ml) post-exercise only the highest alcohol concentration resulted in a small diuretic action. However, the large volume and immediate post-exercise situation provided a larger total alcohol dose and likely greater stimulus for fluid retention in their study. Perhaps surprisingly, only one study has examined fluid balance responses to alcohol in a euhydrated

state (Hobson and Maughan 2010). These authors reported a 12% greater diuresis following the ingestion of 1L of lager beer containing 4% alcohol, compared to the ingestion of the same volume of a non-alcoholic control beer. This diuretic action was blunted when the body is hypohydrated. In addition, Eggleton (Eggleton 1942) noted that the diuretic response to alcohol was proportional to the ingested dose and that there was a large variability in response between individuals: she also noted that the response was blocked by administration of pituitary extract, which contains AVP.

The HI values presented here are based on the hydration status at 2 h after the end of the drink ingestion period. This time point was chosen for 4 reasons. This was the time at which drinks began to show differences. Secondly, the majority (82%) of urine output over the 4 hour period had been passed by this point. Thirdly, in a typical day, most people would expect not to have an interval longer than 2 h between drinks, and any subsequent food or fluid ingestion would over-ride the effects of the initial drink. Fourthly, for the drinks used in the present study it made little difference to the calculated HI whether this was based on the first 2 h or on the whole 4 h collection period.

Although the results of the present study relate only to the acute effects of a large bolus of fluid over the subsequent four hours, there is evidence to support the suggestion that the results may be extrapolated to a longer time scale. Grandjean et al (Grandjean, Reimers et al. 2000) had subjects consume water or water plus varying combinations of beverages, including carbonated, caffeinated cola and coffee. They observed no significant differences in the effect of various combinations of beverages on 24 h hydration status. In addition, Tucker et al. (Tucker, Ganio et al. 2014) recently presented a preliminary report to suggest that 24 h hydration status was not different when subjects drank only water or a variety of drinks, including water, cola and fruit juice, provided that an adequate total volume was consumed.

Maintaining an adequate hydration status is associated with a decreased risk of a range of adverse outcomes (Manz and Wentz 2005). In addition to the volume consumed, it is clear that the nature of ingested fluids will have important consequences for the maintenance of hydration status. The present study describes a novel tool to enable the objective description of the effectiveness of beverages to maintain long term hydration status. It is reproducible, with a coefficient of variation of about 18% and the pattern of response for a range of commonly-consumed beverages is consistent with what is known about the effects of their constituents on water balance. The high coefficient of variation was due to inter-individual

differences to hydration responses when participants were euhydrated. An appreciation of the HI of a beverage has relevance to individuals where long term maintenance of fluid balance is important, such as professions where fluid availability is limited, or in older adults. There is also a clear application to industry, where this tool could be employed to label products to indicate the hydration potential of beverages. Due to the complexity of the commercially-available beverages used in this study, it was not possible to directly determine the relative influence of individual drink components on fluid balance (e.g. electrolyte content, energy density). Future studies should apply this model to further examine the significance of these nutrients in isolation, as well as to assign HI values to a wider range of commercially-available beverages.

## **PART B: HYDRATION POTENTIAL OF COMMONLY CONSUMED DRINKS IN AN OFFICE-WORKING ENVIRONMENT**

### **4b.1 Introduction**

It has been widely demonstrated that dehydration is related with reductions in vigour, energy levels, concentration, alertness, and increases in fatigue, confusion, anger, and tension (Sharma, Sridharan et al. 1986, Shirreffs, Merson et al. 2004, Lieberman, Bathalon et al. 2005, Petri, Dropulic et al. 2006, Pross, Demazieres et al. 2013). Thus, maintaining euhydration in a working environment is a relevant factor to consider in terms of wellbeing and productivity. It is also required by law that employers must provide access to drinkable water to their employees (HSE 2006).

The European Food Safety Authority (EFSA) has established some fluid adequate intake guidelines. These suggest that male adults should ingest 2.5 litres and female adults 2.0 litres of fluid obtained from food and fluid per day. EFSA also recommends that 80% of the daily fluid intake should come from beverages, meaning that the recommended daily intake of fluids from beverages is 1.6 litres and 2 litres for males and females, respectively. This can be translated as drinking six to eight glasses of fluids, which might include a beverages such as coffee, tea, fruit juices or others.

In the United Kingdom, the Health and Safety Executive recommends that when working hard or at a high rate in heat stress conditions employees should consume around 250 ml of fluid every 15 minutes (HSE). The recommendation to employees is to drink small amounts



throughout the working day and not just during meal times or in response to thirst to meet the daily fluid intake recommendations. Different occupations, such as drivers, medical staff working on surgeries in the theatre, or call centre staff, might find it difficult to take a break during the working day to drink. Shirreffs et al (Mear SA 2014) assessed hydration status and fluid intake during work in 156 employees (including office workers, teachers, firefighters, among others). They observed that a large proportion of the studied group were hypohydrated at the start of the working day and remained hypohydrated at the end of the shift.

The Institution of Occupational Safety and Health recommends particularly for call centre staff to increase hydration awareness; and suggests to the employers to provide a free re-fillable drinks bottle (IOSH 2016). However, it has been observed in different call centres and workplaces that employers have tried to establish policies to limit the time that their workers spend on toilet breaks (Norton 2002) . Norton found half of call centre employees postponed toilet breaks because of management and related issues, and one in four reported to defer several times a week. Call centre workers, teachers and nurses were particularly badly affected (Norton 2002). If the employees spend longer in the toilet, this can affect even their monthly wage. In Norway, in 2012, there was a protest from call centres employees when the workplaces set a high technology surveillance system that alerted the managers when the employees were away from their desks for a toilet break for longer than 8 minutes (Sparks 2012). More recently in the UK there were complaints from workers in a call centre when their employer established a policy that stated that their toilet breaks were limited to just seven minutes a day (Farnie 2015). However, the employees unions and the work inspectors have developed some documents to make employers aware about the rights of workers to have a toilet break when needed and without detriment. For example, in the UK, the Trades Union Congress has stated that not being able to urinate or defecate when the employee needs it can cause a range of health issues including digestive tract problems and kidney infections for example (TUC 2010). In the particular case of call centre workers they should be encouraged to regularly drink water to help reduce voice strain; but they are often discouraged from using toilets frequently or avoid breaks themselves. There can be strategies that maintain hydration while reducing the urine output that could prove to be effective at reducing time away from their desks.

Several factors are known to influence the hydration potential of drinks such as: volume ingested, ingestion rate, macronutrient composition, water content, electrolyte and caffeine

content. However, relatively little is known about the impact of fluid composition on fluid balance during normal daily living/office working situations. (Shirreffs and Maughan 2000). In a recent multicentre study we demonstrated that under resting and euhydrated conditions in a young healthy active male population, the macronutrient composition and also the electrolyte balance of ingested beverages are key to reducing urine production and aiding fluid retention over a 4 hour follow-up period. We observed that orange juice, milk and oral rehydration solution are the beverages with a better hydration potential (Maughan, Watson et al. 2016). Based on these results, the aim of the present study was to investigate the effect of 4 different commonly consumed beverages on urine output and net fluid balance during a field study conducted over 3 hours in a group of call centre workers of mixed age and gender during normal office working hours. We hypothesized that milk would be the beverage with the best hydration potential, retaining the fluid for longer in the call centre workers and reducing their urine production.

## 4b.2 Methods

Twenty-four participants working in the call centre volunteered to participate in the field study. A randomised beverage table was generated to allocate each participant to ingest one of the 4 test drinks. Written consent was obtained from all the subjects. Participants arrived to the office in a euhydrated state, 1 hour following the ingestion of 500ml of water and overnight fasted. For the total sample of  $n=24$  (males  $n=7$  and females  $n=17$ ), the mean  $\pm$  SD characteristics were as detailed in **Table 4-3**.

After an overnight fast of at least 8h, volunteers were asked to ingest 500 ml of water 2h before coming to the office. When the participants arrived to work, they were asked to empty their bowels if needed and they were asked to empty their bladder. The urine was collected to record the mass and a 5ml sample was retained for further analysis. Body mass was recorded while they were wearing 1 layer of clothing and without shoes. Participants ingested 1 L of the assigned test drink over a period of 60 minutes (i.e. 250 ml every 15 min) while they were seated at their desks. Office temperature was maintained at 23 degrees. As in our previous studies (Maughan, Watson et al. 2016), a fixed volume was chosen as most of the beverages are served in standard volumes independently of the body mass of who is ingesting it. The test beverages were still water, coffee (prepared according to the protocol detailed in Maughan (Maughan, Watson et al. 2016)), orange juice, and semi-skimmed milk.

Beverage nutrient composition is presented in **Table 4-4**. Participants were asked to empty their bladder at the end of the drinking period and again at the end of each hour of the study period. Urine mass was recorded and a 5ml sample was collected each time. Participants were informed that if they needed to urinate before the hour was complete it was collected and added to any further urine produced by the end of the corresponding hour. Two hours after drinking the final urine was collected, participants' body mass was recorded once again. **(Figure 4-6)** Net fluid balance and beverage hydration index were calculated. Urine volume and electrolyte composition were assessed by mass and flame photometry analysis respectively. Urine samples were also analysed for osmolality by freezing point depression. All samples were analysed in duplicate and a mean of the duplicate was used. The main outcome was the cumulative urine mass after ingestion of each test beverage and these data were used to calculate the beverage hydration index (BHI) for each drink. All statistical analysis was completed using a statistical software package (SPSS statistics 21 for Windows). Net fluid balance, cumulative urine mass, changes in body mass, BHI and net electrolyte balance were analysed by paired t test. Statistical significance was accepted at  $P < 0.05$ . Data are presented as mean  $\pm$  SDs.

**Table 4-3 Participant characteristics on entry into the study for those that completed the data collection.**

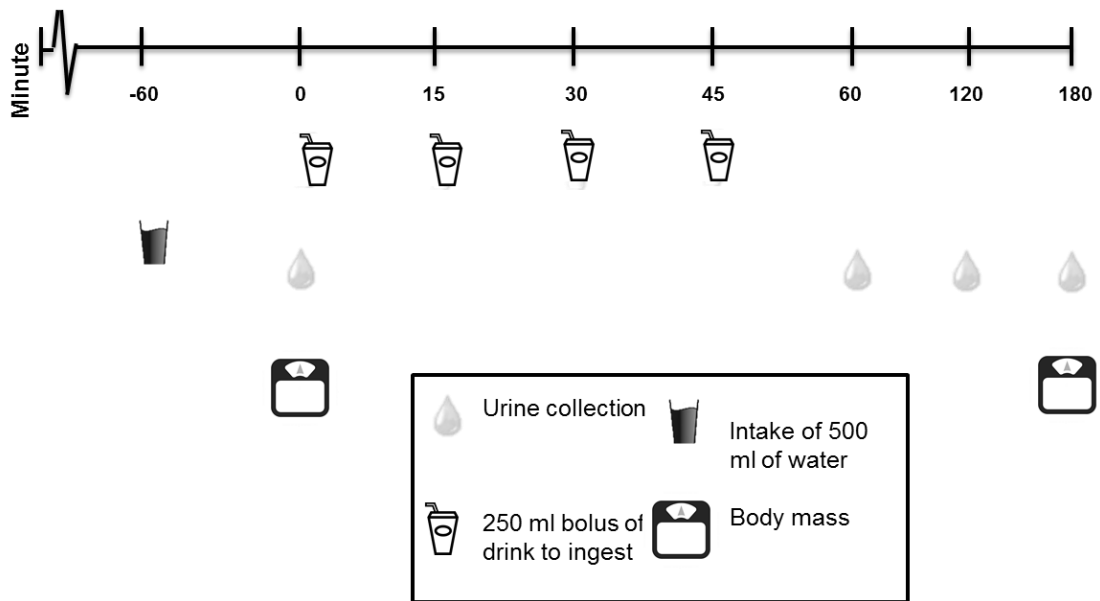
Values are mean (SD).

Group	Age (y)	Height (m)	Body mass (kg)	BMI (kg/m <sup>2</sup> )
Males (n=7)	31.3 (10.4)	1.76 (0.08)	93.5 (18.5)	29.9 (4.4)
Females (n=17)	33.1 (9.8)	1.68 (0.09)	77.1 (13.1)	27.4 (3.7)
All	32.6 (9.7)	1.70 (0.09)	82.1 (16.4)	28.2 (4.0)

**Table 4-4 Water, energy, macronutrient and sodium and potassium content of tested beverages.**

Macronutrients data were obtained from the product labels and electrolytes data were obtained through flame-photometry analysis.

<b>Drink</b>	<b>Water content (%)</b>	<b>Energy (kcal/ L)</b>	<b>Carbohydrate (g/100ml)</b>	<b>Fat (g/100ml)</b>	<b>Protein (g/100ml)</b>	<b>Sodium (mmol/L)</b>	<b>Potassium (mmol/L)</b>
Water	100	0	0.0	0.0	0.0	0	0
Coffee	99	4	0.1	0	0	1	7
Orange juice (OJ)	89	470	10.5	0.1	0.5	1	33
Semi-skimmed milk	89	500	4.8	1.8	3.6	19	40



**Figure 4-6 Study design schematic. Test beverages were water, black coffee, orange juice and semi-skimmed milk (2% fat).**

### 4b.3 Results

The study was conducted in a single day of data collection in October 2015. The room temperature was 23 C. From the 23 participants, 6 ingested water, 6 ingested coffee, 6 drank orange juice (OJ) and 5 consumed semi-skimmed milk. From the data of the initial 24 volunteers, one participant was removed from the analysis as total urine volume collected was not consistent with normal renal function or some of the total volume of each void was discarded and the participant provided just a sample (total urine output was about 200ml over 3 hours after drinking 1L of fluid in a euhydrated state). Initial hydration status assessment indicated that all the participants were euhydrated on arrival at work (urine osmolality  $324 \pm 186$  mOsm/kg). The urine output and the net fluid balance immediately after drinking and 1h after drinking were not significant different between any of the groups. Two hours after the ingestion of the tested beverages, cumulative urine mass was lower and net fluid balance was more positive than for still water after the ingestion of semi-skimmed milk (**Figure 4-7A**). ) Milk ingestion also maintained body mass at the end of the follow up period (change in body mass pre to post trial 0.1(0.2)kg) while the most negative change in

body mass pre to post trial was after ingesting coffee (-0.4(0.2)kg). The differences in body mass pre to post trial for water and for orange juice were also negative (-0.2(0.2) and -0.1(0.1) kg respectively). Net fluid balance was positive at 2 hr after milk ingestion (203(181) ml) and was significantly different ( $p < 0.05$ ) from water (-7(108) ml) and coffee (-143(173) ml) but not orange juice (123(141)) (**Figure 4-7B**). The mean (SD) total urine mass loss over 2 hours for still water was 1007(108) g and was significantly different to milk 797(181) g ( $P < 0.05$ ). Urine losses with orange juice (953(246) g) and coffee (1067(164) g) were not different to water, but coffee was also different to milk ( $p < 0.05$ ). (**Figure 4-8A**)

When BHI was calculated, BHI was significantly higher on milk than water (**Figure 4-8B**). The test beverages had different water content varying from 100% to 89% (**Table 4-4**). These differences cause variations in the amount of water that participants ingested. For this reason, the BHI was recalculated considering the different amount of water ingested in each of the trials. The BHI values corrected for the water content are presented in **Figure 3C**.

The tested beverages were also different in their electrolytes content (**Table 4-4**) thus the sodium and potassium net balance was also different between trials. Net sodium loss occurred with all the beverages but was greater after the ingestion of orange juice compared with water and milk, and was greater with coffee than milk. Net potassium loss occurred following the ingestion of water and coffee but there was positive net potassium balance with orange juice and milk (**Figure 4-9A and 4-9B**). As observed in our previous study (Maughan, Watson et al. 2016) the beverages with highest sodium or potassium content were those with the highest BHI, i.e. milk and orange juice. The estimated insensible losses in this group were 115(389)g.

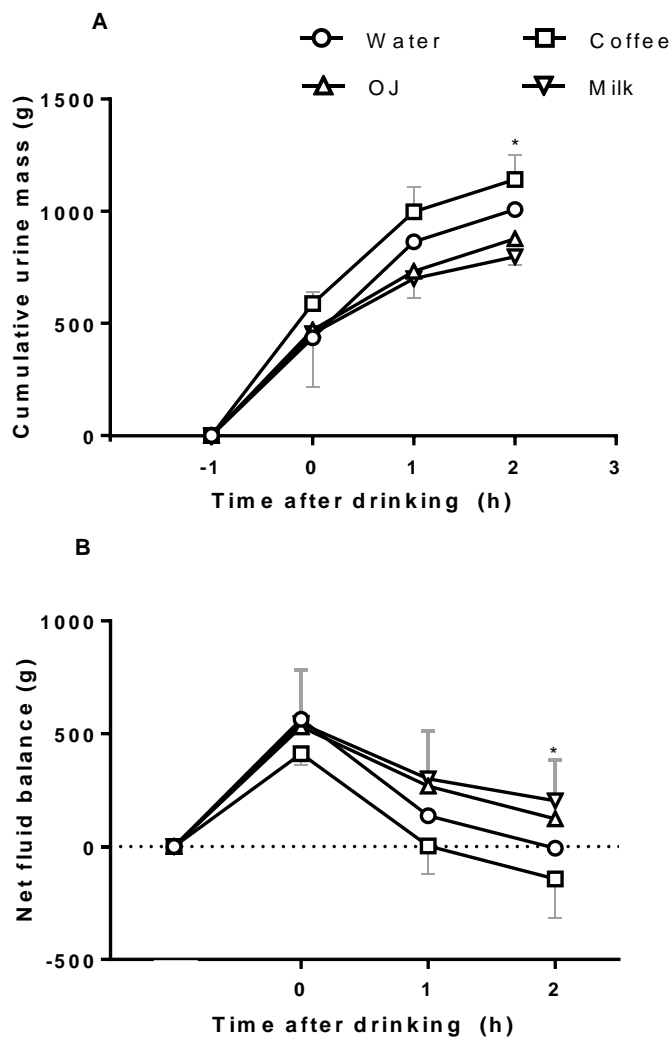


Figure 4-7 Cumulative urine mass (A) and net fluid balance (B) following ingestion of water, coffee, orange juice (OJ) and milk.

Values are mean (SD). \* indicates significant difference from water and coffee on the milk.

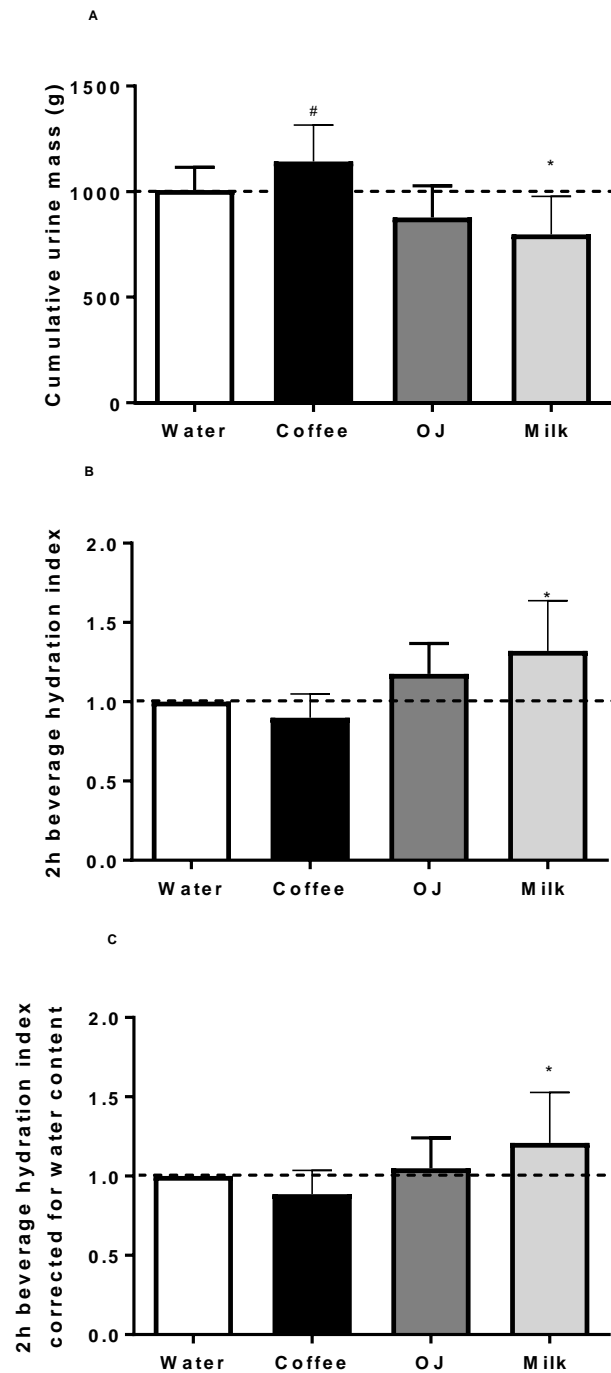


Figure 4-8 Total urine loss over 2 hours following ingestion of water, coffee, orange juice (OJ) and milk (A), 2 hours beverage hydration index (BHI) for each of the test beverages (B) and 2 hours beverage hydration index corrected for water content (C)..

Values are mean (SD). \* indicates significant difference from water and # indicates significant difference from milk. .



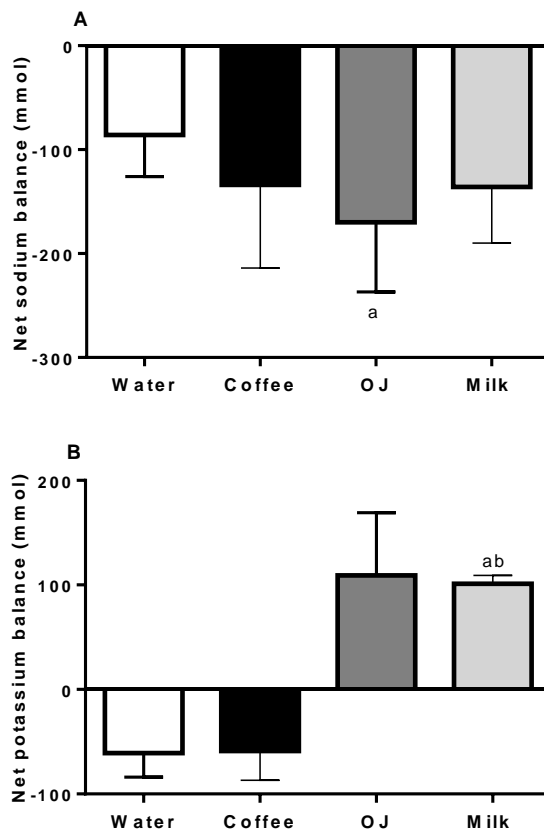


Figure 4-9 Net sodium balance (A) and net potassium balance (B) over 2 hours following ingestion of water, coffee, orange juice (OJ) and milk.

Values are mean (SD). a, b, c, d indicates significant difference from water, coffee, orange juice (OJ) and semi-skimmed milk, respectively.

#### 4b.4 Discussion

The purpose of this field study was to replicate our previous research in a normal working environment, and to investigate if the results that we observed in the laboratory could be applied to a typical living/working situation. Furthermore, the study aimed to extend the previous work by studying a group that included females and people of different body mass and age. The study also provided an insight into the hydration status of a group of call centre workers during their normal working hours. We observed that our results from the previous study can have a practical application in an office environment as milk had a better hydration potential than water, orange juice and coffee regardless of the sex and/or body mass of the participants. The beverages that were tested in this study (still water, orange juice, coffee and semi-skimmed milk) were chosen because they are viable drinks to have at work and some of them are popular drink choices in the office environment. A variety of beverages can help to maintain fluid balance during office working shifts. In the present study, when compared with water, milk ingestion reduced urine output and helped to maintain a positive net fluid balance 2 hours following beverage ingestion. The beverage hydration index (BHI) is a tool that helps to identify beverages that promote long term fluid retention and maintenance of fluid balance for a prolonged period. A beverage with a higher BHI means that it is more effective at maintaining fluid balance. The results from this field based study closely match the observations from our prior work (Maughan, Watson et al. 2016), revealing that the BHI can be useful under free working conditions in both males and females and regardless of their body mass index. We also observed that, as in the laboratory, the beverages with the highest macronutrient and electrolyte content also had a higher BHI. These observations are beneficial for occupational health professionals as improving hydration status can be an easy intervention contributing in the health and well-being among the call centre workers.

It has been suggested that occupational health and human resources professionals need to make employees aware about the relevance of hydration and how it will impact wellbeing and productivity at work. Also it has been recognised that employees should take a break to drink and also to go to the toilet if desired (Silcox 2015). Most studies that have investigated hydration in the work place have been focused on physical work in the heat. For example, Rossi et al observed that firefighters, due to the clothing they have to wear, and because they are exposed to intense heat, may sweat at a rate up to 2.1L/h (Rossi 2003). Bishop et al studied the effect of performing heavy work at 21 degrees while wearing protective clothing

and observed sweat rates of up to 2.25 L/h (Bishop, Pieroni et al. 1991). Dehydration has also a negative impact on the decision making process and on cognitive performance which can both be associated with a decline in productivity. One of the papers examining call centres workers and hydration stated that the lack of humidity in the environment where employees need to use their voice might demand extra hydration to maintain fluid balance (Wilson 2013). To our knowledge, the present study has been the only one investigating hydration status in call centre workers.

From our observations the ingestion of semi-skimmed milk may be considered a good option for employees in a call centre to maintain a positive net fluid balance over a short period of time. Due to its hydration potential and its fluid retention effect, milk might reduce the number of visits to the toilet following ingestion, however this needs further investigation. The retention of fluid volume following milk ingestion may be important in situations where frequent work breaks need to be avoided. However, it is important to consider the energy content of milk if it is chosen as a way to ensure maintenance of euhydration. The caloric content should be accounted as part of the daily energy intake particularly for those who may be concerned about gaining body mass or who are already overweight/obese (Benelam and Wyness 2010). Milk contains casein and whey proteins in a ratio of 4:1 which elicits a slower digestion and absorption kinetics of these proteins (Bos, Metges et al. 2003). This delay has an impact on delay of diuresis too. On the other hand, milk also contains electrolytes (sodium and potassium) that have an effect on subsequent expansion of blood volume and plasma osmolality [247]. Milk also has the highest energy density (500 kcal/l) and as the rate of gastric emptying declines with increasing energy density of the fluid consumed (McHugh and Moran 1979) this also results in a higher BHI in combination with the electrolytes content.

Coffee and caffeinated beverages are very popular amongst the population and also in the workplace. Caffeinated beverages represent an important fluid source despite being often considered as dehydrating. It is a common belief that caffeine stimulates diuresis (Hammond 2014). For this reason, some people try to avoid the consumption of caffeinated beverages. Caffeine acts as an adenosine receptor antagonist reducing fractional sodium reabsorption in the proximal tubule and in the distal nephron (Killer, Blannin et al. 2014). Passmore et al investigated the effect of the administration of different doses of caffeine (45, 90, 180, 360 mg) on the renal and cardiovascular responses. They observed that urine volume was increased when participants ingested the 360 mg caffeine solution (Passmore, Kondowe et al.

1987). Unpublished data from our multicentre project reported in Chapter 5 demonstrated that a caffeine content up to 400mg/l did not have a negative effect on net fluid balance. The consumption of up to 400 mg/day of caffeine, irrespective of form, is considered as safe in the adult population (except pregnant women)(Tetens 2015). However in the present study, the amount of caffeine that was ingested in the group that consumed coffee was 212mg/l so there was not any increase in the urine production. The practical application of our results are that a moderate consumption of caffeinated drinks (including coffee) can contribute to the daily total fluid intake without having any negative effect on net fluid balance.

Orange juice contains carbohydrate and electrolytes, mainly potassium, which made it the second best option in the present study in terms of the BHI. In our previous study (Maughan, Watson et al. 2016) we observed that the beverages with the highest energy content in the form of carbohydrate, fat or protein had a higher BHI. Orange juice was the drink with the highest energy content (470 kcal/l) after semi-skimmed milk. Orange juice also contained a moderate amount of potassium, contributing to the highest net potassium balance. From our previous study, a high energy content was generally associated with a higher BHI. However when the responses for cola, lager and orange juice were compared, it was suggested that other factors play a meaningful role, e.g. electrolytes and alcohol. We suspect that the high BHI for orange juice can be explained by a combination of factors, not only protein and carbohydrate content.

Another factor to consider when determining hydration status is the insensible losses that occur in an office working environment. On average in this study for the 3 hour period we estimated an insensible water loss of 115(389) g. Despite the likely large variation between participants in the amount of insensible losses, these might be caused by skin losses as well as respiratory water losses through talking on the telephone (Grandjean 2004). Since the working environment was 23 °C differences in clothing worn may impact upon variation in insensible losses between individuals (Benelam and Wyness 2010). This temperature might be quite warm for an office room as it has been stated that one of the fundamental requirements for a working environment is that the place allows employees to perform their work optimally under comfortable conditions (Roelofsen 2002). According to the Health and Safety Executive (HSE) in the UK, the law does not state a minimum or maximum temperature at the workplace, but the temperature in workrooms should normally be at least 16 °C for a sedentary job or 13 °C when physical effort is involved (HSE). The Workplace (Health, Safety and Welfare) Regulations 1992 states that specifically in indoor workplaces

during work hours, the temperature in all workplaces inside buildings shall be reasonable (Britain 1993). There is no legal maximum safe working temperature. The Trade Union Congress (TUC) in the United Kingdom has recommended maximum safe working temperatures: 27 °C for manual workers and 30 °C for sedentary workers (TUC 2006). According to the TUC guidelines, the temperature of the call centre was still in the tolerable range. The trial was performed in October 2015 so it is very probable that during the summer days the temperature of this office can reach temperatures closer to the 30 °C because of the informatics equipment and the glass structure of the building.

Total body water comprises around 50-65% of body weight (Mack and Nadel 2011). Total body water depends on age, sex and body composition. A 70 kg male adult has around 42 litres of total body water (Wang, Deurenberg et al. 1999). It has been observed that total body water expressed as a percentage of total body mass, is highest in infants (~70%) and it declines with ageing. This reduction of total body water over the life span can be explained with an associated increase in body fat. The decrease in total body water is mainly due to decreased intracellular water (Schoeller 1989). This relationship suggests the possibility of having a special recommendation particularly addressed to the older adults as their total body water is normally lower from the young/middle aged adults. In this field study we also had a wider age range (20 to 57 years) that differed from our previous study (Maughan, Watson et al. 2016) where the age range was controlled (19 to 35 years) by excluding participants under 18 and over 35 years old. However, the results from the field study matched the findings from the laboratory controlled study.

Females also usually have more body fat than males and therefore the total body water in adult females is around 50% of the total body mass. This is why the adequate fluid intake recommendation for female adults is normally lower in comparison with the recommendation for their male counterparts. Our previous work was studying a group of male adults with a normal body mass index and under laboratory controlled conditions (Maughan, Watson et al. 2016). In the present study, body composition was not determined for logistical and practical reasons, and the volunteers that took part in this research had a wide variation of body mass index (body mass index of the participants ranged from 19.8 to 38.2 kg/m<sup>2</sup>).

On the other hand, there are some hormonal responses in the females that might affect fluid balance. It has been reported sex difference in terms of fluid balance responses during

prolonged exercise. Eijsvogels et al. (2013) observed differences between genders with a significantly lower fluid intake and larger losses of body mass in men due to dehydration. These authors also observed a higher increase in plasma sodium levels and a higher incidence of hypernatremia in the male participants. It has been observed that females can experience fluid retention during pregnancy and the luteal phase of the menstrual cycle and it is also a side effect of the use of gonadal steroids for contraception or hormone replacement therapy (Stachenfeld 2014, Stachenfeld 2014). Some studies have demonstrated that the osmotic threshold for vasopressin secretion is reset during pregnancy and during the luteal phase of the menstrual cycle (Durr, Stamoutsos et al. 1981, Davison, Gilmore et al. 1984, Spruce, Baylis et al. 1985, Stachenfeld, DiPietro et al. 1999) and this would affect the fluid and electrolyte balance. We did not track the menstrual cycle or the use of contraception or hormonal replacement of our female participants in this study. The age of the female volunteers ranged from 20 to 57 years.

We can conclude that this study confirms that the BHI is a novel tool that allows assessment of the effectiveness of beverages to maintain euhydration in situations that are not laboratory controlled. Although the BHI also seems effective in both males and females and also to a wider age range and in people with different body composition We highly recommend further investigation to confirm that the findings from the previous study (Maughan, Watson et al. 2016) to explore if the BHI is useful in populations that include females and subjects with different body composition. Further research is needed to investigate a larger sample size and possibly other types of working environment to validate the usefulness of the BHI. We also suggest to investigate the effect of body composition and age on the BHI.

## CHAPTER 5. SYSTEMATIC EVALUATION OF CARBOHYDRATE SODIUM, AND CAFFEINE ON THE BEVERAGE HYDRATION INDEX.

### ABSTRACT

This study aimed to systematically examine the influence of carbohydrate, sodium and caffeine content of beverages on the BHI. Three cohorts, each of 12 young, healthy, physically active males (Mean  $\pm$ SD: age  $26 \pm 4$  y; height  $179 \pm 7$  cm; body mass  $77.6 \pm 8.5$  kg; water intake  $2 \pm 0.6$  L/d), ingested 1L of beverages containing four inclusion levels of a single component (sucrose 0, 5, 10 and 20%; sodium 7, 15, 27 and 52 mmol/L or caffeine 0, 50, 100 and 200 mg/L) in a double-blind, crossover manner. Urine output and blood samples were collected at each hour for the subsequent 4h. Cumulative urine output (CUO) was lower ( $803 \pm 241$ g) after the ingestion of a 20% carbohydrate beverage than 0% ( $1129 \pm 231$ g), 5% ( $1119 \pm 225$ g) and 10% ( $1034 \pm 314$ g) carbohydrate beverages ( $P < 0.05$ ). CUO was also lower with 27 mmol/L ( $1104 \pm 339$ g) and 52 mmol/L ( $1012 \pm 322$ g) sodium beverages than 7 mmol/L ( $1375 \pm 280$ g) and 15 mmol/L ( $1306 \pm 295$ g) beverages ( $P < 0.05$ ). However, no differences in CUO were apparent following the ingestion of beverages containing 0 to 400 mg caffeine (CUO: 0mg:  $1390 \pm 238$ g, 50mg:  $1428 \pm 229$ g, 100mg:  $1460 \pm 400$ g, 200mg:  $1429 \pm 258$ g;  $P = 0.83$ ). Thus, the BHI was greater in beverages with higher carbohydrate or higher sodium content, but not influenced by caffeine. After 2h, 20% carbohydrate and 27 mmol/L and 52 mmol/L sodium solutions had significantly higher BHI than their respective control beverages [Median (IQR) BHI: 20% carbohydrate: 1.67(1.37, 4.05); 27 mmol sodium/L: 1.28(1.07, 1.55), 52 mmol sodium/L: 1.27 (1.07, 2.23);  $P < 0.05$ ]. Serum aldosterone and arginine vasopressin were similar and did not change over the time period regardless of the carbohydrate, sodium or caffeine content of the beverages. In conclusion, the carbohydrate content of beverages has no effect on BHI at concentration up to 10% carbohydrate. Sodium content of beverages in concentrations of 27mmol/L and higher can improve the hydration potential of beverages. Caffeine doses in beverages up to 400mg/L do not have an impact upon diuresis when ingested in a euhydrated state.

## 5.1 Introduction

Maintaining adequate hydration status is important to prevent the adverse outcomes that result from acute and chronic hypo- or hyper-hydration (Maughan 2012). There is growing evidence for a link between poor hydration status and a variety of disease states including renal, gastrointestinal, circulatory, and neurological disorders as well as a relationship between hydration status and outcomes in older patients admitted to hospital (Manz and Wentz 2005, El-Sharkawy, Watson et al. 2015). In addition, there is good evidence that hypo-hydration, if sufficiently severe, will adversely influence physical function (Sawka and Noakes 2007), and evidence for an effect of hydration status on cognition and mood (Shirreffs, Merson et al. 2004, Masento, Golightly et al. 2014, El-Sharkawy, Sahota et al. 2015).

Several factors are known to influence the effectiveness of fluid ingestion on maintenance or restoration of fluid balance. This area has been studied widely over the past 25 years, particularly focussed around restoration of exercise-induced dehydration. In particular, the volume of fluid ingested and the sodium content have been observed to be key in restoration and retention of fluid balance following exercise-induced dehydration (Shirreffs and Maughan 2000). In a recent multicentre study we have demonstrated that under resting euhydrated conditions the macronutrient composition as well as electrolyte balance of ingested drinks are key to reducing urine production and aiding fluid retention over a 4 hour follow-up period in young adult males (Maughan, Watson et al. 2016). Our observations highlight that the rate of fluid delivery to the circulation, and the subsequent ability to retain/excrete fluid, is important in categorising the hydration potential of beverages. We were able to classify beverages by their hydration potential (cumulative urine output following ingestion of water divided by cumulative urine output following ingestion of test beverages) and named this the beverage hydration index (BHI).

In our previous study (Maughan, Watson et al. 2016), generally, fluids higher in energy content such as those containing dairy protein (milk) or carbohydrates (orange juice), as well as those with high electrolyte content (milk, orange juice and oral rehydration solution) had a greater BHI. This response is likely due to two primary mechanisms involving both slower rate of gastric emptying and fluid delivery to the circulation (Mahe, Huneau et al. 1992, Calbet and Holst 2004) as well as the influence of electrolytes (particularly sodium) on subsequent expansion of blood volume and a decrease in plasma osmolality (Heer, Baisch et al. 2000). Indeed, the pattern of response in BHI for the range of commonly consumed beverages in



our study (Maughan, Watson et al. 2016) was generally consistent with what is known about the effects of their components on body fluid balance. For some beverages, such as tea and coffee, we observed an equivalent hydration potential when compared to the ingestion of water. This observation maybe considered surprising since there is still considerable general public confusion regarding the potential diuretic actions of caffeine (Hammond 2014). However, the dose administered in our previous work was ~200mg of caffeine, and previous reports have highlighted that up to as much as 350mg of caffeine may not induce a significant diuresis vs. matched volumes of water (Maughan and Griffin 2003, Killer, Blannin et al. 2014).

A limitation of our previous work (Maughan, Watson et al. 2016) is that we assessed commercially-available beverages containing a range of nutrients that may influence fluid balance (e.g. milk, sports drink) and this does not enable identification of the impact of each individual component on the BHI. Therefore, a systematic assessment of key components of beverages and their effect on the beverage hydration index is warranted. Thus, the objective of the present study was to explore the dose-response effects of individual beverage components (sodium, carbohydrate and caffeine) on the hydration potential of beverages (assessed as the BHI) when ingested under conditions of normal daily living. By characterising the effects of these individual components common to many common available drinks on the BHI in euhydrated individuals, we aimed to provide further insight into the classification of beverages by their hydration potential. We hypothesized that increasing the sodium or carbohydrate content in a given beverage would increase the BHI, and that only the highest dose of caffeine would decrease the BHI.

## 5.2 Methods

### 5.2.1 General Study Design

Three separate laboratories (Loughborough, Bangor and Stirling) collaborated to complete this study. At each site, 12 healthy, physically active men to participate in the present study aged between 18 and 35 years were recruited (**Table 5-1**). Using the experimental approach reported previously (Maughan, Watson et al. 2016), each site compared the effect of a control beverage and beverages containing three levels of a single nutrient on post-ingestion fluid balance; Loughborough - caffeine, Stirling - carbohydrate, Bangor - sodium (beverage

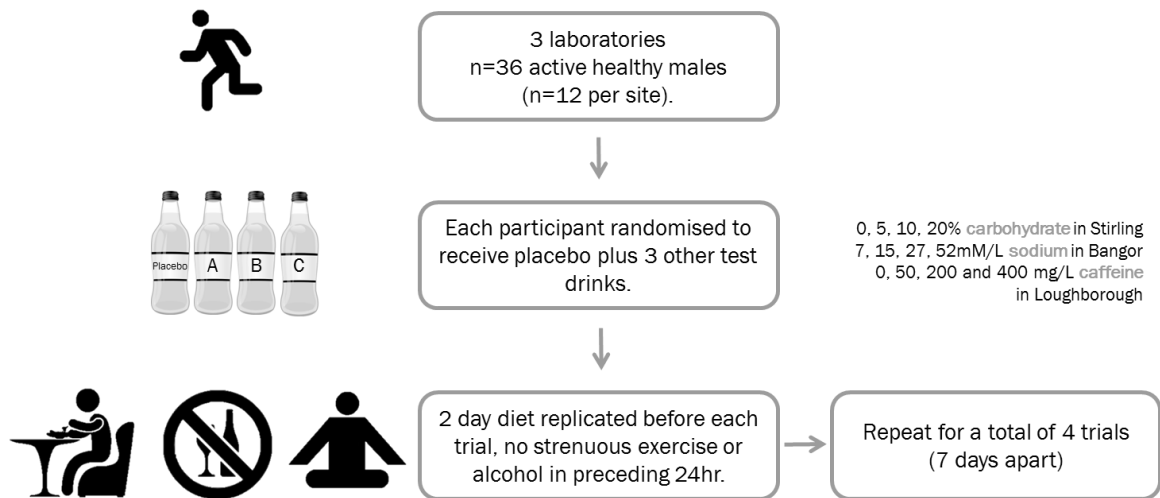
composition for each site is outlined below). These fluid balance data were then used to calculate a beverage hydration index (BHI) relative to the control beverage. Those volunteers with a history of cardiovascular, renal, muscle-skeletal or metabolic disease, as determined from a pre-participation health screen questionnaire, were excluded from participating in the present study. As body mass was used as an index of euhydration, those volunteers currently undertaking an energy-restricted diet and/or exercise plan were also excluded. Participants were asked to record their diet including their fluid intake (household measures technique; (Marr 1971)) as well as any exercise performed, in a diary over the 2 d before the first trial and asked to replicate this before their subsequent visits. Participants were also asked not to perform any strenuous exercise or consume alcoholic beverages in the 24 h preceding all trials. Ethical approval for the study was obtained separately from the local Ethics Committees of the three Institutions involved.

**Table 5-1 Participant physical and fluid intake characteristics.**

	Stirling - Carbohydrate (n = 12)	Bangor - Sodium (n = 12)	Loughborough - Caffeine (n = 12)	All (n = 36)	<i>P value</i>
Age (y)	26 ± 6	25 ± 4	27 ± 2	26 ± 4	0.53
Height (cm)	181 ± 7	179 ± 7	178 ± 7	179 ± 7	0.67
Mass (kg)	77.6 ± 9.3	78.2 ± 7.8	77.1 ± 8.9	77.6 ± 8.5	0.95
BMI (kg/m <sup>2</sup> )	23.9 ± 2.7	24.6 ± 2.2	24.2 ± 1.5	24.2 ± 2.1	0.75
Water intake (L/d)	1.9 ± 0.3	2.2 ± 0.9	1.9 ± 0.5	2.0 ± 0.6	0.42
Caffeine intake (mg/d)	210 ± 142	180 ± 123	206 ± 176	199 ± 145	0.87
Alcohol intake (g/d)	5 ± 6	4 ± 4	3 ± 2	4 ± 4	0.55

Data are Means ± SD.

Figure 5-1 Experimental design



### 5.2.2 Experimental Procedures

Following an overnight fast of at least 8 h, participants emptied their bladder upon waking, retaining an aliquot in a sterile collection tube. One hour before arriving at the laboratory, volunteers were instructed to consume 500 ml of still water (Highland Spring™, Perthshire, UK) over the course of 15 min. Upon arrival in the laboratory, volunteers remained seated in a comfortable environment for 20 min. A 21-g cannula was introduced into a superficial forearm or antecubital vein and a blood sample was collected. Participants were then asked to void their bladder and bowels before measurement of near-nude body mass (underwear only) to the nearest 50 g. Beginning approximately 30 min after arrival at the laboratory participants steadily ingested 1 L of the assigned test beverage over a period of 30 min (2 equal volumes administered 15 min apart). Using the approach employed previously (Maughan, Watson et al. 2016), a fixed volume, rather than a volume relative to body mass, was chosen as most beverages are served and ingested in containers of a standard volume. At the end of the 30 min drinking period, a blood sample was drawn and participants were asked to empty their bladder. This was repeated at hourly intervals, until 4 h post-ingestion. If a participant wished to pass urine before the hour was complete, this urine was collected and then added to any further urine produced at the end of the corresponding hour. After the final urine sample was collected, near-nude body mass was recorded again.

### 5.2.3 Beverages and beverage preparation

The control beverage at all sites consisted of a sugar-free fruit-flavoured beverage prepared according to manufacturer's directions using still water (Highland Spring™, Perthshire, UK). Each site prepared the control beverage, and this same beverage with three inclusion levels of a single nutrient, administered in a randomized, counter-balanced and double-blind manner; Loughborough 50, 200 and 400mg per L of caffeine (BDH, Leicestershire, UK), Stirling 50g, 100g and 200g per L of sucrose (Fine beet sugar, British Sugar Ltd, UK), Bangor 15, 27 and 52 mmol/L Na, as sodium chloride (NaCl; dried vacuum salt, Glacia Fine 60, British Salt Ltd, UK). The control beverage contained 7mmol/L Na (due to the addition of the fruit squash). The osmolalities of the 4 beverages administered at Stirling were 46, 205, 386 and 808 mOsmol/kg; at Bangor were 33, 54, 85 and 138 mOsmol/kg; and at Loughborough were 44, 43, 44 and 44 mOsmol/kg. All beverages were stored at standard refrigerated temperature (4-6°C) until served (**Table 5-2**).

**Table 5-2 Formulation of the ingested drinks**

%CHO	Lemon concentrated (ml)	Sucrose (g)	Water (ml)	Water %
<b>0</b>	100	0	900	100
<b>5</b>	100	50	880	98
<b>10</b>	100	100	830	93
<b>20</b>	100	200	780	88

Sodium (mmol/L)	Lemon concentrated (ml)	Additional Sodium (mmol/L)	Water (ml)	Water %
<b>7</b>	100	0*	900	100
<b>15</b>	100	8*	900	99
<b>27</b>	100	20*	900	99
<b>52</b>	100	45*	900	99

Caffeine (mg/L)	Lemon concentrated (ml)	Caffeine (mg)	Water (ml)	Water %
<b>0</b>	100	0	900	100
<b>50</b>	100	50	900	99
<b>200</b>	100	200	900	99
<b>400</b>	100	400	900	99

\* Lemon concentrated provided 7 mmol/L of sodium.

### 5.2.4 Urine and blood collection, storage and analysis

Collection, handling, and storage of urine and blood samples were undertaken in accordance with the Human Tissues Act. Stored samples were discarded once analysis was completed. Specific sample analysis was undertaken at all sites, unless otherwise stated.

All urine collected during the study was passed into a 1 L plastic container. The volume of each urine pass was determined by measuring the mass on an electronic balance (to the nearest 0.1 g), with the mass of the empty plastic container subtracted to enable the estimation of urine volume. From each urine sample a 5 ml aliquot was dispensed into a plain screw-capped tube that was stored at 4°C. Urine osmolality was measured in duplicate using freezing-point depression method (Gonotec Osmomat, Berlin, Germany at Loughborough and Bangor and Roehbling osmometer, Camlab, UK at Stirling) within 48 h of collection.

11 mL blood samples were drawn into dry syringes and immediately dispensed into a 5 mL serum tube, and 1 mL and 5 mL EDTA tubes. At Stirling, duplicate 100 µL aliquots of whole blood were rapidly deproteinised in Eppendorf tubes containing 1 mL of ice-cold 0.3 N perchloric acid. These were centrifuged and the resulting supernatant used to determine blood glucose concentrations using an appropriate, validated, in-house method (Glucose oxidase method, Instrumentation Laboratory, Italy).

Whole blood in the serum tube was allowed to stand for 1 h at room temperature to clot before centrifugation (10 min, 4°C, 2000-3000 g). Serum was then dispensed into an appropriate storage tube (e.g. Eppendorf) and an aliquot stored at 4°C for measurement of osmolality (Gonotec Osmomat, Berlin, Germany at Loughborough and Bangor and Roehbling osmometer, Camlab, UK at Stirling) and sodium by flame photometry (Bangor). Osmolality assessment of an identical control solution (mean 292 mmol/kg) at each site indicated that the Roehbling osmometer (Stirling) consistently reported a +4 mmol/kg bias compared with the Gonotec osmometer (Loughborough and Bangor). A further serum aliquot was stored at -80°C to enable to measurement of aldosterone and arginine vasopressin concentrations by enzyme-linked immunosorbent assay (all sites; Enzo Life Sciences, Lausen, Switzerland) and caffeine concentrations by HPLC (Loughborough; as previously described (Holland, Godfredsen et al. 1998)).

### 5.2.5 Data and statistical analysis

Participant characteristics at each institution were compared by an ordinary 1-factor ANOVA. Pre-drink hydration status, as assessed by body mass and serum and urine osmolality, was compared by one way ANOVA. To determine consistency of hydration status before beverage ingestion intra-individual coefficients of variation were determined for body mass, serum and urine osmolality. The main outcome measures were cumulative urine mass and net fluid balance after the ingestion of each beverage, presented as BHI. The BHI for each beverage was determined by dividing each individual's cumulative urine mass after the beverage with respective cumulative urine mass for the control beverage (i.e. 0% for carbohydrate, 7 mmol/L for sodium and 0 mg for caffeine). For each beverage component studied, the cumulative urine mass and net fluid balance were compared each hour and between different beverage doses by 2-way repeated-measures ANOVA. Significant main effects and interactions were further explored by Tukey's multiple-comparison tests to determine which beverages differed in cumulative urine mass and net fluid balance. BHI values were not normally distributed and therefore statistical comparison between beverages was made by Friedman test with significant effects further explored by Dunn's multiple comparison tests. To assess the practical meaning of the differences in urine output and net fluid balance observed between beverage doses, the difference was compared with the normal variation determined by a separate repeatability analysis, described in more detail elsewhere (Maughan, Watson et al. 2016). In brief, the repeatability of the cumulative urine mass was equal to a CV of 18% at 2 h and 15% at 3 h (~170 mL). In addition, the meaningfulness of these differences was also calculated with the use of 95% CI of differences between means and Cohen's *d* effect size (Cohen 1988).

To further elucidate how urine output and net fluid balance were altered by beverage content we examined selected aldosterone, arginine vasopressin and serum osmolality responses by one way repeated-measures ANOVA with significant main effects and interactions further explored by multiple-comparisons tests.

All statistical analyses were completed with the use of a computerized statistical software package (GraphPad Prism version 6 for Windows). Statistical significance was accepted at  $P < 0.05$ .

### 5.3 Results

The study sample collection was conducted between September 2014 and December 2014. The study was completed when the target number of participants ( $n = 12$ ) at each institution had finished the study, providing  $n = 12$  observations on each beverage condition. In total,  $n = 40$  were recruited: pre-participation screening excluded  $n = 0$ , loss to follow-up occurred because of vomiting after ingestion ( $n = 2$ ), or because of voluntary withdrawal from the study due to external factors ( $n = 2$ ).

#### 5.3.1 Pre-drink ingestion hydration status

On each trial, hydration status before beverages were ingested indicated euhydration (**Table 5-2**). Hydration status was also consistent as indicated by the intra-individual CV compared with previously published values (Cheuvront, Ely et al. 2010). The CV for body mass was 0.6%, 0.8% and 0.6% for carbohydrate, sodium and caffeine trials, respectively. The CV for serum osmolality was 0.7%, 1.0% and 0.7% for carbohydrate, sodium and caffeine trials, respectively. The CV for urine osmolality was 37%, 39% and 24% for carbohydrate, sodium and caffeine trials, respectively.

Table 5-3 Pre-drink ingestion hydration status.

<b>Stirling – Carbohydrate (n = 12)</b>					
	<b>0%</b>	<b>5%</b>	<b>10%</b>	<b>20%</b>	<b>P</b>
Body mass (kg)	77.5 ± 9.2	77.5 ± 9.4	77.7 ± 9.1	77.5 ± 9.5	0.70
Serum osmolality* (mmol/kg)	295 ± 3	296 ± 2	296 ± 2	295 ± 2	0.77
Urine osmolality (mmol/kg)	524 ± 323	557 ± 209	488 ± 290	664 ± 332	0.38
<b>Bangor – Sodium (n = 12)</b>					
	<b>7 mmol/L</b>	<b>15 mmol/L</b>	<b>27 mmol/L</b>	<b>52 mmol/L</b>	<b>P</b>
Body mass (kg)	78.2 ± 7.8	78.4 ± 8.1	78.5 ± 7.8	78.1 ± 8.2	0.50
Serum osmolality (mmol/kg)	289 ± 3	290 ± 3	291 ± 4	292 ± 4	0.17
Urine osmolality (mmol/kg)†	520 ± 215	544 ± 232	475 ± 201	513 ± 300	0.82
<b>Loughborough – Caffeine (n = 12)</b>					
	<b>0 mg</b>	<b>50 mg</b>	<b>100 mg</b>	<b>400 mg</b>	<b>P</b>
Body mass (kg)	77.3 ± 10.1	77.5 ± 10.1	77.7 ± 10.1	77.3 ± 10.1	0.26
Serum osmolality (mmol/kg)	287 ± 4	289 ± 5	289 ± 6	290 ± 5	0.05
Urine osmolality (mmol/kg)	441 ± 179	486 ± 144	478 ± 163	519 ± 168	0.48

Data are presented as Means ± SD. Notes: \*osmolality assessment of an identical control solution (mean 292 mmol/kg) at each site indicated that the Roehbling osmometer (Stirling) consistently reported a +4 mmol/kg bias compared with the Gonotec osmometer (Loughborough and Bangor).† n = 11 for Bangor urine osmolality analysis.



### 5.3.2 Plasma blood glucose, serum sodium and plasma caffeine responses

Blood glucose was greater after ingesting beverages containing carbohydrate (**Figure 5-1A**,  $P < 0.01$ ). Up to one hour after beverage ingestion, blood glucose remained higher after the 20% carbohydrate beverage than the 0% and 5% beverages. Blood glucose was then similar between beverages for the remainder of the 4 h with exception of the 10% carbohydrate beverage being lower than the 0% and 20% beverages at 2 h. Serum sodium was similar throughout the 4 h period after ingesting beverages of different sodium contents (**Figure 5-1B**,  $P > 0.05$ ). Plasma caffeine content increased in a dose-response manner where beverages with high caffeine content had highest plasma caffeine (**Figure 5-1C**,  $P < 0.01$ ).

### 5.3.3 Urine output and fluid balance responses to carbohydrate

Immediately after the ingestion of the different carbohydrate beverages, urine mass was similar ( $P = 0.12$ ). One, two and three hours after beverage ingestion, cumulative urine output was lower and net fluid balance higher after the 10% and 20% carbohydrate beverages than the 0% and 5% carbohydrate beverages (**Figures 5-2A & 2B**). Throughout the 4 h period, cumulative urine output was lower and net fluid balance higher after the 20% carbohydrate beverage than the 0%, 5% and 10% beverage. The largest differences in urine output and fluid balance were at 2 h. The effect sizes at 2 h compared with the 0% beverage were 1.46 for the 20% carbohydrate beverage and 0.73 for the 10% carbohydrate beverage. The mean differences in urine output compared with the 0% beverage were 500 g (95% CI: 399, 601) for the 20% carbohydrate beverage and 189 g for the 10% carbohydrate beverage (95% CI: 87, 290). These differences in urine output can be considered meaningful as they exceeded the 2 h cumulative urine output and net fluid balance CV calculated previously (Maughan, Watson et al. 2016).

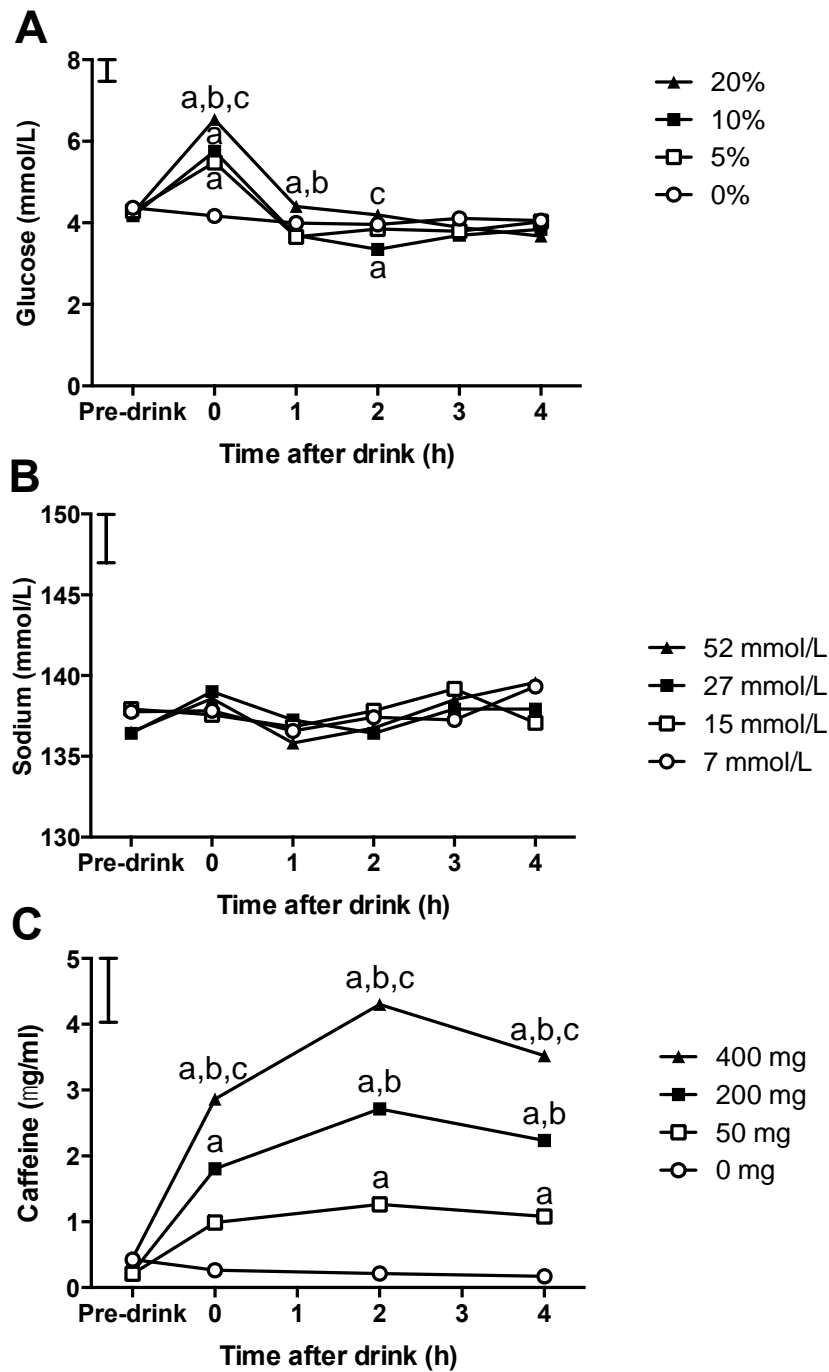


Figure 5-2 Blood glucose (A), serum sodium (B) and plasma caffeine responses (C) after the ingestion of 1 L of various carbohydrate (A), sodium (B) and caffeine (C) content beverages. n = 12 observation in each beverage condition.

Beverages with different responses are identified by Tukey’s multiple comparison test: a, indicates difference to 0% carbohydrate or 0 mg caffeine, b, indicates difference to 5% or 50 mg caffeine, c, indicates difference to 10% or 200 mg caffeine. The vertical error bar in the top left corner represents the overall mean SD during the 4-h collection.

### 5.3.4 Urine output and fluid balance responses to sodium

One hour after ingestion of the different sodium beverages urine mass was similar ( $P = 0.30$ ). Two, three and four hours after beverage ingestion, cumulative urine output was lower and net fluid balance higher after the 27 mmol/L and 52 mmol/L sodium beverages than the 7 mmol/L and 15 mmol/L beverages (**Figures 5-2C & 5-2D**). The largest differences in urine output and fluid balance were at 3 h. The effect sizes at 3 h compared with the 7 mmol/L beverage were 1.06 for the 52 mmol/L beverage and 0.87 for the 27 mmol/L beverage. The mean differences compared with the 7 mmol/L beverage were 372 g (95% CI: 228, 516) for the 52 mmol/L sodium beverage and 300 g (95%CI: 156, 444) for the 27 mmol/L sodium beverage. These differences also exceeded the 3 h cumulative urine output and net fluid balance CV.

### 5.3.5 Urine output and fluid balance responses to caffeine

Urine mass and net fluid balance were similar throughout the 4 h period on all trials after the ingestion of drinks with different caffeine content (**Figures 5-2E & 5-2F**,  $P = 0.83$ ).

### 5.3.6 Beverage Hydration Index

The BHI, an indication of short-term hydration potential of a drink, was greater in drinks with higher carbohydrate and sodium content, but was not affected by caffeine content (**Figure 5-3**). After 2, 3 and 4 h, 20% carbohydrate drink and 27 mmol/L and 52 mmol/L sodium drinks had higher BHI than their respective lowest dose (i.e. 0% for carbohydrate and 7 mmol/L for sodium, **Figure 5-3A & 5-3B**, all differences  $P < 0.05$ ). The BHI for the 20% carbohydrate drink was also greater than that of the 0% drink after 1 h and the 20% carbohydrate drink BHI was greater than the 5% carbohydrate drink at 1, 2 and 3 h (all differences  $P < 0.05$ ). In addition, the BHI for the 52 mmol/L sodium drink was greater than that of the 7 mmol/L sodium drink after 3 and 4 h (all differences  $P < 0.05$ ).

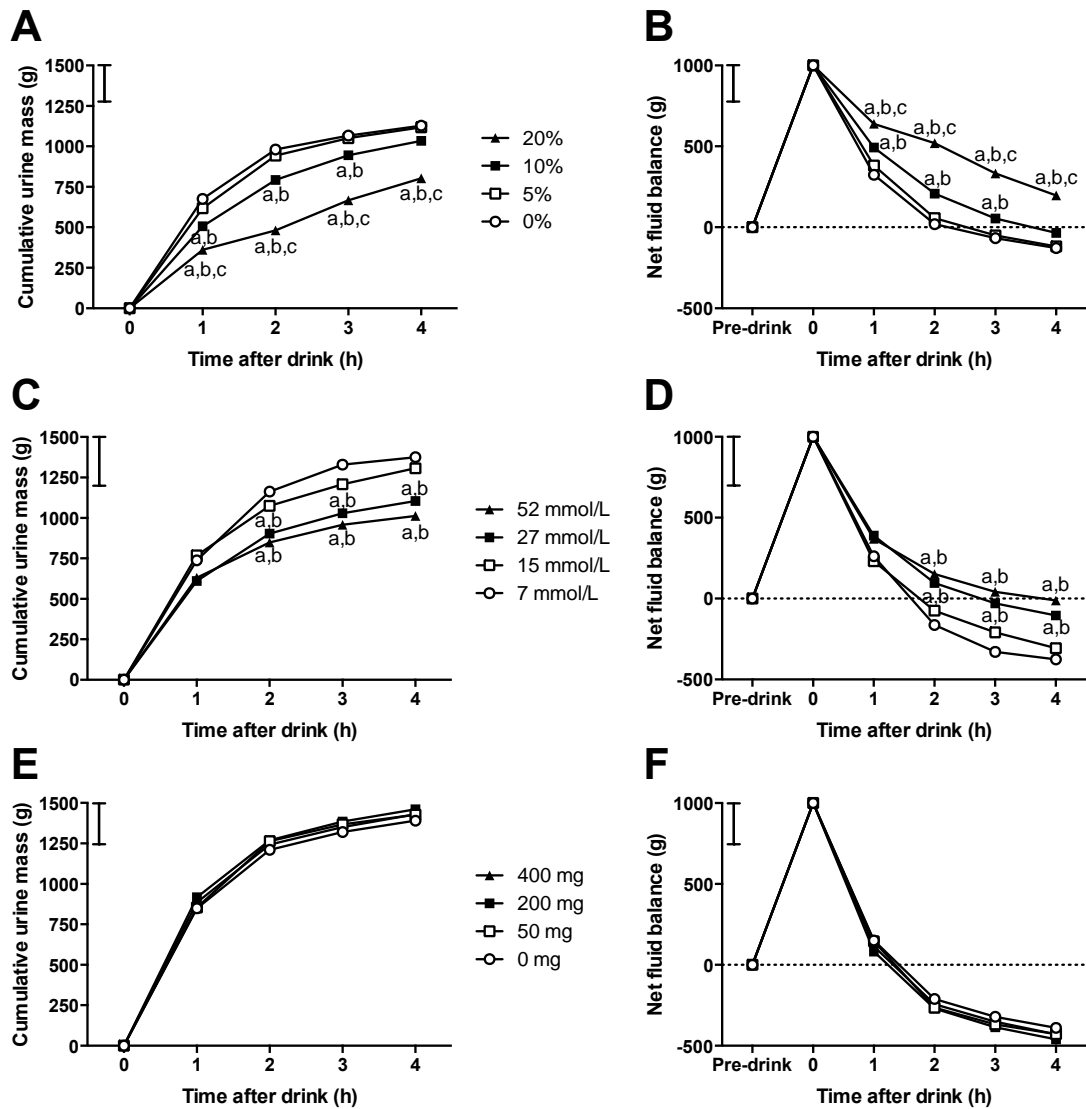


Figure 5-3 Cumulative urine output and net fluid balance after the ingestion of 1 L of various carbohydrate (A & B), sodium (C & D) and caffeine (E & F) content beverages. n = 12 observation on each beverage.

Beverages with different responses are identified by Tukey's multiple comparison test: a, indicates difference to 0% carbohydrate or 7 mmol/L sodium beverage; b, indicates difference to 5% carbohydrate or 15 mmol/L sodium beverage; c, indicates difference to 10% carbohydrate beverage. The vertical error bar in the top left corner represents the mean SD during the 4-h collection.

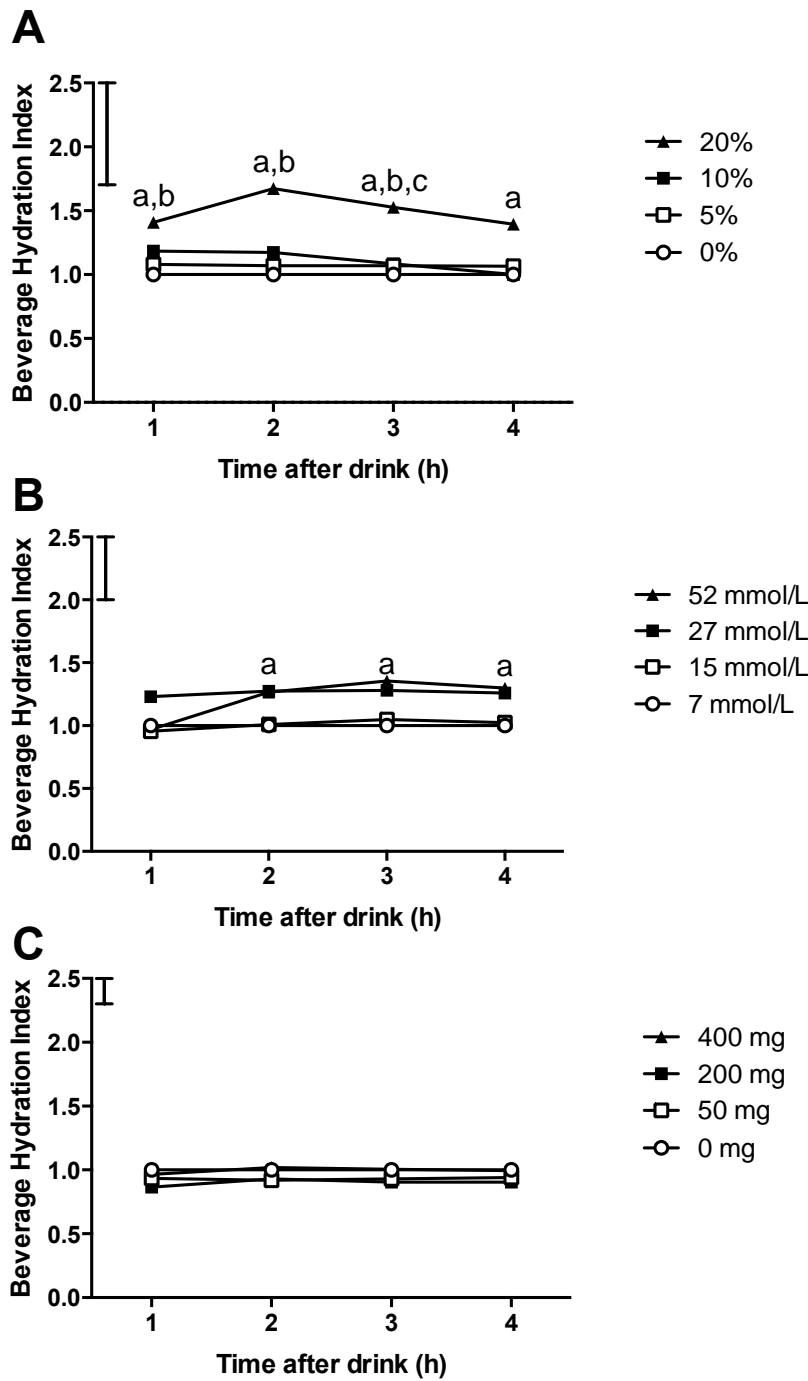


Figure 5-4 Beverage hydration index for various carbohydrate (A), sodium (B) and caffeine (C) content beverages. n = 12 observation on each beverage.

Beverages with different responses are identified by Dunn’s multiple comparison test: a, indicates difference to 0% carbohydrate or 7 mmol/L sodium beverage; b, indicates difference to 5% carbohydrate beverage; c, indicates difference to 10% carbohydrate beverage. These are median data with the mean IQR during the 4-h collection represented by the vertical error bar in the top left corner.

### 5.3.7 Fluid-regulation and redistribution

Throughout the 4 h period, aldosterone and arginine vasopressin concentrations were similar irrespective of the carbohydrate, sodium or caffeine content of beverages (**Table 5-3**). The first hour after ingestion of 10% and 20% carbohydrate content beverages, serum osmolality increased while it was unchanged and similar after 0% and 5% carbohydrate beverage ingestion (**Figure 5-4A**). In contrast, immediately after the ingestion of sodium beverages, serum osmolality decreased (**Figure 5-4B**). The decrease in serum osmolality was greatest after the 52 mmol/L sodium beverage.

**Table 5-4 Fluid-regulatory hormones. Mean aldosterone and arginine vasopressin (AVP) responses after test drink ingestion**

<b>Stirling Carbohydrate (n = 12)</b>					
	<b>0%</b>	<b>5%</b>	<b>10%</b>	<b>20%</b>	<b>P</b>
Aldosterone (pg/ml)	103 ± 31	113 ± 27	100 ± 30	106 ± 34	0.47
AVP (pg/ml)	3.5 ± 0.6	3.4 ± 0.6	3.6 ± 0.6	3.7 ± 0.7	0.50
<b>Bangor Sodium (n = 12)</b>					
	<b>7 mmol/L</b>	<b>15 mmol/L</b>	<b>27 mmol/L</b>	<b>52 mmol/L</b>	<b>P</b>
Aldosterone (pg/ml)	109 ± 41	126 ± 67	150 ± 59	100 ± 62	0.16
AVP (pg/ml)	3.7 ± 0.7	3.6 ± 0.9	3.8 ± 1.2	3.9 ± 0.8	0.79
<b>Loughborough Caffeine (n = 12)</b>					
	<b>0 mg</b>	<b>50 mg</b>	<b>200 mg</b>	<b>400 mg</b>	<b>P</b>
Aldosterone (pg/ml)	90 ± 73	99 ± 64	72 ± 64	87 ± 108	0.60
AVP (pg/ml)	3.5 ± 1.4	3.5 ± 1.1	2.9 ± 0.9	3.8 ± 0.9	0.22

Data are presented as Mean ± SD.

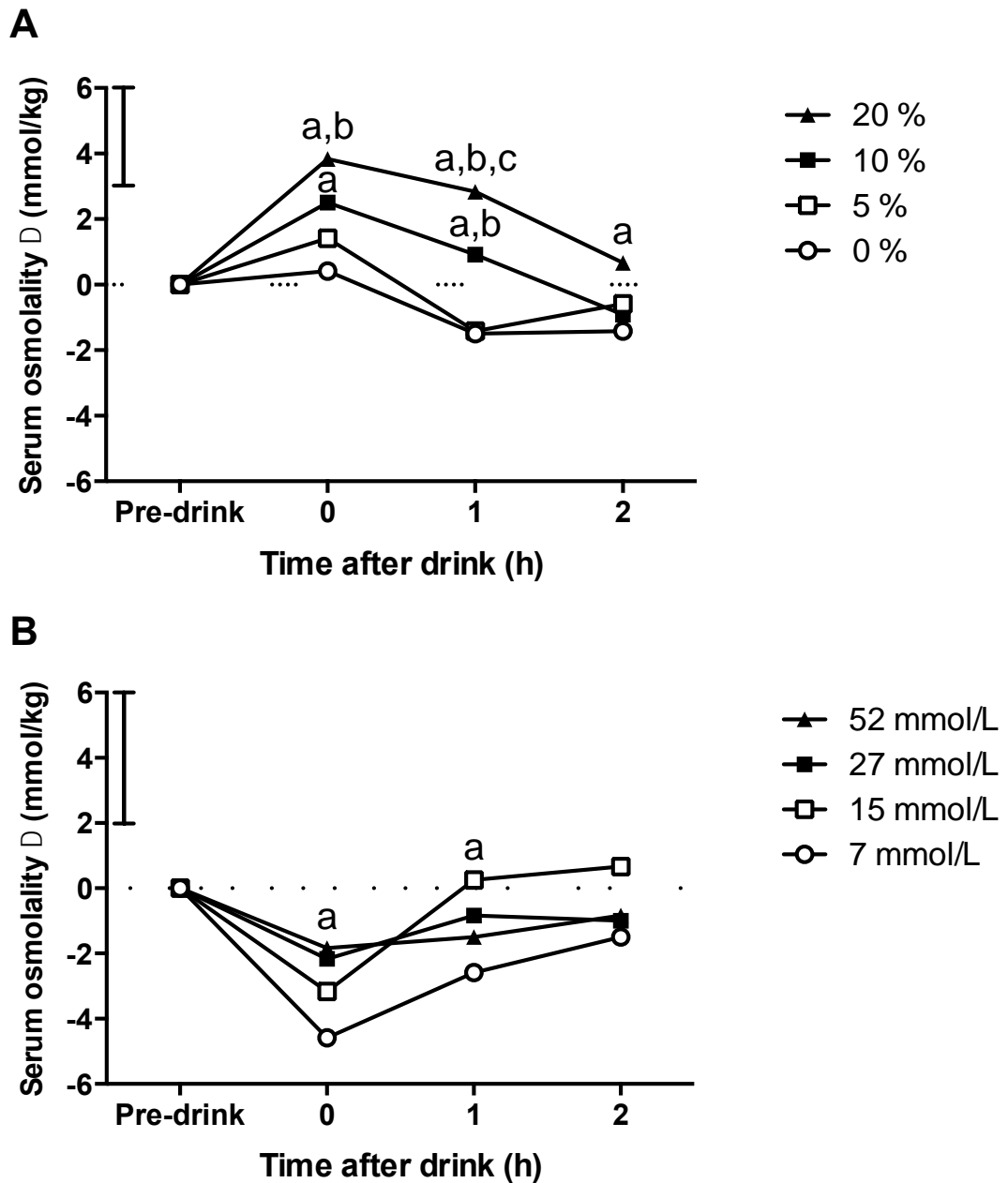


Figure 5-5 Serum osmolality change after the ingestion of 1 L of various carbohydrate (A) and sodium (B) beverages. n = 12 observation on each beverage.

Beverages with different responses are identified by Tukey’s multiple comparison test: a, indicates difference to 0% carbohydrate or 7 mmol/L sodium beverage; b, indicates difference to 5% carbohydrate beverage; c, indicates difference to 10% carbohydrate beverage. The vertical error bar in the top left corner represents the mean SD during the 4-h collection.

## 5.4 Discussion

In our previous work (Maughan, Watson et al. 2016) we were able to quantify the hydration potential of commercially-available drinks using a beverage hydration index (BHI). The BHI largely classified beverages according to expectations based on the known effects of energy density and electrolyte composition on fluid delivery and retention. However, in many cases beverages contained combinations of key components that could influence gastric emptying, intestinal absorption, and fluid retention characteristics (e.g. macronutrients, electrolytes and caffeine). The results of the present study reveal that carbohydrate content up to 5%, caffeine content up to 400mg/L, and sodium content up to 15mM have no effect on fluid balance or the beverage hydration potential assessed as the BHI. However, 10% and 20% carbohydrate beverages and beverages containing 27mmol/L and 52mmol/L sodium result in some delayed diuresis and greater BHI. These data indicate that carbohydrate and sodium thresholds exist with regard to impacting BHI, but that caffeine intake up to the highest dose examined (400mg/L) has no effect on BHI. Given that these test drinks were examined in a euhydrated state, the reduced urine output on these trials likely occurred due to mechanisms involving a combination of gastric emptying and intestinal absorption characteristics, as well as potential effects on renal excretion of the fluid excess.

Early studies investigating factors influencing the rate of fluid delivery to the body water pool identified a role for electrolytes and macronutrients, and their combination, on gastric emptying rate (Hunt and Pathak 1960) and intestinal absorption (Phillips and Summersk. Wh 1967). These studies demonstrated that with low glucose content the addition of sodium to test meals increased the rate of gastric emptying. However, when the glucose content was increased the gastric emptying action of the added sodium was reduced. Other studies have investigated the effects of solute volume, temperature, osmolality, and glucose content of beverages on the rate of gastric emptying (Hunt and Macdonald 1954, Costill and Saltin 1974, Vist and Maughan 1994, Vist and Maughan 1995). These studies observed that even small amounts of glucose (<4% solution) reduced the rate of gastric emptying compared to water, warm/hot fluids reduced gastric emptying compared to cold beverages, and that faster initial emptying rates were reached with higher bolus volumes. These previous studies highlight the important role of beverage components (electrolytes and carbohydrate content) as well as volume and temperature on delivery of fluid to the intestine. These observations suggest that the gastric emptying rate of test drinks in the present study would be reduced with an increasing energy / carbohydrate content (above 4-5% carbohydrate), remain unchanged or



be increased with an increasing sodium content, and likely remain unchanged by increasing caffeine content.

Furthermore, osmolality and solute flux determine net water transport in the small intestine and thus contribute to rate of fluid delivery to the body water pool (Malawer, Ewton et al. 1965). Hypertonic solutions (>300 mOsm/kg) result in transient net water secretion into the intestinal lumen whereas hypotonic solutions (<260 mOsm/kg) stimulate net water absorption (Hunt, Elliott et al. 1992). High carbohydrate solutions with high osmolality will therefore delay gastric emptying, slow delivery of fluid to the intestine, and cause net water secretion into the intestinal lumen. However, Gisolfi et al (Gisolfi, Summers et al. 1992) revealed that water absorption was independent of carbohydrate content in solutions up to 6%. Applying these observations to the present study would suggest that more concentrated sucrose solutions would likely affect both gastric emptying and intestinal water transport (in solutions >8% carbohydrate). Indeed, the pattern of change in serum osmolality in the present study with increased osmolality following the beverage ingestion period in the 10% and 20% sucrose beverage trials would suggest a transient net efflux of water out of the circulation into the gastrointestinal tract with those beverages (**Figure 4**). A net efflux would delay the expansion of the total body water pool and remove the stimulus for a diuretic response, which is reflected in the differences in cumulative urine output over time and the subsequent BHI value obtained. However, it is important to note that despite the positive effect on the BHI score, hypertonic solutions containing 10%-20% carbohydrate may have a potential negative effect on total energy balance throughout the day (Helm and Macdonald 2015).

All of the sodium-containing beverages in the present study were hypotonic and thus should promote gastric emptying and net water uptake from, rather than net efflux into, the intestinal lumen. Although there was no change in serum sodium across all trials, it is likely that increasing the sodium content of beverages aided fluid retention in the body water pool and this became significant in the 27mmol/L and 52mmol/L trials. Initial changes in serum osmolality on the sodium trials would reflect this response, with less reduction in osmolality on the 27mmol/L and 52mmol/L sodium trials compared to control. Thus, the impact of increasing the sodium content of beverages upon total urine output and BHI probably reflects initial differences in gastric emptying rate which induce increases in intestinal water transport and subsequent retention of the fluid in the body water pool.

The principal determinant of permeability, and consequently of water reabsorption, in the collecting ducts of the kidneys is arginine vasopressin (AVP). Aldosterone, produced by the adrenal cortex, also stimulates sodium reabsorption in the cortical collecting ducts. In the present study the responses of aldosterone and AVP to fluid ingestion were similar regardless of the content of carbohydrate, sodium or caffeine within the beverages. AVP and aldosterone also did not change over time during the ingestion or follow-up period. This lack of response may be explained by the participants arriving at the laboratories in a euhydrated state. Indeed, participants achieved euhydration by drinking 500ml of still water 1 hour before their arrival on each trial day. Measurements of serum and urine osmolality in the first samples collected on arrival at the laboratories indicates the absence of dehydration on all trials (**Table 5-2**). If the participants had been under a fluid restriction protocol or exercise-induced dehydration we would likely have observed changes in AVP and possibly also in aldosterone concentrations. Thus, in the present work it seems that differences in urine output between carbohydrate beverages and between sodium-containing beverages are not influenced by differences in renal water or sodium excretion.

Caffeinated beverages are popular, representing an important fluid source, yet are widely considered to induce diuresis and subsequently to promote dehydration. For this reason, the public are regularly advised to avoid consumption of caffeinated beverages, especially at times when hydration status may be under threat. Caffeine acts as an adenosine receptor antagonist reducing fractional sodium reabsorption in the proximal tubule and in the distal nephron (Killer, Blannin et al. 2014) which could lead to increased renal water loss. Previous research exploring the effect of administering different doses of caffeine (45, 90, 180, 360 mg) has observed increased urine volume only when participants ingested 360 mg of caffeine (Passmore, Kondowe et al. 1987). In our study we did not observe any effect of caffeine at a dose of up to 400 mg on the net fluid balance response when ingested in 1L of fluid (**Figure 5-2E & 5-2F**). We can therefore conclude that under the present study conditions, 400 mg was not sufficient to stimulate a measurable diuresis. This lack of effect could be explained by all participants being habitual caffeine users, but clear evidence of an attenuated diuretic effect with regular exposure to caffeine has not been reported. Based on our results we support previous findings that consuming caffeinated beverages (containing up to 400 mg caffeine) does not negatively affect hydration status (Grandjean, Reimers et al. 2000, Armstrong, Pumerantz et al. 2005). The practical application is that caffeinated beverages (containing up

to 400mg of caffeine) can contribute to daily total fluid intake targets without having negative effects on fluid balance.

These data in euhydrated healthy young men demonstrate the important individual roles of macronutrients (carbohydrate) and electrolytes (sodium) on fluid delivery and retention, with no effect of caffeine content in the range of doses studied. The data highlight that the key drivers promoting differences in BHI between the beverages assessed in our study are likely to be delayed gastric emptying rate and intestinal water transport with high carbohydrate doses (20% carbohydrate), and increased fluid retention for higher sodium containing drinks (27 and 52 mmol/L Na<sup>+</sup>). The effect on BHI was observed earlier with the more concentrated carbohydrate beverages (at 1 h) and later with the higher sodium containing beverage (at 2 h), likely reflecting the different effects of the beverage components upon fluid delivery and retention (**Figure 5-3**). These data strengthen our previous observations on BHI and suggest that beverages can obtain a high BHI score due to characteristics from components that delay fluid delivery, or improve fluid delivery and retention. In our previous work the drinks that performed well on the BHI score contained a combination of ingredients that likely targeted both of these mechanisms. Unfortunately, we have not assessed gastric emptying or intestinal water transport in our studies to date but are reliant upon urine output and serum osmolality changes as markers of water redistribution in response to beverage ingestion.

By studying euhydrated individuals we were able to observe the hydration potential of beverages under controlled conditions in which urine production was not limiting to sample collections. Under these circumstances rapid fluid delivery from a beverage to the body water pool in the absence of sufficient electrolytes will lead to rapid diuresis. The present study findings lend support to the recommendation of ingesting a variety of beverages, including caffeinated beverages, alongside water, to meet daily fluid intake requirements. Furthermore, we provide further evidence that the sodium content of a beverage is likely to be a main driver for improved fluid delivery and maintenance of positive net fluid balance. These observations require further exploration in other groups such as older adults who likely have a combination of impaired ability to respond to a fluid overload and a reduced ability to reduce renal water excretion. We also recommend further investigation in potassium content of beverage in young and older adults.

## CHAPTER 6. COMPARISON OF NET FLUID BALANCE RESPONSES TO DIFFERENT DRINK COMPOSITIONS IN YOUNG AND OLDER MEN.

### ABSTRACT

The purpose of the fifth study described in Chapter 6 was to compare net fluid balance (NFB) responses to the ingestion of commonly consumed drinks in young and older men. Aging is associated with a reduced ability to maintain homeostatic control over a variety of body functions, including maintenance of body fluid balance. 24 healthy male participants: 12 young (mean  $\pm$  SD)  $24.5 \pm 4.3$ y and 12 older  $62.7 \pm 6.6$ y were recruited. Initial near nude body mass was recorded after emptying their bladder to provide a urine sample. Participants then consumed a fixed volume (1L, 250ml every 15 min) of water (W, control), fruit juice (F, 0 mmol/L Na<sup>+</sup>; 23 mmol/L K<sup>+</sup>; 21 kcal/100 ml), sports drink (S, 29 mmol/L Na<sup>+</sup>; 2.7 mmol/L K<sup>+</sup>; 28 kcal/100ml) or skimmed milk (M, 19mmol/L Na<sup>+</sup>, 40mmol/L K<sup>+</sup>; 35 kcal/100ml). Participants urinated at the end of the 60-minute drinking period and every hour for the next 3 hours. Urine mass was recorded and a sample obtained for analysis of urine osmolality and electrolytes (Na<sup>+</sup> and K<sup>+</sup>). Blood samples were drawn immediately after the drinking period and every hour for the next 3 hours, for serum osmolality and electrolytes. Initial serum osmolality demonstrated that both groups began euhydrated (young,  $298 \pm 3$ ; old,  $297 \pm 4$  mOsm/kg). Following M ingestion NFB was different to W after 3hours in young ( $+259 \pm 288$  g;  $P < 0.05$ ) but not in older ( $+49 \pm 456$ g) men. No differences in NFB were observed between W and the other drinks in young (F:  $+73 \pm 384$ g, S:  $+47 \pm 271$ g), or older (F:  $-6 \pm 374$ g, S:  $-21 \pm 290$ g) men. Net Na<sup>+</sup> balance was negative with W ( $-578 \pm 279$ mg) and most positive and different to W with ingestion of S ( $+124 \pm 366$ mg;  $P < 0.05$ ) in both groups. Net K<sup>+</sup> balance was positive on F and M ( $+179 \pm 402$ mg;  $P < 0.05$ ) but negative on W and S ( $-741 \pm 321$ mg) in both young and older men. In young adults M helps to maintain positive net fluid balance for longer than other drinks. In older adults this effect of M is not observed despite similar net electrolyte balance responses. Future work should more fully explore these potential differences in fluid balance responses to drink ingestion between young and older adults.

## 6.1 Introduction

Ageing is associated with changes in the homeostatic control systems that regulate fluid and electrolyte balance (Luckey and Parsa 2003). These changes increase the risk of becoming dehydrated or developing problems of fluid overload (Allison and Lobo 2004). The main age-related changes are: reduced thirst due to a decrease in the sensitivity of the volume and osmoreceptors (Kenney and Chiu 2001), loss of lean mass leading to reduction in total body water by 10-15% (Allison and Lobo 2004, Adams, Leitzmann et al. 2014), a reduced capacity to concentrate urine and to handle water and electrolytes efficiently (Lindeman, Tobin et al. 1985), and a decrease in gastrointestinal motility and in gastric secretion that impact water losses in faeces (Russell 1992, Atillasoy and Holt 1993). A factor that can lead to fluid overload is that older healthy adults have an impaired ability to excrete excess fluid promptly due to an age-related reduction in glomerular filtration rate (GFR) (Dontas, Marketos et al. 1972). In addition, pharmacological interventions such as laxatives and diuretics make older adults more vulnerable to shifts in fluid balance that can result in over hydration or, more frequently, dehydration (Mentes 2006).

The risk of dehydration in older adults is exacerbated by cognitive and behavioural changes such as a lack of knowledge or misconceptions concerning effects of drinking or not drinking sufficient fluid (Hooper, Bunn et al. 2014) and limiting or avoiding drinking to reduce the need of urinating (Godfrey, Cloete et al. 2012). It has been observed that in general, older adults tend to consume less water from beverages and from food than younger adults, and previous reports suggest that 30% of adults do not meet the fluid intake requirements established by the European Food Safety Authority (EFSA 2010, Gandy 2012) leaving many individuals prone to chronic dehydration. Furthermore, it has been demonstrated that hydration status has an impact on the clinical outcome of older hospitalized patients (El-Sharkawy, Watson et al. 2015). Dehydration has been associated with 6 times greater risk of in-hospital mortality compared with euhydrated patients (El-Sharkawy, Watson et al. 2015). EFSA has defined the adequate intake of fluids from beverages for older males as 2.0 L/d and for older females as 1.6 L/d.

There are several studies examining interventions to improve hydration status in healthy older adults (Hooper, Bunn et al. 2014, Bunn, Jimoh et al. 2015). However, most studies have investigated interventions to improve hydration and nutritional status in older people with dementia (Abdelhamid, Bunn et al. 2016) or in older adults resident in care homes. A study undertaken by Robinson & Rosher (Robinson and Rosher 2002) demonstrated that the way in which beverages are presented (i.e. the intervention was to use a drinking cart with different colours mugs), and the variety of beverages available, were critical components of a hydration program. In an intervention study of Simmons et al (2001) with nursing home residents demonstrated that a behavioural intervention consisting of verbal prompts to drink was effective in increasing fluid intake in residents. Thus, the availability and accessibility of a variety of hot and cold drinks may help to ensure that older people consume enough fluid (Hooper, Bunn et al. 2014). To date, most intervention studies aimed at improving hydration status in older adults have focused on the fluid ingestion behaviour, but no study has investigated the effect of beverage composition on hydration status in an older adult population.

A recent study completed in our laboratory and in collaboration with Bangor University and Loughborough University (Maughan, Watson et al. 2016) observed that different beverages such as tea, cola, coffee, lager and sports drink were just similarly effective as water in maintaining hydration status in a group of young adults. Other beverages (milk, orange juice and oral rehydration solution) were more effective than water in maintaining hydration status during a seated rest period in young healthy active males (age 18-35 years). These data highlight that electrolyte composition (sodium / potassium) as well as certain macronutrients (i.e. protein/carbohydrate) are important in determining the hydration potential of beverages in young adults. However, no studies have compared the responses to beverages with different macronutrient and electrolyte composition between older adults and young adults. Therefore, we aimed to investigate the impact of beverage composition on maintenance of hydration status in young and older adult males. We hypothesized that the influence of drink components in test beverages (electrolyte content, protein content, carbohydrate content or their combination) would lead to differential responses in net fluid balance and hydration status in older adults compared to young adults, due to physiological changes in homeostatic control that occur with ageing.

The aim of the present study was to assess fluid balance responses to the ingestion of a fixed volume of commonly consumed beverages when in a mild dehydrated state in young and older men. We hypothesized that beverages with higher electrolyte content or high caloric content would have greater fluid retention in young and that these responses might not be observed in the older group.

## 6.2 Methods

The hydration response to the ingestion of 4 commonly-consumed and commercially available beverages was tested. The four beverages assessed were: still water (as control), orange and mango juice (moderate carbohydrate, and potassium), skimmed milk (moderate carbohydrate, protein and electrolytes (sodium and potassium)) and a sports drink (moderate carbohydrate and sodium).

### 6.2.1 Pre-trial standardization/exclusion criteria

Twenty-four participants were recruited, 12 meeting the inclusion criteria for the young group and 12 meeting the criteria for the older group (**Table 6-1**). Those participants with a history of cardiovascular, renal, musculoskeletal or metabolic disease as determined from a health screen questionnaire were excluded. Participants who were under an energy restricted diet and/or exercise plan to lose weight or those consciously attempting to increase muscle mass were excluded because body mass was used as one of the markers of hydration status. Volunteers were asked to record and replicate their food and fluid intake in the two days before each trial and also were asked to refrain from alcohol ingestion and vigorous physical activity 24 hours before all trials.

**Table 6-1 Participant characteristics on entry into the study.**

Values are mean (SD). No significant differences were noted between groups, except for age.

Group	Age (y)	Height (m)	Body mass (kg)	Body mass index (kg/m <sup>2</sup> )	Daily fluid intake (ml/day)	Initial serum osmolality (mOsm/kg)	Initial urine osmolality (mOsm/kg)
Young (n=12)	24.5 (4.3)	1.79 (0.07)	76.7 (9.1)	24.0 (2.3)	1929 (351)	298 (3)	534(301)
Older (n=12)	62.7 (6.6)	1.78 (0.07)	79.2 (9.3)	25.1 (3.4)	1733 (211)	297 (4)	401 (149)

### 6.2.2 Experimental procedure

Participants attended the laboratory on four occasions, each separated by 7 days. They were asked to drink 500 ml of water 1 hour before arriving at the laboratory in an attempt to obtain a euhydrated condition. Participants were also asked to collect a urine sample in a sterile collection container to determine first morning urine osmolality. When the participants arrived at the laboratory (in the morning after an overnight fast), they were asked to empty their bladder/bowels, urine was collected, urine mass was recorded, and a 5ml aliquot was retained for analysis. After this, initial near nude body mass was recorded (in underwear only). An intravenous cannula was inserted into an antecubital vein; participants adopted a seated position for 15 min after which a baseline blood sample was drawn. Participants then consumed a fixed volume (1L, 250ml every 15 min) of water (W, control), fruit juice (F), sports drink (S) or skimmed milk (M). Participants urinated at the end of the 60-minute drinking period and every hour for the next 3 hours. Participants were allowed to urinate at any moment but they were asked to empty their bladder every 60 minutes. When volunteers needed to urinate before the 60 minutes period time, the urine was collected and was combined with the urine produced on the time point. Urine mass was recorded to determine cumulative urine mass every hour and a 5ml aliquot of urine was collected. Blood samples were drawn immediately after the drinking period and every hour for the next 4 hours through the indwelling cannula. All blood samples were drawn from participants once they had remained in a seated position for at least 10 minutes. (**Figure 1**).



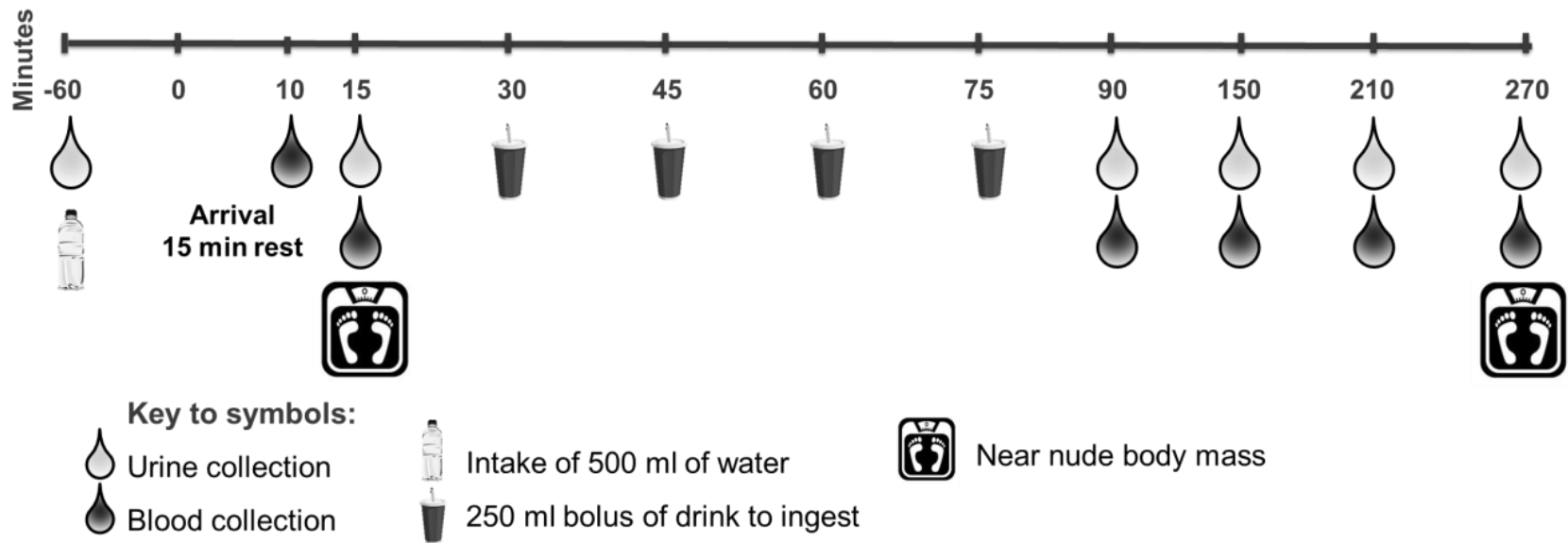


Figure 6-1 Schematic design of experimental trial days

### 6.2.3 Beverages

Test beverages were applied in a randomised counterbalanced order. Standard commercial beverages (Highland Spring still water, Trop50 orange – mango juice, Lucozade and 0.1% fat milk; Tesco) were purchased as a single batch from a single source (for products with a short shelf life) to be used for all trials. The nutrient composition of the test beverages is presented in **Table 6-2**. All beverages were stored at a standard refrigerated temperature (4-6°C) until serving.

### 6.2.4 Blood, serum and urine analysis

Total urine mass was measured over the 3 hours post beverage ingestion and the samples obtained each hour were analysed for urine osmolality and urine electrolyte excretion. All urine collected at each time period was passed into a 2 L plastic container and then the volume was estimated by measuring the mass on an electronic set of scales (to the nearest 1g), with the mass of the empty plastic container subtracted to enable the calculation of the estimation of the urine volume. From each urine sample a 5ml aliquot was stored in a plain screw capped tube. This tube was stored at 4°C for the analysis of urine osmolality and electrolyte (sodium and potassium) determination. Whole blood samples were dispensed in three different tubes: a serum tube that was allowed to stand for 4-5 hours at room temperature to clot before centrifugation (15 min; 10000 rpm; 4°C) and serum was used for serum osmolality and electrolytes and two EDTA tubes, one was stored immediately in an ice bath after blood was dispensed and then it was centrifuged to obtain the plasma that was subsequently stored in an Eppendorf tube at -80°C for later determination of creatinine to calculate creatinine clearance (Devanand and Chithrapavai 2013). The other EDTA tube was stored at room temperature until determination of haemoglobin using cyanmethemoglobin method and haematocrit using a micro haematocrit centrifuge to calculate changes in cell, plasma and blood volumes. Urine and serum osmolality was measured in duplicate with the use of a freezing point depression method (Löser osmometer) within 48 hours of collection. Urine and serum sodium and potassium concentrations were measured in duplicate with the use of flame photometry (PFP7/C Clinical Flame Photometer, Jenway) within 7 days of collection.

**Table 6-2 Water, energy, macronutrient and sodium and potassium content of tested beverages.**

Macronutrients data were obtained from the product labels and electrolytes data were obtained through flame-photometry analysis.

Drink	Water content (%)	Energy (kcal/100ml)	Carbohydrate (g/100ml)	Fat (g/100ml)	Protein (g/100ml)	Sodium (mmol/L)	Potassium (mmol/L)
Water – control (W)	100	0	0.0	0.0	0.0	0	0
Fruit juice (F)	95	23	4.4	0.0	0.3	0	28
Sport drink (S)	94	28	6.4	0.0	0.0	22	3
Skimmed milk (M)	91	35	5.0	0.1	3.4	19	23

### 6.2.5 Data presentation and statistical analysis

Net fluid balance was calculated based on the urine output mass at each time point. All the participants started at zero and then achieved a positive net fluid balance of 1000 (g) based on the fixed volume of the fluid they were asked to drink (1 L). To calculate the fluid balance at any given time point, the total mass of urine (cumulative urine mass) that had been collected following the drinking period was subtracted from the initial fluid balance.

$$\text{Initial Fluid Balance} = \text{IFB} = 1000 \text{ g}$$

$$U_n = \text{urine mass at } n \text{ time point}$$

$$\text{Net fluid balance after 3 hours} = \text{IFB} - (U_1 + U_2 + U_3)$$

The beverage hydration index (BHI), as described by Maughan 2016 (2016), was obtained by dividing the total urine output over a period of time when the control drink was ingested (still water – Highland Spring®) by the total urine output for the same period of time with the intake of another beverage.

$$BHI = \frac{\text{Total urine output when control was ingested}}{\text{Total urine output when a test drink was ingested}}$$

Cumulative urine output at different time points, by beverages between groups were compared using a 3-way repeated measures ANOVA. Participant characteristics, net fluid balance, hydration index and cumulative urine electrolyte loss were analysed by independent t test. All statistical analyses were completed using a statistical software package (IBM SPSS Statistics, Version 21 for Windows and Mac). Statistical significance was accepted at  $P < 0.05$ . Data are presented as means  $\pm$  SDs.

## 6.3 Results

The study was finished when the target number of participants ( $n=24$ , 12 young and 12 older) had completed the study.

### 6.3.1 Pre-drink ingestion hydration status

Mean urine osmolality for the first morning urine collection demonstrated that the young group was slightly dehydrated (American College of Sports, Sawka et al. 2007) while the older group values were in the euhydrated range (764(257) mOsm/kg for young and 595(172)mOsm/kg for older). However, mean serum and urine osmolality obtained before beverages were ingested demonstrated that both groups began mildly dehydrated in each trial (young, 298(3) and 534(301); older, 297(4) and 401(149) mOsm/kg for serum and urine, respectively). Participant characteristics and initial serum and urine osmolality are shown in **Table 6-1**.

### 6.3.2 Urine output and fluid balance

Urine mass did not differ between trials or between groups immediately after the 1hr ingestion period of the beverages for the young group ( $P > 0.69$ ) and for the older group ( $P > 0.15$ ). However, one hour after the ingestion of the beverages, cumulative urine output for W was significantly different to M in the young group and this was sustained at 2 and 3 hrs. Urine output at 1hr post ingestion was significantly less in older compared with young adults after ingesting W.

Total urine mass loss over 2 hours post drink for W was significantly different to M but not for F or S in the young group. No differences were observed between beverages in the older group (Figure 6-2). When observing the changes in body mass pre to post trial after the ingestion of W, F, S and M, there was no significant difference between any of the beverages in the older group, however in the younger group M ingestion led to maintenance of body mass after the 3 hours period.

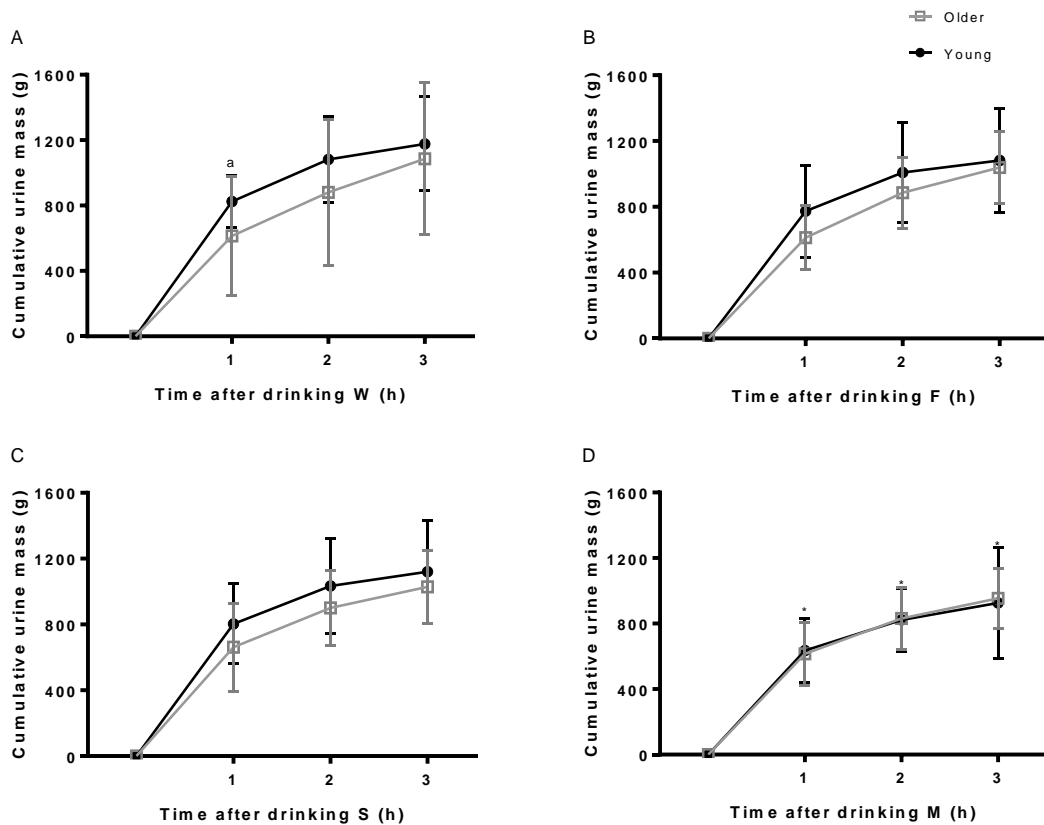


Figure 6-2 Cumulative urine output following the ingestion of water (A), fruit juice (B), sport drink (C) and milk (D).

Values are mean (SD). \* Indicates significant difference from water in young only; *a* indicates significant difference between young and older groups.

6.3.3 Net fluid balance (NFB)

When comparing NFB 3 hours after the ingestion of the different beverages, the response in the older group was observed to be the same with the control (W) and the test beverages (F, S, M). In the young group, following M ingestion, NFB was significantly different after 3 hours (+259(288) g) compared with W (Figure 6-3).

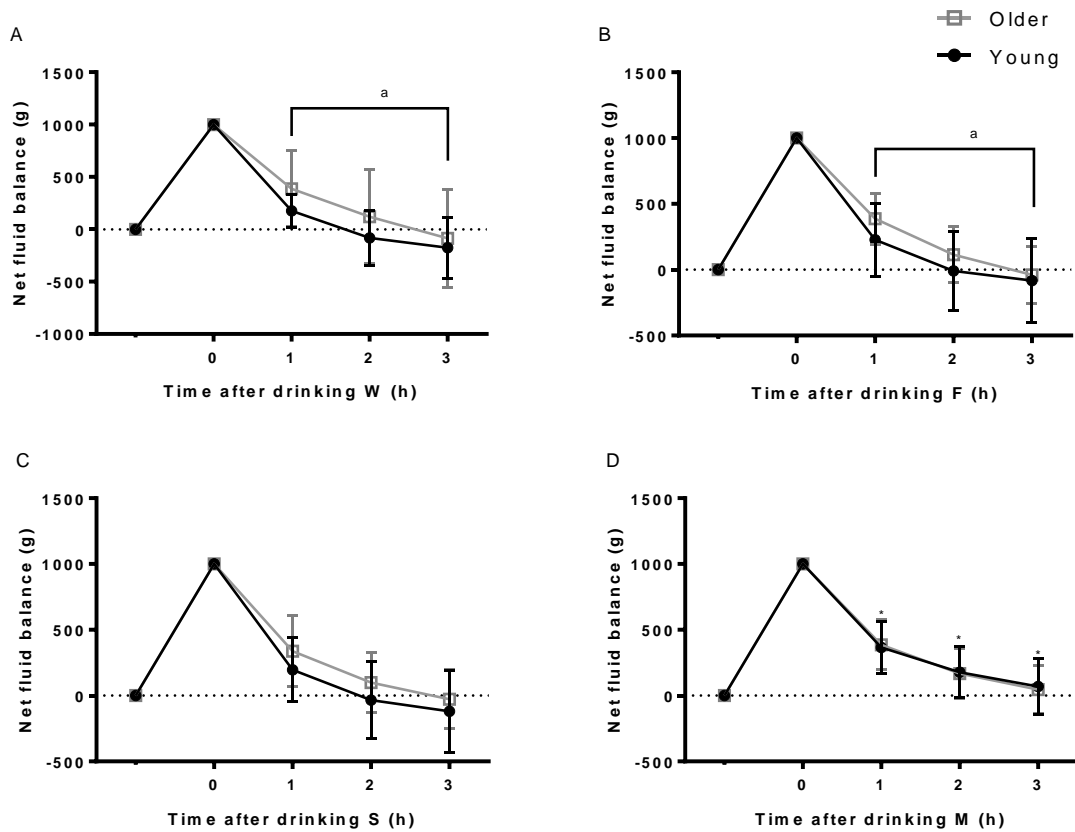


Figure 6-3 Net fluid balance following the ingestion of water (A), fruit juice (B), sport drink (C), and milk (D).

Values are mean (SD). \* Indicates significant difference from water in young only; <sup>a</sup> indicates significant difference between young and older groups.

### 6.3.4 Urine electrolyte excretion and balance

3 hours after beverages were consumed S led to a significant positive sodium balance in comparison with water in the young group. Sodium balance on S was also significantly different than W in the older group (Figure 6-4A). When analysing net potassium balance, F was significantly different than W in both groups being more positive in young. ( $p < 0.05$ ) (Figure 6-4B).

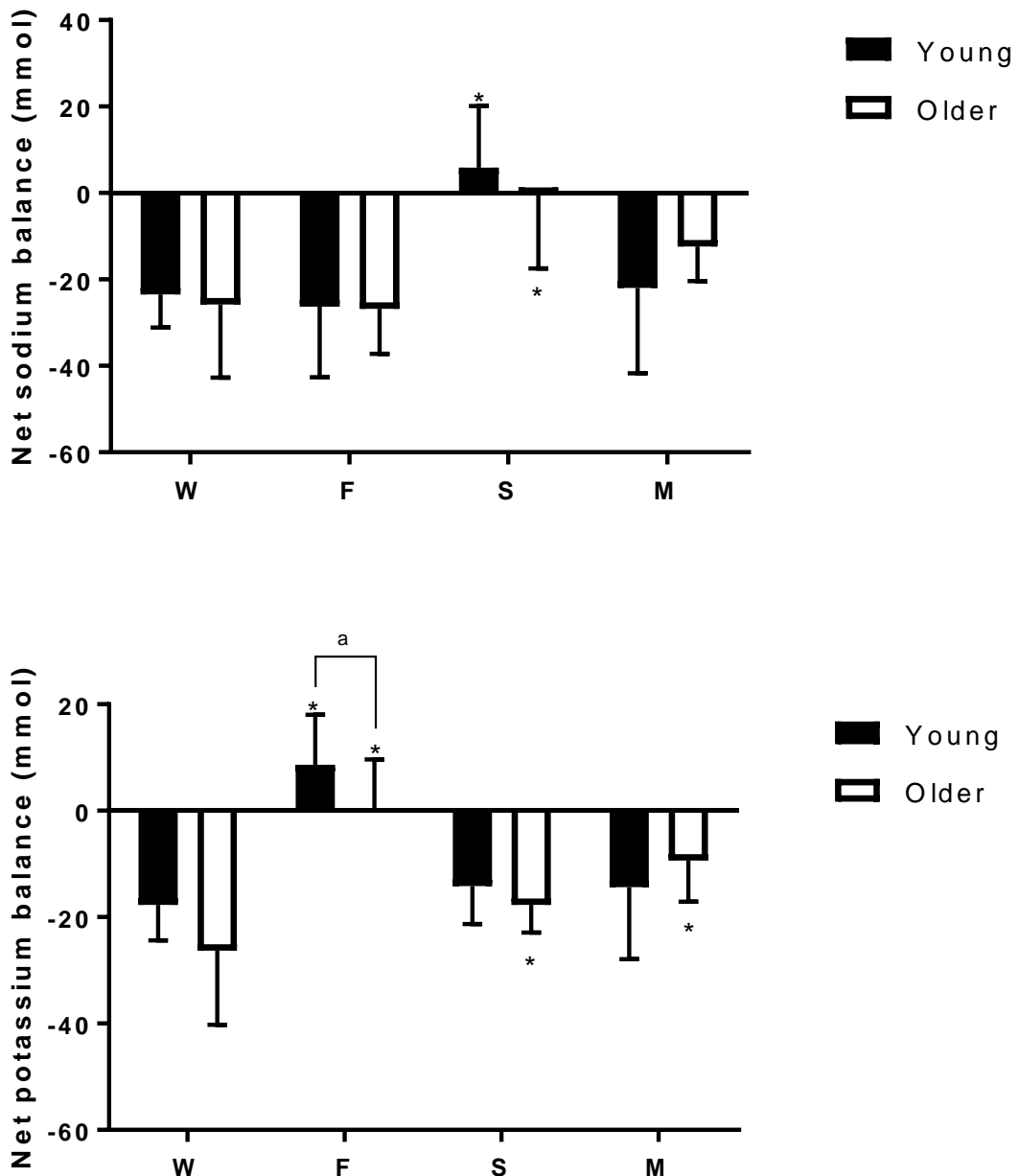


Figure 6-4 Net sodium balance (A) and net potassium balance (B) over 3 hours following the ingestion of water (W), fruit juice (F), sport drink (S), and milk (M), respectively.

Values are mean (SD). \* Indicates significant difference from water in both groups; <sup>a</sup> indicates significant difference between groups.

### 6.3.5 Creatinine clearance

Mean (SD) creatinine clearance was lower in older (65(19) ml/min) than in younger (87(41) ml/min) men. When comparing the creatinine clearance between beverages, there was no significant difference in comparison with W in the young men. However, there was a significant difference in creatinine clearance after ingestion of S in comparison with W in the older group ( $P < 0.02$ ) with a higher creatinine clearance after ingestion of S (Figure 6-5).

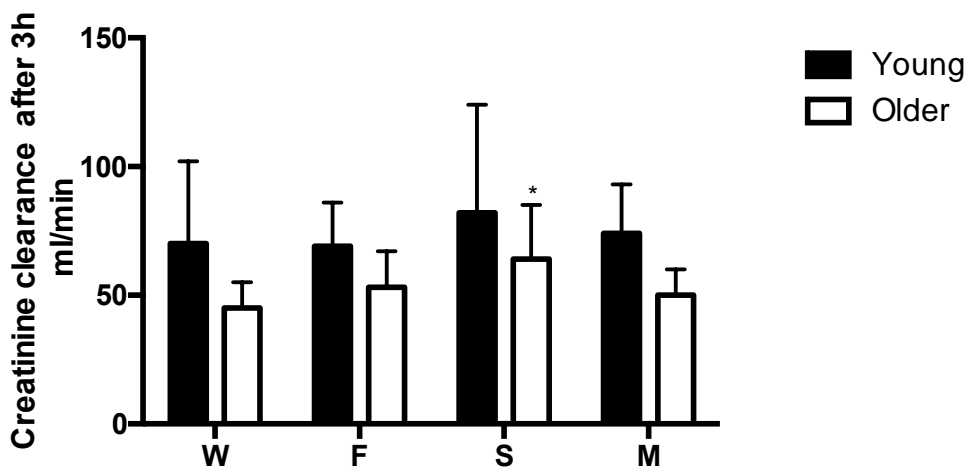


Figure 6-5 Creatinine clearance rate after 3h of ingestion of water (W), fruit juice (F), sport drink (S), and milk (M). Values are mean (SD).

\* Indicates significant difference from water.



### 6.3.6 Beverage Hydration index (BHI)

After 2 h of ingestion, skimmed milk (M) had a higher BHI than still water (W) in the young adult group ( $P < 0.05$ ) (Figure 6-6). The water content of the beverages that were used in this study varied from 100% to 91% (Table 6-2) so it was appropriate to recalculate the BHI to consider the different volumes of water ingested in the different trials (Figure 6-7). The BHI normalized by the drink's water content shows the effect of the drink itself on hydration status excluding differences in total water content. Skimmed milk was still the drink with the highest BHI in the young adults group however it was not significantly different.

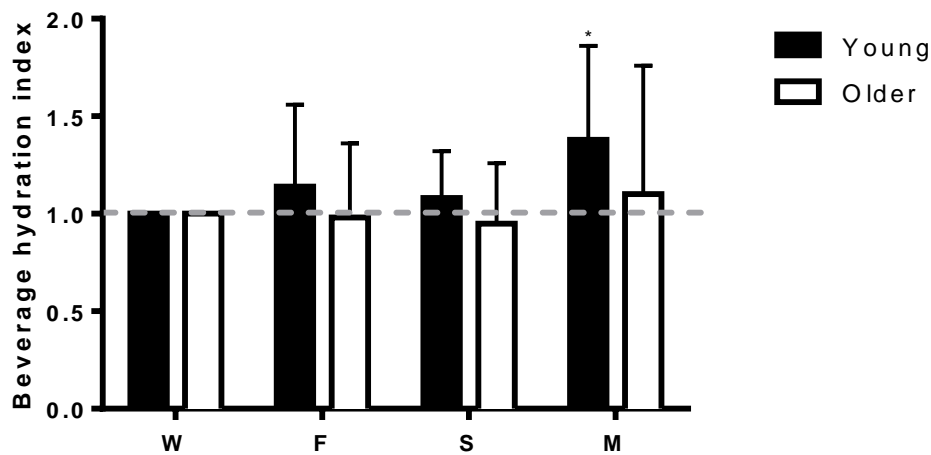


Figure 6-6 Two hours beverage hydration index for water (W), fruit juice (F), sport drink (S), and milk (M) in young and older group.

Values are mean (SD). \* Indicates significant difference from water in corresponding group.

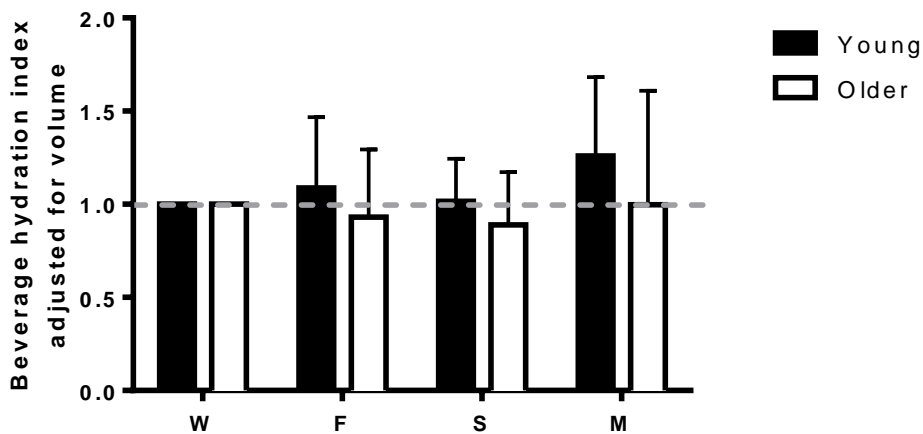


Figure 6-7 Two hours beverage hydration index adjusted for volume for water (W), fruit juice (F), sport drink (S), and milk (M) in young and older group.

Values are mean (SD). The dashed line represents the beverage hydration index for water.

## 6.4 Discussion

In this study we investigated the hydration potential of beverages with different nutrient composition (protein and electrolytes) and assessed their effectiveness to maintain hydration status over the hours post ingestion in young and older adults. The beverage hydration index revealed that water was as good as milk, fruit juice and a sport drink at maintaining net fluid balance over the follow-up period in the older adult group. However, in young adults we replicated the observations from our previous study (Maughan, Watson et al. 2016) by demonstrating that milk was more effective than water at maintaining fluid balance over the follow-up period. There are several factors that could explain these differences between groups such as alterations in renal function, gastric emptying responses, and changes in regulation of electrolyte balance that occur with ageing.

The ability to concentrate urine was measured in healthy people aged 20-79 years in the Baltimore Longitudinal Study of Ageing and revealed that individuals aged 60-79 years had a reduction of ~20% in maximal urine osmolality and 50% decrease in the ability to reabsorb sodium and urea, and to concentrate solutes when compared with younger age groups (Rowe, Andres et al. 1976). After analysing urine osmolality of the participants in the present study, it was observed that there was a 39% lower osmolality of the urine in the older group 3h after drink ingestion when compared with the younger group. Taken together, these past

(Maughan, Watson et al. 2016) and present data suggest that an impaired capacity to concentrate urine in older adults could explain the differences in hydration response between young and old.

Due to a reduced renal excretion capacity older adults are more susceptible to develop dilutional hyponatraemia in the setting of excess water load, or in stress situations such as fever, surgery, illness and/or the intake of medications such as diuretics or those that enhance arginine vasopressin action. The inability to excrete excess water rapidly also means that older people are at risk of water overload and hyponatremia when excess of a solution of water and sodium is given exogenously such as in intravenous fluid therapy (Allison and Lobo 2004). El-Sharkawy et al (El-Sharkawy, Sahota et al. 2014) reported that older surgical patients were at greater risk of having an iatrogenic fluid overload due to inaccurate prescription of fluid. Furthermore, Lindeman et al [22] suggested that the “well known” recommendation of drinking eight glasses of fluids daily (2 L) may be inappropriate in older people because it might contribute to incontinence and/or water overload. In the present study, the volume overload induced by ingesting 1 L of fluid in one hour when in an already euhydrated state appears to lead to an inadequate homeostatic response in older participants compared to the younger group. This is evidenced by lower total urine output in the first 2 hours after ingestion of the fluid load in older than younger adults. Thus, it seems likely that differences between young and older adults in response to water ingestion relates to age related declines in renal function that lead to delayed excretion of the fluid overload. It is also known that GFR remains stable until the age of 40 years, after which GFR declines linearly at an average rate of about 8 ml/min/decade (Silva 2005). A group of researchers in the Baltimore Longitudinal Study of Ageing (Lindeman, Tobin et al. 1985) concluded that one third of older adults did not show any change in GFR but also their data allowed them to determine the decline in creatinine clearance as 0.75 ml/min/year. In the present study we found that mean creatinine clearance was lower in older than in younger men, demonstrating the reduced GFR in the aged group, however there were just two participants in the older group showing a lower GFR out with 1 standard deviation. Thus, it seems likely that differences between young and older adults in response to water ingestion relate to age related declines in renal function that lead to delayed excretion of the fluid overload.

### 6.4.2 Digestibility of protein

Gastrointestinal function is also affected by ageing. It has been demonstrated previously that basal and maximal gastric acid output decrease in ageing human groups (Holt, Rosenberg et al. 1989). Dangin et al (Dangin, Guillet et al. 2003) investigated the utilisation and digestion of different protein sources (whey protein and casein) and observed that in young people casein could be classified as a slow protein due a longer gastric emptying. However in the older group there was an acceleration of the casein emptying rate due to an age related decrease in gastric acid secretions. This reduction in gastric acid secretion probably had an impact on the clotting of casein that would keep it in a liquid form, causing a faster emptying than in the clotted (solid) form. In the present study, there was no difference in net fluid balance, and BHI determination between milk and water trials in the older group, but there was in the young adult group. The response of net fluid balance observed in the young group matched what we previously observed in our previous study (Maughan, Watson et al. 2016) meaning that milk had the best hydration potential in this age group. When the mean for BHI for milk corrected for water observed in the young group in this study was compared with our previous study, there was not a significant difference ( $P=0.76$ ) demonstrating also that the results from our previous study are replicable. This difference in net fluid balance and in BHI response might be because there is a faster emptying in the older group due to a lower gastric acid secretion. However, further study is required to determine which of these reasons explains the observations of our study.

### 6.4.3 Sodium balance

Older adults are prone to fluid expansion when challenged with a volume or sodium overload. Older adults have been reported to have a diminished capacity for renal sodium excretion (Luft, Rankin et al. 1979). Older people have also been shown to require a longer period to excrete a salt load when compared with younger adults. Luft and collaborators (Luft, Rankin et al. 1979) gave participants a 150 mEq sodium diet plus ad libitum salt intake and they examined the effect of volume expansion in whites, blacks and subjects of different ages. They observed that black and  $\geq 40$  year old subjects excreted less sodium than white or  $< 40$  years old participants. In older people the capacity to maintain sodium balance in response to reduced sodium intake is also impaired (Luckey and Parsa 2003). Younger people readily excrete sodium excess given that a rapid increase in GFR is the required mechanism

to deal with acute salt excretion. It is natural that older people (with a normally decreased GFR) will present a limited ability to manage sodium loads and would thus be predisposed to overexpansion of the extracellular fluid compartment. Epstein and Hollenberg (1976) demonstrated that when older and younger subjects were given a low-sodium diet, the older group needed 2 to 3 times more time to balance their sodium excretion. Therefore, an inability to conserve sodium with advanced age might predispose the older adults to instability in the resetting of sodium loss (Shannon, Wei et al. 1986). This can be further explained by the observation of a blunted renin secretion in older people (Weidmann, De Myttenaere-Bursztein et al. 1975). In the present study the beverages that were provided were in the range of 0-22 mEq sodium. This level of sodium intake was not likely sufficient to observe any significant difference between groups or between beverages in the older adult group. Indeed, no differences were noted in the fluid balance or net sodium balance response to the sodium containing sport drink between young and older adults. The mean loss of sodium in urine appeared to be less in older than younger adults after milk ingestion; however, this did not reach statistical significance.

#### 6.4.4 Potassium balance

Potassium balance is also affected by the ageing process. Maintenance of serum potassium concentration within the normal range is influenced by various hormones, GFR, and intracellular translocation of potassium (Schlanger, Bailey et al. 2010). Around 90% of the filtered potassium is reabsorbed by the early to mid-distal tubule while most of the potassium in the urine is derived from secretion of potassium in the mid to late distal tubule and cortical collecting tubule (Biswas and Mulkerrin 1997). Potassium excretion is also reported to be reduced in the aged kidney (Schlanger, Bailey et al. 2010). This is attributed to the combination of low potassium secretion (Musso, Liakopoulos et al. 2006) and high potassium reabsorption (Vander 1991). Also, a decrease in renal mass with age includes reduction in tubular mass, meaning reduced transport pathways for potassium egress. As a result, healthy older people have a reduction in the transtubular potassium gradient compared with younger adults; this means that older people cannot excrete a potassium load as well as younger people (Musso, Liakopoulos et al. 2006). In the present study when comparing the potassium excretion after 3 hours of drink ingestion, there was a significant difference in the young group when they ingested milk in comparison with the potassium excretion after water ingestion ( $p < 0.01$ ). The potassium excretion in young adults after

ingesting fruit juice was similar with the results reported by Maughan et al (2016). There was a significant difference between groups that was observed after ingestion of fruit juice that cannot be explained as an effect of ageing. Kirkland et al (Kirkland, Lye et al. 1983) observed reduced urine sodium and potassium excretion after 24 hours in older adults (mean 66.5 years) in comparison with the younger group (mean age 29.2 years). Age related reduction in renin and aldosterone would be expected to contribute to a higher risk of hyperkalaemia and is reflected by a reduced transtubular potassium gradient in older people (Musso and Oreopoulos 2011). Both of these previous observations do not appear to occur in our sample of older adults.

Through the distal tubules, aldosterone increases sodium reabsorption and facilitates potassium excretion. In this way, aldosterone delivers a major protective mechanism in preventing hyperkalaemia after potassium load (Weidmann, De Myttenaere-Bursztein et al. 1975). It has been also observed that direct aldosterone response to hyperkalaemia is reduced in older people. In healthy older adults, serum potassium is usually normal (Dall and Gardiner 1971), but because of the reduction in plasma renin activity and serum aldosterone, there is a tendency for the aged kidney to excrete less potassium than normal in urine, thereby accounting for higher levels of serum potassium that might lead to hyperkalaemia (Andreucci, Russo et al. 1996). In the present study, we observed higher values of serum potassium in the older group than the values in the young group after the ingestion of the potassium containing beverages (milk and fruit juice) which each provided 23 and 28 mmol/L of potassium, respectively. It is likely that the potassium load provided by these beverages would not be considered an excessive challenge to electrolyte homeostasis with the acute fluid bolus.

These differences in response of electrolyte excretion between age groups may be explained through some of the changes that occur with ageing. There are changes in the renin-aldosterone system in older people (>60y). Older adults have plasma renin activity that is 40-60% lower than in a younger population (Noth, Lassman et al. 1977). This reduction results in a 30-50% decrease in plasma aldosterone concentration (Skott, Ingerslev et al. 1987). Some studies have demonstrated that in older adults, plasma renin activity and urinary aldosterone are diminished in comparison with the young adults, especially after salt restriction (Flood, Gherondache et al. 1967, Epstein and Hollenberg 1976, Hegstad, Brown et al. 1983). These changes in renin and aldosterone can contribute to the development of various fluid and electrolyte abnormalities in older adults primarily by altering sodium balance. However in the

present study no hormone analysis was done. Further investigation is necessary to study the hormonal changes (aldosterone, vasopressin and atrial natriuretic peptide) that might occur when young and older adults ingest beverages with different composition.

We can conclude that milk ingestion helped to maintain positive net fluid balance for longer during seated rest than achieved with any of the other beverages in the young euhydrated adults. In older adults, water was likely effective as milk, fruit juice and a sport drink at maintaining net fluid balance under the same circumstances. It may be speculated that it was necessary to provide a higher sodium or potassium challenge to observe a difference between beverages in older adults due to altered homeostatic control however this could prove problematic given reduce ability to handle disturbances in homeostasis.

Further investigation is needed to find out if there is any key component of beverages that might help to maintain a positive net fluid balance in older people for a longer period of time, helping to improve hydration status in this age group. At the present, we suggest that water is effective to maintain euhydration if older adults would choose this as a beverage of choice for fluid intake.

## **CHAPTER 7.      TIMING AND TYPE OF FLUID INGESTED BY SCOTTISH ADULTS: WHAT INFLUENCES THE ABILITY TO MEET DAILY FLUID INTAKE TARGETS?**

### **ABSTRACT**

The study described in this chapter aimed to investigate the hydration habits of Scottish young (18-35 years old) and older adults (+50 years old), identifying their fluid choices, volume, and preferences in relation to time of day. In this observational study we could analyse the behaviour related with fluid intake of 492 Scottish adults. The participants were also asked about fluid intake in relation with exercise and they were inquired about their dehydration awareness. The results showed that 26.1% of the young females, 30.3% of the young males, 25.8% of the older females and 50.4% of the older males did not meet the EFSA fluid recommendations. After the analysis of the volume and the choices of beverages that the participants reported ingesting throughout the day, it was observed that the difference between those who met and those who did not meet the EFSA adequate intake could be attributed to differences in water ingestion, mainly during the mid-morning (after breakfast until 11 am) and during the early-afternoon (after lunch time up to 5 pm). It was concluded that these moments might be key when implementing interventions to improve hydration status especially in the older population.



## 7.1 Introduction

It has been widely recognized that there is an important relationship between fluid intake and physical health and cognitive performance (Popkin, D'Anci et al. 2010). The prevalence of dehydration in adults has been reported to be around 16-28% (Stookey 2005) depending on age. Older adults experience several physiological and behavioural changes that might lead to dehydration. There may also be a lack of knowledge related to whether older adults drink or do not drink enough fluids (Hooper, Bunn et al. 2014). Importantly, inadequate fluid intake and subsequent dehydration has been reported to be associated with a six times greater risk of in-hospital mortality compared with euhydrated older patients (El-Sharkawy, Watson et al. 2015).

The Panel on Dietary Reference Intakes for Electrolytes and water in the USA has established a recommendation for daily water intake from fluids and foods in adults: 2.7L for women and 3.7L for men (IOM 2005). The European Food Safety Authority (Agostoni, Bresson et al. 2010) released their Scientific Opinion on Dietary Reference Values for total adequate intake of water for adults. They recommend 2.0L for women and 2.5L for men. In both sets of guidelines it is assumed that ~20% of total daily water requirements might come from water content of ingested foods and 80% from a variety of fluids ingested during daily living, indicating that beverage intake is 1.6 and 2.0 litres/day for females and males respectively. The recommendations for young and older adults are identical.

However, age is a factor that can have an impact on fluid balance. Ageing is associated with a reduced ability to maintain homeostatic control over a variety of body functions, including fluid regulation. There are some important changes in older people that impact hydration status such a decrease in muscle mass (Adams, Leitzmann et al. 2014) and impaired renal function (decreased renal sensitivity to vasopressin and a reduction of the renal secretion of renin) (Noth, Lassman et al. 1977) and in drivers of thirst sensation (Kenney and Chiu 2001). The older adult population are therefore at higher risk of developing dehydration than younger people.

There have been several surveys or data analyses investigating the hydration habits of adult populations, and some have compared young and older adults. Zizza et al (2009) analysed the data from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) and they compared the differences in total water intake variables across age groups. They observed that people older than 75 years had a significantly lower total water intake when

compared with young adults. Ferreira-Pêgo et al (2015) and Guelinckx et al (2015) analysed the total fluid intake from 16276 adults aged between 18 and 70 years from several countries. Ferreira- Pêgo et al (2015) evaluated the percentage of individuals meeting the EFSA adequate intake of water from fluids and revealed important differences in total fluid intake between countries and interestingly, with all the countries combined, 40% of men and 60% of women meet the EFSA recommendations. Guelinckx et al (2015) observed that in the United Kingdom the largest contributors to total fluid intake were hot beverages (tea and coffee) and water. Gandy (2012) conducted a study in the United Kingdom (UK) with 1456 children and adults to analyse fluid intake using a 7-days specific intake diary. Gandy observed that 30% of the adults and more than 50% of children did not meet the EFSA water intake recommendations. Gibson & Shirreffs (2013) analysed data from the UK National Diet and Nutrition Survey in adults aged 19 to 64 years. The survey was a nationally representative health and diet survey of 1724 adults living in the UK in 2000/2001. They observed that total water intake from all food and fluid averaged 2.5 L for men and 2.0 L for women. Tea and coffee represented over 40% of the daily intake of beverages. Gibson & Shirreffs also observed that beverage consumption peaked at 8am for women and at 9pm for men (Gibson and Shirreffs 2013). Recently, an online survey carried out by the Royal National Life Boat Institution on more than 2000 respondents in the UK reported that 89% of the population do not drink enough water from fluids to meet the EFSA recommendations, and that 17% of the total responders do not drink water at all during the day (RNLI 2014).

Although all of these studies have provided relevant data relating to the total fluid intake of the UK population, to date no study has focused on the hydration habits in older adults. Gandy concluded that 25% of the adults aged over 18 years did not meet EFSA fluid adequate intake (Gandy 2012). Gibson & Shirreffs found that older adults consumed more beverages in the morning and young adults more in the evening. Also, it was observed that men aged +50 years ingested on average 30% of their total fluid intake before 10 am while young men (19-35 years) drank less than 22% by that time (Gibson and Shirreffs 2013). In the survey developed by the RNLI, 25% of the >55 year old responders reported they did not drink water during the day (RNLI 2014).

Based on the observations of the studies detailed previously, it is important to identify hydration patterns and beverage choices to propose a strategy that might help young and older people to meet EFSA fluid intake recommendations and to maintain euhydration.

Therefore we designed a survey to understand what young and older adults do in real life regarding their fluid intake behaviour and preferences.

The aim of the present study was to explore the hydration habits of young (18-35 years old) and older adults (>50 years old) in Scotland. We also aimed to detect their fluid choices, volume, and preferences in relation to time of day. Another aim of the present study was to identify how many young and older adults met the EFSA fluid intake recommendations, and some of the factors contributing to meeting these guidelines. We hypothesized that the observations of the present study might be useful in helping to target specific time of day and fluid types and volumes that could aid in meeting total intake guidelines.

## 7.2 Methods

### 7.2.1 The questionnaire

We designed a questionnaire containing 30 questions to assess typical daily fluid intake patterns over the course of the previous week. Questions were largely open to give the responders the opportunity to answer freely without biasing their answers. The questionnaire was distributed in Scotland by three different channels: 1) delivery of hardcopies of the audit at University of Stirling Sports Centre to the 50+ fitness classes participants, 2) delivery of hardcopies through Food Train Centres at 7 different locations across the country, and 3) online delivery at the University of Stirling by inviting local students and staff to participate.

The questionnaire had queries of general information (age, gender) and questions regarding any medication/nutritional supplement use in the first section. In the second part, the participants were asked to recall their hydration habits during the previous 7 days and were asked about their choices and volumes of beverages ingested during 7 different periods of the day, including from first thing in the morning upon waking to fluid intake overnight. They were also asked about how they might identify if they were dehydrated and about their physical activity habits and their hydration during exercise. (*See in Appendix the Hydration Habits Audit Document*).

492 responders completed the audit (314 females and 178 males). Responders were separated into groups depending on their age: 18-35 years (n=129) and >50 years (n=303).

Data from participants aged >35-50 years were not used in the data analysis as the numbers were small (n=60) and it enabled an age gap between young and older groups for age associated comparisons. Responders were classified as meeting or not meeting the EFSA fluid adequate intake recommendations for their gender.

### 7.2.2 Data analysis

Data analysis was focused on total fluid intake (TFI) from all beverages reported in the audit. Metabolic water was not included as the aim of the audit was to focus on fluid intake.

Data were first analysed for the individual fluid intake recall. Individual fluid items were classified into 10 different categories: water, tea, coffee, fruit juice, non-caffeinated hot beverages, milk/dairy beverages, sugary soft beverages, diet soft beverages, alcoholic beverages and other. Water included tap water, bottled still water and sparkling water. Tea included black tea. Black coffee and espresso coffee were included in the Coffee category. Fruit juice included fresh fruit juice and bottled fruit juice. Non caffeinated hot beverages included green tea, fruit tea (berry tea, apple and cinnamon tea) and ginger tea. Milk/dairy beverages included skimmed milk, semi skimmed milk, whole milk, chocolate milk, milk added to tea or coffee, drinkable yogurt and yogurt smoothies. Sugary soft beverages included cola and other flavoured carbonated soft beverages. Diet soft beverages contained the soft beverages sweetened with non-caloric sweeteners. Alcoholic beverages included every drink with some percentage of alcohol in them such as beer, wine, spirits and alcopops. When alcoholic beverages were ingested with a mixer, it counted to the correspondent category (sugary soft beverages, diet soft beverages). Those beverages that did not fit in any of the other categories were included in the 'other' grouping.

Respondents were asked to detail what they drank in the different periods of the day and they were asked to report the volume of fluid expressed as mugs, specifying a mug equalled 250ml. Total fluid intake was calculated considering 250ml as a factor for the number of mugs the respondents reported. The times of the day were defined as follows: early morning (first thing in the morning upon waking and until end of breakfast), mid-morning (after breakfast until 11am), lunchtime (after 11am until the end of lunchtime), afternoon (after lunchtime up to 5pm), early evening (after 5pm up to 8 pm, including the evening meal), late evening (from 8pm until bedtime), and overnight (bedtime to first thing in the morning).

### 7.2.3 Statistical analysis

Differences in total fluid intake between groups were assessed using independent T-tests with Bonferroni correction for multiple comparisons. All t tests were 2-tailed. Linear-regression analysis was performed to investigate relationships between total fluid intake and hours of exercise that the respondents reported to practice per week. All statistical tests were undertaken using SPSS version 15.0. Statistical significance was accepted at  $P < 0.05$ . Data are presented as means  $\pm$  SD.

## 7.3 Results

The age of respondents ranged from 18 to 93 years. When divided into young and older age groups the mean (SD) for the young adults was 23(4) y and for the older adults was 64(12) y. When the middle aged adults ( $n=60$ ) were removed from the analysis, the total number of respondents was  $n=432$  (129 in the young group and 303 in the older group (**Table 7-1**)). Regarding the use of medications that may influence fluid balance (i.e. diuretics, blood pressure medication), one participant in the young females group, 63 respondents in the older females group and 31 in the older males group reported to be taking any of this kind of medications.

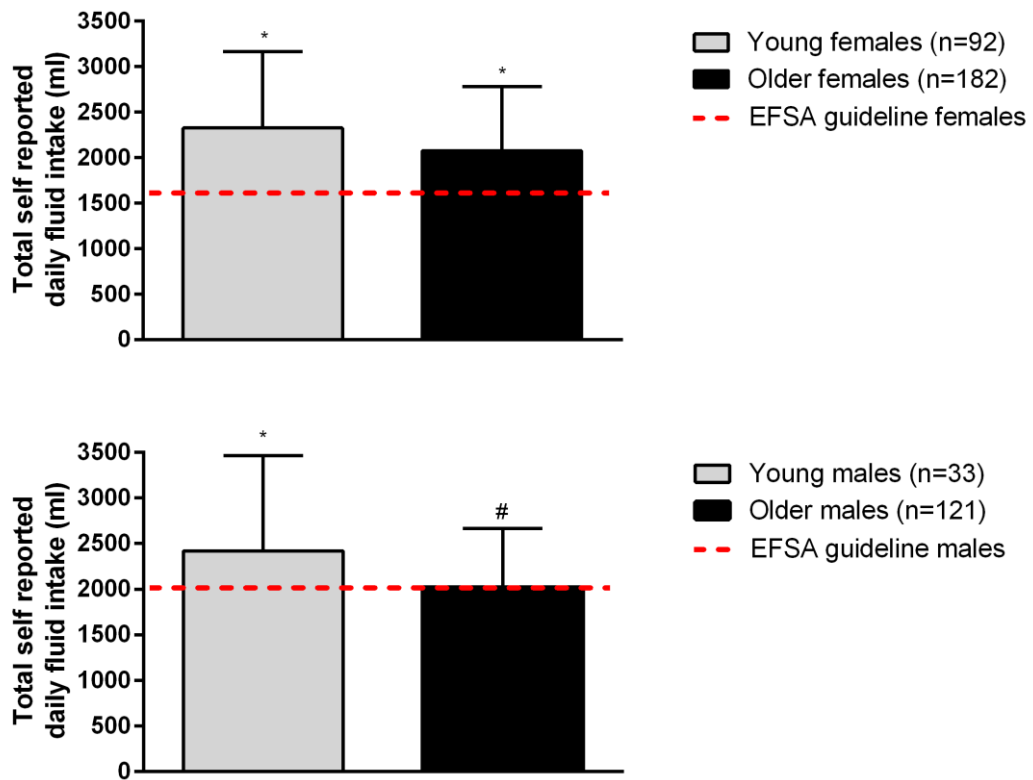
**Table 7-1 Total fluid intake (ml/day) by age and gender group in those meeting or not meeting the EFSA fluid intake recommendations.**

Values are means (SD).

	Meeting EFSA recommendations		Not meeting EFSA recommendations	
	Total fluid intake (ml)	n	Total fluid intake (ml)	n
Young females	2497 (625)	68	1188 (432)	24
Older females	2338 (575)	135	1163 (407)	47
Young males	2870 (955)	23	1338 (323)	10
Older males	2518 (509)	60	1461 (380)	61

### 7.3.1 Total fluid intake

When separated by age and by gender, the mean values for total fluid intake appeared to meet the EFSA recommendation (**Figure 7-1**). Young females reported a daily total fluid intake of 2325(842) ml, older females 2072(705) ml; while young males reported to ingest 2418(1045) ml and the older males 2018(649) ml. However, when examining the data more closely 26.1% of the young females, 30.3% of the young males, 25.8% of the older females and 50.4% of the older males did not meet the EFSA fluid recommendations.



**Figure 7-1** Self-reported total daily fluid intake of older and younger adult females and males.

# indicates significant difference between young and older adult males. \* indicates significant difference from EFSA recommended guideline.

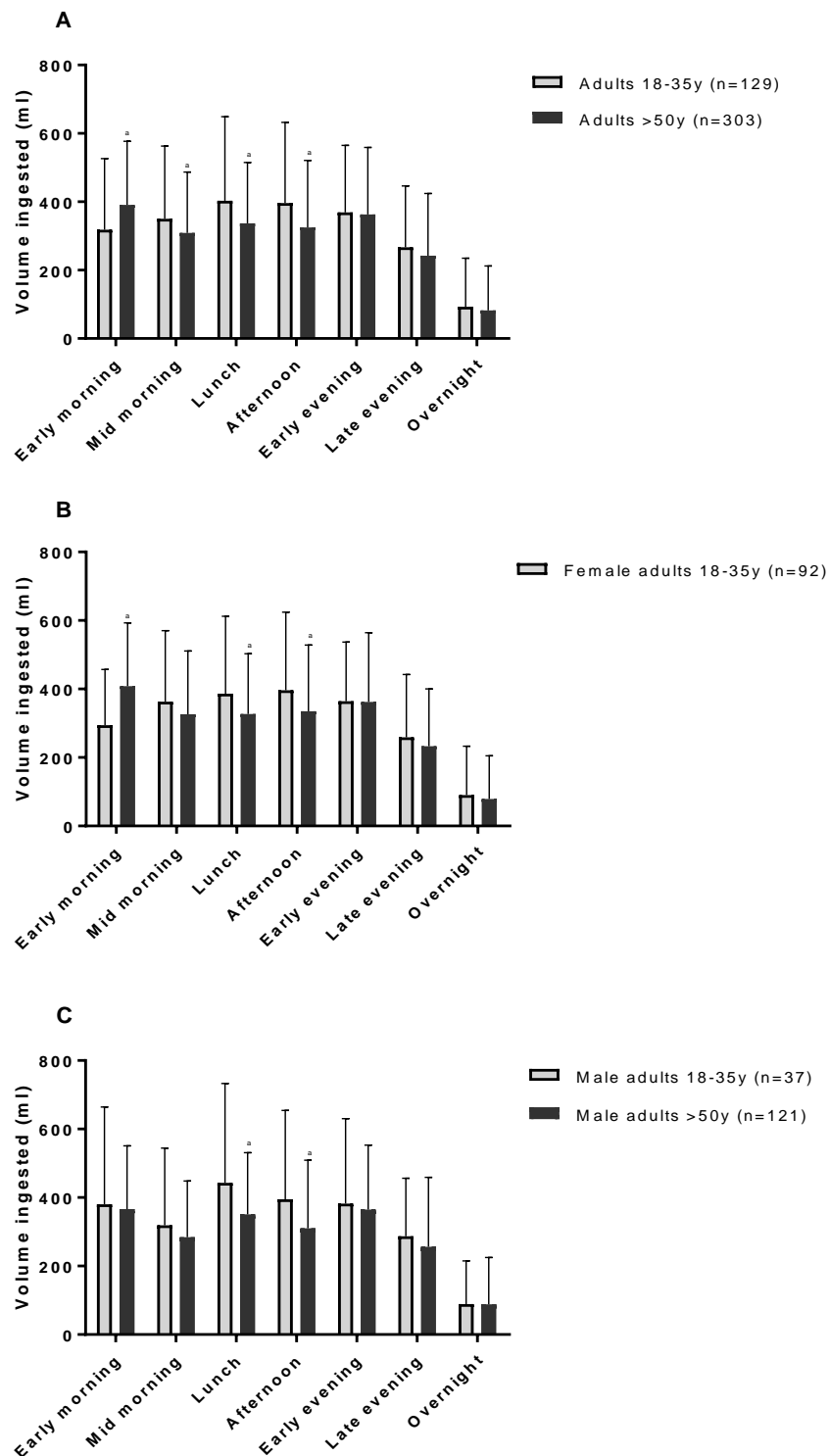
### 7.3.2 Timing of beverages

Young adults' beverage intake peaked at lunch time and during the afternoon. Older adults reported more fluid ingestion in the early morning (before 11 am). When the groups were separated by gender the younger females reported more fluid ingestion during the afternoon, and younger males reported more fluid intake at lunchtime. On the other hand, older females reported more fluids were ingested in the early morning whereas older males reported drinking a higher volume of fluids during the early morning and the early evening (**Figure 7-2**).

### 7.3.3 Type of beverages

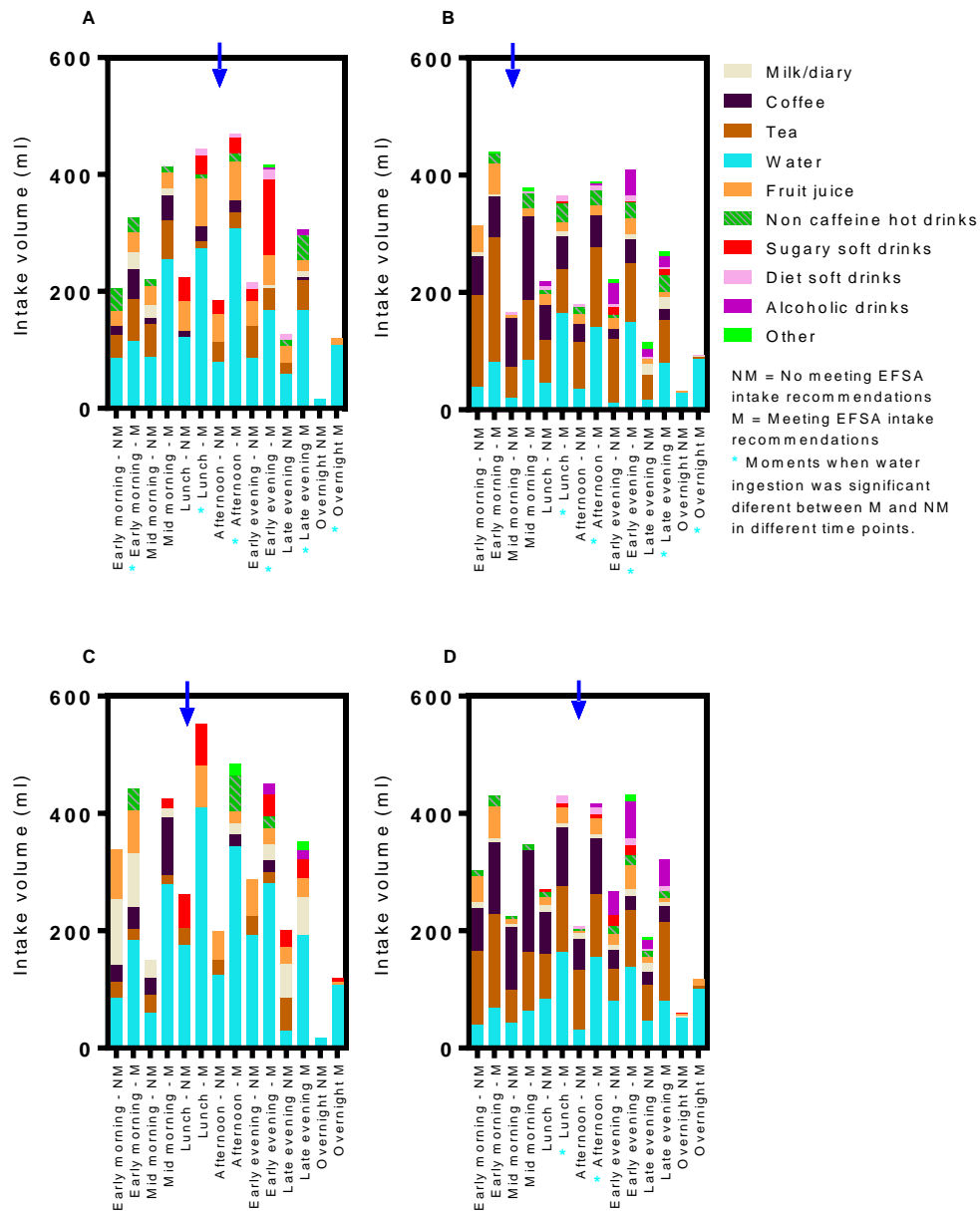
The beverages that the respondents reported at each time point during the day were analysed by age group, by gender and also separated in the categories of meeting or not meeting the EFSA fluid intake recommendations (**Figure 7-3**). The beverages that were reported at each time point were compared between the meeting and the not meeting groups to investigate if there was any drink at any time point during the day that was contributing to meeting or not meeting the guidelines (**Figure 7-3**).





**Figure 7-2 Self-reported fluid intake volumes ingested at different times of the day**

(A) Young adults and older adults, (B) Female young and older adults and (C) Male young and older adults. Values are mean (SD). <sup>a</sup> indicates significant difference between young and older adults groups at that period of the day.



**Figure 7-3 Amount and types of beverages ingested according to periods of the day (mean).**

(A) Females 18-35 years old (n=92); (B) Females >50 years (n=182); (C) Males 18-35 years old (n=33); (D) Males >50 years old (n=121). NM = not meeting EFSA fluid intake recommendations. M = Meeting EFSA fluid intake recommendations. \* Moments when water ingestion was significant different between M and NM in different time points. ↓ Time points when increasing water intake as intervention could convert NM to M.

7.3.4 Influence of habitual physical activity on total daily fluid intake

The impact of weekly habitual physical activity duration upon estimated total daily fluid intake was examined. There was no clear association between activity duration and total fluid intake in any of the groups except for older adult females. Older females demonstrated increased total daily fluid intake in relation to reported longer duration of total physical activity during a typical week (Figure 7-4).

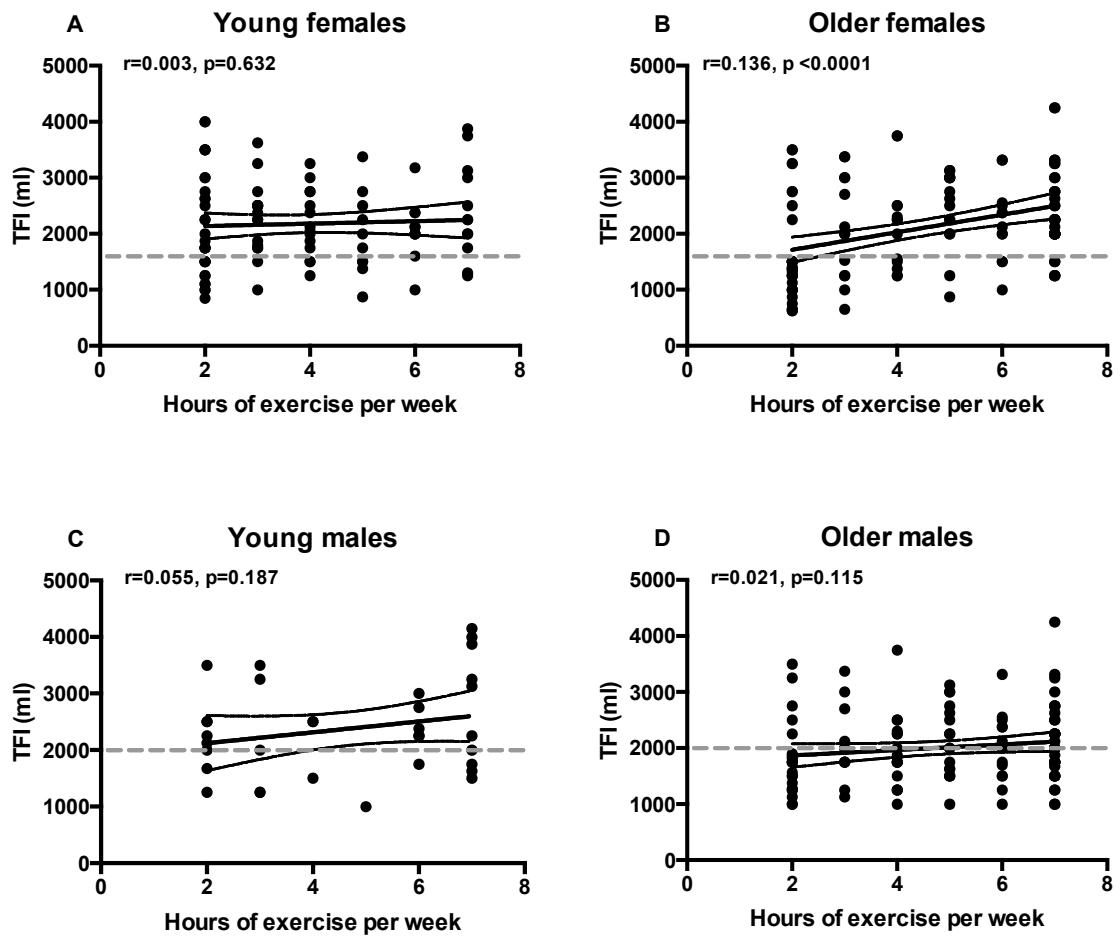
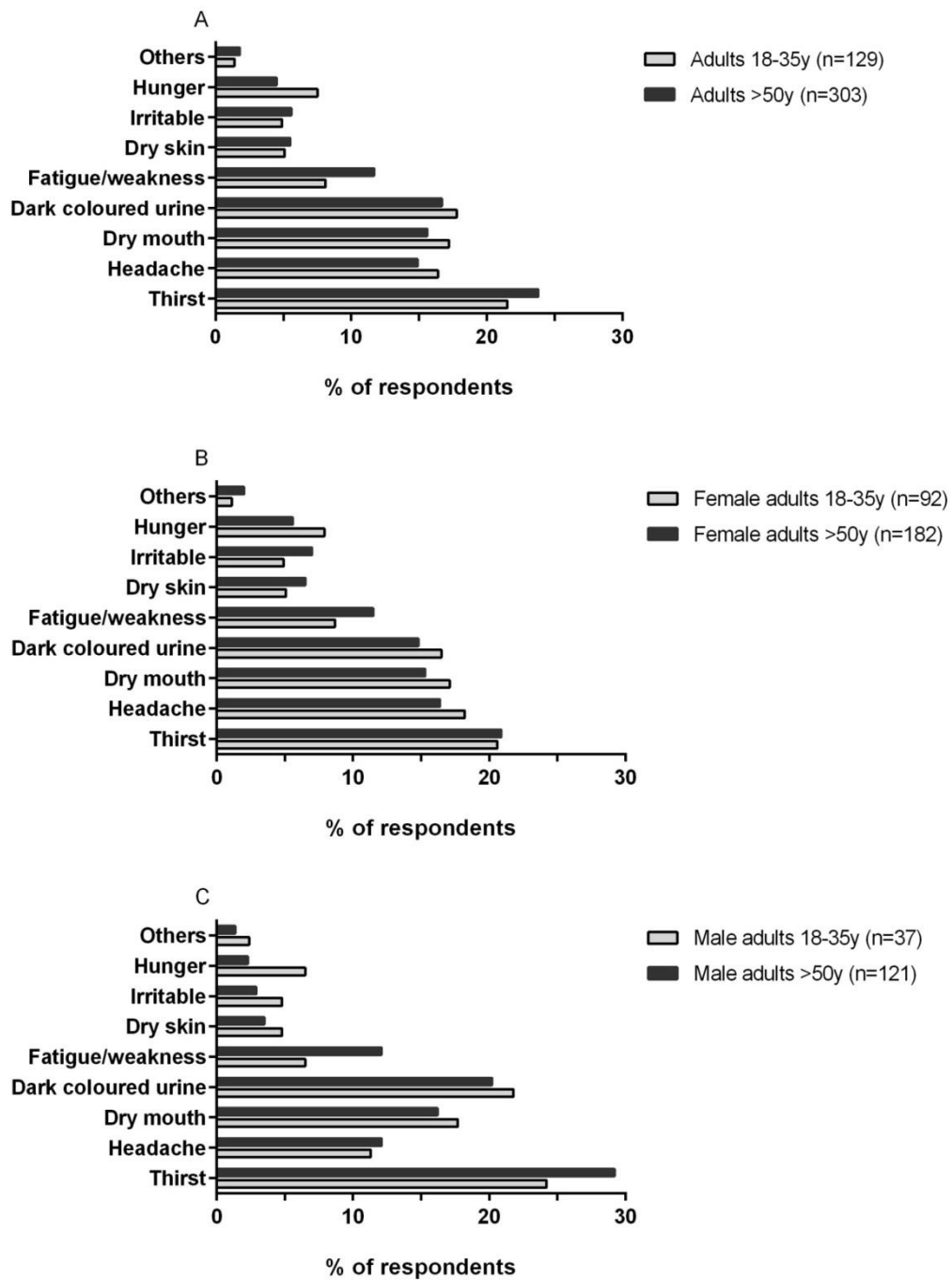


Figure 7-4 Relationship (95% confidence limits shown) between hours of exercise per week and total fluid intake

(A) young females, (B) older females, (C) young males and (D) older mails. The dotted line represents the EFSA fluid adequate intake recommendation.

### 7.3.5 Awareness of dehydration signs and symptoms

The respondents were asked to indicate which signs and symptoms of dehydration they might use to identify if they were dehydrated. The participants could select more than one response and they were also given the possibility to provide an open answer. Thirst had the highest number of responses when data were analysed by gender groups and by age (**Figure 7-5**). In the open responses the participants provided, the young group also reported that they know they are dehydrated if they pinch their skin and it takes longer to return to normality, after a night out drinking alcohol and overheating. The respondents in the older group said other signs of dehydration are pain in kidneys, low urine output, cloudy urine, cramps in legs and feet, feeling light headed, cough and poor mental concentration.



**Figure 7-5 Dehydration awareness reported signs and symptoms**

(A) Young adults and older adults, (B) Female young and older adults and (C) Male young and older adults.

## 7.4 Discussion

The amount of water that people are required to ingest to maintain a euhydrated state can vary between individuals as it depends upon many factors such as age, body composition, body mass, gender, ambient temperature, humidity, clothing worn, physical activity, and health status (Manz and Wentz 2005, Sawka, Chevront et al. 2005). These many factors make it difficult to determine a specific fluid recommendation for individuals within a group.

Scientific groups and committees around the world have attempted to set different fluid intake recommendations (IOM 2005, EFSA 2010). It is important to consider that these recommendations, and also most of the other current guidelines, are based on surveys of food and fluid intake in large groups. However, the health status and lifestyle of the participants in these analyses are not reported. The very popular recommendation of “drink at least eight by eight ounce glasses of water a day” has little scientific support but it has been built into recommendations (Valtin 2002) and closely matches the EFSA guidelines of 1.6 and 2 litres per day for females and males respectively. Some populations (like physically active people or those who live in a hot and humid environment) are to be expected to need larger amounts of fluid.

Data from previous studies investigating fluid intake in the British population (Gandy 2012, Gibson and Shirreffs 2013) did not assess if the older population were meeting the EFSA guidelines. When looking to the overall data we obtained from our survey, the data showed that the mean estimated total fluid intake from both young and older adults, met these recommendations. However, due to the variance noted it was apparent that several participants were not meeting the EFSA adequate fluid intake and were worthy of further analysis.

The findings of our audit revealed that more than 50% of older male adults and 25% of older adult females did not meet the EFSA beverage fluid intake recommendations. These data could have important implications for those individuals considering the negative clinical outcomes that might result if admitted to hospital in a dehydrated state (El-Sharkawy, Watson et al. 2015).

When self-reported fluid intake was analysed at different times during the day, we observed that there were some time points when fluid intake was significantly different between young and older adults (i.e. mid-morning, lunchtime and afternoon). Considering these

results, we suggest that interventions attempting to increase fluid intake in older adults should target these specific time periods of the day, i.e. the recommendation for young females who do not meet daily fluid intake targets is to drink more in the afternoon, for young males is to increase their fluid intake during lunchtime. For older females the recommendation is to try to drink more during mid-morning and for older males to increase their fluid intake during the afternoon.

Food and fluid intake declines with age. Social and physical factors may be part of the explanation for this decrease. Appetite, food and fluid intake are influenced by palatability, so a decline in olfactory and gustatory function due to ageing can affect food and fluid intake (Rolls 1992, Kenney and Chiu 2001). De Castro et al investigated the *ad libitum* fluid intake of young and older subjects. Participants were asked to maintain a food and fluid diary for seven consecutive days. They observed that older people consumed less alcohol than younger groups, but older adults ingested more coffee/tea than younger subjects who ingested more sugar sweetened and diet beverages. They also observed that people over 50 years old tended to ingest fluids earlier in the day than the younger groups (De Castro 1992). The output from our data matches the beverage choice and timing patterns of this previous work. We suggest that this information is relevant to know when considering implementation of strategies to improve hydration status in these age groups. De Castro (de Castro 1988) and Phillips et al (Phillips, Rolls et al. 1984) investigated the spontaneous fluid intake in healthy adults finding that under *ad libitum* conditions neither the amount nor the pattern of fluid ingestion is regulated physiologically to any great extent. They concluded that the amount and timing of spontaneous fluid ingestion in humans is mainly determined by eating. Under normal conditions, water balance is left to regulation by the kidneys (Phillips, Rolls et al. 1984, de Castro 1988, de Castro 1991). It seems that this mechanism also applies to the older adult population. When fluids are readily available independent community-dwelling older adults have similar fluid intakes to younger subjects. Older adults are able to obtain normal levels of fluids by the co-ingestion of fluids with solids under *ad libitum* conditions (De Castro 1992). Thus, it is important to target times of day for fluid intake as it is not just depending on fluid availability.

By analysing the volume and the choices of beverages that the participants reported ingesting throughout the day, we observed that the difference between those who met and those who did not meet the EFSA adequate intake could be attributed to differences in water

ingestion. We consider therefore that it can be suggested that increasing water intake at key points in the day could contribute to helping adults to meet the guidelines.

We also analysed if the physical activity habits reported by the respondents had any effect on their total fluid intake. Leiper et al (1996) investigated the effect of exercise on water turnover in trained middle age men using the doubly labelled water technique. These authors reported that middle aged men who exercise have a faster water turnover rate than a group of sedentary individuals of the same age. These results indicate that fluid intake in subjects who exercise regularly is higher than that of sedentary individuals of the same age group due to a need to replace exercise induced sweat and respiratory water losses (Leiper, Carnie et al. 1996). Considering the particular hydration needs of individuals that exercise vs. those who do not, we considered it could be a factor affecting total fluid intake in the groups we studied. We observed that only in the older female group was there a weak association between total fluid intake and the hours of physical activity completed per week. Thus, for most participants physical activity was not the explaining factor in the variation in TFI. Kenney investigated the effects of age on sweating response and observed that it generally decreases with age (Kenney 1995); this might be a reason to explain why greater amounts of physical activity are not associated with greater estimated total fluid intake in the older groups.

Abdallah et al (Abdallah, Remington et al. 2009) investigated the health care providers' perceived risk factors regarding dehydration and their proposed strategies to promote hydration for community-dwelling older adults. One of their findings was that there is a lack of awareness/education/understanding of dehydration in this particular group. The participants in their study reported being greatly concerned with the lack of awareness and understanding of the risks of dehydration in the community-dwelling older adults and their families. Also some older adults have cognitive impairment or depression that might hamper their understanding of instructions around maintenance of hydration (Abdallah, Remington et al. 2009). In the present study, of the responses related to dehydration awareness, thirst was the most frequent sign reported in all groups, and was the highest rated sign in the older male group. This observation indicates that it is important to educate the older adult population about how to track their hydration status. Thirst is often considered to be impaired in older adults and thus may not be the best overall indicator of hydration status (Rolls and Phillips 1990). It is also important to consider that there are some drug treatments that might alter thirst and cause dehydration (Ahmed and Haboubi 2010). The second most



mentioned dehydration sign that was reported by the older adult groups was dark coloured urine. However, urine colour has little utility to determine if older people are dehydrated (Fortes, Owen et al. 2015). This is because older subjects lose their ability to concentrate the urine as part of the ageing process (Epstein 1996). It is important to generate awareness of how to track hydration in this particular group. Increasing public awareness of the risks related with dehydration is warranted to promote good hydration habits for older adults in the community. Recently, Burchfield et al (2015) proposed a new user friendly hydration marker to assess hydration. This technique consists of 24h urine collection recording the volume for each void. Void number might be utilised as a simple hydration tool for the general population (Burchfield, Ganio et al. 2015). We propose that this technique should be investigated by relating the number of voids in 24h with other hydration markers (urine osmolality or serum osmolality for example) in older people to determine if a correlation is observed. If simple volume and number of void information can be diagnostic then it could be easily applied to this age group.

Most of the fluid intake recommendations are based on population food intake surveys that present the data in only one way which may not be appropriate for people of different characteristics (e.g. age, sizes, physical activity, etc.). Other methods such as mass adjusted volumes or volumes based on energy intake might be considered to set fluid intake guidelines.

A limitation of the present study is that these data are self-reported so that might impact upon the estimates of fluid intake. There are many data collection methods that can be useful in this kind of study such as surveys, questionnaires, interviews and observational data (Gray 2013, Green and Thorogood 2013). A survey is the process of collecting and analysing the data and the questionnaire is the set of questions used to gather the information. Questionnaires like the one we used in this study have many advantages: they are low cost in terms of time and money, their inflow of data is quick even from large samples, respondents can complete the questionnaire when it is convenient for them, data analysis of closed questions is relatively easy as they can be coded quickly and, there is a lack of interview bias (Gillham 2008). However, questionnaires also have some disadvantages. Most people prefer verbal communication than written word. Also most of the time there is no opportunity to clear up any ambiguous answer. Respondents may provide imprecise answers but the researchers are not in a position to detect this. In contrast, face to face interview might reveal essential problems through observing body language or the verbal tones of the

respondent (De Vaus 2013). Considering all the potential advantages and disadvantages we believe a survey / questionnaire is still a useful tool to obtain hydration habits data. Further studies including the analyses of health/medical status, environmental conditions, body composition and the measurement of hydration biomarkers during the day, such as urine osmolality over 24h, or over specific time points are needed.

Another weakness of the present study is that we did not validate the audit. However, we will validate the audit as an assessment tool of hydration habits providing food scales for acute measurement of fluid ingestion before the submission of the manuscript of the present study to any journal.

We conclude that fluid intake recommendations and guidelines should consider the time of intake of beverages, and the type of fluid, particularly in the older adult groups. Our data indicates that adding a glass of water at two or three specific moments during the day could make the difference between meeting and not meeting the current fluid intake guidelines for free living young and older adults.

## **CHAPTER 8. GENERAL DISCUSSION**

Adequate hydration in the human body is essential for health and it is indispensable for life. Water has numerous functions and it is involved in multiple reactions in the human body. There are several factors and mechanisms that tightly regulate body fluid balance. In a series of studies the present thesis explored the role of hydration in assessment of body composition, and the impact of beverage composition on the hydration potential of beverages in young and older adults under specific experimental conditions and in a real life situation. The thesis ended with an examination of the typical fluid intake habits of young and older Scottish adults.

### **Summary of the studies and synopsis of findings in relation to other relevant literature**

In Chapter 3, the findings demonstrated the relevance of performing body composition measurements in a euhydrated state. With this study we addressed the question of the relevance of hydration status to obtaining accurate body composition estimations, particularly from DXA scans. We concluded that hydration status is an important factor that alters the results of body composition estimation by DXA because it has a direct impact on fat free soft tissue mass estimates. The analysis and understanding of the effects of hypohydration on body composition estimation in an athletic population is highly relevant for sport nutrition practitioners and for researchers when assessing body fat and/or fat free soft tissue changes in response to training or nutrition interventions. It is highly recommended that one of the guidelines to perform a DXA scan is to ask the athlete to drink 500ml of water 2 hours before the scan to ensure euhydration and to obtain a reliable estimation of body composition.

Most of the studies that have used a DXA scan to estimate body composition have been focussed on standardising body positioning, the clothing worn, and more recently the food intake in athletic and non-athletic populations (Nana, Slater et al. 2011). The study reported in Chapter 3 is novel because it is the first one that investigated the effect of hypohydration on the body composition estimations by DXA in an athletic population. Many studies that have estimated body composition by DXA in athletic populations did not provide any information on the hydration status of their participants (Prior, Cureton et al. 1997, Calbet, Moysi et al. 1998, Ballard, Fafara et al. 2004, Silva, Minderico et al. 2006, Loftin, Sothorn et al.

2007, Larsson and Henriksson-Larsen 2008, Espana Romero, Ruiz et al. 2009, Moon, Eckerson et al. 2009, Campion, Nevill et al. 2010, Terzis, Spengos et al. 2010, Nana, Slater et al. 2012). Other studies have reported in their experimental protocol that the participants were euhydrated before the DXA scan but they did not provide any detail on hydration marker assessment (De Lorenzo, Bertini et al. 2000, Andreoli, Monteleone et al. 2001, Andreoli, Melchiorri et al. 2004). Clark et al reported in their study that participants were encouraged to ingest adequate fluids to maintain euhydration and they assessed urine specific gravity considering euhydration as USG <1.025 g/ml (Clark, Sullivan et al. 2007). Santos et al (2010) described in their methodology that they performed the scans when the participant was in a “neutral hydration”. These authors determined this state if the voided urine was pale and also they confirmed it comparing the laboratory body mass with the mass for the previous 3 days (Santos, Silva et al. 2010). Nana et al have recommended a protocol to standardise the conditions under which DXA scans should be performed (Nana, Slater et al. 2016). The authors denominated this protocol as “Best”. The “Best” protocol suggests that participants should be fasted overnight and presented after have been to the toilet to empty their bladder/bowels. In this protocol the subjects should not have exercised on the morning of the scan. Participants should wear underwear and remove all jewellery and metal objects. However, Nana et al did not mention any recommendation as part of this protocol related with hydration. However, an overnight fast prior to performing the DXA scan would normally result in a decline in total body water of around 1% of body mass (Shirreffs, Merson et al. 2004) resulting in altered results of fat free soft tissue mass estimation. We consider that the assessment of hydration status by urine markers such as osmolality or specific gravity, in addition to body mass, is useful to compliment the standardised DXA scan protocol. Thus, we would recommend the ingestion of 500 ml of water 2 hours before the scan to ensure euhydration. A 2 hr pre measurement time point allows for any excess fluid to be excreted without leading to a hypohydrated state. Since the study in Chapter 3 was published in 2015, three studies to date have cited our paper and they have implemented strategies to ensure euhydration when their participants have been scanned by DXA (Alexander 2015, Milsom, Naughton et al. 2015, Turnagöl 2016). So, this practice is being adopted and probably makes their data (mainly fat free soft tissue mass estimations) more valid and reliable as the researchers are ensuring their participants are euhydrated before performing scans.

To improve and maximise the precision of the body composition estimation by DXA scans it is important to have a standardised scanning protocol. Ideally subjects should be scanned in a

fasted, rested and euhydrated state. This standardised scanning protocol will reduce test-retest error and will provide more valid results when assessing changes in fat free soft tissue mass induced by training or nutritional interventions in athletes.

The study detailed in Chapter 4 - Part A was the first part of a multicentre project between University of Stirling, Bangor University, and Loughborough University. This study addressed to the question of how different commonly ingested beverages affect urine output and fluid balance and based on this we developed the beverage hydration index (BHI) by studying a euhydrated young healthy active male population under resting conditions. The BHI was defined as the total renal excretion following ingestion of water divided by total renal excretion following ingestion of the test beverage (still water). In this study it was demonstrated that orange juice\*, skimmed milk, whole milk and an oral rehydration solution were the best beverages in terms of longer fluid retention in the body (higher BHI, \* when BHI was not corrected for the water content). Importantly, these observations largely followed what was already known about the impact of beverage composition differences (macronutrients and electrolytes) on fluid delivery and retention. As such, we concluded that the BHI could be considered a useful tool for classification of beverages by their hydration potential. In Part B of this Chapter, a field study (University of Stirling only) demonstrated that the results from the laboratory study could be replicated in a field trial conducted in an office-working environment. We also demonstrated that inclusion of females and participants with different body mass index and different ages may not alter the hydration potential responses and ranking of beverages. These findings support the wider application of the BHI as a tool to classify beverages. However, further investigation of the application of the BHI in different groups such as children and females is required to confirm its usefulness.

Our published paper about the development of a BHI (Maughan, Watson et al. 2016) has already been cited a couple of times. Ruxton et al (Ruxton 2016) have referred to our data to support the fact that tea may offer a healthy source of hydration as there was no significant differences in its hydration potential/BHI in comparison with water. There has also been considerable media interest in the work from articles in the New York Times (Burfoot 2016) through to the New Zealand Listener (Pellegrino 2016) as well as featuring on a BBC Science programme (The Truth about Healthy Eating).

In Chapter 5, a systematic evaluation of several key beverage components was completed. This study was the second part of the multicentre study with Stirling, Bangor, and Loughborough. With this study we assessed the question of what is the impact of specific beverage components on urine output and fluid balance. University of Stirling investigated the outcome on short-term hydration potential of beverages with different concentrations of carbohydrate using beverages containing 50, 100, and 200 g/L of sucrose. Bangor University analysed the hydration potential of beverages with different sodium content (15, 27 and 52 mmol/L of sodium). Loughborough University examined the effect of different caffeine doses on the hydration potential of beverages using drinks containing 50, 200 and 400mg/L of caffeine. The BHI was calculated for the different beverages in the three study centres. BHI was higher in beverages with higher carbohydrate (20%) and sodium (27 and 52 mmol/L) content but it was not affected by even the highest caffeine content (400mg/L).

The experimental study presented in Chapter 6 observed, as demonstrated previously, that milk helped to maintain positive net fluid balance for a longer period in comparison with other beverages (fruit juice, sports drink and water) in euhydrated young males at rest. However, this effect of milk was not observed in older adult males. This can likely be explained by the physiological changes due to ageing such as: changes in renal and gastrointestinal function. Interestingly, water was as good as milk, fruit juice and a sports drink to maintain a positive net fluid balance in older adults under the same circumstances. The results of this study suggest that water is a good option to maintain euhydration in older adults. However, it is necessary to investigate if there is any key component of beverages that might help to improve hydration status in this particular group.

In the past 2 or 3 decades, most of the hydration related studies have been focused on rehydration after exercise. However, there was little research concerning hydration for people who are not exercising. For this reason we considered it timely to study hydration in typical consumers simulating a daily-living situation. A previous study that examined rehydration post exercise (James et al (2011)) investigated the effects of a drink with carbohydrate and milk protein (25g/L) on rehydration after exercise in the heat. They compared a carbohydrate-milk protein solution with energy (carbohydrate) and electrolyte matched solution. Participants were asked to start the trials in a euhydrated state. The researchers achieved this by asking the volunteers to drink 500 ml of still water 1.5 h before coming into the laboratory. In our studies we replicated the part of the protocol that James et al used to ensure euhydration in the studies reported in Chapters 4 (parts A and B), 5 and

6. James et al concluded that a solution with carbohydrate-milk protein (25g/L of milk protein) is better retained than the carbohydrate solution after exercise induced dehydration when a volume equivalent to 150% of the body mass loss was ingested. The authors concluded that milk protein might be more effective than just carbohydrate to increase fluid retention. In the studies reported in Chapters 4 part A, 4 part B and in the young group studied in Chapter 6 we observed that under resting conditions, milk was the beverage that scored the best hydration index. Although these studies were undertaken in different conditions (rest) our data were similar to the observations of James et al (James, Clayton et al. 2011) meaning that the combination of casein protein in milk with electrolytes (sodium and potassium) likely slows gastric emptying and aids longer fluid retention during rest and after exercise.

The influence of beverage sodium content on post exercise rehydration has been studied previously (Shirreffs and Maughan 1998). These authors observed that a concentration of 100mmol/L of sodium was more effective than concentrations of 0, 25 or 50 mmol/L of sodium to maintain net fluid balance after exercise when a volume equal to 150% of the body mass loss was ingested. In comparison with our results under resting conditions, we observed that sodium containing beverages with a concentration of 27 and 52mmol/L of sodium were the best at improving fluid retention, and having a positive impact on the net fluid balance. This difference between exercise and rest might be due to the sodium losses through sweat or hormonal alterations induced by exercise. Another important difference to consider between the studies is that Shirreffs & Maughan's participants were asked to ingest 150% of the mass loss after exercise and in our study participants ingested a fixed volume of 1 L.

Evans et al (Evans, Shirreffs et al. 2009) observed that a hypertonic solution of 10% glucose and electrolyte (osmolality =  $667 \pm 12$  mOsm/kg) was more effective to rehydrate and maintain euhydration after an exercise-induced dehydration of  $1.9 \pm 0.1\%$  of body mass in comparison with 2% and 0% glucose solutions (osmolality =  $193 \pm 5$  mosm/kg and  $79 \pm 4$  mosm/kg respectively). In the study reported in Chapter 5 we reported that more concentrated carbohydrate solutions (20% sucrose solution) would likely delay gastric emptying rate and intestinal water transport and thus improve BHI without necessarily providing effective replacement of the body water pool. The knowledge generated in these chapters has allowed identification of the hydration potential of different beverages when

ingested in a euhydrated state, and the dose-response effect of some of the key components driving an improved BHI.

Dehydration is caused by not drinking enough fluid that leads to an increased osmolality of extracellular fluids (Cheuvront, Kenefick et al. 2013). As reported in Chapter 7, a high proportion of older adults do not meet the adequate fluid intake recommendations. Stookey (2005) reported that the prevalence of hypertonicity in community dwelling older adults may be as high as 60%, indicating cell dehydration. Hypertonic plasma is also a predictor of adverse outcomes. Clark et al (2013) investigated retrospectively if an intervention of drinking 500ml of water before each of the three main daily meals over 12 weeks, along with a hypocaloric diet, had an effect on the glucose homeostasis in an older population. These authors analysed the data for fasting plasma glucose and insulin, calculated HOMA-IR and plasma copeptin concentrations. These blood markers were associated with urinary specific gravity, water intake and body mass. They observed improvements in fasting insulin for the participants in the group that ingested water. Our study reported in Chapter 6 observed that water, fruit juice, sport drink and skimmed milk had the same efficiency to maintain net fluid balance in euhydrated older adults. These results may suggest that if the volume of ingested fluids (including water, hot and cold beverages and the water contained in food) is increased to meet the adequate fluid intake recommendations it would likely also help to improve glucose homeostasis in older adults.

On the other hand, ageing has been related with a reduction in protein status that is reflected in some health complications such as: higher risk of falls and fractures; increased risk of infections; hospital admissions; and diminished immune system. All of these factors increase the potential for morbidity and mortality (Wolfe 2012). Some older adults struggle to meet their protein requirements as their appetite is reduced or because they have dentition problems that can be an impediment to eating meat for example (Chernoff 2004). It has also been observed that protein requirements for older people, to maintain lean body mass, may actually be increased in comparison with younger people (Campbell, Trappe et al. 2001). Milk is a source of multiple nutrients, including high quality protein, electrolytes and vitamins. Barr et al (2000) investigated the effect of a nutrition intervention where participants were advised to increase their milk intake by 3 cups per day or to maintain their usual diet for 12 weeks. They observed that the group that ingested more milk significantly increased their energy, protein, vitamins A, D and B12 and other micronutrient intakes. Thus, milk ingestion had a positive effect on their nutritional status. They concluded that older



adults could increase milk ingestion to improve their nutrient intakes. Our observations can further support these recommendations as milk intake will also have a positive effect on hydration status. Improving hydration status will be beneficial for their health status and for the prevention of the complications of dehydration.

In Chapter 7 our questionnaire of beverage intake habits revealed that a high proportion of the older adult population (particularly older males) do not meet the current European guidelines for intake of water from beverages. Prolonged inadequate intake of fluids may lead to health complications. It has been demonstrated that chronic low fluid intake may be an important factor in the pathogenesis of conditions like urolithiasis (Borghini, Meschi et al. 1996), urinary tract infection (Nygaard and Linder 1997), chronic kidney disease (Wang, Wu et al. 2008, Torres, Bankir et al. 2009) and bladder cancer (Michaud, Spiegelman et al. 1999). In the older adult population a low fluid intake is generally related to a worsening of outcome when they are admitted to hospital (El-Sharkawy, Watson et al. 2015). In our observational study we could analyse the behaviour related with fluid intake of a group of 492 Scottish adults. We investigated what young and older adults actually drink in terms of when and how much fluid is ingested. We also asked them about their habits of fluid intake in relation with exercise and inquired about their dehydration awareness. From the results obtained in this study, the key difference between those participants meeting or not meeting the EFSA adequate fluid intake guidelines was their water intake. It was observed that this was mainly due to lower intake during the mid-morning (after breakfast until 11 am) and the early-afternoon (after lunch time up to 5 pm).

A recently published study by Athanasatou et al (2016) retrospectively investigated the water intake of a sample of Greek adults utilising two different methods. In part A of their study (n=1092, 43 ± 18 years (19.6% were 65-75 y), 48.1% males), the participants completed a questionnaire called "Water Balance Questionnaire" (WBQ), a semi-quantified food and fluid frequency questionnaire focused on evaluating fluid intake for three days. In part B of their study (n=178, 37±12 years (42.1% were 40-64 y and the rest were ≤39 y), 51.1% males), a different sample of participants was asked to record their water, beverage and food intake for 7 days. They observed that when they used WBQ to assess water intake, it resulted in higher estimates than when the assessment was done through the seven day diaries. The authors attributed the differences between study A and B to several factors. The WBQ was delivered to 1092 participants while the 7 day diaries were distributed to 178 subjects. Also, WBQ was designed specifically to record fluid intake including a food frequency

questionnaire with more than 20 beverage related questions. WBQ asked about the ingested volumes as the number of glasses while the seven day diaries used continuous data (ml per drinking occasion). Another factor that could influence these differences was that the participants that used the seven day diaries were also asked to collect 24h urine for all seven days and this urine collection in study B might have altered their normal drinking behaviour. Using both approaches, the researchers observed that water was the beverage ingested in the largest volume, followed by hot beverages and milk. According to their observations, they determined the compliance with EFSA adequate intake guidelines, and found some discrepancies. When data from part A was used, 83% of females and 74% of males met the EFSA fluid intake recommendations. However, when data from study B was analysed it was observed that only 62% of females and 40% of males complied with the EFSA adequate fluid intake. These differences should be considered when assessing fluid intake of a sample or a population. However, the selection of the instrument or the approach that will be used to assess fluid intake habits will depend on multiple factors such as human and economic resources.

When comparing their data with the results we obtained from Chapter 7 the key difference is that Athanasatou et al (2016) did not separate the participants of their study into those meeting or not meeting the EFSA recommendations. We consider that it is relevant for any research related with hydration habit assessment through food/fluid diaries or questionnaires to do this categorisation when calculating mean fluid intake. If the data is not categorised as meeting or not meeting the criterion, it can often seem that a higher percentage of respondents are complying with the EFSA adequate fluid intake. For example from our data, when data were separated by age and by gender, the mean values for all groups were over the EFSA fluid intake daily recommendation. However, when we separated these data into meeting or not meeting the recommendations, the results highlighted those who did not meet the recommendations. Athanasatou et al (2016) also did not do any classification of their data based on the age of participants.

Malisova et al (2016) recently published their work in which they studied water intake and hydration indices in healthy European adults in “The European Hydration Research Study (EHRS)”. Their participants were from Spain, Germany or Greece. Total water intake was assessed from seven day food and fluid diaries. The researchers also collected 24 h urine samples over seven days for hydration marker analyses. Blood samples were collected for blood indices. The study was novel because researchers obtained data from blood hydration

indices for 573 volunteers and also it was the first time that 24 h urine samples were collected and analysed over 7 days in a hydration study of this magnitude. They observed that in their sample, approximately 60% were euhydrated and 20% were dehydrated over the period of 7 days. Despite the study being named the EHRS, the authors acknowledged that the results they presented are from three countries and cannot be generalizable to the entire European population. They suggested further studies in different countries and populations. Malisova et al (2016) reported the mean total water intake of their volunteers as  $2.75 \pm 1.01$  L/day. The researchers mentioned that water intake guidelines are often complex and not always harmonized. They reported that food water content contributed to 24% of the total water daily intake. We consider that this study highlights the relevance of including a hydration marker from urine or blood to complement the data obtained through a questionnaire or an interview. However, as acknowledged by Baron et al (Baron, Courbebaisse et al. 2015) to date, there is not a consensus on the best methods to assess hydration status, particularly in large populations. Although the instruments (questionnaires/audits) for data collection were different, when our results were compared with Malisova et al (2016), overall data (considering the data from young, middle-aged and older adults) showed that the Scottish population tended to drink less than the German, Spanish and Greek populations. One reason that might explain this is that we did not assess specifically the fluids from food (i.e. soups, broths, stews, raw fruits and vegetables) and possibly we missed a considerable portion of the fluid intake as the respondents were asked that particular aspect of their fluid intake. This also could be related with the climate, considering it is normally colder in Scotland than in Germany, Spain or Greece. Tani et al (2015) investigated the effect of the season and air temperature on fluid intake from beverages and from foods in Japanese adults. The research included a wide range of temperatures (from 11.3 °C during the winter to 31.5 °C during the summer). They observed that fluid intake from beverages was positively related with air temperature, while the fluid intake from foods was inversely related with air temperature. This might mean that the adequate fluid intake guidelines should be different depending on the regular weather of each place. This is particularly true for Europe as there are big climate differences between countries. Further research is needed to investigate hydration habits in different countries in Europe with different types of weather.

The study described in Chapter 7 provided relevant information about beverage choices, time of ingestion and dehydration awareness, by gender, in different age groups from a sample of

the Scottish adult population. This information could be very useful to guide development of strategies to improve hydration status in young and older adults. It can be considered as a pilot study to develop further research. A follow-up study using a questionnaire to investigate the hydration habits (including choices, volume and timing of ingestion) in different age groups in combination with blood/24 h urine sample analyses for hydration markers would add to the existing literature. Based on the results of this first phase, we suggest an interventional study where participants who do not meet the EFSA guidelines increase their water consumption at a targeted time of day to meet the EFSA adequate fluid intake recommendations. Another study that is needed is to develop a hydration status indicator that can be useful to detect hypohydration in older people. The outcome from the questionnaire showed that, for example, dark urine colour and thirst are the dehydration signs most frequently mentioned. However, dark urine colour is not a good indicator of hydration status for older people because of their impaired capacity to concentrate urine. For this reason we would also suggest to test and implement the number of urine voids and urine volume per void in 24 h (Burchfield, Ganio et al. 2015) in conjunction with 24 h urine osmolality (Perrier, Buendia-Jimenez et al. 2015) as physiological indicators of hydration status. These markers could then be correlated with total fluid intake in young and older adults as suggested by Perrier et al (Perrier, Rondeau et al. 2013). Another variable that could be interesting to measure is AVP. It has been demonstrated that AVP has a function as a disease predictor for diabetes and cardiometabolic risk and that adequate fluid intake may decrease AVP and copeptin (a stable surrogate marker of AVP release) (Enhörning, Wang et al. 2010, Johnson and Armstrong 2013, Guelinckx, Vecchio et al. 2016). Thus, measuring copeptin in subjects who do not meet the EFSA adequate fluid intake before and after a targeted fluid intake intervention might provide a more objective measure of intervention success.

### **Thesis limitations and future directions**

The data presented in this thesis add novel and relevant information to the literature that will help the advancement of knowledge in the field of hydration for healthy and free living population. However, there are some limitations that should be considered.

- For the study reported in Chapter 3, while hydration status is clearly important for improving reliability and validity of body composition estimation through DXA, there are other factors that need further investigation including: impact of diet

composition on the gastrointestinal contents, menstrual cycle phase, and carbohydrate loading/depletion.

- For the studies described in Chapters 4a, 4b, 5 and 6, the fact that participants started the trials in a euhydrated status can be considered as a limitation. Further research is needed in when participants are mildly dehydrated as that will simulate better the habitual behaviour. Further investigation is needed to study older people when dehydrated to assess beverages with different compositions to determine which are the best for restoring fluid balance. Further work is particularly needed since older people are prone to present with fluid overload as well as dehydration. Also, more research is required to understand the effect of different types and amount of protein on gastric emptying. Approaches to inhibiting or prolonging the clotting of the casein protein would enable manipulation of BHI to meet the needs of different situations. For the studies reported in Chapter 4a and 5, the participants ingested 1 litre of the test beverage in 30 minutes what would seem unlikely in real life. For this reason, for studies 4b and 6, we changed the ingestion period to 60 minutes as an attempt to mimic real life. Another limitation of the study reported in Chapter 4a is that we tested 12 beverages and still water as control. This number of beverages were selected from the wide range of products that are available in the market. We considered these 12 beverages were representative of different components. However, we suggest testing a wider range of beverages to obtain further BHI information on commonly consumed products. This could eventually enable the inclusion of BHI as part of the nutritional labelling. Including BHI information on products would make the consumer more aware of the hydration potential and would improve understanding of fluid requirements in the body. However, BHI labelling would also alert consumers to high energy, high salt formulations and may thus not be supported by industry. Another possible study would be to verify if the BHI applies to other populations such as children and females. Further research could also be developed around the hydration potential of different graded alcoholic beverages and to assign a BHI for different volumes of alcohol.
- The data presented in Chapter 7 have the limitation of have been obtained from a 7 day diet diary completed through a recall methodology. We acknowledge that the audit should have been validated before using it as a hydration habits assessment

tool. However we will validate the audit before submitting the data to a scientific journal.

- Future studies should also consider interventions to increase fluid intake to help meet the recommendations and therefore hydration status in the older adults. Based on the results of the audit, we suggest an intervention study where addition of 500ml of water (divided into two 250ml portions) on top of normal fluid intake during the day could improve habitual daily fluid intake. We also propose the investigation of other methods to determine adequate fluid intake recommendations and guidelines based on body composition or total energy expenditure estimations to have a more individualised approach according to each person's needs.

### **Concluding summary**

Adequate fluid intake plays a role in maintaining health in all the stages of life. The data reported in this thesis revealed the relevance of euhydration when assessing body composition to obtain valid and reliable results and the impact of beverage components such as carbohydrate, sodium, caffeine and alcohol on hydration status in young and healthy older adults. The beverage hydration index is a tool that can be useful to identify the hydration potential of commonly consumed beverages considering that the ingestion of a variety of beverages can help to maintain euhydration in the general healthy population.

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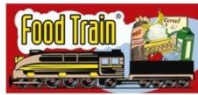
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## **APPENDIX**




### Hydration Habits Audit

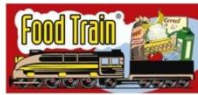
The Health and Exercise Sciences Research Group – School of Sport with the support of Food Train is conducting an audit on hydration habits in adults to inform future studies focused on hydration interventions.

We would really appreciate it if you could help us by answering the following questions (the audit will take less than 10 minutes to complete).

#### **Instructions:**

Please select the most appropriate answer for each question. The questions about fluids refer to fluids that you drink rather than fluids contained in food.


1. Gender:
  - Male
  - Female
2. Age: \_\_\_\_\_years
3. Are you taking any medication that could influence your body water content (e.g. blood pressure drugs, diuretics, etc.)?
  - Yes (please specify) \_\_\_\_\_
  - No
4. Are you taking any nutritional supplement (e.g. multivitamins, minerals, etc.)?
  - Yes (please specify) \_\_\_\_\_
  - No
5. How many mugs of fluid do you typically drink per day? (Each mug  equals 250 ml)
  - Less than 4 mugs
  - 4-5 mugs
  - 6-7 mugs
  - 8-9 mugs
  - 10-11 mugs
  - More than 11 mugs



**Thinking about the last week...**

6. What do you typically choose to drink in the morning (first thing in the morning on waking and with breakfast)?


\_\_\_\_\_

7. How many mugs of this drink would you typically consume? (1 mug  = 250 ml)

\_\_\_\_\_

8. Following the first morning drinks, what do you typically drink before 11 am?


\_\_\_\_\_

9. How many mugs of this drink would you typically consume? (1 mug  = 250 ml)

\_\_\_\_\_

10. What do you typically choose to drink after the midmorning (after 11 am) until the end of your lunchtime?


\_\_\_\_\_

11. How many mugs of this drink would you typically consume? (1 mug  = 250 ml)

\_\_\_\_\_

12. What do you typically choose to drink during the afternoon (after lunchtime up to 5 pm)?


\_\_\_\_\_

13. How many mugs of this drink would you typically consume? (1 mug  = 250 ml)

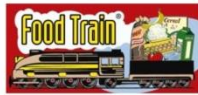
\_\_\_\_\_

14. What do you typically choose to drink during the early evening (after 5 pm), up to and including your evening meal (up to 8 pm)?

\_\_\_\_\_

15. How many mugs of this drink would you typically consume? (1 mug  = 250 ml)


\_\_\_\_\_



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16. What do you typically choose to drink before bedtime (from 8pm until bedtime)?

---

17. How many mugs of this drink would you typically consume? (1 mug  = 250 ml)

---

18. Do you typically make fluid available during the night (bedtime to first thing in the morning)? If yes, please state the type of fluid here.

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19. If you do drink during the night how many mugs of this drink would you typically consume? (1 mug = 250 ml)

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20. For any hot drinks such as tea or coffee do you add sugar/milk?

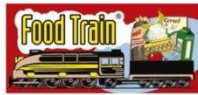
- Yes                      If so, how much? \_\_\_\_\_
- No

21. For any dairy based drinks (milk, yogurt, etc.) do you have any concern about intolerances?

- Yes                      If so, please give us details \_\_\_\_\_
- No

22. Do your drinking habits change between weekdays and weekends (alcohol intake, social drinks, etc.)?

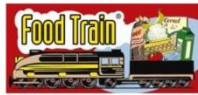
- Yes                      If so, please give us details \_\_\_\_\_
- No



23. If you drink alcoholic drinks, how many units of alcohol do you have per week?

- I don't drink alcohol
- Less than 5 units per week
- 5-10 units per week
- 11-15 units per week
- 16-20 units per week
- More than 20 units per week

<p><b>1.5</b> units</p>  <p>Small glass red/white/rosé wine (125ml, ABV 12%)</p>	<p><b>2.1</b> units</p>  <p>Standard glass red/white/rosé wine (175ml, ABV 12%)</p>	<p><b>3</b> units</p>  <p>Pint of higher-strength lager/beer/cider (ABV 5.2%)</p>	<p><b>1.7</b> units</p>  <p>Bottle of lager/beer/cider (330ml, ABV 5%)</p>
<p><b>3</b> units</p>  <p>Large glass red/white/rosé wine (250ml, ABV 12%)</p>	<p><b>2</b> units</p>  <p>Pint of lower-strength lager/beer/cider (ABV 3.6%)</p>	<p><b>2</b> units</p>  <p>Can of lager/beer/cider (440ml, ABV 4.5%)</p>	<p><b>1.5</b> units</p>  <p>Alcopop (275ml, ABV 5.5%)</p>
<p><b>1</b> unit</p>  <p>Single small shot of spirits* (25ml, ABV 40%)</p>			



24. Is there a particular time of day when you try to drink more fluids?

Yes (Please select one):

- In the morning
- In the afternoon
- In the evening

If so, please explain why:

\_\_\_\_\_

No

25. Is there a particular time of day when you restrict yourself from drinking?

Yes (Please select one):

- In the morning
- In the afternoon
- In the evening

If so, please explain why:

\_\_\_\_\_

No

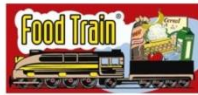
26. What factors influence when and how much you drink? Please select the answer(s) that apply:

- Thirst
- Mealtimes
- My schedule for drinking
- Social environment
- Availability of fluids
- Other(s) – Please specify

27. Do you know how much fluid from drinks is recommended for you each day?

Yes If so, please give us details \_\_\_\_\_

No



28. How would you tell if you were dehydrated and in need of some fluids?

(Please select the answer (s) that apply)

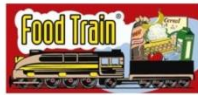
- Feel thirsty
- Headache
- Dry mouth
- Dark coloured urine
- Fatigue or weakness
- Dry skin
- Irritable
- Hungry
- Other(s) – Please specify \_\_\_\_\_

29. How many hours per week do you routinely engage in physical activity/sport?

- I don't practice any physical activity
- Less than 2 hours per week
- 2 to 3 hours per week
- 3 to 4 hours per week
- 4 to 5 hours per week
- 6 to 7 hours per week
- More than 7 hours per week

30. If you take part in exercise, what do you drink while being active? (if you don't practice any physical activity, you do not need to answer this question)

- I don't drink anything
- Water
- Sport drink
- Other(s) – Please specify \_\_\_\_\_



**Contact details**

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Food Train Region: \_\_\_\_\_

Phone number: \_\_\_\_\_

Email: \_\_\_\_\_

**Thanks for your help!**



## **PUBLICATIONS**

# Errors in Dual Energy X-Ray Absorptiometry Estimation of Body Composition Induced by Hypohydration

Nidia Rodriguez-Sanchez and Stuart D.R. Galloway

Dual energy x-ray absorptiometry (DXA) is a popular tool to determine body composition (BC) in athletes, and is used for analysis of fat-free soft tissue mass (FFST) or fat mass (FM) gain/loss in response to exercise or nutritional interventions. The aim of the current study was to assess the effect of exercise-heat stress induced hypohydration (HYP, >2% of body mass (BM) loss) vs. maintenance of euhydration (EUH) on DXA estimates of BC, sum of skinfolds (SF), and impedance (IMP) measurements in athletes. Competitive athletes (23 males and 15 females) recorded morning nude BM for 7 days before the first main trial. Measurements on the first trial day were conducted in a EUH condition, and again after exercise-heat stress induced HYP. On the second trial day, fluid and electrolyte losses were replaced during exercise using a sports drink. A reduction in total BM ( $1.6 \pm 0.4$  kg;  $2.3 \pm 0.4\%$  HYP) and total FFST ( $1.3 \pm 0.4$  kg), mainly from trunk ( $1.1 \pm 0.5$  kg), was observed using DXA when participants were HYP, reflecting the sweat loss. Estimated fat percent increased ( $0.3 \pm 0.3\%$ ), however, total FM did not change ( $0.1 \pm 0.2$  kg). SF and IMP declined with HYP (losses of  $1.5 \pm 2.9\%$  and  $1.6 \pm 3\%$  respectively) suggesting FM loss. When EUH was maintained there were no significant changes in BM, DXA estimates, or SF values pre to post exercise, but IMP still declined. We conclude that use of DXA for FFST assessment in athletes must ensure a EUH state, particularly when considering changes associated with nutritional or exercise interventions.

**Keywords:** fat-free soft tissue mass, fat mass, hydration, exercise, DXA

Body composition assessment is an important part of athlete monitoring; and is widely used to assess changes following exercise or nutritional interventions. Athletes competing in gravitational, weight class and aesthetic sports often reduce their body mass / fat mass, or maintain it as low as possible to gain a competitive advantage. In extreme cases, athletes could develop severe medical problems sometimes with fatal consequences (Nattiv et al., 2007). Considering these practices, the International Olympic Committee Medical and Scientific Commission set up a Working Group on Body Composition, Health and Performance to determine whether optimum body composition and/or minimum values for body fat content and body water content could be established (Sundgot-Borgen et al., 2013). Publications arising from the IOC working group highlight that greater understanding of factors influencing all aspects of body composition estimation is important (Ackland et al., 2012; Meyer et al., 2013; Sundgot-Borgen et al., 2013).

Dual energy x-ray absorptiometry (DXA) was originally designed to measure specific bone regions (bone mineral density of hip and spine) of older adults,

has been used for over two decades, and is considered the gold standard technique for these assessments (Blake & Fogelman, 2009). More recently DXA has become a popular and accessible tool to determine fat and lean tissue composition. Many factors affect body composition estimation by DXA, one of which is soft tissue hydration. DXA scanning assumes soft tissues are normally hydrated for accurate partitioning into fat and lean fractions (Plank, 2005) and that there is a constant hydration status of fat-free soft tissue mass (73%; Pietrobelli et al., 1998). However, hydration of fat-free soft tissue mass can range from 67–85% (Moore & Boyden, 1963). Acute changes in hydration status can therefore alter fat-free soft tissue mass DXA estimates (Lohman et al., 2000). Several clinical studies suggest DXA is able to detect small individual changes in total mass and soft and lean tissue mass in healthy adults and patients (Going et al., 1993; Kohrt, 1995, 1998; Pietrobelli et al., 1998). To date, the effect of hypohydration followed by rehydration on DXA estimates of fat-free soft tissue mass and fat mass has only been analyzed in a nonathletic group using a 24 hr fluid restriction protocol (Going et al., 1993) and has not used the most recent scanning technology. Therefore, analyzing and understanding the effects of hypohydration on body composition estimation in an athlete population could be very important for sports nutrition practitioners and researchers. This is particularly true when assessing minimum body fat criteria and/or fat-free soft tissue

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mass changes in response to nutritional or exercise interventions.

Studies on the influence of daily activities, meal ingestion, and acute exercise on body composition estimates have already been performed in healthy controls (Horber et al., 1992) and more recently on athletes (Nana et al., 2013; Nana et al., 2011). Furthermore, previous work has not compared DXA estimates with skinfolds/impedance analysis outcomes following an acute fluid deficit. We hypothesize that DXA estimates of fat-free soft tissue mass will track fluid balance deficits incurred during exercise-heat stress and that control of hydration status is a crucial part in assessment of body composition in athletes.

## Methods

We recruited 38 participants (23 males, 15 females) from different athletic clubs representing the range of physiques found among athletic populations. The study was approved by the University of Stirling Research Ethics Committee and the NHS East of Scotland Research Ethics Committee. Participants were excluded from the study if they were older than 40 years (older than typical athletic population on whom our research is focused) or not currently training / competing in their sport. Participants were involved in a range of sports (running, cycling, rowing, rugby, boxing, football, gymnastics, triathlon, martial arts, rock climbing, and tennis). Subject characteristics were age  $28.1 \pm 5.5$  years, height  $172.6 \pm 9.3$  cm, and stable baseline body mass  $69.5 \pm 10.6$  kg. Females were asked to complete all laboratory visits during the same menstrual phase to avoid potential changes in body fluid and body mass. To achieve this we obtained menstrual cycle phase history information from them before, and during participation in the study.

### Early Morning Body Mass Measurement

Participants were provided with a set of scales (Seca Quadra 808, Birmingham, UK) to record body mass for 7 days before the first test in their own homes. The scales were individually calibrated against known mass (range: 0–90 kg) before use. Calibration correlation coefficients were 0.99–1.00. Morning, fasted, nude body mass was recorded after emptying bladder and bowels to establish stability of body mass over the period before starting the trials. To reduce potential variance all participants used the same set of scales throughout the entire study period. No correction was applied to mass recordings to account for the slight differences between sets of scales.

### Study Design Overview

Participants attended the laboratory on three occasions. The first visit was for prescreening, signing consent and issuing of scales for daily body mass recording. The second visit was 1 week later and was the first main trial day (Day 1) involving anthropometric measurements,

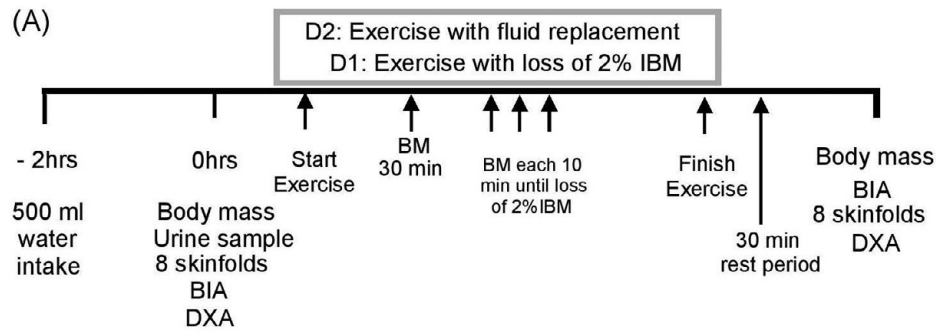
impedance analysis, and then DXA scanning. These measurements were conducted on entering the laboratory in a euhydrated condition, and again after a period of exercise-heat stress aimed at producing a fluid deficit of  $\geq 2\%$  of the initial body mass. A further week later participants attended for a final visit (Day 2) in which we repeated all of the measurements and the same exercise-heat stress work period as Day 1, but fluid losses and estimated energy/glycogen usage were replaced using a carbohydrate-electrolyte sports drink (Gatorade) to maintain body mass (Figure 1A).

### Standardized Baseline Conditions

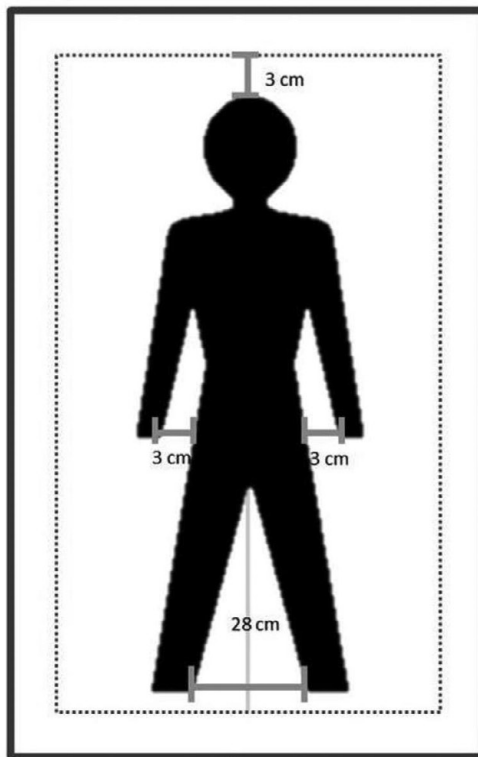
For both days of testing, participants were asked to attend to the laboratory in the morning after fasting for at least eight hours, without doing strenuous exercise or ingesting alcoholic beverages the previous day. Participants were instructed to drink 500 ml of water 2 hr before entering the laboratory to ensure euhydration. On arrival at the laboratory, participants emptied their bladder and bowels and provided a urine sample for initial hydration status assessment. Urine samples were measured for osmolality using a freezing point depression osmometer (Roebing, Camlab, UK). Initial nude body mass and anthropometric measures including stature and 8 skinfolds (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, and medial calf) using a Harpenden caliper were recorded. Land marking and measurements were done by the same International Society for the Advancement of Kinanthropometry (I.S.A.K.) Level 3 trained anthropometrist following the International Standards for Anthropometric Assessment (Stewart et al., 2011). Impedance was then estimated using a single frequency (50 kHz) bioelectrical impedance analysis device (Bodystat 1500) with participants in a supine position. All readings were obtained within 1 min of adopting the supine posture. This procedure was to avoid erroneous impedance readings through fluid shifts that occur with prolonged periods in this position (Shirreffs & Maughan, 1994). Following these initial measurements participants were carefully positioned for one whole body DXA scan.

### DXA Scan

Body composition was measured from a whole body scan using a narrowed fan-beam DXA (iDXA GE Healthcare) with analysis performed using GE Encore 13.40.038 Software (GE Healthcare). All scans were performed and analyzed by the same trained technician. iDXA was calibrated with phantoms as per manufacturer guidelines each day before measurement. All scans were undertaken using the standard thickness mode; automatically chosen by the software. Subjects wore minimal clothing (underwear) and removed jewelry and metallic objects for scans. We established a protocol for undertaking whole body scans which emphasized consistency in the positioning of subjects on the scanning area of the DXA instrument (Figure 1B).



(B)



**Figure 1** — Study design (A). The exercise time varied depending on each subject and positioning protocol (B) for dual energy x-ray absorptiometry (DXA): Subjects were centrally aligned in the scanning area of the DXA instrument, we measured 3 cm from the distance of the top line drawn on the surface of the bed to the vertex of the head of the participants, the hands were in a prone position and we ensured that the distance between the thumbs and the legs was 3 cm, we placed a foam block between their feet which was transparent under the DXA scan to maintain a constant distance between the feet of 28 cm in each scan. All the distances were measured with a metric ruler in each scan. The scans were analyzed automatically by the software, with regions of interest subsequently confirmed by the technician before data analysis. IBM= initial body mass.

### Exercise Protocol

Following scanning, participants undertook an exercise-heat stress protocol. On Day 1, exercise was performed without any food/fluid ingestion. Exercise was conducted on a stationary cycle ergometer (Monark 874E) in a warm

environment ( $26.4 \pm 0.9$  °C) with participants wearing a plastic bin bag and warm clothing to enhance heat stress. The protocol consisted of 30 min of cycling at a predetermined fixed load ( $160 \pm 25$ W (males),  $94 \pm 16$ W (females)) and pedal cadence (70 rpm) followed by subsequent 10 min bouts. Between exercise bouts

participants were asked to dry themselves off before undertaking a nude body mass measurement. Nude body mass measurement was required to ensure sweat in clothing did not influence mass loss assessment, and all measures were made with participants behind a privacy screen. Repeated bouts of cycling were performed until a 2% body mass loss was achieved. Activity duration, heart rate, power, and pedal cadence were recorded during exercise. Following a 30 min rest period to cool down, shower, and empty bladder we obtained final nude body mass, skinfold, impedance, and DXA measures.

On Day 2 participants replicated the exact intensity and duration of exercise performed on Day 1, with ingestion of a known volume of sports drink (Gatorade) to replace the fluid losses experienced on Day 1. On Day 2, the aim was to maintain initial body mass and euhydration status.

### Statistical Analysis

Statistical analyses were conducted using Minitab, version 16.1.0. For tabulated and graphical data, mean and standard deviation (*SD*) values were used. Differences related to pre- or postexercise scanning, gender, or hydration status were tested using repeated measures analysis of variance and general linear model. *P* values less than .05 were considered significant. Reliability measures for pre- and postexercise anthropometric measurements, impedance and DXA scans also were conducted. Paired *t* tests were used to assess whether absolute differences existed between preexercise measurements or measurements pre- and postexercise on Days 1 and 2.

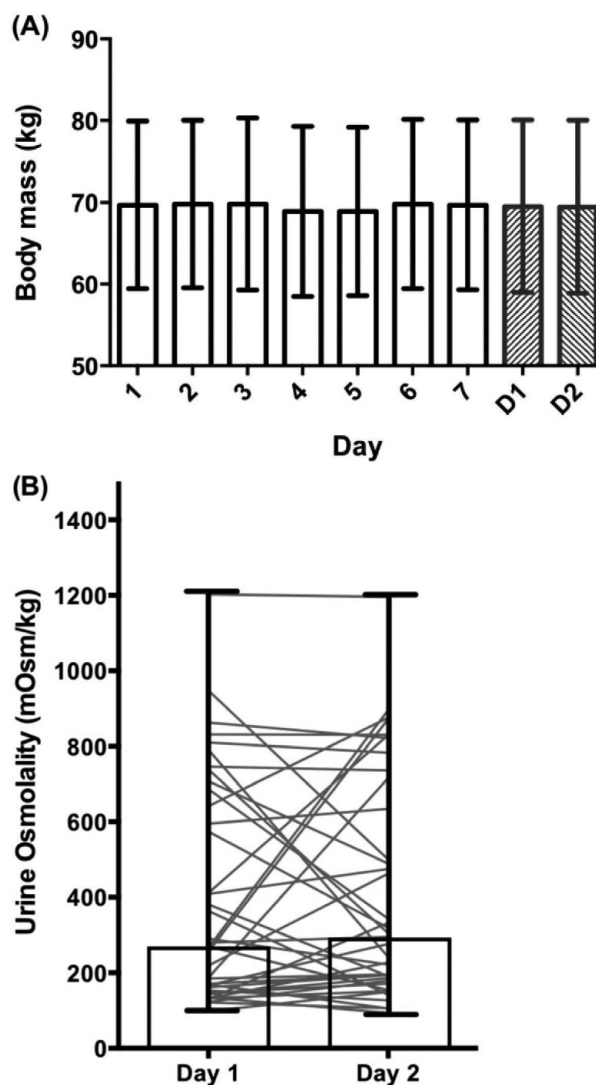
We also compared the results from repeat DXA scans and calculated the coefficient of variation (*CV*); defined by the *SD* of difference in duplicate measurements expressed as a percentage of the overall mean data (Hopkins, 2000).

## Results

Participants were of 8 different nationalities. Analysis of menstrual cycle phase history (female participants) revealed 40% ( $n = 6$ ) were in follicular phase, 53% ( $n = 8$ ) in luteal phase and 7% ( $n = 1$ ) presented amenorrhea confirmed by a sports medicine physician.

### Reliability of Baseline Measures and Conditions Before and During Each Trial Day

Participant body mass was not different across the 7 days preceding the trials and on trial days (Figure 2A). Urine osmolality (Day 1, 268 mOsm/kg [min: 98, max: 1203]; Day 2, 290 mOsm/kg [min: 94, max: 1196]) demonstrated that most participants were generally well hydrated based on ACSM euhydration criteria (American College of Sports Medicine, 2007). However, urine osmolality values were sometimes variable within individuals



**Figure 2** — Mean (*SD*) nude body mass (A) of the participants over 7 days before beginning the trials and also the body mass before exercise on both trial days (D-1 and D-2). No differences in nude body mass were observed across days. Urine osmolality (B) determined on attending the laboratory on Day 1 and Day 2 for initial hydration status assessment. Values are shown as the median (range) for  $n = 38$ . Individual data are also plotted in the figure.

between trials (Figure 2B) despite following the pretrial water ingestion criteria.

All other preexercise data, including DXA measurements, were consistent between trial days. When analyzing *CV* for the DXA body composition estimates between the preexercise scans on Day 1 and on Day 2, data were considered reliable. We found trivial *CV* of bone mineral density, fat expressed as a percentage, whole body tissue mass, whole body fat mass, whole body fat-free soft tissue mass and estimated body mass demonstrating minimal variability between Day 1 and Day 2 (Table 1). Room

temperature (Day 1;  $26.4 \pm 0.9$  °C, Day 2;  $26.7 \pm 0.9$  °C) and relative humidity (Day 1;  $37 \pm 5\%$ , Day 2;  $38 \pm 6\%$ ) were similar between trials. Average exercise duration for 2% body mass reduction was  $60.9 \pm 12.1$  min (males:  $55.7 \pm 11.2$ ; females:  $69.0 \pm 8.5$  min). Average exercising heart rate was  $155 \pm 13$  (Day 1) and  $150 \pm 14$  beats per minute (Day 2) representing  $81 \pm 7$  and  $78 \pm 8\%$  of age predicted maximum heart rate, respectively (Table 2).

### Effects of Exercise Induced Hypohydration on Body Mass and Estimates of Body Composition

Exercise on Day 1 led to a mean body mass reduction of  $1.6 \pm 0.4$ kg ( $2.3 \pm 0.4\%$  hypohydration). Gender differences in losses were  $1.8 \pm 0.3$ kg (males) and  $1.3 \pm 0.3$ kg (females); representing  $2.4 \pm 0.4\%$  and  $2.2 \pm 0.3\%$  hypohydration, respectively. On Day 2 the mean fluid intake to match sweat losses was  $1.5 \pm 0.4$ L ( $1.6 \pm 0.3$  L, males;  $1.2 \pm 0.3$  L, females) and body mass was maintained during the exercise period.

DXA body composition values (including bone mineral density (BMD) and fat, lean and total mass), sum of skinfolds, and impedance are summarized in Table 3. On Day 1 there was a statistically significant reduction of  $1.5 \pm 0.4$ kg (2.2%) in total tissue mass and  $1.3 \pm 0.4$ kg (2.5%) in fat-free soft tissue mass from pre- to postexercise (Figure 3). However, significant increases in fat mass percentage were observed following hypohydration ( $0.3 \pm 0.3\%$ ) with no change in absolute fat mass (kg). With fluid replacement on Day 2 there were no significant changes in any DXA estimates.

Differences in body segment composition (arms, legs and trunk) were also assessed pre- and post-exercise using DXA data (Figure 4). On Day 1, trunk (tissue fat percentage increased ( $0.5 \pm 0.7\%$ ) while total tissue mass ( $1.2 \pm 0.5$ kg) and lean tissue mass ( $1.1 \pm 0.6$ kg) decreased, with no changes in segment composition pre- postexercise noted on Day 2. Sum of skinfolds was significantly lower on Day 1 (Table 3) following hypohydration ( $1.5 \pm 2.9\%$ ; all participants). Analyzed by gender the reduction in males was  $1.4 \pm 3.5\%$  and in females  $1.6 \pm 2.0\%$ .

**Table 1 Baseline Conditions Before Exercise for Each Trial Day Showing Percentage Change (% $\Delta$ ) and Coefficient of Variation (CV %) Between the Day 1 and Day 2 Preintervention Values**

Measurement	Pre Day 1	Pre Day 2	% $\Delta$	CV (%)
Body mass (kg)	69.5 (10.6)	69.5 (10.6)	-0.09	0.99
Sum of skinfolds (mm)	89.6 (35.4)	88.4 (34.0)	-1.08	6.97
Impedance ( $\Omega$ )	504 (66)	507 (69)	0.68	21.08
Bone mineral density ( $\text{g}/\text{cm}^2$ )	1.243 (0.142)	1.239 (0.139)	-0.30	1.05
Whole body tissue (% Fat)	20.9 (7.1)	20.8 (7.1)	-0.08	3.30
Whole body tissue (kg)	67.1(10.2)	67.0 (10.3)	-0.11	1.17
Whole body fat (kg)	13.8 (4.6)	13.8 (4.5)	-0.40	3.92
Whole body fat-free soft tissue mass (kg)	53.3(10.4)	53.3 (10.4)	0.01	1.24
Bone mineral content (kg)	2.9 (0.6)	2.9 (0.6)	0.05	1.24
DXA estimated total mass (kg)	70.0 (10.6)	69.9 (10.7)	-0.09	1.17

**Table 2 Exercise and Environmental Characteristics Recorded on Each Trial Day**

Characteristics	Day	All ( $n = 38$ )	Males ( $n = 23$ )	Females ( $n = 15$ )
Heart rate during exercise (bpm)	1	155 (13)	156 (14)	154 (11)
	2	150 (14)	152 (14)	146 (13)
% age predicted max heart rate	1	81 (7)	82 (8)	80 (6)
	2	78 (8)	80 (8)	76 (7)
Power (W)	1	134 (39)	160 (25)	94 (16)
	2	135 (39)	160 (26)	96 (14)
Room temperature (°C)	1	26.4 (0.9)	26.4 (1.0)	26.4 (0.6)
	2	26.7 (0.9)	26.6 (1.0)	26.9 (0.7)
Relative humidity (%)	1	37 (5)	37 (5)	35.6 (2.9)
	2	38 (6)	38 (5)	38.3 (7.0)
Exercise duration (min)	1 & 2	60.9 (12.1)	55.7 (11.2)	69.0 (8.5)

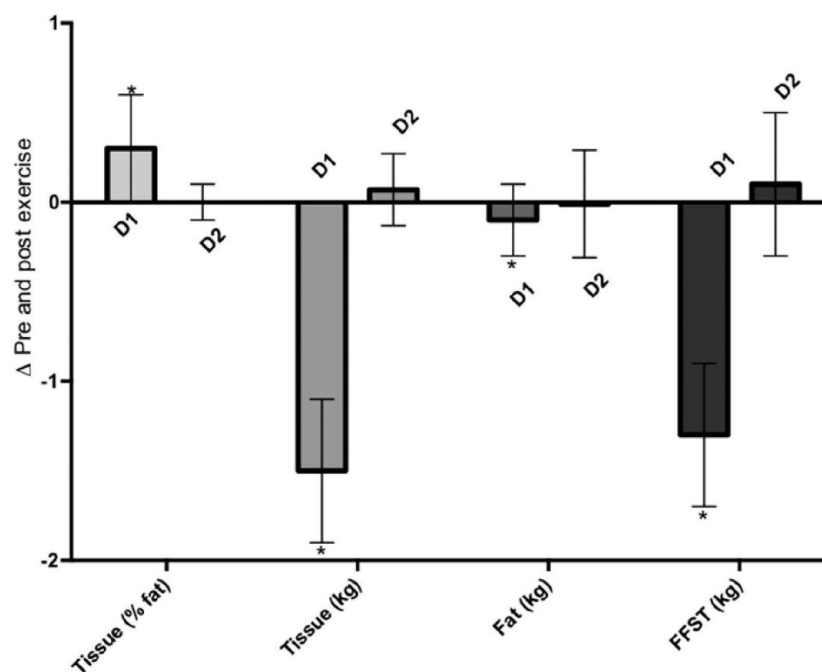
Note. Day 1 Refers to the Hypohydration Trial. Day 2 Refers to the euhydration trial.

**Table 3** Body Composition Data Analyzed on Each Trial Day

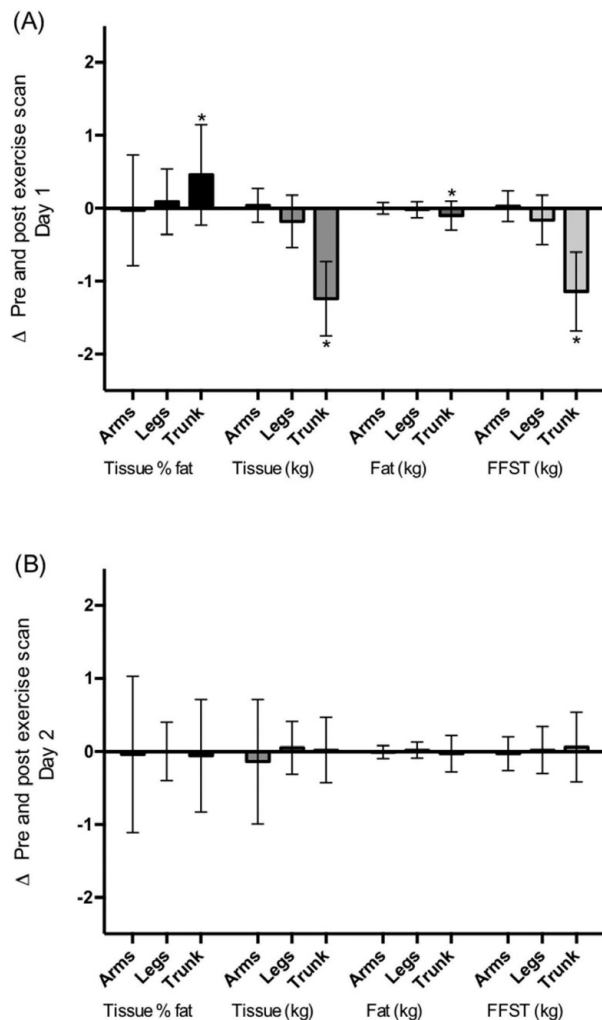
Measurement	Day	Preexercise	Postexercise	% $\Delta$	Mean Difference (95% CI)
Body mass (kg)	1	69.5 (10.6)	67.9 (10.3)*	-2.28	-1.6 (1.5, 1.7)
	2	69.5 (10.6)	69.5 (10.6)	0.05	0.0 (-0.1, 0.0)
Sum of skinfolds (mm)	1	89.6 (35.4)	88.2 (34.6)*	-1.48	-1.4 (0.6, 2.2)
	2	88.4 (34)	88.0 (33.2)	-0.25	-0.4 (-0.4, 1.1)
Impedance ( $\Omega$ )	1	504 (66)	495 (64)*	-1.60	-8 (-4, -12)
	2	507 (69)	498 (61)*	-1.63	-9 (-3, -15)
BMD (g/cm <sup>2</sup> )	1	1.243 (0.142)	1.241 (0.100)	-0.19	-0.002 (-0.002, 0.006)
	2	1.239 (0.139)	1.239 (0.100)	-0.01	0.032 (-0.060, 0.003)
Tissue (% fat)	1	20.9 (7.1)	21.2 (7.2)*	0.28	-0.3 (-0.4, -0.2)
	2	20.8 (7.1)	20.8 (7.1)	-0.39	-0.0 (-0.1, 0.00)
Tissue (kg)	1	67.1 (10.2)	65.6 (10.0)*	-2.19	-1.5 (-1.4, -1.6)
	2	67.0 (10.3)	67.1 (10.3)	0.11	-0.1 (-0.1, -0.1)
Fat (kg)	1	13.8 (4.6)	13.7 (4.5)	-0.92	-0.1 (-0.1, -0.2)
	2	13.8 (4.5)	13.7 (4.5)	-0.10	-0.1 (-0.1, 0.1)
Fat-free soft tissue mass (kg)	1	53.3 (10.4)	51.9 (10.2)*	-2.54	-1.3 (-1.2, -1.5)
	2	53.3 (10.4)	53.4 (10.5)	0.15	-0.1 (-0.2, 0.0)
BMC (kg)	1	2.9 (0.5)	2.9 (0.5)	-0.33	-0.0 (-0.0, -0.0)
	2	2.9 (0.5)	2.9 (0.5)	-0.21	-0.0 (-0.0, -0.0)
Estimated mass (kg)	1	70.0 (10.6)	68.5 (10.4)*	-2.12	-1.5 (1.4, 1.6)
	2	69.9 (10.7)	70.0 (10.7)	0.10	0.1 (0.1, 0.0)

Note. Pre- and postexercise values, percentage change pre to post exercise (% $\Delta$ ), and mean difference (95% confidence interval) between pre and postexercise values are shown. Day 1 refers to the hypohydration trial; Day 2 refers to the euhydration trial.

\*Indicates significant difference from preexercise.



**Figure 3** — Absolute changes from pre- to postexercise induced hypohydration on Day 1 and on Day 2: change in fat percentage (tissue %fat), change in tissue mass (tissue, kg) and change in fat mass (Fat, kg) and fat-free soft tissue mass (FFST, kg) estimated by DXA. (\*indicates significant difference)



**Figure 4** — Changes in DXA body composition in different body segments. Part A shows absolute changes between pre- and postexercise on Day 1. Part B presents the differences between pre and post exercise on Day 2. (\*indicates significant difference)

On Day 2, sum of skinfolds decreased by  $0.3 \pm 2.5\%$  (full group) and  $0.1 \pm 2.7\%$  and  $0.8 \pm 2.1\%$ , respectively for males and females, but there was no significant interaction effect. Impedance was significantly reduced by hypohydration on Day 1 (Table 3) from  $504 \pm 66$ – $495 \pm 64$  W. On Day 2 there was also a significant reduction in impedance from  $507 \pm 69$ – $498 \pm 61$  W.

## Discussion

This is the first study in a trained athlete population to examine the effects of combined exercise-heat stress and accompanying hypohydration on DXA estimates of whole and regional body composition. This type of intervention related to hydration status (hypo- and

hyper-hydration) has only been analyzed previously in nonathletic groups using 24 hr fluid restriction or dialysis (Going et al., 1993; Horber et al., 1992). In the current study, baseline measures (initial body mass, initial hydration status, sum of skinfolds, impedance and body composition), and environmental conditions were all consistent and demonstrated that under these experimentally controlled conditions estimates of body composition are reliable. In our study, exercise-induced hypohydration reduced total mass, total tissue mass and fat-free soft tissue mass estimates from DXA. With maintenance of euhydration we did not observe any significant differences in estimations and measurements from pre- to postexercise.

A recent study investigated the effects of exercise and ad libitum meal/fluid intake on DXA estimates of body composition in cyclists (Nana et al., 2013). The authors observed these factors are associated with changes in mean estimates of total and regional body composition that range from trivial to small but substantial (Nana et al., 2013). The loss of body mass examined in the current study represents a common level of hypohydration that could be presented as a nonoptimal hydration strategy or as part of an intentional dehydration to “make the weight” in category classified sports (Ackland et al., 2012; Klungland Torstveit & Sundgot-Borgen, 2012; Sundgot-Borgen & Garthe, 2011). With hypohydration we observed a significant reduction in total tissue and fat-free soft tissue mass determined by DXA from pre- to postexercise that was not evident when euhydration was maintained. Sum of skinfolds and impedance data demonstrated reductions from pre- to postexercise on the hypohydration trial suggesting a loss in fat mass that was not evidenced in DXA scan data. Skinfold results correspond with previous studies which have shown hydration affects elasticity and compressibility of tissues modifying the measurement of skinfolds (Ward, Rempel, & Anderson, 1999). The output from impedance matches with previous findings which demonstrated small fluid changes (gains or losses) could be misinterpreted as changes in fat content of an athlete (Saunders et al., 1998).

DXA changes in body composition by region in the pre- to postexercise scans on the hypohydration trial revealed changes were mainly localized to the trunk region. The localization of effects to the trunk could be explained by losses in specific body fluid compartments, particularly blood volume. A reduction in blood volume would lead to reduced central blood volume when lying in a supine position for scanning, as the flow from splanchnic and renal circulations is redistributed (Kenney, 2008; Rowland, 2001; Rowland & Roti, 2004). Although previous studies have found an effect of gender (Buehring et al., 2013), the current research observed the significant differences with hypohydration were consistent between genders. When euhydration was maintained there was no significant change in body mass, whole body or regional DXA scan indices, or sum of skinfolds from pre- to postexercise. A previous study in nonathletes observed, follow-



ing intake of 0.8–2.4 L of water, that the determination of bone mineral content and of fat mass by DXA were not affected, while estimation of fat-free soft tissue mass in the trunk region was considerably increased (Horber et al., 1992). The present work adds to this literature by demonstrating the magnitude of change in fat-free soft tissue mass estimates with a 2% hypohydration in an athlete population.

Participants were asked to carefully control baseline conditions before arrival in the laboratory to achieve reliable measurements for body composition from DXA, skinfolds, and impedance analysis. The values obtained were clearly consistent between preexercise assessments on the two trial days. This suggests our attempts to avoid variation in baseline body mass and composition, by controlling hydration status, dietary intake, and prior exercise, were effective. In female participants we ensured they completed the trials during the same phase of their menstrual cycle as body mass may fluctuate throughout the menstrual cycle. Findings from a previous study on 41 females demonstrated average body mass increased by 0.3% between follicular and luteal phases (Pliner & Fleming, 1983). Research suggests an increase in body mass during luteal phase is not attributable to fluid retention; but rather an alteration in energy intake (Chihal, 1990; Pliner & Fleming, 1983; Tomazo-Ravnik, 2006). By considering menstrual cycle phase we could track the unique differences between trials with changes in hydration status from pre- to postexercise alone.

In conclusion, this research provides additional guidance for future use of DXA in athletes, such as ensuring athletes are euhydrated before scanning. Assessment of hydration status should be considered optimal practice for test-retest scans, and consideration should be given to menstrual cycle phase in females. Thus, by controlling hydration status before scanning practitioners can more accurately evaluate fat-free soft tissue mass changes in athletes as part of nutritional or exercise interventions.

### Acknowledgments

The authors wish to thank Dr. Ian Rollo from Gatorade Sports Science Institute UK who provided the drinks used in the study. The study did not receive funding from any external source and was conducted as part of the postgraduate research work of Nidia Rodriguez Sanchez.

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# A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index<sup>1</sup>

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## ABSTRACT

**Background:** The identification of beverages that promote longer-term fluid retention and maintenance of fluid balance is of real clinical and practical benefit in situations in which free access to fluids is limited or when frequent breaks for urination are not desirable. The postingestion diuretic response is likely to be influenced by several beverage characteristics, including the volume ingested, energy density, electrolyte content, and the presence of diuretic agents.

**Objective:** This study investigated the effects of 13 different commonly consumed drinks on urine output and fluid balance when ingested in a euhydrated state, with a view to establishing a beverage hydration index (BHI), i.e., the volume of urine produced after drinking expressed relative to a standard treatment (still water) for each beverage.

**Design:** Each subject ( $n = 72$ , euhydrated and fasted male subjects) ingested 1 L still water or 1 of 3 other commercially available beverages over a period of 30 min. Urine output was then collected for the subsequent 4 h. The BHI was corrected for the water content of drinks and was calculated as the amount of water retained at 2 h after ingestion relative to that observed after the ingestion of still water.

**Results:** Total urine masses (mean  $\pm$  SD) over 4 h were smaller than the still-water control ( $1337 \pm 330$  g) after an oral rehydration solution (ORS) ( $1038 \pm 333$  g,  $P < 0.001$ ), full-fat milk ( $1052 \pm 267$  g,  $P < 0.001$ ), and skimmed milk ( $1049 \pm 334$  g,  $P < 0.001$ ). Cumulative urine output at 4 h after ingestion of cola, diet cola, hot tea, iced tea, coffee, lager, orange juice, sparkling water, and a sports drink were not different from the response to water ingestion. The mean BHI at 2 h was  $1.54 \pm 0.74$  for the ORS,  $1.50 \pm 0.58$  for full-fat milk, and  $1.58 \pm 0.60$  for skimmed milk.

**Conclusions:** BHI may be a useful measure to identify the short-term hydration potential of different beverages when ingested in a euhydrated state. This trial was registered at [www.isrctn.com](http://www.isrctn.com) as ISRCTN13014105. *Am J Clin Nutr* 2016;103:717–23.

**Keywords:** fluid balance, dehydration, rehydration, euhydration, electrolytes, macronutrients, gastric emptying, intestinal absorption, renal excretion, urine

## INTRODUCTION

Water intake is episodic, whereas losses are continuous. Under normal free-living conditions, homeostatic mechanisms mean

that body water balance fluctuates over the course of a normal day, but generally returns to the same point over a 24-h cycle (1). Consequently, large fluid deficits are uncommon for the majority of the population, but knowledge of beverages that can maintain hydration status over a longer period may be of interest to those who wish to stay hydrated in situations in which free access to fluid is limited or when frequent breaks for urination are not desirable (2–5). Although several studies have examined the effectiveness of beverages for postexercise rehydration (6), the protocols employed do not represent a common situation for the majority of the population. Thus identification of beverages that promote longer-term fluid retention and maintenance of fluid balance for prolonged periods under euhydrated conditions would be of real clinical and practical benefit.

Adequate daily water intake is defined in the United States by the Institute of Medicine (7) at 3.7 L for men and 3.0 L for women and in Europe by the European Food Safety Authority (8) as 2.5 L for men and 2.0 L for women. The distribution of fluids over the course of the day and their composition, however, also may be important in determining how well an individual is able to maintain an adequate hydration status. The volume and composition of ingested drinks have a strong influence on the rates at which they empty from the stomach and are absorbed in the small intestine, thus affecting their entry into the body water pool (9). Beverage components are also metabolized and excreted on different time scales (9). These various factors are likely to result in different hydration status profiles in the first few hours after ingestion of different beverages. It should therefore be possible to assign a beverage hydration index (BHI) to each drink that will define the hydration response to any particular drink, in much the same way as the glycemic index defines the blood glucose response to ingestion of foods (10). In the case of a BHI, the cumulative volume of urine passed over a fixed period of time is in effect the AUC for renal water excretion. The urine volume passed relative to a standard treatment (still water) can therefore be calculated as the BHI of a beverage.

<sup>1</sup> Supported by a grant from the European Hydration Institute.

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Received May 12, 2015. Accepted for publication November 24, 2015.

First published online December 23, 2015; doi: 10.3945/ajcn.115.114769.

Therefore, the aim of the present study was to assess fluid balance responses to the ingestion of a fixed volume of commonly consumed beverages ingested when in a euhydrated state, with a view to establishing the feasibility of a BHI. We hypothesized that drinks containing a high electrolyte content or high energy content would have greater fluid retention and thus a higher BHI than plain water. Conversely, drinks containing nutrients with known diuretic actions, such as alcohol and caffeine, may have lower BHI values.

## METHODS

### General study design

Three separate laboratories (Loughborough, Bangor, and Stirling) collaborated to test 72 recreationally active, healthy male subjects. Ethics approval for the study was obtained separately from the ethics committees of the 3 institutions involved.

A randomization table was generated based on each participant undertaking a maximum of 4 experimental trials, which included water plus 3 other test drinks administered in a randomized fashion, and was based on each experimental site's assessing all available test drinks ([www.randomization.com](http://www.randomization.com)). Rehydration study data (11, 12) informed the sample size estimates and indicated a minimum sample size for each test drink of  $n = 12$ . Although not a cluster randomized trial, we factored in an additional sample size weighting to account for possible increased variance because of data collection across 3 different sites. The final sample size estimate based on 80% power with mean total urine output of 900 mL, pooled SD of 300 mL, and a mean difference of 220 mL, detectable at an  $\alpha$  level of 0.05, required a total of  $n = 15$  observations per drink. We therefore aimed to recruit  $n = 30$  at each site; allowing for loss to follow-up, this ensured completion of  $n = 24$  at each site, giving  $n = 17$  observations on any given test drink.

### Pretrial standardization/exclusion criteria

At each site, 24 healthy, physically active men between 18 and 35 y of age were recruited. For the total sample of  $n = 72$  the mean  $\pm$  SD characteristics were the following: age  $24 \pm 4$  y, height  $178 \pm 6$  cm, body mass  $77.3 \pm 9.9$  kg, and water intake  $2.0 \pm 0.8$  L/d (**Table 1**). Those with a history of cardiovascular, renal, musculoskeletal, or metabolic diseases, as determined from a preparticipation health screen questionnaire, were ex-

cluded. Because body mass was used as an index of euhydration, those currently undertaking an energy-restricted diet and/or exercise plan also were excluded. Participants were asked to record their diet, including their fluid intake (household measures technique), as well as any exercise performed, in a diary over the 2 d before the first trial and asked to replicate this before their subsequent visits. Participants also were asked not to perform any strenuous exercise or consume alcoholic beverages in the 24 h preceding all trials.

### Experimental procedures

After an overnight fast of at least 8 h, participants emptied their bladder on waking, retaining an aliquot in a sterile collection tube. One hour before arriving at the laboratory, volunteers were instructed to consume 500 mL still water (Highland Spring) over the course of 15 min. On arrival in the laboratory, volunteers remained seated in a comfortable environment for 10 min. A single 5-mL blood sample was collected via venipuncture from an antecubital vein, and blood was dispensed into a serum tube. Participants were then asked to void their bowels and bladder before measurement of near-nude body mass (underwear only) to the nearest 50 g behind a screen. Approximately 30 min after arrival at the laboratory, participants then ingested 1 L of the assigned test drink over a period of 30 min (4 equal volumes administered 7.5 min apart). A fixed volume, rather than a volume relative to body mass, was chosen, because most drinks are served and ingested in containers of a standard volume. Participants were asked to empty their bladder at the end of the drinking period and again at the end of each hour of the study period. If a participant requested to pass urine before the hour was complete, this was collected and then added to any further urine produced at the end of the corresponding hour. After the final urine sample was collected, near-nude body mass was recorded once again.

### Drinks and drink preparation

Each participant consumed still water (Highland Spring) and 3 of the following drinks in a randomized, counter-balanced order: sparkling water (Highland Spring), cola (Coca-Cola), diet cola (Diet Coke), sports drink (Powerade; Coca-Cola), oral rehydration solution (ORS) (Dioralyte; Sanofi), orange juice (Tesco Everyday Value), Lager beer (Carling), hot black coffee (Nescafe Original), hot black tea (PG tips), cold black tea (PG tips), full-fat milk (3.6% fat; Tesco) or skimmed milk (0.1% fat; Tesco). The nutrient composition of the test drinks is presented in **Table 2**.

All cold drinks were stored at a standard refrigerated temperature (4–6°C) until serving. Tea, coffee, and ORS were prepared according to the manufacturer's instructions and were prepared with still water (Highland Spring still water). Hot black coffee and black tea were brewed with freshly boiled still water (Highland Spring) and served at 60°C, with the temperature being maintained in a hot water bath. Cold black tea was brewed in the same manner, then stored and served at 4–6°C. The ORS was prepared and stored and also served at 4–6°C. A 5-mL sample of each drink preparation was portioned into aliquots in plain tubes. All drinks were tested for osmolality, sodium, and potassium after preparation within 48 h and 5 d after collection, respectively.

**TABLE 1**

Participant physical characteristics and daily water intake at each of the 3 study sites and for combined data (all sites)<sup>1</sup>

	Bangor (n = 24)	Loughborough (n = 24)	Stirling (n = 24)	All sites (n = 72)	P
Age, y	24 $\pm$ 4	26 $\pm$ 3	25 $\pm$ 5	25 $\pm$ 4	0.05
Height, cm	177 $\pm$ 7	180 $\pm$ 6	179 $\pm$ 7	178 $\pm$ 6	0.48
Body mass, kg	76.2 $\pm$ 12.3	77.4 $\pm$ 7.3	78.3 $\pm$ 9.8	77.3 $\pm$ 9.9	0.77
BMI, kg/m <sup>2</sup>	24.2 $\pm$ 3.3	24.0 $\pm$ 1.6	24.5 $\pm$ 2.6	24.2 $\pm$ 2.6	0.77
Water intake, L/d	2.0 $\pm$ 0.9	2.0 $\pm$ 0.6	2.1 $\pm$ 0.8	2.0 $\pm$ 0.8	0.75

<sup>1</sup>Values are means  $\pm$  SDs. P values shown were obtained from an ordinary 1-factor ANOVA.

**TABLE 2**

Water, energy, and macronutrient content (carbohydrate, fat, and protein) of drinks was obtained from drink labels, whereas osmolality, sodium, potassium, and caffeine content were determined by in-house analysis<sup>1</sup>

Drink	Water content, %	Energy, kcal/L	Carbohydrate, g/100 mL	Fat, g/100 mL	Protein, g/100 mL	Osmolality, mmol/kg	Sodium, mmol/L	Potassium, mmol/L	Caffeine, mg/L
Still water	100	0	0	0	0	2	0	0	0
Sparkling water	100	0	0	0	0	7	1	0	0
Cola	89	420	10.6	0	0	432	2	0	96
Diet cola	100	4	0	0	0	23	2	0	127
Sports drink	96	160	3.9	0	0	297	21	4	0
ORS	97	80	1.8	0.1	0	229	55	20	0
Orange juice	89	470	10.5	0.1	0.5	570	1	33	0
Lager	94	330	2.2	0	0.4	774	1	6	0
Coffee	99	4	0.1	0	0	34	1	7	212
Tea	100	0	0	0	0	16	1	4	179
Cold tea	100	0	0	0	0	18	1	5	179
Full-fat milk	88	640	4.7	3.6	3.2	286	18	41	0
Skimmed milk	91	350	5.0	0.1	3.4	282	19	40	0

<sup>1</sup>ORS, oral rehydration solution.

### Urine and serum analysis

All urine collected during the study was passed into a 1-L plastic container. The volume of each urine pass was determined by measuring the mass on an electronic balance (to the nearest 0.1 g), with the mass of the empty plastic container subtracted to enable the estimation of urine volume. From each urine sample, a 5-mL aliquot was dispensed into a plain screw-capped tube. This was stored at 4°C for the analysis of urine osmolality and sodium and potassium concentrations. Urine and serum osmolality was measured in duplicate with the use of the freezing-point depression method (either Gonotec Osmomat or Advanced Instruments) within 48 h of collection. Urine sodium and potassium concentrations were measured in duplicate with the use of flame photometry (Corning Flame Photometer) within 5 d of collection. Collection, handling, and storage of urine and serum were in accordance with the Human Tissues Act. Stored samples were discarded once satisfied analysis was completed.

Whole blood in the serum tube was allowed to stand for 1 h at room temperature to clot before centrifugation (10 min; 4°C; 2000–3000 × g). Serum was then dispensed into an appropriate storage tube (e.g., Eppendorf) and stored at 4°C for measurement of osmolality.

To help ensure consistency in the data analyzed across sites, 7 independently prepared quality control solutions were also analyzed in replicates of 10 by each research group. These contained undisclosed concentrations of sodium and potassium and a measured osmolality. Two-way random-effects intraclass correlation coefficient analysis suggested good agreement between the different institutions for osmolality, sodium, and potassium analysis in which the intraclass correlation coefficients were all ≥0.999. In addition, Bland-Altman limits of agreement analysis indicated that bias between any 2 institutions was <2% for osmolality, <1% for sodium, and <2% for potassium.

### Data and statistical analysis

Participant characteristics, pretrial participant preparation, and urine responses to the still water trial from each institution were initially compared by an ordinary 1-factor ANOVA. To confirm that hydration status was similar before each trial, serum and

urine osmolality were compared between drinks by repeated-measures ANOVA.

The main outcome measure was cumulative urine mass after ingestion of each drink. This was also expressed as a BHI for each beverage by dividing each individual's cumulative urine mass after still water with cumulative urine mass for each other test drink consumed. Individual hour cumulative urine mass and BHI of each drink was compared by paired *t* test to determine which drinks differed from still water.

To assess the practical meaning of the BHI differences observed between still water and each of the test drinks, the difference was compared with the normal variation determined from a separate repeatability analysis. For this purpose 12 participants ingested the same drink on 2 occasions. The drinks used for this repeatability analysis were the same as those used in the present study. The repeatability of the BHI was equal to a CV of 18% (~180 mL). In addition, the meaningfulness of group differences was also calculated with the use of Cohen's *d* effect size (13) and 95% CI of differences between means.

Although a fixed volume of each of the test drinks was consumed, the presence of other components in some of these drinks means the water content of drinks varied from 88% to 100% (Table 2). It might therefore be argued that the BHI should be corrected for the differences in water intake. If, however, the aim was to estimate the effects of the different drinks on body water content, then the uncorrected values would be more appropriate. For clarity the data have been expressed both ways.

All other secondary outcome measures (net fluid balance, BHI corrected for water content, and cumulative urine electrolyte loss) were analyzed by paired *t* test.

All statistical analyses were completed with the use of a computerized statistical software package (GraphPad Prism version 6 for Windows). Statistical significance was accepted at *P* < 0.05. Data are presented as means ± SDs.

### RESULTS

The study was conducted between February and August 2014. The study was completed when the target number of participants (*n* = 72) had finished the study, providing *n* = 17 observations on

each test drink in total across the 3 sites, with  $n = 72$  observations on water. In total,  $n = 86$  participants were recruited, preparticipation screening excluded  $n = 1$  participant, and  $n = 85$  were randomly assigned. Loss to follow-up occurred because of vomiting after ingestion of the tea ( $n = 6$ ) and ORS ( $n = 1$ ) or because of voluntary withdrawal from the study due to external factors ( $n = 6$ ).

### Institutional comparison of pretrial standardization and urine output response to a standard drink

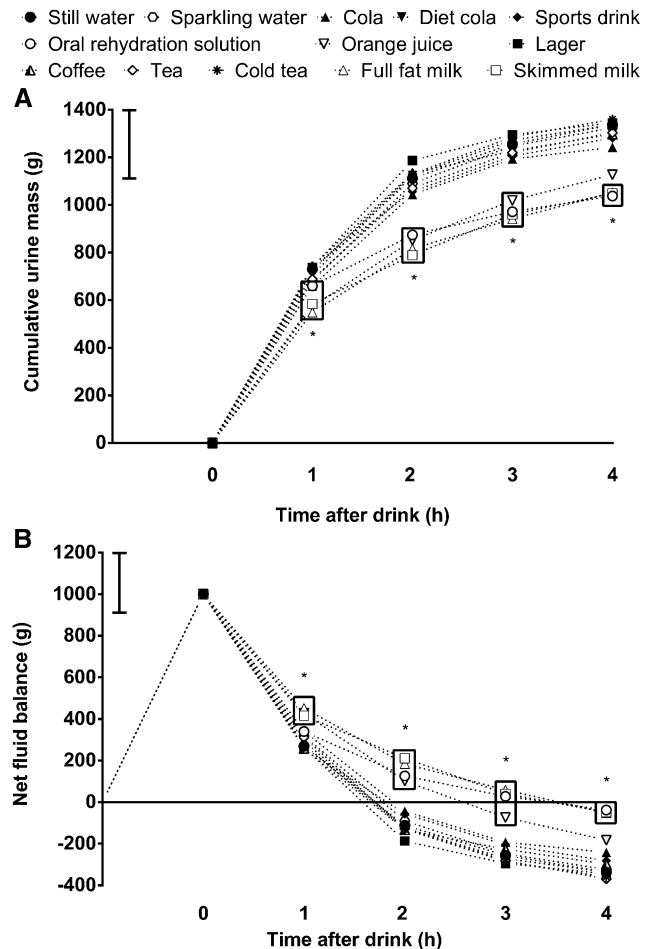
Before ingestion of drinks in the still-water trial, body mass, serum osmolality, and urine osmolality were not different, suggesting that participants' preparation before trials was similar at each institution (Table 3). We also confirmed that cumulative urine mass after the still-water drink trial was similar at each institution, which further suggests that the participants in the 3 institutions had similar fluid regulation (Table 3). It was therefore deemed reasonable to combine the data from the 3 institutions for the main study.

### Predrink ingestion hydration status

Serum osmolality ( $293 \pm 6$  mmol/kg,  $P = 0.88$ ) and urine osmolality ( $582 \pm 265$  mmol/kg,  $P = 0.56$ ) was similar immediately before drinks were ingested in each trial.

### Urine output and fluid balance

Urine mass did not differ between trials immediately after the ingestion of the drinks ( $P > 0.19$ ). One hour after the ingestion of the drinks, cumulative urine mass was lower and net fluid balance was higher than for the still water drink after the ingestion of full-fat milk ( $P < 0.01$ ), skimmed milk ( $P < 0.01$ ), and ORS ( $P < 0.01$ ) (Figure 1). Two and three hours after drink ingestion, cumulative urine mass was lower and net fluid balance was higher than for the still water drink after the ingestion of full-fat milk ( $P < 0.01$ ), skimmed milk ( $P < 0.01$ ), ORS ( $P < 0.01$ ), and orange juice ( $P < 0.05$ ). Four hours after drinks



**FIGURE 1** Cumulative urine mass (A) and net fluid balance (B) after ingestion of 1 L of various commonly consumed and commercially available drinks [ $n = 17$  observations on each test drink, except for orange juice and diet cola ( $n = 16$ ) and tea ( $n = 15$ )]. Drinks with different responses to still water were identified by paired  $t$  test analysis at each time point and highlighted in rectangular boxes;  $*P < 0.05$ . The vertical error bar in the top left corner represents the overall mean SD for all drinks during the 4-h collection.

were ingested, cumulative urine mass was lower and net fluid balance was higher for full-fat milk ( $P < 0.01$ ), skimmed milk ( $P < 0.01$ ), and ORS ( $P < 0.01$ ), but not orange juice ( $P = 0.06$ ). The effect sizes at 4 h for cumulative urine output compared with still water were 1.04 for full-fat milk, 0.85 for skimmed milk, and 1.09 for ORS (all large effects), with an effect size of 0.65 for orange juice (a medium effect). The mean differences in cumulative urine output were 294 g (95% CI: 154, 434) for full-fat milk, 339 g (95% CI: 190, 489) for skimmed milk, and 362 g (95% CI: 222, 505) for ORS.

### BHI

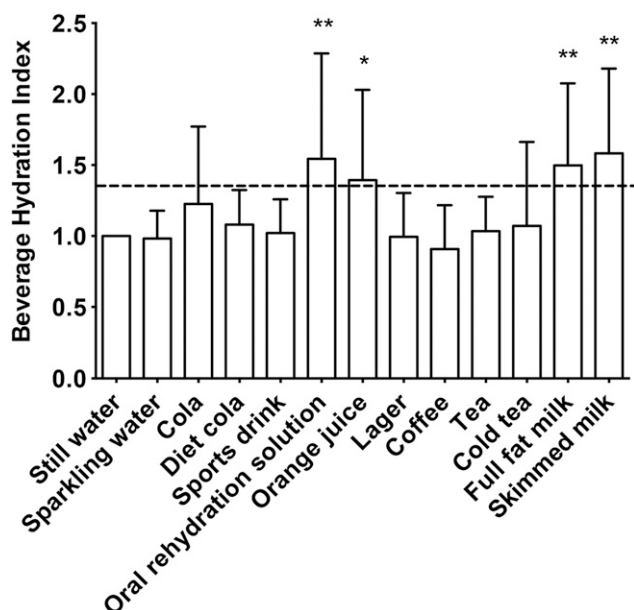
After 2 h, full-fat milk, skimmed milk, ORS, and orange juice had a higher BHI than still water (all differences  $P < 0.05$ ) (Figure 2). The effect sizes at 2 h were 1.22 for full-fat milk, 1.37 for skimmed milk, 1.03 for ORS, and 0.87 for orange juice (all large to very large effects). The higher BHI between still water and full-fat milk, skimmed milk, ORS, and orange juice also exceeded twice the CV of the BHI measure. Mean

**TABLE 3**

Institutional comparison of pretrial standardization and urine output response to a standard drink<sup>1</sup>

	Bangor ( $n = 24$ )	Loughborough ( $n = 24$ )	Stirling ( $n = 24$ )	$P$
Preingestion of still water				
Body mass, kg	$76.1 \pm 12.3$	$76.7 \pm 7.3$	$78.2 \pm 9.7$	0.76
Serum osmolality, mmol/kg	$293 \pm 8$	$291 \pm 4$	$295 \pm 3$	0.14
Urine osmolality, mmol/kg	$564 \pm 243$	$607 \pm 302$	$538 \pm 176$	0.62
Postingestion of still water				
Urine mass, g	$1341 \pm 360$	$1337 \pm 352$	$1333 \pm 288$	0.99

<sup>1</sup>Values are means  $\pm$  SDs.  $P$  values shown were obtained from a 1-factor repeated-measures ANOVA. No differences were observed between institutions for body mass, serum osmolality, or urine osmolality immediately before still-water ingestion or 4-h cumulative urine mass after 1 L still water ingestion, suggesting at each institution that participants' preparation for trials was similar and that participants in the 3 institutions had similar fluid regulation.



**FIGURE 2** BHIs for 13 commonly consumed and commercially available drinks. Drinks with different responses to still water were identified by paired *t* test analysis: \* $P < 0.05$ , \*\* $P < 0.01$ . The dashed line represents twice the CV of the BHI measure. Values are means  $\pm$  SDs of  $n = 17$  observations on each test drink, except for orange juice and diet cola ( $n = 16$ ) and tea ( $n = 15$ ). BHI, beverage hydration index.

differences for 2 h BHI values were 0.50 (95% CI: 0.20, 0.80) for full-fat milk, 0.58 (95% CI: 0.28, 0.89) for skimmed milk, 0.54 (95% CI: 0.16, 0.93) for ORS, and 0.39 (95% CI: 0.05, 0.73) for orange juice. Additionally, full-fat milk, skimmed milk, ORS, and orange juice BHIs were greater than that for still water at 3 and 4 h after drink consumption ( $P < 0.05$ ).

### BHI corrected for water content

The water content of the drinks used in this study varied from 100% to 88% (Table 2), and consequently the amount of water ingested varied between drinks. It might be appropriate therefore to recalculate the BHI to take into account the different volumes of water ingested in the different trials. The BHI values presented in **Figure 3** have been normalized by the drinks' water content to reflect the effect of the drink itself on hydration status excluding the differences in water content. As was the case without the correction for drink water content, the corrected BHI for full-fat milk ( $P = 0.02$ ), skimmed milk ( $P < 0.01$ ), and ORS ( $P = 0.01$ ) were higher than that for still water. The effect sizes for corrected BHI data at 2 h were 0.89 for full-fat milk, 1.14 for skimmed milk, and 0.98 for ORS (all large effects). The mean differences for corrected 2-h BHI were 0.32 (95% CI: 0.06, 0.58) for full-fat milk, 0.44 (95% CI: 0.16, 0.72) for skimmed milk, and 0.50 (95% CI: 0.13, 0.87) for ORS. The BHI for orange juice was, however, no longer different from still water ( $P = 0.11$ ), with an effect size of 0.60 (a medium effect) and a mean difference of 0.24 (95% CI:  $-0.06$ , 0.54).

### Urinary electrolyte excretion and balance

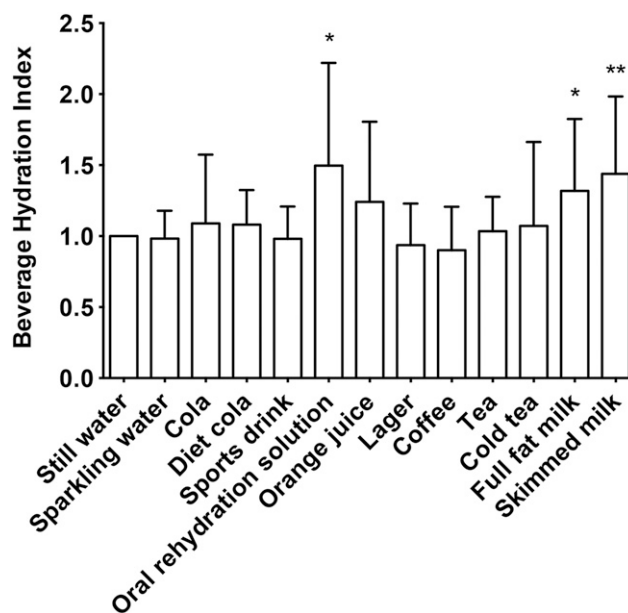
Several drinks had greater sodium or potassium balances than still water 2 h after drinks were consumed (**Figure 4**). Drinks

with positive sodium or potassium balances were typically those with the highest BHI. That is, ORS had a positive sodium balance (Figure 4A), whereas orange juice and full-fat and skimmed milk had positive potassium balances (Figure 4B).

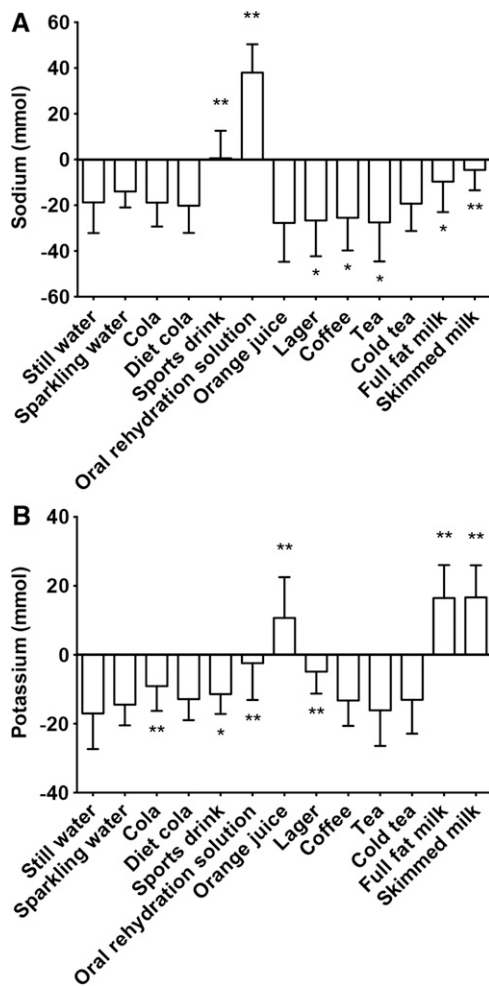
### DISCUSSION

Adequate hydration status may be associated with a decreased risk of a range of adverse outcomes, including urologic, gastrointestinal, circulatory, and neurological disorders (14, 15). In addition, maintenance of euhydration is important for the preservation of physical and mental function (4, 5, 15, 16). Consequently, identification of beverages that promote longer-term fluid retention and maintenance of fluid balance for prolonged periods would be of real clinical and practical benefit in situations in which free access to fluids is limited, or when frequent breaks for urination are not desirable (2–5). In this study we propose a novel tool to enable the objective assessment of a beverage's effectiveness to maintain hydration status over a period of time after ingestion. The calculated BHI revealed that drinks containing the highest macronutrient and electrolyte contents were the most effective at maintaining fluid balance.

The differences noted in the urine volume and calculated BHI during the monitoring period might be attributed in part to differences in the water content of the different drinks. Stahl et al. (17) recognized that the amount of water present in a fixed volume of beverage varies because of the presence of other nutrients, meaning the amount of water available to influence hydration status can markedly differ, an observation these authors termed the "postabsorptive hydration index." The water content of the test beverages in the present study ranged from 100% for



**FIGURE 3** BHIs for 13 commonly consumed and commercially available drinks after correction for water content of drink ingested. Drinks with different responses to still water were identified by paired *t* test analysis: \* $P < 0.05$ , \*\* $P < 0.01$ . Values are means  $\pm$  SDs of  $n = 17$  observations on each test drink, except for orange juice and diet cola ( $n = 16$ ) and tea ( $n = 15$ ). BHI, beverage hydration index.



**FIGURE 4** Sodium (A) and potassium (B) net balances 2 h after ingestion of 1 L of various commonly consumed and commercially available drinks. Drinks with different responses to still water were identified by paired *t* test analysis: \**P* < 0.05, \*\**P* < 0.01. Values are means ± SDs of *n* = 17 observations on each test drink, except for orange juice and diet cola (*n* = 16) and tea (*n* = 15).

still water to 88% for full-fat milk. Correction of the urine output to account for differences in the volume of water ingested made little difference to the relative BHI responses (Figures 2 and 3), suggesting that such a correction may not be required when considering drinks with characteristics similar to those used in the present study.

In addition to variations in the water content of a beverage, the present BHI model recognizes that the presence of additional nutrients in a beverage also will influence the retention of fluid substantially, meaning that beverages with similar water contents may display markedly different effects on long-term hydration status. There are several elements of a beverage that might affect fluid balance in the hours after ingestion: the macronutrient content, the electrolyte (primarily sodium and potassium) content, and the presence of diuretic agents (primarily caffeine and alcohol). Ingested drinks with a high energy content, whether in the form of carbohydrate, fat, protein, or alcohol, will empty from the stomach more slowly than energy-free drinks and will thus potentially reduce or delay the diuresis that follows in comparison with the ingestion of a bolus of still water (11, 18). This effect has

the potential to contribute to the retention of ingested fluids within the body water space. The drinks in the present study with the highest energy density were full-fat milk (640 kcal/L), orange juice (470 kcal/L), lager (330 kcal/L), cola (420 kcal/L), and skimmed milk (350 kcal/L). High energy content was generally associated with a high BHI, but a comparison of the responses to cola, lager, and orange juice suggest that other factors also play a meaningful role (e.g., electrolytes and alcohol).

In the present study, no water or salt deficit was induced before the beginning of the study. Acute administration of a bolus of water plus sodium chloride or other sodium salts results in a transient increase in total body water; this hyperhydration is prolonged relative to that observed after the intake of still water (19). In the present study, the ORS and milk drinks contained relatively high concentrations of sodium and potassium, the orange juice contained a moderate amount of potassium, and the remaining drinks contained relatively trivial concentrations of these electrolytes. It is notable that the drinks with the highest electrolyte content tended to have the highest BHI.

The known diuretic effects of caffeine and alcohol, because of their action in inhibiting the release of arginine vasopressin (20, 21), would influence the response to ingested drinks that contain caffeine or alcohol. An acute dose of <250–300 mg caffeine is unlikely to have a measurable effect on urine output, although such an effect is likely to be seen when the dose exceeds ~300 mg (22). In line with these observations, we did not observe an impact from moderate caffeine intake (96–212 mg) on net fluid balance in the present study. Furthermore, the alcohol content of the lager did not increase diuresis over other drinks, but the alcohol may have countered the hypothesized positive influence of energy density on the BHI. Perhaps surprisingly, only one study has examined fluid balance responses to alcohol in a euhydrated state (23). That study reported a 12% greater diuresis after the ingestion of 1 L lager beer containing 4% alcohol compared with the ingestion of the same volume of a nonalcoholic control beer.

The BHI values presented here are based on the net fluid balance at 2 h after the end of the drink ingestion period. This time point was chosen for 4 reasons. First, this was the time at which drinks began to show differences. Second, the majority (82%) of urine output over the 4-h period had been passed by this point. Third, in a typical day, most people would expect not to have an interval longer than 2 h between drinks, and any subsequent food or fluid ingestion would override the effects of the initial drink. Fourth, for the drinks used in the present study, it made little difference to the calculated BHI whether this was based on the first 2 h or on the whole 4-h collection period.

Although the results of the present study relate only to the acute effects of a large bolus of fluid over the subsequent 4 h, there is evidence to support the suggestion that the results may be extrapolated to a longer time scale. Grandjean et al. (24) had subjects consume water or water plus varying combinations of beverages, including carbonated, caffeinated cola and coffee. They observed no significant differences in the effect of various combinations of beverages on 24-h hydration status. In addition, Tucker et al. (25) recently suggested that 24-h hydration status was not different when subjects drank only water or a variety of drinks, including water, cola, and fruit juice, provided that an adequate total volume was consumed.

In summary, the present study describes a novel tool to enable the objective assessment of the effectiveness of beverages to



maintain hydration status. The BHI is reproducible and the pattern of response for a range of commonly consumed beverages is consistent with what is known about the effects of their constituents on water balance. An appreciation of the BHI has relevance for individuals for whom long-term maintenance of fluid balance is important, such as in professions in which fluid availability is limited (3–5), as well as in older (2) or incapacitated (15) patients. There is also a clear application to industry, where this tool could be employed to label products to indicate the hydration potential of beverages. Because of the complexity of the commercially available beverages used in this study, it was not possible to directly determine the relative influence of individual drink components on fluid balance (e.g., electrolyte content and energy density). Future studies should apply this model to further examine the significance of these nutrients in isolation, as well as to assign BHI values to a wider range of commercially available beverages.

The authors' responsibilities were as follows—RJM: conceived of the project and had primary responsibility for the final content; RJM, PW, PAAC, NPW, SJO, NR-S, and SDRG: developed the overall research plan; PW, NPW, and SDRG: had study oversight; PAAC, AD, and NR-S: conducted the research and analyzed the samples; NPW and SJO: performed the statistical analysis; and RJM, PW, NPW, and SDRG: wrote the manuscript with PAAC, SJO, and NR-S. RJM is chair of the Scientific Advisory Board for the European Hydration Institute. PW has received funding in the last 3 y from the European Hydration Institute for other hydration-related research. None of the other authors reported a conflict of interest related to the study.

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**Abstract accepted for mini-oral presentation at the European College of Sport Science  
Annual Congress in Amsterdam, The Netherlands, July 2014.**

## **ERRORS IN THE ESTIMATION OF BODY COMPOSITION INDUCED BY HYPOHYDRATION**

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### **INTRODUCTION**

Dual energy X-ray (DXA) is a popular tool to determine body composition (BC) in athletes, and is increasingly used for segmental analysis of lean mass and fat mass gain/loss in response to exercise or nutritional interventions (Ackland et al., 2012). Previous studies with athletes have assessed the effect of daily activities, exercise and meal intake in athletes on DXA BC estimates (Nana et al., 2011, 2013). The aim of this study was to analyse the effect of fluid losses incurred through exercise-heat stress ( $> 2\%$  of BM loss) on DXA estimates of BC and on skinfolds (SF) and impedance (IMP) measurements in athletes.

### **METHODS**

Competitive athletes (23 males and 15 females) recorded their morning nude BM for 7 days prior to first main trial which involved SF, IMP and DXA scanning. Measurements were conducted in euhydrated (EU) condition, and after a period of exercise-heat stress aimed at inducing a fluid deficit of  $\geq 2\%$  of the initial BM (HYP). During second trial day, we repeated all of the measurements and the same exercise-heat stress work period from first day, but fluid and electrolyte losses and estimated energy/glycogen usage were replaced using a carbohydrate-electrolyte sports drink to maintain BM at an EU state.

### **RESULTS**

A reduction in total BM ( $1.6 \pm 0.4$  kg;  $2.3 \pm 0.4\%$  HYP) and total lean mass ( $1.3 \pm 0.4$  kg), mainly from a reduction in trunk lean mass ( $1.1 \pm 0.5$  kg), was observed using DXA when participants were HYP. Fat percent increased ( $0.3 \pm 0.3\%$ ), however, total fat mass did not change ( $0.1 \pm 0.2$  kg). SF and IMP dropped when the participants lost  $\geq 2\%$  of their initial BM (losses of  $1.5 \pm 2.9\%$  and  $1.6 \pm 3\%$  respectively). When athletes replaced fluid losses during the exercise-heat stress, DXA BC estimates did not change from pre to post exercise. The sum of SF and IMP demonstrated reductions from pre to post exercise suggesting a loss in fat mass that was not evidenced in DXA scan.

## **DISCUSSION**

With HYP we observed a considerable significant reduction in total tissue and lean mass determined by DXA when the pre exercise scan was compared with the post exercise scan. Changes in BC by region in the pre-post exercise scans revealed the main changes presented with HYP were localised in the trunk region. When EU was maintained there were no significant changes in BM, DXA estimates, IMP or sum of SF from pre to post exercise.

The suggestion for future use of DXA is to ensure athletes who will be scanned are EU and even doing a prior assessment of hydration status, particularly when assessing lean mass in athletes as part of nutritional or exercise interventions and also when researchers are evaluating lean mass as part of their investigations

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**Abstract accepted for poster presentation at the American College of Sports Medicine Annual Meeting in Boston, United States, June 2016.**

**HYDRATION POTENTIAL OF COMMONLY CONSUMED DRINKS IN AN OFFICE-WORKING ENVIRONMENT**

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Several factors are known to influence the hydration potential of drinks such as: volume ingested, ingestion rate, macronutrient composition, water content, electrolyte and caffeine content. However, relatively little is known about the impact of fluid composition on fluid balance during normal daily living / office working situations. **PURPOSE:** To investigate the effect of 4 different commonly consumed drinks on urine output and net fluid balance over 3 hours in office-workers. **METHODS:** Twenty-three participants (euhydrated, males (n=7) and females (n=16), age: (mean(SD) males 31.3(10.4) y; females 33.1(9.8) y), BMI: males 29.9(4.4); females 27.4 (3.7), arrived at work in a euhydrated state. After emptying their bladder and recording of body mass they ingested 1 L of fluid over the following hour as either water, coffee, orange juice or semi-skimmed milk. Energy content of the drinks was 0 kcals/L (water), 4 kcals/L (coffee), 470 kcals/L (orange juice) and 500 kcals/L (milk). Urine output was collected immediately following, and each hour for 2 hours following, fluid ingestion for volume and electrolyte analysis. On completion a final body mass was obtained. **RESULTS:** The mean(SD) total urine mass loss over 2 hours for still water was 1007(108) g and was significantly different to milk 797(181) g ( $P<0.05$ ). Urine losses with orange juice (953(246) g) and coffee (1067(164) g) were not different to water, but coffee was also different to milk ( $p<0.05$ ). Net fluid balance was positive at 2 hr after milk ingestion (203(181) ml) and was significantly different ( $p<0.05$ ) from water (-7(108) ml) and coffee (-67(164) ml). Net Na<sup>+</sup> balance was significantly different from water (-495(207) mg) after ingestion of orange juice (-973(298) mg) and milk (-295(253) mg). Net K<sup>+</sup> balance was significantly different from water (-315(64) mg), also after ingestion of orange juice (576(171) mg) and milk (901(118) mg). **CONCLUSIONS:** A variety of drinks can be ingested during normal daily living / working to help maintain fluid balance. However, ingestion of milk led to a reduced urine output compared with the other drinks, most likely due to its casein protein and electrolyte content. The retention of fluid volume following milk ingestion may be important in situations where frequent work breaks need to be avoided.

**Abstract accepted for poster presentation at the American College of Sports Medicine Annual Meeting in Boston, United States, June 2016.**

**HOW DO DIFFERENT DRINKS AFFECT BODY FLUID BALANCE: DOES AGE MAKE A DIFFERENCE?**

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Aging is associated with a reduced ability to maintain homeostatic control over a variety of body functions, including maintenance of body fluid balance. **PURPOSE:** To compare fluid balance responses to the ingestion of commonly consumed drinks in young and older men. **METHODS:** 16 healthy male participants: 8 young (mean (SD) 25.1(5.2) y and 8 older 60.1(5.8) y were recruited. A venous cannula was inserted and a baseline blood sample was obtained. Initial near nude body mass was recorded after emptying their bladder to provide a urine sample. Participants then consumed a fixed volume (1L, 250ml every 15 min) of water (W, control), fruit juice (F, 0 mmol/L Na<sup>+</sup>; 23 mmol/L K<sup>+</sup>; 21 kcal/100 ml), sports drink (S, 29 mmol/L Na<sup>+</sup>; 2.7 mmol/L K<sup>+</sup>; 28 kcal/100ml) or skimmed milk (M, 19mmol/L Na<sup>+</sup>, 40mmol/L K<sup>+</sup>; 35 kcal/100ml). Participants urinated at the end of the 60-minute drinking period and every hour for the next 3 hours. Urine mass was recorded and a sample obtained for analysis of urine osmolality and electrolytes (Na<sup>+</sup> and K<sup>+</sup>). Blood samples were drawn immediately after the drinking period and every hour for the next 3 hours, for serum osmolality and electrolytes. **RESULTS:** Initial serum osmolality demonstrated that both groups began euhydrated (young, 298(3); old, (297(4) mOsm/kg). Mean(SD) total urine mass loss over 3 hours for W (1256(222) g) was significantly different (p<0.01) to M (876(358) g) but not to F (1139(354) g) or S (1216(262) g) in the young group. No difference in total urine mass loss was observed between drinks in the older group (W, 1217(507) g; F, 1124(160) g; S, 1100(228) g; and M, 974(137) g). There was no difference in Na<sup>+</sup> net balance with M in the older group (-0.30(0.16) g compared to young (-0.44(0.38) g). Net K<sup>+</sup> balance in both young and old, respectively, was different following M ingestion (+0.09(0.62) and +0.46(0.35) g) compared to W (-1.10(0.52) and -1.22(0.97) g) and S (-1.11(0.61) and -0.71(0.57) g) but was not different between groups. **CONCLUSIONS:** In young adults M helps to maintain positive net fluid balance for longer than other drinks. In older adults this effect of M is not observed despite similar net electrolyte balance responses. Future work should more fully explore these potential differences in fluid balance responses to drink ingestion between young and older adults.

**Abstract accepted for presentation at the International Sports Exercise and Nutrition Conference in Newcastle, United Kingdom, December 2016.**

**SYSTEMATIC EVALUATION OF CARBOHYDRATE, SODIUM, AND CAFFEINE CONTENT OF DRINKS ON THE BEVERAGE HYDRATION INDEX.**

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The beverage hydration index (BHI) is a novel tool to allow the objective description of the hydration potential of beverages (Maughan 2016). The urine volume passed relative to a standard beverage can be calculated as the BHI. The present study aimed to systematically examine the influence of carbohydrate, sodium and caffeine on the BHI. Three cohorts, each of 12 young, healthy, physically active males [Mean(SD): age 26(4) y; height 179(7) cm; mass 77.6(8.5) kg; water intake 2(0.6) L/d], ingested 1L of beverages containing four inclusion levels of a single component (sucrose 0, 5, 10 and 20%; sodium 7, 15, 27 and 52 mmol/L or caffeine 0, 50, 100 and 200 mg/L) in a double-blind, crossover manner. Urine output and blood samples were collected at each hour for the subsequent 4h. Cumulative urine output (CUO) was lower (803(241)g) after the ingestion of a 20% carbohydrate beverage than 0% (1129(231)g), 5% (1119(225)g) and 10% (1034(314)g) carbohydrate beverages ( $P<0.05$ ). CUO was also lower with 27 mmol/L (1104(339)g) and 52 mmol/L (1012(322)g) sodium beverages than 7 mmol/L (1375(280)g) and 15 mmol/L (1306(295)g) beverages ( $P<0.05$ ). However, no differences in CUO were apparent following the ingestion of beverages containing 0 to 400 mg caffeine [CUO: 0mg: 1390(238)g, 50mg: 1428(229)g, 100mg: 1460(400)g, 200mg: 1429(258)g;  $P=0.83$ ]. Thus, the BHI was greater in beverages with higher carbohydrate or higher sodium content, but not influenced by caffeine. After 2h, 20% carbohydrate and 27 mmol/L and 52 mmol/L sodium solutions had significantly higher BHI than their respective control beverages [Median (IQR) BHI: 20% carbohydrate: 1.67(1.37, 4.05); 27 mmol sodium/L: 1.28(1.07, 1.55), 52 mmol sodium/L: 1.27 (1.07, 2.23);  $P<0.05$ ]. Serum aldosterone and arginine vasopressin were similar and did not change over the time period regardless of the carbohydrate, sodium or caffeine content of the beverages. In

conclusion, the carbohydrate content of beverages has no effect on BHI at concentration up to 10% carbohydrate. Sodium content of beverages in concentrations of 27mmol/L and higher can improve the hydration potential of beverages. Caffeine doses in beverages up to 400mg/L do not have an impact upon diuresis when ingested in a euhydrated state.

**CHARACTER COUNT (not including spaces): 1924**