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Dehydration
assessment techniques and effects on endurance performance and well-being

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DEHYDRATION:

ASSESSMENT TECHNIQUES AND EFFECTS ON ENDURANCE PERFORMANCE AND WELL-BEING

by

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AUTHOR'S DECLARATION

This work had not been previously accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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This thesis is the product of my own investigations, except where otherwise stated. Other sources are acknowledged giving explicit references.

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SUMMARY

The purpose of this thesis was to examine: 1. the diagnostic accuracy of routine clinical and saliva hydration markers to identify both intracellular and extracellular dehydration in an elderly hospitalised cohort; 2. the diagnostic accuracy of traditional and novel hydration marker to identify mild intracellular and extracellular dehydration in young healthy participants; 3. whether stimulating saliva improved the diagnostic accuracy and practicality of hydration assessment using saliva; 3. if mild intracellular and extracellular dehydration had a differential effect on endurance exercise performance and affective well-being.

All routine physical signs, urine and saliva flow rate showed poor diagnostic accuracy for detecting either form of dehydration. In contrast, saliva osmolality demonstrated moderate diagnostic accuracy to distinguish both dehydration types, and may have utility for the assessment of both intracellular and extracellular dehydration in older individuals.

Although stimulating saliva reduced collection time by 85%, stimulation reduced the diagnostic accuracy of both saliva flow rate and saliva osmolality to identify progressive mild to modest intracellular dehydration evoked by exercise and heat-stress.

Diagnostic accuracy to identify mild intracellular dehydration was perfect for urine colour and specific gravity, near perfect for plasma osmolality, and fair for LF-HF, saliva osmolality and flow rate. Diagnostic accuracy to identify mild extracellular dehydration was generally poor, with the exception of postural heart rate change. A combination of thirst, urine colour and postural heart rate change improved dehydration diagnosis by discriminating between dehydration types.

Endurance performance was worse after mild extracellular than intracellular dehydration. Markers of affective well-being were unaltered after extracellular dehydration, but cardiovascular and ventilatory strain appeared higher during exercise. In contrast, intracellular dehydration caused disruption to affective well-being, but did not alter cardiovascular and ventilatory parameters. Dehydration type appears to alter the magnitude and mechanism by which dehydration impairs endurance performance.

Key words: **Hydration assessment, hypovolaemia, hyperosmolality, hypohydration, endurance performance, mood.**

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PUBLICATIONS

I was involved in all aspects of protocol design, data collection, data analyses and preparation of the following thesis chapters. However, I also gratefully acknowledge input from other named authors for each publication. The following is a list of publications arising from the material presented in this thesis.

Full papers

Fortes, M.B., Owen, J.A., Barker, P., Bishop, C., Elghenzai, S., Oliver, S. and Walsh, N.P. (2015) Is this elderly patient dehydrated? Diagnostic accuracy of hydration assessment using physical signs, urine and saliva markers. *Journal of the American Medical Directors Association.* 16(3), pp. 221-228.

Conference communications

Owen, J.A., Edwards, E.T., Walsh, N.P. and Oliver, S.J. (2013). Mild hypertonic and isotonic hypohydration: effects on endurance exercise performance in a temperate environment (invited oral presentation). *4th Hydration Network Meeting of the European Hydration Institute, Reial Acadèmia de Medicina i Cirurgia de Catalunya, Barcelona, 26th June.*

Owen, J.A., Fortes, M.B., Walsh, N.P. and Oliver, S.J. (2012). The utility of hydration markers to identify and track modest hypertonic and isotonic dehydration (Poster presentation). *International Sport and Exercise Nutrition Conference, Northumbria University, Newcastle-upon-Tyne, 13-14th December.*

Owen, J.A., Edwards, E.T., Walsh, N.P. and Oliver, S.J. (2012). Endurance performance in a temperate environment after mild hypertonic and isotonic hypohydration (Poster presentation). *International Sport and Exercise Nutrition Conference, Northumbria University, Newcastle-upon-Tyne, 13-14th December.*

Owen, J.A., Fortes, M.B., Edwards, E.T., Walsh, N.P. and Oliver, S.J. (2012). Effect of mild hypertonic and isotonic dehydration on utility of hydration markers and human performance (Poster presentation). *3rd Hydration Network Meeting of the European Hydration Institute, Verona, Italy, 13th September)*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD	axillary dryness
ADH	antidiuretic hormone or arginine vasopressin (AVP)
AFI	*ad libitum* fluid intake
AI	adequate intake (fluid)
ANOVA	analysis of variance
AUC_{ROC}	area under the ROC curve
AVP	arginine vasopressin or antidiuretic hormone (ADH)
BIA	bioelectrical impedance analysis
BIS	bioelectrical impedance spectroscopy
BM	body mass
BML	body mass loss
BP	blood pressure
BUN	blood urea nitrogen
CE	cycle ergometry
cm	centimetre
CO	cardiac output
CON	control trial
Cr	creatinine
CR	capillary refill
CV_A	analytical coefficient of variation
DEH	dehydration

DM	dry mucous membranes
ECF	extracellular fluid
ED	extracellular dehydration
F	female
FI	fluid intake
g	gram
h	hour
Hg	mercury
HR	heart rate
HSD	honest significant difference
HYD	normal hydration
ICF	intracellular fluid
ID	intracellular dehydration
kcal	kilo calorie
kg	kilogram
km	kilometre
L	litre
LF-HF	low-frequency to high-frequency heart rate variability ratio
M	male
m	metre
min	minute
mg	milligram
mL	millilitre

mm	millimetre
mOsmol	milliosmole
NBM	nude body mass
NFI	no fluid intake
NN	the interval between successive normal heart beats
pH	power of hydrogen (measure of acidity/alkalinity)
Posm	plasma osmolality
PNS	parasympathetic nervous system
PPO	peak power output
RH	relative humidity
RMSSD	the square root of the mean squared difference of successive NN intervals
ROC	receiver operator characteristic
RPE	rating of perceived exertion
s	second
SBP	systolic blood pressure
SD	standard deviation
SDNN	standard deviation of NN intervals
SE	sunken eyes
SEM	standard error of the mean
SFI	some fluid intake
SFR	saliva flow rate
SNS	sympathetic nervous system

Sosm	saliva osmolality
ST	skin turgour
STARD	standards for reporting diagnostic accuracy
STIM	stimulated saliva
SV	stroke volume
TBW	total body water
Tc	tachycardia
Tosm	tear osmolality
TTE	time to exhaustion
TM	treadmill
TWI	total water intake
USG	urine specific gravity
UK	United Kingdon
Uosm	urine osmolality
UrC	urine colour
US	United States
VAS	visual analogue scale
$\dot{V}O_{2max}$	maximal oxygen uptake
W	watts
˚C	degrees Celsius

GLOSSARY OF TERMS

Dehydration	the state of being in fluid deficit. *Alternative term - hypohydration.*
Normal hydration	sigmoidal fluctuation of hydration status within homeostatic limits. *Alternative term - euhydration.*
Fluid loss	the process of losing body fluid. *Alternative term – dehydration.*
Fluid gain or fluid-intake	the process of gaining body fluid. *Alternative term –rehydration is a term used to describe fluid gain from a dehydrated state.*
Intracellular dehydration	occurs when the concentration of body fluid-loss is hypo-osmotic with respect to plasma. This result in a hyperosmotic plasma and either no change or a transient decrease in ECF volume. *Alternative terms – hypertonic dehydration, hypertonic hypovolaemia, hypertonic euvolaemia, water-loss dehydration.*
Extracellular dehydration	occurs when the concentration of body fluid-loss is iso-osmotic with respect to plasma, due to a greater loss of electrolytes. Resulting in an unchanged (or little change in) ECF osmolality but larger ECF volume contraction. *Alternative terms – isotonic dehydration, isotonic hypovolaemia, volume depletion, water and solute-loss dehydration.*

THESIS FORMAT

A general introduction highlights the causes, types and prevalence of dehydration in different populations and settings. In addition, the negative consequences of mild dehydration are discussed and the importance of hydration assessment as a countermeasure to these negative consequences (**Chapter 1**). A literature review then provides a brief background to pertinent research and the broad aims of the thesis (**Chapter 2**). A general methods chapter outlines the common procedures and analyses performed in the subsequent experimental studies (**Chapter 3**). The thesis includes data from one prospective cohort study and two independent experimental studies. The first study investigates the diagnostic accuracy of saliva hydration markers to detect dehydration in a hospitalised, elderly cohort (**Chapter 4**). The second experimental study examines the utility and diagnostic accuracy of novel stimulated saliva parameters as potential markers of dehydration (**Chapter 5**). The third experimental study is divided into two chapters. The first investigates the effects of mild intracellular and extracellular dehydration on the diagnostic accuracy of hydration assessment markers in young healthy persons **(Chapter 6)**, whilst the second examines the effects of these mild dehydration types on endurance exercise performance and well-being (**Chapter 7**).

CHAPTER ONE

General Introduction

The maintenance of human fluid balance is normally well-maintained through hormone-mediated renal water conservation and by fluid intake in response to thirst. Nonetheless, a deficit in total body water, or dehydration, is common across populations and settings due to numerous factors that affect fluid-loss and fluid intake. For example, fluid-loss is increased with sweating, which is compounded by illness such as fever, or with exercise, physical activity, wearing protective clothing and thermal stress; and excessive urination which occurs with some commonly prescribed medications (e.g. diuretics), decreasing kidney function with age, and exposure to austere environments, such as high-altitude or cold-exposure. Often, fluid-intake is insufficient to prevent dehydration when there is limited access to fluid, reported in some military and athletic settings; or when the thirst sensation is blunted, which is observed with ageing and when humans are exposed to external stressors such as heat, cold, altitude and exercise.

The negative consequences of dehydration are well documented and include acute kidney injury, cardiovascular instability, heat-exhaustion and the condition can be fatal at severe magnitudes of dehydration (fluid deficit > 10% of body mass). Although the research is unclear, some authors believe that even mild to modest dehydration (equivalent to 1-3% of body mass) is associated with detrimental effects on human performance and well-being (Armstrong, Costill, & Fink, 1985; Dougherty, Baker, Chow, & Kenney, 2006; Ganio et al., 2011; Gopinathan, Pichan, & Sharma, 1988; Thomas, Tariq, Makhdomm, Haddad, & Moinuddin, 2004). Notably, these magnitudes of dehydration are likely common across populations and settings and therefore suitable interventions to counteract these potential negative consequences are necessary.

A useful countermeasure to halt the progress of more serious fluid-deficit related illnesses involves simple and inexpensive oral rehydration determined by the accurate identification of dehydration. Currently, no consensus exists on the diagnostic superiority of a single hydration marker (Armstrong, 2005). The lack of progress in the development of hydration markers may be due to the complexity of dehydration, which is poorly defined and often treated as a single condition (Crecelius, 2008). An underappreciated fact is that dehydration primarily manifests in one of two types or a combination of the two. A reduction in total body water due primarily to a water deficit, results in an intracellular dehydration. Whereas, a primary loss of both solute and water is termed extracellular dehydration (Nadel, Pedersen, & Maddock, 1941). Importantly, the cause of dehydration determines the composition of fluid and electrolytes lost and therefore the type of dehydration that develops (Kozlowski & Saltin, 1964). Further, the distinct physiological differences between these types of dehydration will likely determine the impact on human performance and well-being and decide the assessment and treatment process (Cheuvront & Kenefick, 2014).

Currently, the effect of dehydration type on the diagnostic performance of dehydration markers is unclear. Further, the specific physiological characteristics of dehydration types may also explain some of the equivocal findings regarding the effects of mild to modest dehydration on endurance exercise performance and well-being.

CHAPTER TWO

Literature review

Water is fundamental to life and plays a key role in supporting cellular metabolism, biochemical reactions, circulatory function, thermoregulation, and other physiological functions including, nerve transmission, transport of oxygen and nutrients and muscular contraction. Due to this association with other physiological systems the regulation of human fluid-electrolyte balance is a fundamental aspect of human homeostasis.

2.1 Human fluid balance and fluid requirements

Total body water (TBW) ranges between 45-75% of total body mass with variability primarily dependent on body composition (Watson, Watson, & Batt, 1980). The distribution of TBW is divided between the intracellular and extracellular space, each comprising approximately 65 and 35% of TBW, respectively. Of this extracellular fluid, 75% is interstitial and located mainly in lean tissue with the remaining 25% located in the intravascular space as plasma, which represents about 8% of TBW (Institute of Medicine 2004). These fluid spaces are not static volumes, as daily fluctuations in fluid-loss and fluid gain result in dynamic osmotically regulated fluid exchange between compartments.

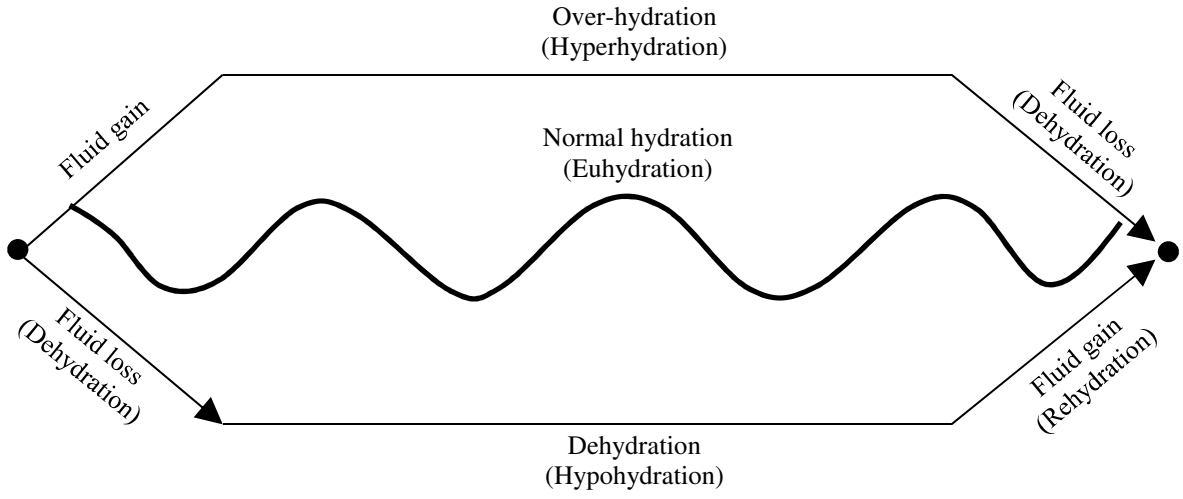


Figure 2.1 Fluid balance and body hydration terminology (Adapted from Greenleaf, 1992)

This daily fluctuation in body water is indicative of normal hydration, or euhydration, where hydration status remains tightly controlled within homeostatic limits (daily variation of < 1% of body mass; Cheuvront et al., 2004) and is represented by a sinusoidal wave indicating daily fluid loss and fluid gain (**Figure 2.1**). When fluid losses are beyond this normal fluctuation (daily variation >1% of body mass) the body is described as being in a state of dehydration i.e. a deficit of TBW. Conversely, when fluid gain outweighs fluid losses a state of over hydration or hyperhydration occurs. Fluid balance and regulation is a complex process and there is currently ambiguity in the literature regarding terminology to describe hydration as a process and state (Cheuvront, Kenefick, Charkoudian, & Sawka, 2013; Cheuvront & Kenefick, 2014; Maughan, Shirreffs, & Watson, 2007; Thomas et al., 2008). Clarification of the terms used within this thesis are summarised in the glossary of terms (**Page xxiii**), a diagrammatic representation of the dynamic nature of fluid balance (**Figure 2.1**) and further descriptions of types of dehydration and related terms are also described further in this review of the literature (**Section 2.2**).

Fluid losses from TBW include; insensible water loss via the skin, the lungs, and in faeces with the largest and most variable routes of water loss found in sweat and urine. Water balance is achieved when water losses are compensated by fluid gain through metabolic water production and from food and fluid intake. Metabolic water production accounts for only a small fraction of total water gain and is proportional to energy expenditure (about 250-400 ml/day; Sawka et al., 2005). The water content of food is highly variable and difficult to track on a day-to-day basis, and accounts for approximately 20–30% of total water intake (Institute of Medicine, 2004). Thus, the majority of total fluid intake occurs through drinking. When *ad libitum* access to fluids is available, body fluid balance is regulated tightly on a daily basis due to the sensations of thirst and sodium appetite. This is achieved by a complex

interaction between neuroendocrine and renal responses to blood volume and osmolality changes. In summary, an increase in the osmolality of the extracellular fluid space (ECF), from a meal-induced addition of sodium or loss of hypo-osmotic fluid through sweating, draws water from the intracellular space (ICF) to re-establish extracellular osmolality. In tandem, osmoreceptor neurons in the central nervous system detect this increase in osmolality. Once stimulated they signal to other parts of the brain to initiate the act of searching for water and then the behavioural act of drinking. At the same time as the search for water is initiated, the activated brain osmoreceptors stimulate magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus to liberate anti-diuretic hormone (ADH or arginine vasopressin, AVP) from their axon terminals in the posterior pituitary into the blood stream. The ADH then passes to the kidney where it stimulates aquaporin 2 accumulation in the outer wall of the collecting duct cells. This facilitates the passage of water down a concentration gradient across the otherwise impermeable wall into the medulla of the kidney for uptake by the blood, and thus reduces water loss and increases renal retention of water. A decrease in water from the ECF resulting in a decrease in blood volume (primarily from plasma) is detected by baroreceptors in the aortic arch, carotid sinus and great veins, initiating the search for water and sodium in order to restore blood volume. At the same time, volume and pressure receptors in the juxtaglomerular apparatus of the kidney sense the decrease in perfusion pressure which stimulates the release of renin. This activates the renin- angiotensin-aldosterone system (RAAS), which ultimately results in the release of aldosterone from the adrenal gland (see **Figure 2.3** for a summary of the RAAS). Aldosterone has two principle functions, one to increase sodium absorption in the kidney via an action in the distal tubule and collecting duct through stimulation of the sodium potassium ATPase pump, and the other to sensitise certain specific areas of the brain to the circulating levels of angiotensin II which stimulates the search and ingestion of sodium i.e. sodium

appetite. The ingestion of water only has the effect of reducing blood osmolality around the physiological set point (i.e. 280 – 295 $mOsm \cdot L^{-1}$). The ingestion of both sodium and water restores blood volume to initial values (For a more detailed summary of the neuroendocrine regulation of fluid balance see **Figure 2.2**). In the ideal physiological system thirst and sodium appetite regulates blood solute concentration (osmolality) and blood volume so that homeostasis is maintained. However, humans do not always respond appropriately to thirst and sodium appetite signals (Thornton, 2010). Pre- and post-natal stimuli can challenge the programming of thirst and sodium appetite during development including; diet, hydromineral challenge (e.g. excessive vomiting during pregnancy), alterations to endocrine regulation (e.g. RAAS) due to disease or medication such as steroid hormones, which alter water and salt intake into adulthood (Perillan et al., 2015). In addition, non-regulatory social and behavioural factors e.g. access to latrines, fear of incontinence, mobility issues and occupational time-constraints can affect habitual fluid-intake and water turn-over during daily living. In response, many governments and organisations have attempted to develop various recommendations for total water intake (TWI). These recommendations for adequate daily water intake (AI) range from 1.7 to 3.5 litres per day, with average TWI of 2.5 $L \cdot d^{-1}$ for adult males and 2.0 $L \cdot d^{-1}$ for adult females (Gibson & Shirreffs, 2013; Institute of Medicine, 2004; Le Bellego et al., 2010). Nevertheless, in the UK 33% of male and 23% of adult females have a low TWI. In addition, these fluid intake recommendations are largely based on population-based median water intakes and lack evidence linking the volume of fluid intake to the assessment of hydration status making the accurate prescription of individual daily fluid intakes problematic. To compound matters further habitual fluid intake has both psychological, physiological, social and environmental drivers and research has shown variation in timing of beverage consumption, beverage type, water content of food and age differences in TWI (Gibson & Shirreffs, 2013).

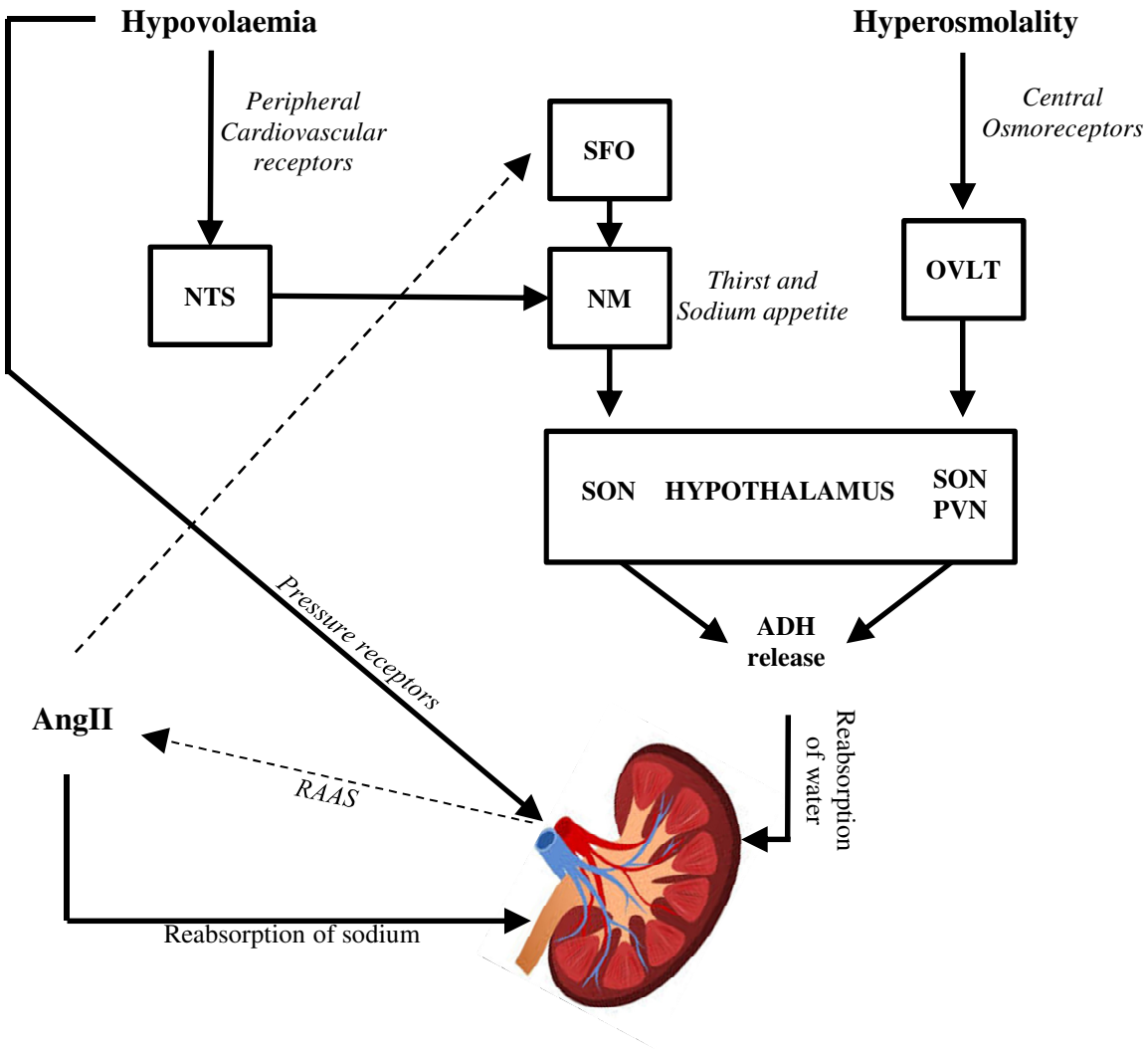


Figure 2.2 Neuroendocrine regulation of body fluid (Adapted from Stachenfeld 2010).

Osmotic information from the OVLT (organum vasculosum of the lamina terminalis), located outside the blood-brain barrier (BBB), is transmitted neurally to the hypothalamus and results in thirst, drinking and AVP release. The NM is utilized by both the subfornical organ (SFO) and the nucleus of the solitary tract (NTS), structures at the centre of sodium appetite and thirst regulation. Changes in volume are initiated by the kidneys, and stimulated by angiotensin outside the BBB (**see Figure 2.3**). The SFO sends a message across the BBB to the NM, which then initiates volumetrically controlled thirst and drinking responses. Atrial baroreceptors also send a signal to the NTS. In addition to stimulating thirst, angiotensin II (AngII) stimulates the SFO and causes fluid regulating hormones to be secreted by the pituitary and adrenal glands, increases blood pressure and eventually causes the kidney to stop secreting sodium and water, which, in turn, decreases both salt appetite and water intake. The paraventricular (PVN) and supraoptic nuclei (SON), both located in the hypothalamus, signal the release of ADH by the posterior pituitary.

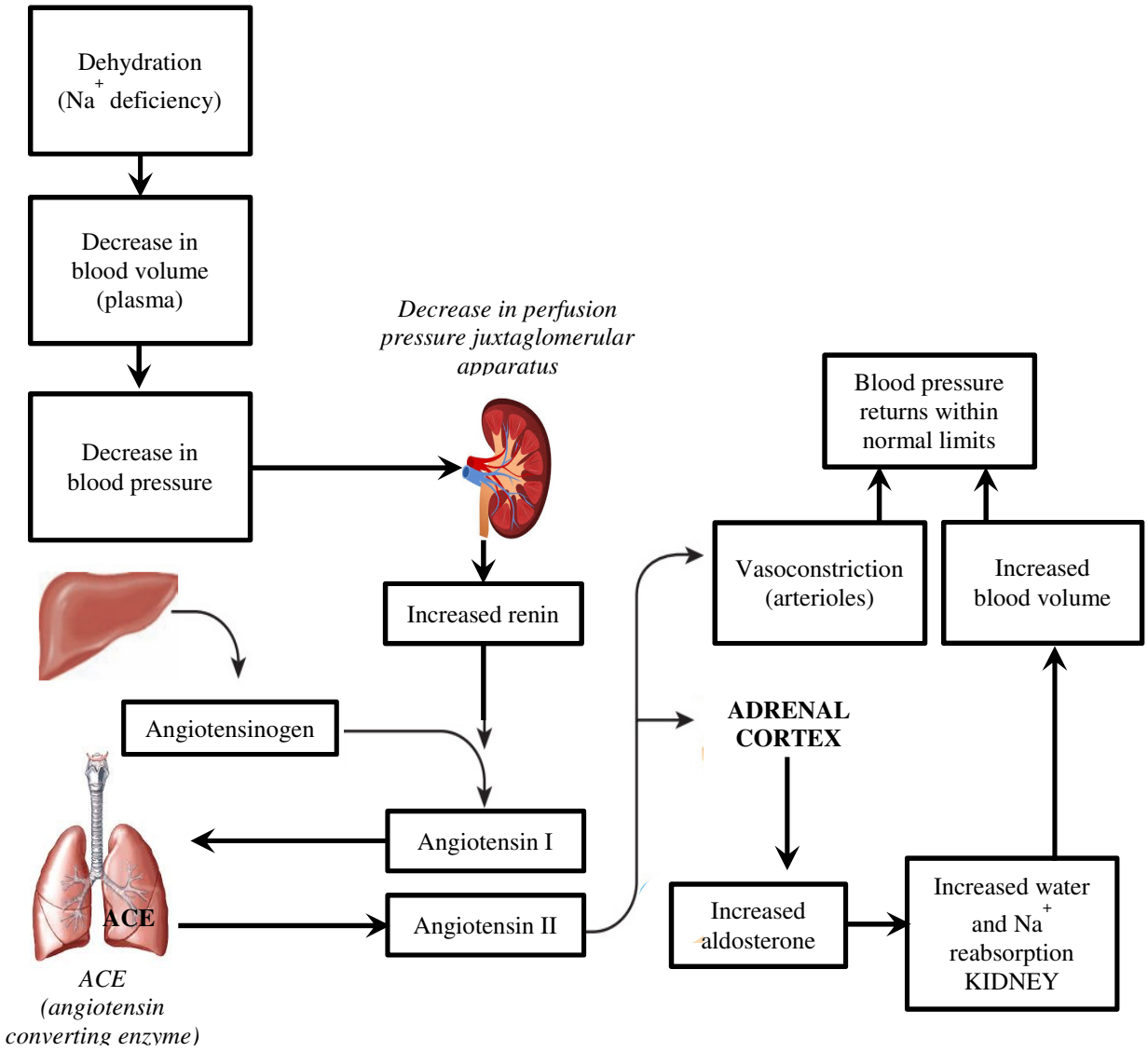


Figure 2.3 Renin-angiotensin-aldosterone system (RAAS)

A reduction in renal blood flow in response to a decrease in blood volume is sensed in the kidney which results in the juxtraglomerular cells converting prorenin into renin which is secreted into the circulation. Plasma renin then carries out the conversion of angiotensinogen released by the liver to angiotensin I (AngI). AngI is subsequently converted to angiotensin II (AngII) by the enzyme angiotensin-converting enzyme (ACE) found in the lungs. AngII is a potent vasoactive peptide that causes blood vessels to constrict, resulting in increased blood pressure. AngII also stimulates the secretion of the hormone aldosterone from the adrenal cortex. Aldosterone causes the tubules of the kidneys to increase the reabsorption of sodium and water into the blood. This increases the volume of fluid in the body, which also increases blood pressure.

2.2 Prevalence, causes and types of dehydration

When fluid-loss outweighs fluid intake, the result is a deficit in TBW. As described previously, this can cause dehydration which can be caused by numerous factors that depend on activity level, diet, the environment and population, disease state and age.

2.2.1 Prevalence of dehydration

Dehydration is likely common in daily living but may be more prevalent in certain populations which include; athletes during training, competition and when making weight (Artioli et al., 2010; Maughan, Shirreffs, Merson, & Horswill, 2005; Whiting, Maughan, & Miller, 1984), military personnel during training and operations (Nindl, Leone, & Tharion, 2002), and persons working or travelling in austere environments, such as high altitude or cold environments (Pitts et al. 1944; Rogers et al., 1964; Frayser et al., 1975; Hubbard et al., 1984;Vidiendal, 1992). Furthermore, illness such as fever, vomiting and diarrhea cause dehydration and are commonly reported in both athletes and military personnel (Swain, 1994; Kasper et al., 2012). It is also well established that the risk of dehydration is increased with age. In the US, hospitalisation of the aged due to dehydration has risen steadily over the last few decades with admissions exceeding 250,000 each year since 2001 (Hall & Defrances, 2003). Indeed, 7% of all hospital admissions of persons aged 65 years or over involved dehydration as a diagnosis (Warren et al., 1994). Reports of dehydration in the elderly are also common in the UK and Europe in both clinical and community settings (Hall & Defrances, 2003; DeFrances & Podgornik, 2006). However, the true prevalence of dehydration in these populations remains unclear, as no standard assessment method has been agreed to determine and individual's hydration status. The development of hydration markers is therefore fundamental if dehydration prevalence is to be accurately determined.

2.2.2 Types of dehydration

One reason for the difficulty in accurately assessing fluid-deficits, is that there is little consensus on the correct definition of dehydration and the term is often used to encompass many conditions relating to a fluid deficit resulting from a loss of water with or without solute (Thomas et al., 2004). Indeed, numerous terms are used to describe dehydration including; fluid deficit, volume depletion or extracellular volume depletion, hyper-osmotic or hypertonic volume depletion , iso-osmotic or isotonic volume depletion and hypo-osmotic or hypotonic volume depletion (Weinberg & Minaker, 1995; Mange et al., 1997; McGee et al., 1999; Sarhill et al., 2001; Thomas et al., 2008; **see Glossary of Terms, Page xxvi**). Fundamentally, a deficit of TBW is characterised by the tonicity of fluid remaining in the body and whether there is a depletion of intracellular and / or extracellular fluid spaces. In essence dehydration exists as one of two main types, or some combination of the two (Nadel et al., 1941a; Nadal et al., 1941b). When the concentration of fluid-loss is hypo-osmotic with respect to plasma, an osmotic gradient is created between the ICF and ECF. As described previously, this draws water from the ICF to the hyperosmotic ECF causing equilibration of fluid and osmolality across these spaces. The characteristics of this type of dehydration are a relatively large loss of ICF volume and hyperosmotic ECF. This type of dehydration is also called intracellular dehydration, hyperosmotic-hypovolaemia, hyperosmotic-euvolaemia or water-loss dehydration. Conversely, when the concentration of fluid-loss is iso-osmotic with respect to plasma due to a greater loss of electrolytes with water, a relatively larger reduction of the ECF space occurs. The osmotic gradient to draw fluids from the larger ICF is absent, and there is no change in ECF or ICF osmolality. Due to the reduction in ECF relative to the other fluid spaces this type of dehydration is called extracellular dehydration and also referred to as iso-osmotic-hypovolemia, volume depletion, or water and salt-loss dehydration. If there is a combination of hypo and iso-osmotic fluid-loss, a mixed dehydration ensues

(Cheuvront & Kenefick, 2014). Therefore, the type of dehydration is determined by the composition of fluid and electrolytes lost. Notably, an underappreciated fact is that the composition of fluid and electrolyte loss is dependent on the cause of dehydration (Kozlowski & Saltin, 1964). Clearly, the absence of standardised assessment techniques to identify dehydration according to type can lead to confusion, misdiagnosis and inappropriate treatment (Thomas et al., 2004).

Table 2.1 Physiological characteristics and causes of extracellular and intracellular dehydration

	Extracellular dehydration	**Intracellular dehydration**
Blood solute concentration	↔	↑↑
Blood volume	↓↓	↔ (or ↓)
Composition of fluid lost (in relation to plasma)	Iso-osmotic	Hypo-osmotic
Electrolyte loss	↑↑	↑
Causes of dehydration type	Illness (e.g. secretory diarrhoea or vomiting). Natriuresis and diuresis from cold or altitude exposure and water immersion. Diuretic medication	Hypotonic sweat-loss compounded by thermal stress, exercise, physical activity or protective clothing. Prolonged periods of inadequate fluid intake or fluid restriction.

2.2.3 Causes and types of dehydration in healthy individuals

At rest, when fluid is freely available, fluid-intake triggered by the sensation of thirst is likely to prevent the development of severe dehydration (Nielsen, 1974; Verbalis, 2003). Despite this, healthy humans do not always respond fully to thirst signals (Thornton, 2010). Indeed,

accurate control of human fluid-balance is difficult to achieve and often fluid-intake is inadequate to compensate for the obligatory daily sources of fluid-loss which can lead to mild to modest dehydration (equivalent to 1-3% of body mass) (Armstrong, 2007; Le Bellego et al., 2010). These obligatory hypo-osmotic fluid-losses are primarily from sweat and respiratory losses (0.4-1.0 and 0.2-0.4 L, respectively), evoking an intracellular dehydration. Further, these fluid deficits often occur over days rather than minutes or hours. Consequently, the decrease in TBW occurs with little or no net-change in ECF volume, also called euvolaemia, due to the osmotic drive between the hyper-osmotic ECF and ICF (Oliver, Laing, Wilson, Bilzon, & Walsh, 2008; Shirreffs, Merson, Fraser, & Archer, 2004). Intracellular dehydration evoked over a prolonged period is also observed in some occupational and athletic settings where the availability of fluid is restricted e.g. during military manoeuvres or when athletes are trying to make weight (Artioli et al., 2010; Nindl et al., 2002).

Intracellular dehydration can also manifest in active populations due to numerous external stressors that augment fluid-loss. For example, thermal and metabolic heat-stress, during exposure to hot environments, during exercise, due to protective clothing or with illness such as fever, causes an increase in fluid-loss through sweat. The rapid loss of hypo-osmotic fluid results in a hyper-osmotic ECF and larger decrease in ECF volume compared with intracellular dehydration evoked over a prolonged period of time. The decrease in ECF volume, or hypovolaemia, is directly related to the sweat rate and to the magnitude of thermal and metabolic heat-stress. Therefore, in order of magnitude this transient hypovolaemia will be greater with exercise in the heat followed by passive heat exposure and then exercise in temperate climates (Caldwell, Ahonen, & Nousiainen, 1984; Kozlowski & Saltin, 1964; Nielsen, 1974). On a cautionary note, exercise-stress will also result in hypovolaemia due to

increased interstitial forces. This is highlighted by the rapid decrease in plasma volume that occurs at the onset of exercise that cannot be related to dehydration. Further, within one-hour a large proportion of the plasma volume declines are restored, even in the absence of fluid replacement (Fortney, Nadel, Wenger, & Bove, 1981; Nadel, Fortney, & Wenger, 1980). The incidence of intracellular dehydration due to thermal and metabolic heat stress is further increased by the delay in complete rehydration following fluid-loss, which is consistently observed in humans (Adolf, 1947; Greenleaf & Sargent, 1965). This phenomenon, termed voluntary dehydration, has been observed in the heat following fluid-loss through sweating (Rothstein, Adolph, & Wills, 1947) or restricted access to fluid (Black, McCance, & Young, 1944) and is thought to be due to a heat-stress mediated decrease in sensitivity to thirst allied to a negative alliesthesia for certain drinking fluids (Greenleaf & Sargent, 1965; Hubbard et al., 1984). Indeed, research suggests that the sensation of thirst is only initiated when dehydration equivalent to 2% of body mass is reached, and after the initiation of drinking thirst is quenched before normal hydration is achieved (Cheuvront & Sawka, 2005; Greenleaf, 1992).

Exposure to cold, cold-water immersion or high-altitude also results in an increase in acute fluid-loss primarily through excessive urination, or diuresis, often accompanied by an increased loss of solutes in the urine such as sodium and potassium; termed natriuresis and kaliuresis respectively (Haditsch, Roessler, & Hinghofer-Szalkay, 2007; Lennquist, 1972). This increased loss of water and electrolytes in the urine results in a rapid loss of fluid from the ECF and evokes an extracellular dehydration. The mechanisms for the cold-induced-diuresis (CID) remain undefined, but may be due to a pressure diuresis resulting from an increased systemic arterial and renal blood pressure (Wallenberg & Granberg, 1967). Conversely, endocrine changes may be responsible for the hypoxic-diuretic-response (HDR),

principally through decreases in the hormones of the renin-angiotensin-aldosterone system and increases in atrial naturetic peptide (Frayser et al., 1975). Together with an increased fluid and electrolyte loss there is also an attenuation of thirst during exposure to cold and high-altitude that may be more pronounced than that observed with thermal stress (Frayser et al., 1975; Rogers et al., 1964). The reduced sensation of thirst is observed both at rest and during exercise regardless of hydration state. Although mechanisms for the altitude induced reduction in thirst are unclear, peripheral vasoconstriction mediating an increase in central blood volume is thought to be responsible for the cold-induced reduction in thirst-sensation (Kenefick, Hazzard, Mahood, & Castellani, 2004). The excess urinary loss of water and electrolytes coupled with a potential decrease in fluid intake due to a blunted thirst response may give rise to a mixed dehydration type, specifically a combination of intracellular and extracellular dehydration (Cheuvront & Kenefick, 2014). Finally, travel and exposure to austere environments can further compound dehydration as illnesses such as vomiting and secretory diarrhea are common, which evokes an extracellular dehydration due to the loss of water and electrolytes (Cheuvront & Kenefick, 2014).

2.2.4 Causes and types of dehydration in older people

Many age-related changes result in an increase in fluid-loss and a decrease in fluid-intake, which increases dehydration risk in the older person (El-Sharkawy, Sahota, Maughan, & Lobo, 2014). Some of the underlying reasons for the increased fluid-losses is a decline in kidney function (Rowe et al., 1976). Renal senescence reflects irreversible structural and functional changes associated with the ageing kidney. Amongst other changes, there is a loss of renal mass due to glomerular sclerosis and glomerular loss. This impairs the ability to retain sodium and, therefore, water, thus predisposing the patient to dysnatraemia and

hypovolaemia. Furthermore, reduced tubular function and the medullary concentration gradient are also impaired in an aged kidney, diminishing the ability of the kidney to concentrate urine. Age related reduction in renal blood flow has also been reported; this contributes to loss of nephrons as a result of ischaemia. These changes impair the ability of the kidney to control water and electrolyte balance, predisposing to dehydration and electrolyte abnormalities, particularly in situations of physiological stress.

Endocrine changes that affect fluid and electrolyte homeostasis have also been reported in older adults. Decreases in circulating renin and aldosterone with a concurrent increase in serum atrial naturetic peptide (ANP) concentration, result in an increase in both sodium and water excretion by the kidney (Miller, 1997; Weidmann, De Myttenaere-Bursztein, Maxwell, & De Lima, 1975). Serum ANP can be nearly five-times higher in older adults than in the young. ANP inhibits renin secretion from the juxtaglomerular cells, therefore, limiting the conversion of angiotensinogen to angiotensin I and II, and reducing the activity of the renin-angiotensin-aldosterone system (**see Figure 2.3 for mechanism of the RAAS**). There is conflicting evidence suggesting increased as well as decreased serum ADH concentrations with age. As previously mentioned, ADH acts to stimulate aquaporin which allows the passage of water across cell membranes and thus helps to conserve body water. The normal diurnal variation results in increased plasma concentrations of ADH at night, but in older adults there is loss of the nocturnal rise in ADH concentrations which contributes to the high prevalence of nocturia. This along with an age-related decline in renal sensitivity to ADH, reduces the kidney's ability retain body water (Miller & Shock, 1953).

Although these physiological changes result in a compromised ability to conserve body water compared with younger adults, another factor associated with the increased incidence of

dehydration in the older person is a general reduction in fluid-intake with age (Phillips, Johnston, & Gray, 1993). It is well recognised that healthy elderly individuals do not respond to dehydration with appropriate thirst compared to healthy younger individuals (Phillips et al., 1991). Investigations have shown a slower restoration of fluid homeostasis in older compared with younger people following exercise-induced dehydration (Mack et al., 1994) and fluid restriction (Phillips et al., 1991). Indeed in the later study although both age groups experienced an increased thirst sensation and water intake following hypertonic saline infusion (0.855 M) over a 2-h period, the older men reported feeling less thirsty and drank less room-temperature water (Phillips et al., 1991). Conversely in a similar study, osmotically stimulated increases in thirst were unaffected by age (Stachenfeld, Mack, Takamata, DiPietro, & Nadel, 1996). Notably in this study, there was a 19% increase in plasma volume in both groups following saline infusion compared with a 9% plasma volume expansion in the young group only in the study by Phillips et al. In addition, Phillips tested participants in the supine position compared with Stachenfeld who had participants in the upright position, meaning that the baroreceptors were loaded before the saline infusion. In a further study, thirst rating was similar between older and younger men with hyperosmolality and hypovolaemia induced by exercising in the heat (Stachenfeld, Dipietro, Nadel, & Mack, 1997). However, subsequent recovery of plasma volume with head-out water immersion only suppressed thirst rating in the younger men. This suggests a diminished sensitivity to volume-mediated thirst with ageing which may be related to age-related differences in atrial naturetic peptide (Kenney & Chiu, 2001).

In addition to these age-related physiological and hormonal changes, behavioural factors dependent on physical and mental health can result in a decrease in fluid-intake. For example, older people suffering from chronic physical or mental disability have been shown to have a

lower daily body water turnover and lower fluid intake and are at a higher risk of being dehydrated (Haveman-Nies, De Groot, & Van Staveren, 1996; Leiper, Primrose, Primrose, Phillimore, & Maughan, 2005). An increase in chronic and acute illness such as dysphagia and diabetes and particularly diabetic ketoacidosis are associated with the incidence of dehydration (Kayser-Jones & Pengilly, 1999; Spira, Gowrishankar, & Halperin, 1997). Other precipitants for dehydration may include demographic, social, environmental and mobility issues (Lavizzo-Mourey et al., 1988; Weinberg & Minaker, 1995; Sanservo, 1997). Clearly, the diminished thirst sensitivity is a major predisposing factor in the development of intracellular dehydration in the elderly person. However, extracellular dehydration is also likely to be common in this population due to the increased water and electrolyte loss from the kidney that occurs with age. Furthermore, the elderly are more likely to be prescribed medication, such as diuretics, antihypertensives, anti-depressants and non-steroidal anti-inflammatory drugs (NSAID) that can cause diuresis and natiuresis (Caswell, Jarvis, Dalton, & Gagic, 1998; Walter & Lenz, 2011). Indeed, the incidence of chronic extracellular dehydration may be exacerbated in this population due to the insensitivity of volume-mediated extracellular thirst (Stachenfeld et al., 1997).

Both young and older people may also commonly consume other diuretic beverages such as caffeine and alcohol. Indeed, a recent study has shown age-related differences in the UK consumption of these beverages, with younger people consuming more alcohol and older people consuming more caffeine (Gibson & Shirreffs, 2013). However, a meta-analysis of the effects of caffeine of hydration status suggested that although large doses of caffeine (2-3 cups of coffee or 5-8 cups of tea) resulted in a stimulation of urine output, there was no evidence to suggest that the diuresis resulted in fluid-loss beyond the volume of beverage drunk (Maughan & Griffin, 2003). In addition, alcohol appears to increase diuresis only when

persons are normally hydrated and not when dehydrated, and this diuresis increases with the concentration of alcohol for similar volumes of beverage (Hobson & Maughan, 2010; Shirreffs & Maughan, 2000). Therefore it appears that moderate intake of both these beverages are unlikely to exacerbate the prevalence of dehydration.

2.3 Consequences of dehydration

Severe dehydration is clearly detrimental to health, and body fluid losses in excess of 10% of body weight can be fatal (Adolf, 1947). Many laboratory based studies have also reported that moderate dehydration (fluid deficit ≥ 4% of body mass) consistently impairs human well-being and performance e.g. endurance exercise performance, cognitive function and subjective feelings. The findings of these studies have shown that factors relating to well-being such as cognitive function, mood and subjective feelings are consistently disrupted following dehydration evoked by passive or active heat exposure (Cian et al., 2000; Cian, Barraud, Melin, & Raphel, 2001; Gopinathan et al., 1988; Sharma, Sridharan, Pichan, & Panwar, 1986). It has consistently been shown that the dehydration-mediated decrement in endurance performance increases with the magnitude of fluid-deficit, and is generally larger with increasing environmental temperature (Ladell, 1955; Craig & Cummings, 1966; Walsh et al., 1994; Cheuvront et al., 2005; Kenefick et al., 2010; Sawka, et al., 2012; Nybo et al., 2013). However, fluid-deficits ≥ 4% of body mass are unlikely to develop regularly in daily living or during athletic or occupational endeavours in temperate environments when fluid is freely available. Furthermore, a major limitation of most studies conducted to date is the inability to determine the effects of dehydration independent of the effects of thermal stress (Grandjean & Grandjean, 2007). This is important as hyperthermia alone is known to increase cardiovascular strain, alter skeletal muscle metabolism, reduce cerebral blood flow,

alter central nervous system activity, attenuate muscular force generation and increase perception of effort (Febbraio, 2000; Nybo & Nielsen, 2001b; Nybo & Nielsen, 2001a; Nybo et al., 2002). In addition, a major limitation of research examining the effects of dehydration is the method of manipulating hydration status which typically involves diuretic medication, exercise in hot environments with limited or restricted fluid intake. Unfortunately, none of these methods allows for a blinding of the treatment, with participants clearly aware of the hydration status under which they are performing. Recently attempts have been made to overcome these design issues with the manipulation of hydration status by intravenous infusion (Maresh et al., 2001; Wall et al., 2013). Such methods offers the unique ability to eliminate the placebo effect from influencing the performance outcome, as participants can be blinded to the amount of fluid re-infused after dehydration. Nevertheless, the following sections **(2.3.1 - 2.3.3)** will highlight research that has examined the effect of mild to modest dehydration on human well-being and performance in the absence of hyperthermia.

2.3.1 Effects of mild to modest dehydration on human well-being

Human well-being is poorly defined within hydration research and generally difficult to assess (Lieberman, 2010). Consequently, much of the literature examining the effects of dehydration on well-being have focussed on measures of affective well-being (Wilson & Morley, 2003). Affective well-being can be defined as the experience of feeling or emotion; as such proxy measures include the self-reported measures of mood and subjective feelings (Schwarz & Clore, 1983). Of the limited research available, mood and subjective feelings appear to be inconsistently disrupted by mild to modest dehydration (**Table 2.2**). For example, a reduction in body weight of up to 2.8% evoked by running on a treadmill for two hours resulted in greater feelings of fatigue but no change in mood compared with normal hydration (Cian et al., 2000). When mild to modest dehydration in the range of 1.0 to 2.7% of

body mass was evoked by prolonged fluid restriction of 13-36 h, there was an increase in fatigue, tiredness and thirst, with a decrease in alertness, concentration and vigour (Pross et al., 2013; Shirreffs et al., 2004; Szinnai, Schachinger, Arnaud, Linder, & Keller, 2005). In a series of more recent studies in males and females, participants were randomised to one of three conditions; exercise-induced dehydration with a diuretic, exercise-induced dehydration with a placebo or normal hydration with a placebo (Armstrong et al., 2012; Ganio et al., 2011). Following these procedures, males and females with a fluid deficit of ≥ 1.0% of body mass exhibited an increase in fatigue compared with participants with normal hydration. Notably, females experiencing a mild fluid-deficit also perceived an increased anger and decreased vigour and concentration.

The inconsistent results from the literature may be due to differences in the methods of assessing mood and subjective feelings or due to gender differences in the populations tested. In addition, many studies have been confounded by the restriction of caffeine, since withdrawal produces adverse effects on cognitive function and mood (Juliano & Griffiths, 2004; Lieberman, 2007). However, an important distinction between these studies is the methods used to cause dehydration which would have resulted in different types of dehydration. For example, prolonged fluid restriction for 13 to 37 h would have evoked an intracellular dehydration (Pross et al., 2013; Shirreffs et al., 2004; Szinnai et al., 2005). Whereas, prior exercise with fluid restriction would have resulted in an intracellular dehydration with a temporary hypovolaemia (Armstrong et al., 2012; Cian et al., 2000, 2001; Ganio et al., 2011). In contrast, exercise induced dehydration with either diuretics or placebo would have resulted in a cohort with a combination of mild intracellular, extracellular and mixed dehydration (Armstrong et al., 2012; Ganio et al., 2011). The potential mechanisms by which dehydration may affect markers of well-being include; distraction by discomfort

following periods of dehydration, changes to brain neurotransmitter concentration e.g. serotonin with dehydration , blood-electrolyte disturbances on local brain regions and hypovolaemia-mediated reductions in cerebral perfusion (Popova et al., 2001; Di & Tasker, 2004; Wilson & Morley, 2003 Cheuvront & Kenefick, 2014). Therefore, it is plausible that the distinct physiological characteristics of dehydration types may have differential effects on measures of affective well-being. However, the independent effects of dehydration type on measures such as mood and subjective feelings and whether the mechanisms for these changes are a direct influence on the central nervous system or psychological (i.e. feelings of discomfort) are currently unknown. Increasing our understanding of the effects of dehydration on measures of well-being could be applied to health care, education, and athletic settings where research into adjunctive measures aimed at limiting adverse effects of dehydration is warranted (Wilson & Morley, 2003).

Table 2.2 A summary of studies examining the effect of mild to modest dehydration on measures of affective well-being in the absence of hyperthermia.

Study	Method of evoking dehydration	Magnitude of dehydration, %BML	Population, Number, gender (mean age, years)	Tests of well-being	Findings
Cian et al., 2000	Exercise (TM 60% $\dot{V}O_{2max}$ for 2 h) + fluid restriction	DEH, 2.7 HYD, 0.4	8, M (27)	VAS (subjective ratings of mood and fatigue)	Increase in fatigue on DEH vs. HYD. No difference in ratings of mood between trials.
Cian et al., 2001	Exercise (TM 65% $\dot{V}O_{2max}$ for 2 h) + fluid restriction	DEH, 2.7 HYD, 0.0	7 M (25)	VAS (subjective ratings of mood and fatigue)	Increase in fatigue on DEH vs. HYD. No difference in ratings of mood between trials.
Shirreffs et al., 2004	Fluid restriction (37 h)	DEH: 13 h, 1.0 24 h, 1.8 37 h, 2.7 HYD: 0.6 at each time-point	15, 9 M, 6 F (30)	VAS (battery of subjective feelings)	Increase in thirst, dry mouth and a decrease in concentration and alertness following 13, 24 and 37 h on DEH vs. HYD. Increase in unpleasant mouth taste and head soreness after 24 and 37 h on DEH vs. HYD.

Szinnai et al., 2005	Fluid restriction (37 h)	DEH, 2.6 HYD, 0.8	8, F (25)	5-point Likert scales (alertness and tiredness) VAS (subjective feelings of thirst, concentration)	Increase in thirst and tiredness and a decrease in alertness and ability to concentrate on DEH vs. HYD.
Ganio et al., 2011	Diuretic (40 mg furosemide) + exercise (TM 5.6 km/h at a 5% gradient) + fluid restriction; **or** Placebo + exercise (as above) + fluid restriction	DEH: DIU + EX + FR, 1.7 PL + EX + FR, 1.4 Pooled, 1.6 HYD, 0.0	26, M (20)	Profile of mood states (mood) VAS (subjective feelings of concentration, headache)	Increase in fatigue and tension on DEH vs. HYD. No difference in other mood states or subjective feelings between trials.

Armstrong et al., 2012	Diuretic (40 mg furosemide) + exercise (TM 5.6 km/h at a 5% gradient) + fluid restriction; **or** Placebo + exercise (as above) + fluid restriction	DEH: Pooled, 1.4 HYD, 0.1	25, F (23)	Profile of mood states (mood) VAS (subjective feelings of concentration, headache)	Increase in total mood disturbance, anger and fatigue with a decrease in vigour and concentration on DEH vs. HYD.
Pross et al., 2013	Fluid restriction (23 h)	NR	37, F (25)	Profile of mood states (mood) Karolinska sleepiness scale Bond and Lader scale VAS (thirst, sleep)	Increase in thirst, fatigue, confusion and general sleepiness, and a decrease in vigour, alertness and calmness on DEH vs. HYD.

Abbreviations: BML, body mass loss; NR, not reported; DEH, dehydration; HYD, normal hydration; $\dot{V}O_{2max}$ maximal oxygen consumption; TM, treadmill; M, male; F, female; DIU, diuretic; EX, exercise; FR, fluid restriction; PL, diuretic placebo; VAS, visual analogue scale.

2.3.2 Effect of mild to modest dehydration on endurance exercise performance

Exercise performance is multifaceted and can be impacted by dehydration in numerous ways. The majority of studies support the notion that dehydration impairs endurance exercise performance, but that intermittent team sport and strength/power performance are affected to a lesser degree (Ali, Gardiner, Foskett, & Gant, 2011; Cheuvront, Carter, & Sawka, 2003; Judelson et al., 2007; R. Maughan & Burke, 2012; McGregor, Nicholas, Lakomy, & Williams, 1999; Owen, Kehoe, & Oliver, 2012; Sawka & Noakes, 2007). As previously mentioned, it has consistently been shown that the dehydration-mediated decrease in endurance exercise performance increases with the magnitude of dehydration and environmental temperature (Ladell, 1955; Craig & Cummings, 1966; Walsh et al., 1994; Cheuvront et al., 2005; Kenefick et al., 2010; Sawka et al., 2012; Nybo et al., 2013). However, there have been some equivocal findings regarding the effect of more commonly experienced mild to modest dehydration on endurance performance in temperate environments. As it is currently unclear whether dehydration of this magnitude is a problem when exercising in these climates this will be the focus of this thesis, for a more comprehensive review of the literature on the effect of dehydration on exercise the reader is directed towards a recent review by Cheuvront & Kenefick (2014).

Studies investigating the effects of dehydration on endurance exercise performance fall into two main categories: those where dehydration develops during exercise, called exercise-induced-dehydration and those that evoke dehydration before exercise begins. The latter method has recently received criticism due to a perceived lack of validity (Goulet, 2012). However, these alternate methodologies do have specific relevance to athletic and occupational settings. For example, exercise-induced-dehydration is more relevant for those involved in events where athletes usually begin exercise with normal hydration. Whereas,

investigations that evoke dehydration before exercise are more relevant to scenarios where athletes may restrict fluid to make weight, fail to replace fluid losses between exercise sessions or in the case of recreational athletes are unable to achieve normal hydration before exercise due to social or occupational constraints (Cheuvront et al., 2003). The reliability, sensitivity, and ecological validity of tests commonly used as a measure of endurance exercise performance have also generated considerable controversy in recent years (Maughan, 2012). Constant speed or power tests to volitional exhaustion (time-to-exhaustion) and time-trial protocols are commonly employed to measure endurance exercise performance. Some believe that time-trial protocols are superior testing methodologies due greater ecological validity and smaller intra-individual variability (Goulet, 2012; Jeukendrup, Saris, Brouns, & Kester, 1996). However, a recent study has shown that time-to-exhaustion and time-trial-protocols are comparable in terms of sensitivity when examining the effect of hypoxia on endurance performance (Amann, Hopkins, & Marcora, 2008). The authors also concluded that these findings were also likely to extend to other factors that affect performance e.g. dehydration. The conclusions of this study can be explained by the larger intervention-effect on performance when using constant-power tests, which compensates for the inherent larger intra-individual variability of the test (Maughan, 2012). Consequently, this section of the thesis will highlight research that has focused on dehydration induced before exercise using either time-to-exhaustion or time-trial protocols. In addition, research that has evoked more commonly experienced mild to modest dehydration during exercise will be examined. From the limited studies available, it remains unclear whether mild to modest dehydration evoked before exercise has a detrimental effect on endurance exercise performance in a thermoneutral environment (see **Table 2.3**). For example, the time to complete a 5000 and 10000 m run on an outdoor track was increased by approximately 7% compared with normal hydration after mild dehydration equivalent to 1.5% of body mass was

evoked by diuretics (Armstrong et al., 1985). Another study reported that dehydration equal to 3% of body mass after 3 h of passive heat exposure resulted in an 8% decrease in work completed during a 30 min time trial on a cycle ergometer (Cheuvront et al., 2005). Conversely, similar magnitudes of dehydration induced by either exercise and fluid restriction or 48 h of prolonged fluid restriction had no effect on 15 min or 30 min time trial performance on a cycle ergometer or treadmill (Daries et al., 2000; McConell et al., 1999; Oliver et al., 2007). Notably, these studies used different methods to evoke dehydration, which probably resulted in different types of dehydration. Currently, the effect of dehydration type on endurance exercise performance is unknown.

The means by which dehydration may limit endurance performance are not fully understood. Likely mechanisms centre on the central and peripheral effects of hypovolaemia and hyper-osmolality and include; increased perceived exertion, greater cardiovascular, respiratory and thermoregulatory strain, alterations to metabolism and more recently it has been suggested that disruptions to measures associated with affective well-being such as increased subjective discomfort from increased thirst and head-soreness may also affect endurance performance (Cheuvront & Kenefick, 2014; Sawka & Noakes, 2007). Although contradictory view-points exist, it is likely that dehydration-mediated fatigue during endurance exercise is a combination of these factors (Marcora, 2009;Williamson, 2010; Amann, 2012). Therefore, it is plausible to suggest that the relative contribution of these psycho-physiological mechanisms may be dependent on the type of dehydration. For example, the greater hypovolemia associated with extracellular dehydration would be expected to cause lower stroke volume and cardiac output during exercise and the subsequent greater cardiovascular strain would reduce oxygen delivery to the working muscle (González-Alonso, Calbet, & Nielsen, 1998; González-alonso, Mora-rodríguez, & Coyle, 2000). Reduced oxygen delivery

to the working muscle is also associated with higher pulmonary oxygen consumption, ventilation and respiratory fatigue, which has been shown to cause earlier termination of high-intensity endurance exercise (Amann, 2012a; González-Alonso, et al., 1998). In addition, hypovolemia and hyper-osmolality independently impair thermoregulatory function (Fortney, Wenger, Bove, & Nadel, 1984; Nadel et al., 1980; Takamata, Nagashima, Nose, & Morimoto, 1997) and it has been suggested that hypovolemia in conjunction with a higher rectal temperature can reduce stroke volume, cardiac output, oxygen uptake and endurance performance even in temperate environments (Cheuvront et al., 2005). Hyperthermia also increases muscle glycogen utilization (Febbraio, 2000) and therefore metabolism may also be affected by dehydration type. A more recent theory suggests that disruptions to measures of affective well-being may influence endurance performance (Cheuvront & Kenefick, 2014; Williamson, 2010).

Table 2.3 A summary of studies examining the effect of mild to modest dehydration evoked before exercise on endurance exercise performance in a temperate environment.

Study	Method of evoking dehydration before performance test	Magnitude of dehydration, %BML	Environment during performance test, ^{o}C (%RH)	Performance test	Performance results
Armstrong et al., 1985	Diuretic (40mg furosemide)	DEH vs. HYD 1500 m, 1.5 5000 m, 1.2 10000 m, 1.6 TM TTE, NR	16 (32)	Track race: 1500 m 5000 m 10000 m TM, TTE at 10000 m race pace	7% increase in time to complete 5000 and 10000 m track race on DEH vs. HYD. No difference in 1500 m track race on DEH vs. HYD. 6% reduction in TM, TTE on DEH vs. HYD.
McConell et al., 1997	Exercise (CE 69% $\dot{V}O_{2max}$ for 120 min) + fluid restriction	NFI, 3.2 SFI, 1.8 FI, 0.1	21 (43)	CE, TTE at 90% $\dot{V}O_{2max}$ immediately after exercise with fluid restriction.	48% reduction in CE, TTE on NFI vs. FI. No difference in CE, TTE between SFI vs. NFI and FI trials.

McConell et al., 1999	Exercise (CE 80% $\dot{V}O_{2max}$ for 45 min) + fluid restriction	NFI, 1.9 SFI, 1.0 FI, 0.0	21 (41)	CE, TT for 15 min immediately after exercise with fluid restriction.	No difference in TT work completed between trials.
Daries et al., 2000	Exercise (TM 65% $\dot{V}O_{2max}$ for 90 min) + fluid restriction	SFI, 3.2 AFI, 2.6 FI, 1.3	25 (55)	TM, TT for 30 min immediately after exercise with fluid restriction.	No difference in TT work completed between trials.
Cheuvront et al., 2005	Passive heat exposure (45°C, 50% RH for 3 h) + fluid restriction	DEH vs. HYD DEH, 3.0 HYD, 0.4	20 (50)	CE, TT for 30 min 3 h after passive heat exposure.	8% reduction in work completed during CE, TT on DEH vs. HYD trial.
Oliver et al., 2008	Fluid restriction (48h)	DEH vs. HYD DEH, 3.2 HYD, 0.6	20 (59)	TM, TT for 30 min after 48 h of fluid restriction or fluid intake.	No difference in TT work completed between DEH and HYD trials.

Abbreviations: BML, body mass loss; NR, not reported; RH, relative humidity; DEH, dehydration; HYD, normal hydration; $\dot{V}O_{2max}$ maximal oxygen consumption; TM, treadmill; CE, cycle ergometry; TTE, time to exhaustion; TT, time trial; NFI, no fluid intake; AFI, *ad libitum* fluid intake; SFI, some fluid intake < sweat loss; FI, fluid intake ≥ sweat loss.

Although evidence is limited, an increase in thirst has been postulated as a potential mechanism by which dehydration can negatively affect endurance performance (Sawka & Noakes, 2007). Specifically, thirst may modify endurance exercise performance by increasing perceived exertion to limit changes to brain-osmolality during exercise. As previously discussed in **Section 2.3.1**, mild to modest dehydration has been shown to sometimes disrupt measures of affective well-being including; increase feelings of fatigue and tiredness, and reduced vigour and concentration (Armstrong et al., 2012; Ganio et al., 2011; Pross et al., 2013; Shirreffs et al., 2004). Notably, recent evidence suggests that increased fatigue, albeit caused by cognitively demanding tasks, decreases subsequent endurance performance via increased perceived exertion (Marcora, Staiano, & Manning, 2009; Pageaux, Marcora, & Lepers, 2013). Whether increases in subjective discomfort and disruptions to mood are specific to dehydration type is unknown, and the impact of these disruptions on endurance performance is also unclear.

2.3.3 Effect of mild to modest dehydration in an elderly population

Dehydration is a significant problem in the elderly. A diagnosis of dehydration during hospital admission is associated with the presence of one or more co-morbidity, longer hospital stay and additional future hospitalisation (Xiao, 2004). Once admitted mortality rate can be as high as 17% in the first month and may exceed 30% in the following twelve months (Warren et al., 1994). The annual cost of care for the older adult with a primary diagnosis of dehydration is estimated at $1.1 to $1.4 billion in the US (Jeanie Kayser-Jones et al., 2003). However, due to the lack of standardised guidelines in the assessment of dehydration these financial estimations should be treated with caution. Specific adverse health outcomes associated with dehydration in older people include falls, fractures, confusion, delirium, heat

stress, kidney failure, pressure ulcers, poor wound healing, suboptimal rehabilitation outcomes, infections, seizures, drug toxicity, and reduced quality of life (Mentes, 2006; Thomas et al., 2008; Wakefield, Mentes, Holman, & Culp, 2008). Consistent data from high quality prospective studies indicate that both intracellular and extracellular dehydration pose a health problem to the elderly person. For example, a raised serum osmolality is associated with increased risk of mortality in a general elderly US population, UK stroke patients and US older people with diabetes (Wachtel et al., 1991; Bhalla et al., 2000; Stookey, 2005). In addition, hospitalisations for volume depletion exceed 260,000 admissions per year in the US, and are associated with a case fatality rate of 3 to 10% (McGee et al., 1999). There is also increasing evidence that even mild dehydration is associated with the development of various morbidities in the elderly (Manz & Wentz, 2005). For example, mild dehydration has been shown to increase the risk of urinary stones, or urothithiasis, and is associated with an increase in urinary tract infection (UTI) (Siener & Hesse, 2003; Wilde & Carrigan, 2003). It has also been shown that constipation is relieved with increased fluid intake in adults or elderly people with dehydration (Arnaud, 2003). There may be an association, although not necessarily a causal one, between a low habitual fluid intake and cancers of the bladder, and colon (Michaud et al., 1999; Shannon, White, Shattuck, & Potter, 1996). Links with other disease, including diabetes, metabolic syndrome and cardiovascular disease have also been postulated, but currently the evidence is unclear (Bilz, Ninnis, & Keller, 1999; Burge, Garcia, Qualls, & Schade, 2001; Chan, Knutsen, Blix, Lee, & Fraser, 2002).

2.4 Assessment of dehydration

A useful countermeasure to the potential detrimental effects of mild to modest dehydration is appropriate oral rehydration initiated by the identification of the fluid-deficit using hydration

markers. Numerous hydration markers have been proposed that either measure absolute fluid volumes or which indirectly identify hydration status. Current developments suggest that these hydration markers vary greatly in their applicability due to differences in practicality, accuracy and validity (Armstrong et al., 2013). To be of practical use, markers of dehydration should be safe and instrumentation should be portable and require little technical expertise (Armstrong, 2007). Optimal hydration monitoring may be required daily or every few hours and therefore methods should provide an immediate result, be inexpensive and non-invasive. Notably, hydration markers should have the accuracy to identify mild dehydration. This is important as dehydration should ideally be identified before larger fluid-deficits develop because the severity of consequences on health and human performance is related to the magnitude of dehydration. However, there is controversy regarding the accuracy and validity of hydration markers that may be borne from differences in research design and the statistical methods used to evaluate marker performance, making comparison across studies difficult (Muñoz et al., 2013). The clinical performance of a laboratory test can be described in terms of diagnostic accuracy, or the ability to correctly classify subjects into clinically relevant sub-groups (Zweig & Campbell, 1993). Indices of diagnostic accuracy are routinely used in clinical chemistry when assessing the performance of novel disease markers (Weinstein, Obuchowski, & Lieber, 2005). However, their use within hydration marker research is a recent development which warrants further examination (Cheuvront, Ely, Kenefick, & Sawka, 2010). In addition, the development of valid markers has been impeded by differences in the dehydration protocol adopted (Muñoz et al., 2013). As previously described the type of dehydration will be dependent on the protocol or cause of dehydration. Currently, the effect of different types of dehydration on hydration marker diagnostic accuracy is unclear.

2.4.1 Techniques measuring absolute fluid volumes: changes in body mass, dilution and bioelectrical impedance

Previous research has shown that changes in hydration status can be tracked by serial measurements of nude body mass (NBM) as any change for up to72 h essentially reflects a change in body water when individuals are in energy balance (Cheuvront et al., 2004). The estimated change in hydration status is usually calculated as the difference in weight during the assessment period expressed as a percentage of the initial body mass (BM, i.e. ΔBM/initial BM x 100). Using the change in BM to estimate a change in hydration status is based upon the assumption that 1 kg weight lost is equal to 1 L of TBW lost. To ensure this assumption is not violated corrections should be made for food and fluid-intake and sweat trapped in clothing which will artificially inflate BM leading to underestimations of TBW lost. Following heavy sweating it is therefore imperative to assess nude body mass (NBM) after individuals have towelled. Day-to-day changes in first morning BM have been suggested to reflect changes in hydration status (Armstrong et al., 2010; Cheuvront et al., 2004). Measures of first morning BM has been reported to vary by no more than 0.7% day-to-day in free-living sedentary persons and individuals undergoing daily exercise and heat acclimatisation for periods of up to 9 days (Cheuvront et al., 2004; Grandjean, Reimers, Haven, & Curtis, 2003). Based upon these investigations it has been suggested that a change in day-to-day first morning BM of greater than 1% indicates dehydration (Casa, Clarkson, & Roberts, 2005).

Dilution and bioelectrical impedance techniques enable the assessment of absolute body fluid compartment volumes including: blood, plasma, ECF, ICF and TBW. With a reported sensitivity of 0.8 L dilution techniques are popularly regarded as the gold standard for assessing TBW (Armstrong, 2005). Blood and plasma volume can also be measured by

isotope or dye dilutions and have been shown to track TBW losses associated with dehydration (Harrison, 1985). Bioelectrical impedance analysis (BIA) and bioelectrical impedance spectroscopy (BIS) provide alternative methods to evaluate TBW, ICF and ECF volumes. Absolute TBW, ICF and ECF volumes determined by BIS have been shown to correlate strongly with dilution methods, in a controlled laboratory condition with normally hydrated individuals ($r > 0.9$, Armstrong et al., 1997).

2.4.2 Limitations of techniques measuring absolute fluid volumes

Although useful as a reference index in tightly-controlled laboratory studies, serial changes in BM are not a practical way of assessing hydration status. Over a period of days, other factors that affect body mass must also be accounted for, such as food and fluid intake, sweat-loss and rehydration following exercise and activity level. In addition, over longer periods changes in BM are likely to be affected by gross changes in body composition which may invalidate any estimation of hydration status. Dilution techniques are also impractical as a routine hydration assessment measure due to both the cost and the technical and time-consuming biochemical analysis. Although impedance techniques have been shown to be simple and non-invasive, some have questioned the validity of these methods. Differences in individual assessment of TBW between dilution and impedance measures were shown to range from -5.4 to 2.9 L (Armstrong et al., 1997). Moreover, impedance techniques have been reported to underestimate fluid losses during dehydration compared with dilution methods (O'Brien, Baker-Fulco, Young, & Sawka, 1999). These investigations suggest that impedance techniques may not possess sufficient sensitivity to identify mild to modest dehydration. Furthermore, impedance techniques are invalidated by changes in skin temperature, body fluid-electrolyte concentration and subject posture making these

techniques unsuitable to assess individual hydration status following or during exercise (O'Brien et al., 1999).

Another major limitation of measures reflecting absolute fluid volumes is that they cannot be used to evaluate an individual's hydration status with a single measurement. Rather, these markers are only useful in measuring a change in hydration status with serial measurements. For example, a single measure of BM, ECF or ICF is not an indication of hydration status. In most situations the use of single measures when assessing hydration status is favourable to serial measurements, as it reduces the number of assessments that need to be carried out, removes the need for a baseline measure of hydration status, and allows the development of universal dehydration thresholds. Therefore, for the purpose of this thesis, markers showing promise to identify hydration status with a single measure will be examined including haematological markers, urinary measures, saliva parameters, tear measurements, evaluation of clinical physical signs and cardiovascular based assessments (Bartok, Schoeller, Sullivan, Clark, & Landry, 2004; Cheuvront et al., 2012; Fortes et al., 2011a; Popowski et al., 2001; Shirreffs & Maughan, 1998; Vivanti, 2010; Walsh, et al., 2004).

2.4.3 Haematological markers of hydration status

Hypo-osmotic fluid-loss and the resulting haemo-concentration that accompanies intracellular dehydration, provides the diagnostic distinction for the use of haematological markers such as blood osmolality (serum or plasma), serum sodium, haematocrit and change in plasma volume. Of these potentially useful hydration markers, the osmolality of blood measured by freezing-point depression osmometry is the most widely investigated. Blood (plasma) osmolality has been shown to identify acute intracellular dehydration evoked by exercise and heat-stress in young healthy males (Popowski et al., 2001; Oppliger, Magnes, Popowski, & Gisolfi, 2005). In a similar population, blood (serum and plasma) osmolality has also been

reported to be an effective marker of chronic intracellular dehydration following prolonged fluid restriction (Shore et al., 1988; Shirreffs et al., 2004; Oliver et al., 2008). The sensitivity of this marker to detect dehydration equivalent to~1% of body mass in these studies has resulted in it being popularly regarded as a 'gold-standard' measure of hydration status (Cheuvront & Sawka, 2005; Cheuvront et al., 2013; Cheuvront & Kenefick, 2014). Furthermore, blood osmolality has a high to near perfect diagnostic accuracy to distinguish between normal hydration and dehydration evoked by exercise and heat-stress (Area under the ROC curve (AUC_{ROC}) between 0.91-0.95; see **Chapter 3** for explanation) (Bartok et al., 2004; Cheuvront et al., 2010; Muñoz et al., 2013). However, in these studies the diagnostic accuracy of blood osmolality was calculated for magnitudes of dehydration ranging between 1.8 to 7% of body mass. Therefore, the diagnostic accuracy of blood osmolality to mild intracellular dehydration is currently unknown. Nonetheless, some authors have questioned the practicality of this marker because haematological derived measures require invasive blood samples to be collected by a qualified phlebotomist and use of a centrifuge and osmometer which make these markers inappropriate for multiple hydration assessments. In addition, blood osmolality is the primary physiological stimulus used to regulate water balance, and is closely regulated by homeostatic systems around a set point of between 280 to 290 $mOsmol \cdot kg^{-1}$ (Institute of Medicine, 2004). Although data is limited, the tight regulation of blood osmolality is thought to be the reason why the diagnostic performance of this marker appears to decrease when fluid-loss occurs over a more prolonged period of time (Muñoz et al., 2013). Further, plasma osmolality measurements have been reported to be poor predictors of changes in hydration status when a single, fasted morning blood sample is collected, and appears to remain constant with modified fluid intake and across a wide range of fluid intakes (Institute of Medicine, 2004; Perrier et al., 2012; Armstrong et al., 2013).

Other haematological-based hydration markers such as serum sodium, haematocrit and plasma volume have received less attention. Plasma sodium provides an alternative to measuring osmolality, as any change in osmolality is primarily a reflection of a change in blood sodium concentration (Cheuvront & Sawka, 2005). Consequently, serum sodium has been shown to have utility in identifying acute and chronic intracellular dehydration equivalent to ≥ 1% of body mass evoked by exercise and heat-stress or prolonged fluid restriction (Bartok et al., 2004; Hamouti, Del, & Mora-rodriguez, 2013; Shirreffs et al., 2004). However, blood osmolality appears to be a more sensitive than serum sodium in young healthy individuals following acute sweat loss during exercise in the heat, possibly due to the greater variability in serum sodium across hydration states (Bartok et al., 2004). Indirect measurement of blood and plasma volume change may be estimated by measuring haematocrit and haemoglobin concentration (Dill & Costill, 1974). However, these measures are only applicable in the short-term only (hours) and the sampling and analysis must be standardised because haematocrit and haemoglobin have been reported to be altered by changes in posture, use of a tourniquet during blood collection and the method of analysis needed to assess haematocrit (Harrison, 1985; Maughan, Leiper, & Shirreffs, 2001; McNair, Nielsen, Christiansen, & Axelsson, 1979). Caution must also be taken when using automated analysers as changes in plasma osmolality have been shown to affect measures of haematocrit and therefore estimated plasma volume change (Watson & Maughan, 2014). In summary, the measurement of blood osmolality is considered the best haematological marker of hydration status. However, the diagnostic accuracy of blood osmolality to identify mild intracellular dehydration is unclear. In addition, this marker is unlikely to identify extracellular dehydration because iso-osmotic fluid-loss will result in an unremarkable blood osmolality.

2.4.4 Urinary hydration markers

The endocrine response to dehydration alters renal function and urine composition and volume which are the fundamental principles providing the framework for using urinary parameters such as urine colour, osmolality and specific gravity as body fluid markers of dehydration. Urine concentration has been shown to be a valid and reliable assessment technique to differentiate between normal hydration and dehydration (Armstrong et al., 1994; Shirreffs & Maughan, 1998). For example, first morning urine osmolality measures were shown to be sensitive to dehydration equal to approximately 2% BML evoked by exercise and heat stress with restricted overnight fluid intake (Shirreffs & Maughan, 1998). Urine osmolality, specific gravity and colour also identify modest intracellular dehydration evoked by exercise alone and exercise and heat stress (Armstrong et al., 1998; Armstrong et al., 1994). Furthermore, urine colour, osmolality and specific gravity have been shown to be early markers of chronic intracellular dehydration during fluid restriction of between 13-24 h (Pross et al., 2013; Shirreffs et al., 2004). The diagnostic accuracy of urine hydration parameters has been reported as moderate to near perfect for urine osmolality and high to near perfect for urine specific gravity and is comparable to the diagnostic performance of blood osmolality (AUC_{ROC} of 0.80 to 0.98 and 0.89 to 0.98, for urine osmolality and specific gravity respectively; (Bartok et al., 2004; Cheuvront et al., 2010; Muñoz et al., 2013).

A commonly cited limitation of urinary indices of hydration status is that they have been shown to lag behind changes in hydration status during acute active dehydration and periods of rehydration (Kovacs, Senden, & Brouns, 1999; Popowski et al., 2001). This might be explained by the impact of an increase in sympatho-adrenal system activity on urine production during exercise compared with at rest (Zambraski, 1996; Melin et al., 2001). Specifically, an increase in sympathetic nervous system (SNS) activity has been suggested to

cause vasoconstriction of the glomerular afferent arterioles which decrease renal blood flow which in turn decreases urine flow (Wade, 1996; Zambraski, 1996). The reduction in urine flow is accompanied by an impaired renal concentrating ability which may be responsible for the inferior sensitivity of urine indices during exercise. Another consideration when using urine hydration parameters is the cause and type of dehydration. Acute and chronic intracellular dehydration causes the kidneys' to conserve water and hence there is a rise in the concentration of urine (ions and urea). However, the volume depletion which accompanies extracellular dehydration may also improve the kidney's ability to concentrate urine by decreasing medullary blood flow (Sands & Layton, 2009). In addition, during osmotic diuresis caused by exposure to austere environments, and certain illnesses and medications there is an increased urinary loss of both water and ions. This mild increase in urine concentration may be identified by osmolality, which specifically assesses all dissolved particles such as ions. Therefore, some urine hydration parameters may have utility as markers of both mild intra- and extracellular dehydration. However, the diagnostic performance of urine markers has not been systematically investigated for mild dehydration and across different types of dehydration.

2.4.5 Saliva hydration parameters

An increase in unstimulated whole saliva osmolality and decrease in saliva flow rate has been shown to identify acute intracellular dehydration equivalent to~1% of body mass, following active heat-stress in young healthy participants (Walsh et al., 2004). In a similar population, saliva osmolality and flow rate also identified chronic intracellular dehydration following 48 h of fluid restriction (dehydration equivalent to 3% of body mass; Oliver et al., 2008). Notably, both these markers were also able to identify modest extracellular dehydration

following 48 h of prolonged fluid and energy restriction (dehydration equivalent to 3% of body mass; Oliver et al., 2008). This suggests that saliva hydration markers may have utility to identify dehydration regardless of type. In support of this, saliva osmolality had moderate diagnostic accuracy to discriminate between normal hydration and mixed dehydration (3.1% of body mass) evoked by a combination of diuretic administration and 12 h of fluid restriction (Ely et al., 2014). However, recent research has been contradictory regarding the diagnostic performance of saliva osmolality (Cheuvront et al., 2010; Muñoz et al., 2013). Saliva osmolality displayed poorer diagnostic performance than plasma osmolality and urine hydration parameters to identify intracellular dehydration ranging between 2 to 7% of body mass evoked by exercise and heat-stress (Cheuvront et al., 2010). In contrast, when dehydration was evoked by the same method saliva osmolality had comparable if not superior diagnostic accuracy to haematological and urinary markers to identify intracellular dehydration between 1-5% of body mass (Muñoz et al., 2013). However, in these two studies data from a range of magnitudes of dehydration were pooled (dehydration between 1-7% of body mass). Therefore, the diagnostic performance of saliva hydration markers to identify mild intra- and extracellular dehydration is currently unclear.

A potential mechanism for the secretion of smaller amounts of more concentrated saliva may lie in the dehydration-mediated changes in ECF osmolality. In short, for water to move from plasma through the acinar cells to form primary saliva, a trans-acinar cell sodium gradient must be generated (Ship & Fischer, 1997). During intracellular dehydration the ECF sodium concentration increases and this is reflected in a graded increase in plasma osmolality with progressive dehydration (Popowski et al., 2001). As a result, a greater sodium concentration must be generated across the salivary acinar cell to drive fluid into the acinar lumen to form primary saliva. An increase in ECF sodium concentration during intracellular dehydration

might therefore result in the production of smaller amounts of more concentrated saliva. However, this does not account for the utility of saliva hydration parameters in identifying modest extracellular dehydration (Oliver et al., 2008). Salivary gland function is predominantly under the control of the autonomic nervous. Reduced parasympathetic activity both from an increase in blood solute concentration and a decrease in blood volume may explain this observation.

The non-invasive and rapid nature of saliva-collection makes its use as a marker of hydration attractive across many settings e.g. field-based and clinical. However, some have questioned the utility of saliva hydration parameters based on the inherent variability of the markers, the time consuming collection procedure in some populations and the fact that food and fluid consumption, certain xerogenic drugs (e.g. anticholinergic and sympathomimetic drugs) and disease (Sjogren's syndrome, cystic fibrosis) can alter saliva flow rate and potentially confound osmolality measures (Cheuvront et al., 2010; Ely, Cheuvront, Kenefick, & Sawka, 2011; Scully, 2003; Taylor et al., 2012).

2.4.6 Cardiovascular marker of hydration

Although markers of intracellular dehydration exist, fewer markers of extracellular dehydration have been proposed. Markers of extracellular dehydration should aim to identify the hypovolaemia that is associated with this type of dehydration. Hypovolemia is known to impair cardiovascular function and therefore markers that identify these changes might have utility as markers of extracellular dehydration. Possible assessments include, resting or postural changes in blood pressure and heart rate. Although commonly used in clinical settings, and shown to identify moderate and severe hypovolemia (e.g. blood loss: McGee et

al., 1999), few well-controlled laboratory investigations have examined these as markers of hydration status (Cheuvront et al., 2012) and no studies have examined whether these markers are suitable to identify mild extracellular dehydration.

2.4.7 Clinical assessment of hydration status in the elderly patient

Historically the clinical assessment of dehydration can be difficult, especially in the elderly and young infants, and rarely predicts the exact degree of dehydration. Clinical assessment may be particularly inaccurate when assessing patients with mild to modest dehydration and often relies on clinical examination of physical signs and symptoms (Hoxha et al., 2014; Thomas et al., 2004). In addition, recognising dehydration in clinical populations is difficult due to poor monitoring, the challenges in recording accurate fluid balance that accounts for actual input and output as well as insensible fluid loss which is influenced by the disease process and the limited knowledge of frontline staff surrounding hydration (El-Sharkawy et al., 2014).

On admission to hospital and before biochemical analysis of blood, the diagnosis of dehydration is often carried out by clinical examination (Thomas et al., 2008). Physical signs of dehydration such as orthostatic intolerance, decreased skin turgor, tachycardia and dry oral mucosa are associated with intracellular dehydration in elderly patients, but lack diagnostic performance (Chassagne, Druesne, Capet, Ménard, & Bercoff, 2006). The presence of dry axilla have been found to have a positive predictive value (specificity of 82%; Eaton et al., 1994). Similarly the presence of a dry furrowed tongue and dry oral mucous membranes have been shown to be useful signs of dehydration (Vivanti et al., 2008). However, dry mouth can be misleading as many older patients are mouth breathers and medication can lead to

symptoms of dry mouth e.g.anticholinergic (Weinberg & Minaker, 1995). In general, although physical signs of dehydration may be used in initially identifying dehydration, these classical signs are irregularly present in patients with dehydration and should be interpreted with caution (Chassagne et al., 2006). To further compound their use in the clinical setting, the utility of physical signs to identify multiple types of dehydration has not yet been investigated. Urine markers of hydration are also used as an initial screen for dehydration on admission to hospital. Urine hydration markers, including urine colour, specific gravity and osmolality, have been shown to correlate well with measures of serum sodium and blood urea nitrogen to creatinine ratio (BUN:Cr) (Wakefield, Mentes, Diggelmann, & Culp, 2002). However, others have criticised the use of these markers in clinical populations due to factors including; a lack of association with serum osmolality, urine collection is not always possible in elderly populations, urine parameters may be confounded by renal disease and diabetes and urine markers have been shown to be poor markers of hydration status in elderly and critically ill patients (Fletcher, Slaymaker, Bodenham, & Vucevic, 1999; Spira et al., 1997; Angela Vivanti et al., 2008; Weinberg & Minaker, 1995)

As previously described plasma or serum osmolality is often considered a superior marker of intracellular dehydration. However, in some circumstances, the direct measurement of plasma or serum osmolality, via determination of freezing point depression, is not routinely undertaken because of the cost implications e.g. in UK hospitals the measurement of plasma or serum osmolality is uncommon (Siervo, Bunn, Prado, & Hooper, 2014). In the absence of direct measurements assessment of effective osmolality in the clinical setting is sometimes achieved using routine measures of serum electrolytes and glucose without urea nitrogen concentration (Thomas et al., 2008). However, the utility of calculated blood osmolality as a marker of dehydration in an elderly clinical population has not been systematically

investigated (Siervo et al., 2014). A further common measure of dehydration in the hospitalised elderly patient is the assessment of blood urea nitrogen (BUN) to creatinine ratio (BUN:Cr). The BUN:Cr ratio is a routine clinical marker of renal function (Thomas et al., 2004). The relatively constant excretion of creatinine and the variable balance between excretion and reabsorption of BUN, give a typical BUN/Cr ratio of between 10 and 20:1 (Dossetor, 1966). BUN reabsorption is an inverse function of urine flow rate and is mediated by AVP, thus a BUN:Cr ratio > 20 may be used for assessing dehydration *per se* as it is stimulated by either osmotic or volume stimuli (Thomas et al., 2004). Although these haematological markers rely on an invasive blood sample and biochemical analyses, which is costly and time-consuming, they can be used as reference standards to investigate the utility and diagnostic accuracy of less-invasive hydration assessment tools.

2.4.8 Potential novel markers of hydration status

As previously outlined it is unlikely that any single marker of hydration will be suitable across dehydration types and all populations and settings. Therefore, research examining the utility of novel markers is warranted. Thirst is governed by both changes in plasma osmolality and volume and therefore it may identify both intracellular and extracellular dehydration. Popular belief within exercise sciences however suggests that thirst is too insensitive as a hydration measure. Specifically, studies suggest that thirst does not change until dehydration is equal or greater than 2% body mass loss and that this insensitivity is even greater in children and the elderly (Kenefick, Cheuvront, Leon, & Brien, 2012). Although these criticisms are valid they can be applied to most hydration markers. On the contrary at rest, which is the most appropriate time to determine an individual's hydration status, thirst has been shown to identify mild hypohydration (~1-2% of body mass), which is better or

compares favourably with any other hydration marker (Armstrong et al., 2014; Shirreffs et al., 2004). Possibly because it is believed to be an insensitive measure thirst has unfortunately received very little systematic investigation as a hydration marker (Armstrong, 2012). As a result, the diagnostic accuracy of thirst to identify mild intra- and extracellular dehydration is unknown.

Recently, it has been shown that tear osmolarity may be useful as a marker of intracellular dehydration (Fortes et al., 2011). Particularly exciting features of this hydration marker are the non-invasive collection, immediate result (<30 s) and similar excellent sensitivity as plasma osmolality. Water and electrolytes are major components of tear fluid that is secreted from the lacrimal gland, and tear fluid has been reported to be iso-osmotic with plasma. Therefore, dehydration may increase tear osmolarity by decreasing tear secretion rate or residual tear volume (Sollanek et al., 2012). Unlike plasma, urine and saliva markers the utility of these tear markers to identify intracellular dehydration, evoked over a prolonged period, is unknown. Further, as tear fluid is suggested to be isotonic with plasma it may be hypothesised that tear osmolarity is unlikely to identify extracellular dehydration, although this is currently unclear (Tiffany, 2003). In addition, it is also unclear whether tear osmolarity has utility in an outdoor athletic or occupational setting where sunlight, wind, movement convection, sweat (in the eyes) and other factors may complicate these measurements (Sollanek et al., 2012).

Hypovolemia and hyper-osmolality associated with dehydration have been shown to independently increase sympathetic nervous system activity (SNS) and decrease parasympathetic activity (PNS) (Charkoudian, Eisenach, Joyner, Roberts, & Wick, 2005; Triedman, Cohen, & Saul, 1993). Although no research exists, this may form the basis for the

use of measures of heart rate variability as markers of fluid-deficit. Indices such as the standard deviation of NN intervals (SDNN: an index of total heart rate variability), the square root of the mean squared difference of successive NN intervals (RMSSD: an index of parasympathetic activity) and low to high frequency power ratio (LF/HF: an index of sympatho-vagal balance) may prove to be a useful non-invasive assessment technique.

2.5 General aims

With this information in mind the general aims of this thesis were to examine: 1. the diagnostic accuracy of physical signs, urinary and saliva hydration assessment markers in a hospitalised elderly population; 2. the effect of stimulating saliva on its diagnostic accuracy and practicality as a hydration marker; 3. the diagnostic accuracy of a battery of established and novel markers of hydration to two types of mild dehydration; and 3. The effect of two types of mild dehydration on endurance exercise performance and markers of affective well-being.

CHAPTER THREE

General Methods

3.1 Ethical approval

Before the start of all experiments, ethical approval was granted by the Institutional Ethics Committee (School of Sport, Health and Exercise Sciences, Bangor University). Both written and verbal explanations of procedures involved in each experiment were given to subjects and they were informed of their right to withdraw at any time. After any questions they had related to the experiment had been answered, each subject gave their full written and verbal consent to participate. Additionally, for the experiment described in **Chapter 4**, ethical approval was granted by the North West Wales Research Ethics Committee (Ref: 11/WA/0023).

3.2 Anthropometry

Height was recorded without footwear using a wall stadiometer (*Bodycare Ltd, Warwickshire, UK*) and body mass by digital platform scales, which had a precision of 50 g (*Model 705, Seca, Hamburg, Germany*). To monitor changes in body mass following dehydration evoked by exercise and heat-stress, fluid restriction and diuretic medication, participants removed all clothing and were instructed to remove any sweat from the body with a towel before weighing (**Chapter 5, 6 and 7**).

3.3 Determination of maximal oxygen consumption ($\dot{V}O_{2max}$) and peak power output (PPO)

For the determination of $\dot{V}O_{2max}$ and PPO participants performed one of two different continuous incremental exercise tests to volitional exhaustion on either a stationary cycle ergometer (**Chapter 5**, *Monark 814e, Varberg, Sweden*) or on an electomagnetically braked cycle ergometer (**Chapter 6 and 7**, *Excalibur Sport, Lode, Groningen, Netherlands*). In the first protocol, following a 5-min warm-up at 70 W (60 W for females), participants began

cycling at 175 W (120 W for females), with increments of 35 W (30 W for females) every 3 min until volitional exhaustion. During the final 30 s of the test, expired gas was collected into a Douglas bag and analysed for O_2, CO_2 (Servomex 5200, Crowborough, UK), and VE (Harvard Apparatus, Edenbridge, UK) (**Chapter 5**). The second protocol included a 2-min warm-up at 50 W, followed by 50 W increments every 2-min until volitional exhaustion, which was defined as a decrease in cadence below 60 revolutions per minute (rpm) for more than 5 seconds despite strong verbal encouragement (Marcora, Bosio, & de Morree, 2008) (**Chapter 6 and 7**). Maximal exertion was confirmed by heart rate (Polar Electro, Kempele, Finland), perceived exertion (CR-10 scale; Borg, 1998) and expired gas analysis (MetaLyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany). For both protocols peak power output was calculated according to the equation of Kuipers (1985). Participants completed all cycle ergometer tests in the same cycling position, which was determined by them on the first exercise test and recorded by the experimenters for replication in all subsequent exercise tests.

3.4 Blood, urine and saliva collection and analysis

3.4.1 Blood

Blood samples were collected from an antecubital (**Chapters 4, 5, 6 and 7**) or dorsal metacarpal vein (**Chapter 4**) without venestasis into one serum separation vacutainer (**Chapter 4**), and one lithium heparin coated tube (Becton Dickinson, Oxford, UK) (**Chapter 4, 5, 6 and 7**). Blood in the serum-seperation tube was assessed for blood urea nitrogen and creatinine concentration at the hospital biochemistry department (*Olympus AU 2700 chemistry immuno analyser, Beckman Coulter, USA*) (**Chapter 4**). Blood from the lithium heparin coated tube was immediately used to determine hemoglobin and heamatocrit in triplicate using an automated analyser (B-Hemoglobin, Hemocue, Angelholm, Sweden) and

by the capillary method, respectively (**Chapter 6 and 7**). For the capillary method, capillary tubes were filled to approximately three questers full then sealed by placing into sealing clay at a 90^{o} angle. The tubes were then paced in a microheamatocrit centrifuge and spun for 5 min. After, haematocrit was determined using a microhaematocrit reading device. Blood and plasma volume changes were then estimated as previously described (Dill & Costill, 1974). The remaining blood in the lithium-heparin tube was centrifuged immediately at 1500 g for 10 min at 5°C. The plasma was aspirated and triplicate measurements of osmolality were made immediately using a freezing point depression osmometer (Model 330 MO, Advanced Instruments, Massachusetts, USA) **(Chapter 4, 5, 6 and 7)**. If any of the intra-sample values differed by greater than 1% a further sample was measured and the mean of the four samples was recorded. Standard control solutions (290 mOsm/kg) were assessed daily to ensure the osmometer functioned within acceptable limits of precision (±2 mOsm/kg). The analytical coefficient of variation (CV_A) for repeated sample plasma osmolality measurements across the experimental chapters was 0.4%.

3.4.2 Urine

Urine samples were collected in 24-h containers and aliquoted into universal tubes and immediately analysed for urine colour by an 8-point colour chart (Armstrong et al., 1994) and urine specific gravity (USG) using a handheld refractometer in duplicate (Atago URC-Osmo refractometer, Japan) (**Chapters 4, 5, 6 and 7**). Urine osmolality was measured immediately in triplicate by a freezing point depression osmometer (Model 3300, Advanced Instruments, USA) (**Chapters 6**). The analytical coefficient of variation (CV_A) for repeated sample urine osmolality measurements across the experimental chapters was 0.2%.

3.4.3 Saliva

For sixty minutes before saliva collection participants refrained from consuming any food or drink, using tobacco or oral hygiene **(Chapters 5 and 6)**. Where this was not possible, fluid was restricted in the 10 min before saliva sampling to prevent any potentially interfering influence of fluid intake on saliva flow or composition (Ely et al., 2011) **(Chapter 4)**. Unstimulated and stimulated whole-saliva samples were collected using two procedures. In **Chapters 4 and 5** saliva was collected using a pre-weighed Versi-sal® collection device (Oasis Technology, USA). Saliva was collected by placing the Versi-sal® collection device under the tongue. After 4 min, the collection device was inspected for volume of saliva by weighing it immediately (to the nearest milligram) and subtracting the pre-weight. If the volume was insufficient for osmolality analysis (< 25µl), the swab was replaced under the tongue for a further 4 min **(Chapter 4)**. In **Chapter 5** the collection device was placed under the tongue for a predetermined duration (from a habituation session) that provided adequate saliva volume without fully saturating the swab. In **Chapter 6**, saliva was collected by the drool method by allowing saliva to passively pool in the bottom of the mouth and drip off the lower lip into a pre-weighed universal tube. At the end of the collection period the participants expectorated any remaining saliva into the universal tube (Navazesh, 1993). Saliva collection time was standardised to a five minute collection period for all participants. For all saliva collection methods, participants firstly swallowed in order to empty the mouth of residual saliva, before saliva collection was performed and accurately timed. During saliva collection participants sat upright leaning forwards slightly with the eyes open and with minimal oral-facial movement.

In addition, in **Chapter 5** after five minutes rest stimulated whole-saliva sample was collected using the same saliva collection procedures. Stimulation of whole-saliva was

achieved by the application of 250 µL of a citric acid (2% w/v) to the dorsal lateral surface of tongue (Ghezzi, Lange, & Ship, 2000). Similar to unstimulated saliva collection, after five seconds the participant swallowed the citric acid solution and any residual saliva before collection began.

Saliva volume was assessed in duplicate by weighing the Versi-sal® collection device or universal tube immediately (to the nearest milligram) and subtracting the pre-collection weight. By assuming the density of saliva to be 1.00 $g \cdot mL^{-1}$, unstimulated and stimulated saliva flow rate were calculated by dividing the volume collected by the time of collection (Cole & Eastoe, 1988). Saliva was collected from the Versi-sal® device by centrifugation or directly from the universal tube following the drool saliva collection method. Unstimulated and stimulated saliva osmolality was measured immediate in duplicate using a freezing point depression osmometer (model 330 MO, Advanced Instruments, Norwood, Massachusetts, USA). The analytical coefficient of variation (CV_A) for repeated sample saliva osmolality measurements across the experimental chapters was 0.7% (0.4 mOsm/kg).

3.5 Statistical analyses

Specific statistical analyses used are detailed within each chapter. The diagnostic accuracy of hydration markers to discriminate between normal hydration and dehydration was calculated in **Chapters 4, 5 and 6**.

3.5.1 Diagnostic accuracy

The performance of a laboratory test can be described in terms of diagnostic accuracy, or the ability to correctly classify participants into clinically relevant sub-groups e.g. dehydrated or

normal hydration. Diagnostic accuracy refers to the quality of the information provided by the classification device and should be distinguished from the actual practical value, of the information (Zweig & Campbell, 1993). Diagnostic tests such as sensitivity and specificity, area under the receiver operating characteristic (ROC) curve (AUC_{ROC}), predictive values, odds ratios and likelihood ratios have been widely accepted as the standard method for describing and comparing the accuracy of medical diagnostic tests (Altman & Bland 1994; Altman & Bland, 1994; Deeks & Altman, 2004). However, research utilising these diagnostic tests to examine the performance of hydration markers is limited (Bartok et al., 2004; Cheuvront et al., 2012, 2010; Fortes et al., 2011b; Muñoz et al., 2013). Many of these diagnostic tests rely knowing the prevalence of the condition being investigated. In the absence of accurate data on the prevalence of different types of dehydration across different populations, diagnostic accuracy was determined by receiver operating characteristic (ROC) analysis (MedCalc Software bvba, Ostend, Belgium). A useful feature of this test of overall diagnostic accuracy is that sensitivity, specificity and AUC_{ROC} are independent of disease prevalence (Park, 2004).

Receiver operating characteristic (ROC) curves display the relationship between sensitivity (true-positive rate) and 1-specificity (false-positive rate) across all possible threshold values that define a disease or positivity of a condition (Liu, Li, Cumberland, & Wu, 2005). To perform ROC analysis hydration state was dichotomized where the hydration marker values for normal hydration were compared to dehydrated values. Area under the ROC curve (AUC_{ROC}) was used as the global measure of diagnostic accuracy, as previously recommended (Zweig & Campbell, 1993), with binomial exact 95% confidence intervals for each AUC_{ROC}. The values for AUC_{ROC} can range from 0.5 to 1.0, where 0.5 indicates that the hydration marker has no better ability to discriminate between normal hydration and

dehydration than chance. Conversely, 1.0 indicates that the marker is perfect at discriminating between normal hydration and dehydration i.e. perfect identification of correct hydration state (Zweig & Campbell, 1993). Qualitative AUC_{ROC} descriptors that relate to diagnostic accuracy are poor (0.6), fair (0.7), moderate (0.8), high (0.9), near perfect (0.95) and perfect (1.0) (Obuchowski, Lieber, & Wians, 2004). For hydration markers that had an AUC_{ROC} statistically greater than 0.5, the Youden's Index was used to objectively generate a dehydration threshold (Schisterman, Perkins, Liu, & Bondell, 2005). Previous studies have reported plasma, urine and saliva parameters have a diagnostic accuracy equal to or greater than an AUC_{ROC} of 0.7 (Bartok et al., 2004; Cheuvront et al., 2010). In practice this fair to moderate diagnostic accuracy means that a marker discriminates correctly between normal hydration and dehydration at least 70% of the time (Zweig & Campbell, 1993). The sensitivity and specificity of each threshold was then also calculated. To compare the diagnostic accuracy between hydration markers the AUC_{ROC} of each marker was compared by a method analogous to the paired *t* test that accounts for the correlation between samples from the same individual (Hanley & McNeil, 1983).

CHAPTER FOUR

Diagnostic accuracy of physical signs, urine and saliva hydration markers to identify dehydration in an elderly hospitalised cohort.

4.1 Summary

This prospective phase I exploratory study investigated the diagnostic accuracy of routinely used physical signs, urine and saliva dehydration markers in a hospitalised older cohort. One hundred and thirty older adults (59 males, 71 females, mean ±SD age = 78 (9) y) admitted to an emergency department or acute medical care unit underwent a hydration assessment within 30min of admittance. This comprised seven physical signs of dehydration (tachycardia (>100bpm), low systolic blood pressure (<100mmHg), dry mucous membrane, dry axilla, poor skin turgor, sunken eyes, and long capillary refill time (>2s)), urine colour, urine specific gravity (USG), saliva flow rate (SFR) and saliva osmolality. Plasma osmolality (Posm) and the blood urea nitrogen to creatinine ratio (BUN:Cr) were assessed as reference standards of hydration, with 21% of participants classified with intracellular dehydration (Posm >295mOsm/kg), 19% classified with extracellular dehydration (BUN:Cr >20) and 60% classified with normal hydration. All physical signs showed poor diagnostic accuracy (area under the receiver operating characteristic curve, $AUC_{ROC} < 0.6$)) for detecting either form of dehydration. Neither urine colour, USG, nor SFR could discriminate hydration status ($AUC_{ROC} = 0.49\text{-}0.57$, $P>0.05$). In contrast, saliva osmolality demonstrated moderate diagnostic accuracy ($AUC_{ROC} =0.76$, $P<0.001$) to distinguish both dehydration types (70% sensitivity, 68% specificity for intracellular dehydration, 78% sensitivity, 72% specificity for extracellular dehydration and 76% sensitivity, 68% specificity for dehydration *per se*). Saliva osmolality demonstrated superior diagnostic accuracy compared with physical signs and urine markers, and may have utility for the assessment of both intracellular and extracellular dehydration in older individuals.

4.2 Introduction

Dehydration in older adults is a significant clinical problem. However, there is an absence of National Institute of Health and Care Excellence (NICE) clinical guidelines for the assessment of hydration status. Nevertheless, biochemical assessments from blood sampling such as; plasma osmolality, electrolytes and blood urea nitrogen to creatinine ratio (BUN:Cr) represent criterion methods of identifying dehydration in a clinical setting (Mange et al., 1997; McGee et al., 1999; Thomas et al., 2008; Hooper et al., 2012). However, blood sample collection is invasive and laboratory analysis is time-consuming, often delaying the course of treatment by hours. To aid an initial diagnosis of dehydration before requesting biochemistry confirmation, clinicians may use a variety of simple screening measures, albeit in a non-systematic way, that may include; urinary parameters, orthostatic blood pressure change, patient history, and/or presenting signs and symptoms of dehydration (Chassagne et al., 2006; McGee et al., 1999; Vivanti et al., 2008; Wakefield et al., 2002). Nevertheless, these screening methods are often characterised by poor diagnostic performance (Eaton et al., 1994; Fletcher et al., 1999; McGee et al., 1999; Rowat, Graham, & Dennis, 2012; Shimizu et al., 2012; Weinberg & Minaker, 1995). To confound hydration assessment further, the term 'dehydration' is poorly defined and is used to characterize many water and solute deficits relating to whole body fluid deficits. In order to simplify clinical practice researchers have suggested the classification of clinical dehydration into 2 distinct types; intracellular and extracellular dehydration. Intracellular dehydration is typically defined as a plasma osmolality >295 mOsm/kg (Hooper et al., 2012). Extracellular dehydration, which may be isotonic or hypotonic because of equal, or greater proportional loss of solutes than water, is typically defined as a BUN:Cr >20 in the absence of hypertonicity (Stookey, 2005). To the best of our knowledge, there are few, rigorous studies that have investigated the diagnostic accuracy of clinical physical signs and urine indices to detect both intracellular and

extracellular dehydration in hospitalised older adults using a criterion reference method, and none which have simultaneously assessed the utility of any hydration marker to assess both types of dehydration.

In a series of studies, in young healthy adults, we have shown that rapid measurements made from non-invasive collection of saliva fluid can be used to identify mild to modest intracellular dehydration (Walsh, et al., 2004a; Walsh, et al., 2004b; Oliver et al., 2008), and may have utility in identifying extracellular dehydration (Oliver et al., 2008). The utility of these novel saliva markers of dehydration have not yet been examined in a clinical, older adult population, although encouragingly, the presence of a dry tongue was identified as the clinical sign most strongly associated with dehydration in an elderly cohort, albeit demonstrating only 64% sensitivity (Vivanti, 2010). To this end, the purpose of this prospective study was to determine, and compare, the diagnostic accuracy of clinical physical signs routinely used in hospital setting, along with saliva (flow rate and osmolality) and urine indices (colour and specific gravity), to detect intracellular and extracellular dehydration in a hospitalised, older adult cohort using primary reference standards; plasma osmolality and BUN:Cr.

4.3 Methods

Participant characteristics

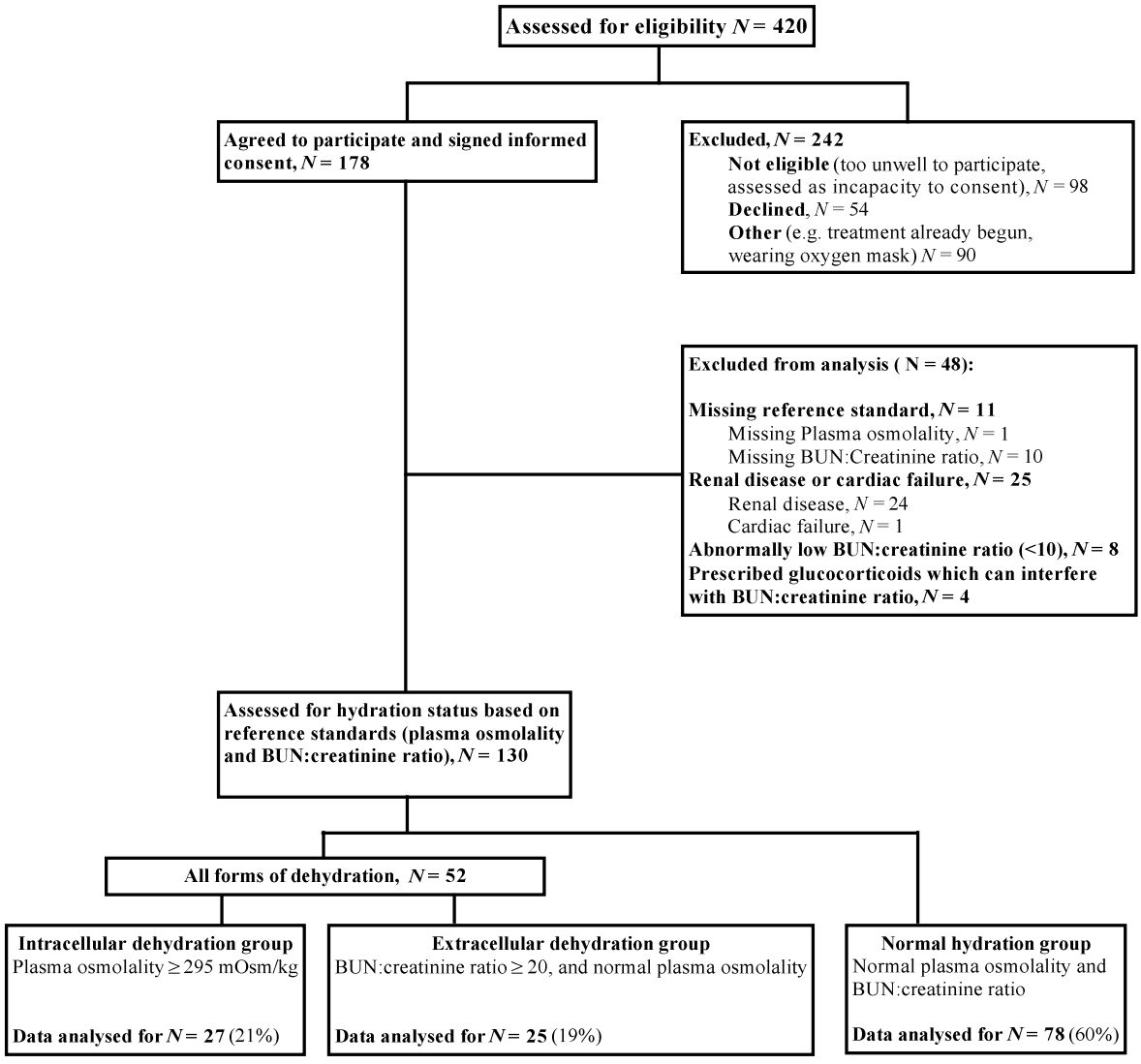
A convenience sample of one hundred and thirty older adults (59 males, 71 females, mean ±SD age = 78 (9) y) adults over 60 years of age admitted consecutively to either the emergency department or acute medical care unit of Gwynedd Hospital, Bangor, with any primary diagnosis and capacity to consent were enrolled between May and November 2011 during the times the investigators were available (09:00h – 17:00h, Monday-Friday). Participant exclusion criteria included: oral trauma or dental surgery within 14 days, swallowing problems, salivary gland tumours, if they were deemed too unwell by the medical staff to participate in the study, if they were assessed as not having capacity to consent, or if they had already begun any form of medical treatment or rehydration therapy (oral or intravenous). Participant flow through the study is depicted in **Figure 4.1**.

Experimental design and procedures

The study was conducted as a phase I, prospective, hospital-based cross-sectional study. Phase I studies are defined as exploratory, in which a new test is first evaluated in a clinical setting to determine whether the test has any ability to discriminate diseased from non-diseased patients (Obuchowski et al., 2004). All measures of hydration status were performed within 30 minutes of admission, with no disruption to routine care in the following order; examination of physical signs of dehydration, collection of saliva, blood and urine. For the reference standards of whole body hydration assessment, a blood sample was collected by the clinical research fellow or a specialist phlebotomist and analysed for plasma osmolality and BUN:Cr. For consistency, all physical examinations and assessment of confidential medical information was carried out by the same clinical research fellow (a junior doctor with five years clinical experience), who was blinded to the results of the reference standards and the

saliva and urine index test results when conducting the physical examination. Saliva and urine samples were collected, and analysed by an independent research assistant who had been trained in the handling and assessment of saliva and urine samples, and who was blinded to the physical examination results. Details of the patients' medical condition, history and medication were recorded retrospectively after the reference and index test results had been established.

Figure 4.1: Participant flow through the study.



NOTE: Abbreviations: BUN, blood urea nitrogen

Assessment of hydration status

Reference standards: Blood was collected and plasma osmolality was measured immediately and serum blood urea nitrogen and serum creatinine were assessed at the hospital clinical biochemistry department using an automated biochemistry analyser as described in **Chapter 3**.

Index tests

Clinical assessment of physical signs of dehydration: The clinical assessment were carried out by the clinical research fellow and consisted of seven physical signs of dehydration that are routinely used in Gwynedd Hospital and reported in other published studies (McGee et al., 1999; Vivanti et al., 2008); tachycardia (resting heart rate >100 beats per minute), low resting systolic blood pressure (<100mmHg), dry mucous membrane (inside of the cheek, dry *v*s. wet), axillary dryness (assessed by palpating the armpit, dry *v*s. moist), poor skin turgor (measured by pinching the skin on the dorsum of the hand and observing if the tissue fold returned to normal immediately), presence of sunken eyes as assessed by the clinical research fellow, and long capillary refill time (> 2s, assessed by holding the patients hand at heart level and blanching the participant's right index finger using moderate pressure and assessing the length of time for the return of normal colour). Each physical sign was assessed with the participant rested and seated upright and assessed dichotomously.

Saliva and urine sample collection and analysis: Saliva and urine samples were collected and from these samples saliva flow rate, saliva osmolality and urine specific gravity were measured as described in **Chapter 3**.

Statistical analysis

The desired sample size for dehydrated participants (n = 20 intracellular dehydration only) was calculated using the following equation:

$$n \geq \frac{(1.96)^2 p(1 - p)}{x^2}$$

Where p = desired sensitivity (70%) as a proportion, and x = desired confidence interval (20%) as a proportion (Banoo et al., 2006). A desired sensitivity of 0.70 was chosen because a minimum sensitivity of > 0.60 has been suggested for promising hydration markers (Hooper et al., 2012). Assuming a prevalence of intracellular dehydration (plasma osmolality ≥ 295mOsm/kg) of 17% (Thomas et al., 2004), and allowing for an approximate one-third exclusion rate from data analysis (due to missing reference tests, and co-morbidities that preclude the use of the reference standards), a total of 178 participants were recruited into the study. Medical records for participants were accessed after enrolment, and due to potential influencing effects on the reference standards assessed in this study, participants with a history of renal disease (n = 24), or who were in cardiac failure (n = 1) were excluded from data analysis. Participants were also excluded from data analysis if the reference tests were not available (n = 11), if they had an abnormally low (<10) BUN:Cr which may be indicative of renal disease or the syndrome of inappropriate antidiuretic hormone (n = 8), or if they were taking glucocorticoid medication (n = 4) which affects the validity of the BUN:Cr (Thomas et al., 2008). Based on the reference standards, participants with a presenting plasma osmolality ≥295mOsm/kg were classified as having intracellular dehydration (Stookey, 2005; Hooper et al., 2012). Of the remaining participants, those with a BUN:Cr ≥20 in the absence of hypertonicity were classified as having extracellular dehydration, and the remaining participants formed the normal hydration control group (normal plasma osmolality and BUN:Cr) (Stookey, 2005).

To assess the diagnostic accuracy of saliva and urine indices, and clinical physical signs for assessment of hydration status, both intracellular, and extracellular dehydration groups were separately compared with the normal hydration control group. Both dehydration groups were also combined to form a generic dehydration group for comparison with normal hydration. For all clinical physical sign data, urine colour, USG, SFR and saliva osmolality the area under the receiver operating characteristic curve (AUC_{ROC}) was used as a measure of global diagnostic accuracy (**see Chapter 3**). In addition, hydration marker cut-offs and the sensitivity and specificity of these cut-offs were calculated where appropriate (**Chapter 3**).

4.4 Results

4.4.1 Participant characteristics

A total of 178 participants were enrolled into the study (n = 85 males, n = 93 females) with mean age ±SD 78 (9) yr. After exclusions, data were analysed for n = 130 participants (n = 59 males, n = 71 females; mean age 78 (9), range 60-101yr), of which n = 27 (21%) were classified as having intracellular dehydration, n = 25 (19%) were classified with extracellular dehydration, and n = 78 (60%) were classified with normal hydration. Of the 27 participants in the intracellular dehydration group, 10 also had an elevated BUN:Cr (≥20). There were no differences between the groups for age (**Table 4.1**). By design, participants with intracellular dehydration had elevated plasma osmolality, and participants with extracellular dehydration had elevated BUN:Cr compared with the normal hydration control group (**Table 4.1**).

4.4.2 Feasibility of collecting index tests

All clinical physical sign assessments were conducted in all 130 participants. All participants were able to produce a saliva sample with the exception of 4 participants (1 with intracellular dehydration, 2 with extracellular dehydration, and 1 with normal hydration). For these 4 participants SFR was recorded as zero, and SFR data was therefore analysed for n = 130. However, there was sufficient saliva (> 25 µl) to assess saliva osmolality in only 98 (75%) participants, and in participants with low SFR saliva collection was time-consuming (> 10 min for 25 µl). Urine samples were not collected for n = 45 participants, who were unable to urinate within an appropriate time frame (within 30 minutes), whilst 1 participant provided a urine sample containing blood, confounding interpretation. Urine colour and specific gravity were therefore analysed for n = 84 (65%).

Table 4.1. Group data for age, and reference blood, and urine and saliva index hydration measures (values represent mean (standard deviation)).

		Intracellular dehydration only (*n* = 25)	**Extracellular dehydration only (*n* = 27)**	**Normally hydrated controls (*n* = 78)**	***P* value (one-way ANOVA)**
	Age (Yr)	78.3 (9.6)	80.1 (9.6)	76.3 (7.7)	0.14
Reference tests	Posm (mOsm/kg)	299 (6)†	283 (6)	283 (9)	< 0.001
	BUN:Cr	18.8 (5.5)	24.3 (4.7)‡	15.7 (2.6)	< 0.001
Index tests	USG	1.017 (0.006)	1.016 (0.007)	1.016 (0.006)	0.77
	Urine colour	4.1 (1.6)	3.9 (1.8)	3.9 (1.7)	0.87
	SFR (µl/min)	56 (55)	86 (183)	77 (90)	0.57
	Sosm (mOsm/kg)	136 (58)*	140 (66)*	92 (45)	< 0.001

NOTE: Abbreviations: BUN:Cr; blood urea nitrogen to creatinine ratio. † Significantly greater than extracellular dehydration and the group with normal hydration ($P < 0.001$). ‡ Significantly greater than intracellular dehydration and normal hydration groups ($P < 0.001$). * Significantly greater than normal hydration group ($P < 0.01$).

4.4.3 Diagnostic accuracy of clinical physical signs

Diagnostic data for all 7 clinical physical signs for both types of dehydration are shown in **Table 4.2** and **Figure 4.2**. No clinical physical sign in isolation could discriminate between normal hydration and either form of dehydration (AUC_{ROC} range 0.44-0.57), suggesting that these techniques have poor diagnostic performance as hydration markers in the current population and setting.

Table 4.2. Diagnostic accuracy of clinical signs to determine both forms of dehydration in combination, and separately in older adults >60yr.

	Both types of dehydration	**Intracellular dehydration**	**Extracellular dehydration**
Clinical assessment		**AUC_{ROC} (95% CI)**	
Low systolic BP (< 100 mmHg)	0.53 (0.43-0.64)	0.49 (0.37-0.62)	0.57 (0.44-0.71)
Tachycardia (HR > 100 bpm)	0.50 (0.40-0.60)	0.44 (0.32-0.56)	0.56 (0.43-0.70)
Dry mucous membrane	0.51 (0.41-0.62)	0.51 (0.38-0.63)	0.52 (0.39-0.65)
Axillary dryness	0.54 (0.44-0.64)	0.53 (0.40-0.65)	0.56 (0.43-0.70)
Poor skin turgor	0.55 (0.45-0.65)	0.53 (0.40-0.66)	0.57 (0.44-0.70)
Sunken eyes	0.51 (0.41-0.62)	0.43 (0.35-0.60)	0.56 (0.42-0.69)
Capillary refill (> 2 S)	0.50 (0.40-0.60)	0.52 (0.39-0.64)	0.48 (0.36-0.61)

4.4.4 Diagnostic accuracy of urine and saliva indices

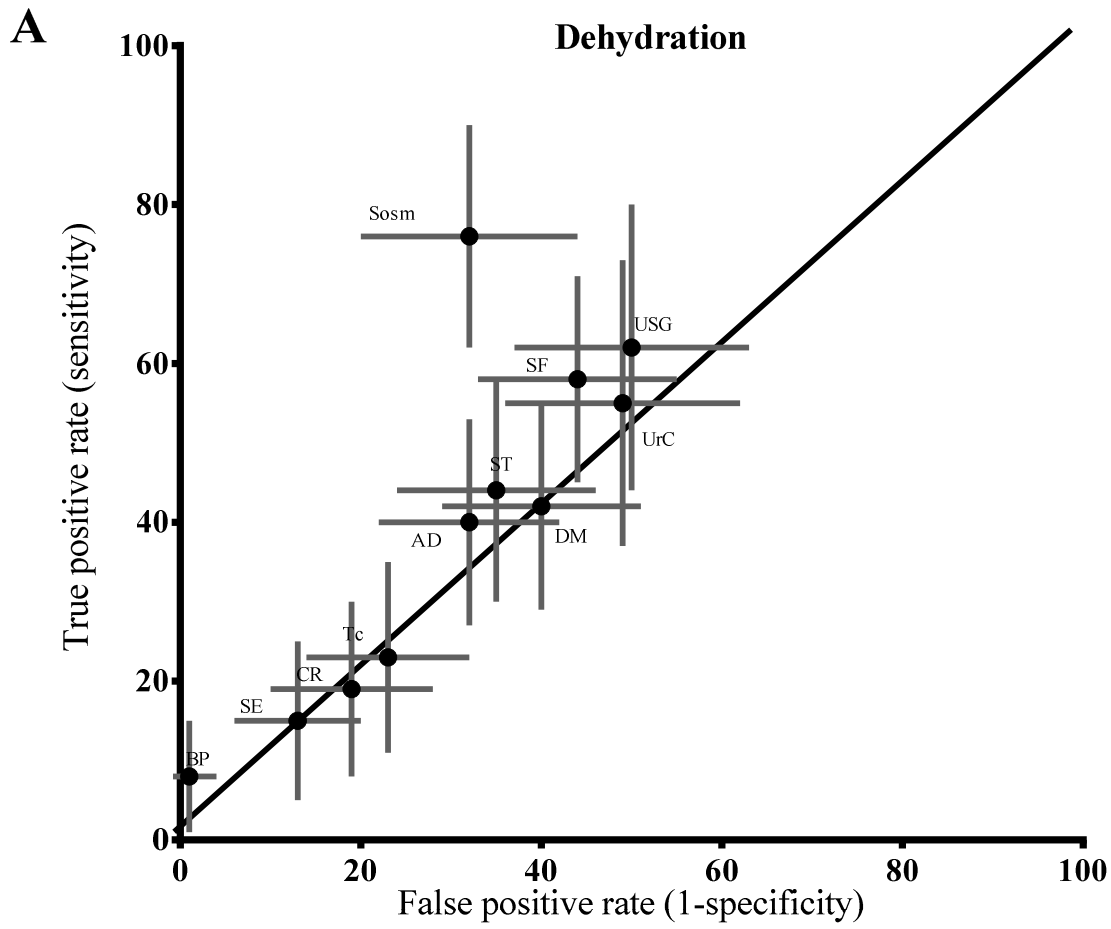
Urine colour, USG and SFR showed no utility as hydration markers as there were no differences in theses indices between any of the three groups for hydration status (**Table 4.1**). Furthermore, when assessed using ROC analyses, neither urine colour, USG or SFR could discriminate between normal hydration and dehydration (AUC_{ROC} ranged from 0.48-0.57, all $P > 0.05$, **Table 4.3**). In contrast, saliva osmolality showed utility as a marker of dehydration as values were greater in participants with both forms of dehydration than those with normal hydration ($P < 0.001$, **Table 4.1**). More importantly, saliva osmolality was also able to distinguish between normal hydration and both types of dehydration separately (AUC_{ROC} = 0.76, $P < 0.01$ individually and combined, **Table 4.3**). The Youden's index derived cut-offs that provided the optimum discrimination between sensitivity and specificity was calculated as: 95, 97, and 94mOsm/kg for intracellular, extracellular, and both forms of dehydration combined, respectively. The diagnostic performance of saliva osmolality to detect dehydration for both forms of dehydration is displayed in Table 4. Saliva osmolality was able to detect intracellular dehydration, extracellular dehydration, and both forms of dehydration combined with a sensitivity of 70, 78 and 76%, and specificity of 68, 72, and 68%, respectively. Importantly, the ability of saliva osmolality to detect dehydration was superior to all clinical physical signs and urine indices for intracellular, extracellular and both types of dehydration in older adults (**Figure 4.2 A, B and C**).

Table 4.3. Receiver operating characteristic (ROC) analysis for urine and saliva indices for the detection of dehydration in older adults (>60yr).

	Both types of dehydration		**Intracellular dehydration**		**Extracellular dehydration**	
Hydration marker	**AUC_{ROC} (95% CI[b])**	***P* value**	**AUC_{ROC} (95% CI[b])**	***P* value**	**AUC_{ROC} (95% CI[b])**	***P* value**
USG ($g \cdot ml^{-1}$)	0.53 (0.39 – 0.66)	0.67	0.55 (0.39 – 0.72)	0.53	0.50 (0.32 – 0.69)	0.98
Urine colour (1-8)	0.52 (0.39 – 0.65)	0.79	0.54 (0.38 – 0.70)	0.61	0.49 (0.41 – 0.67)	0.91
SFR ($\mu l \cdot min^{-1}$)	0.56 (0.46 – 0.66)	0.25	0.55 (0.43 – 0.67)	0.46	0.57 (0.44 – 0.71)	0.28
Sosm ($mOsm \cdot kg^{-1}$)	**0.76** (0.66 – 0.86)	**<0.001**	**0.76** (0.66 – 0.87)	**<0.001**	**0.76** (0.62 – 0.89)	**0.001**

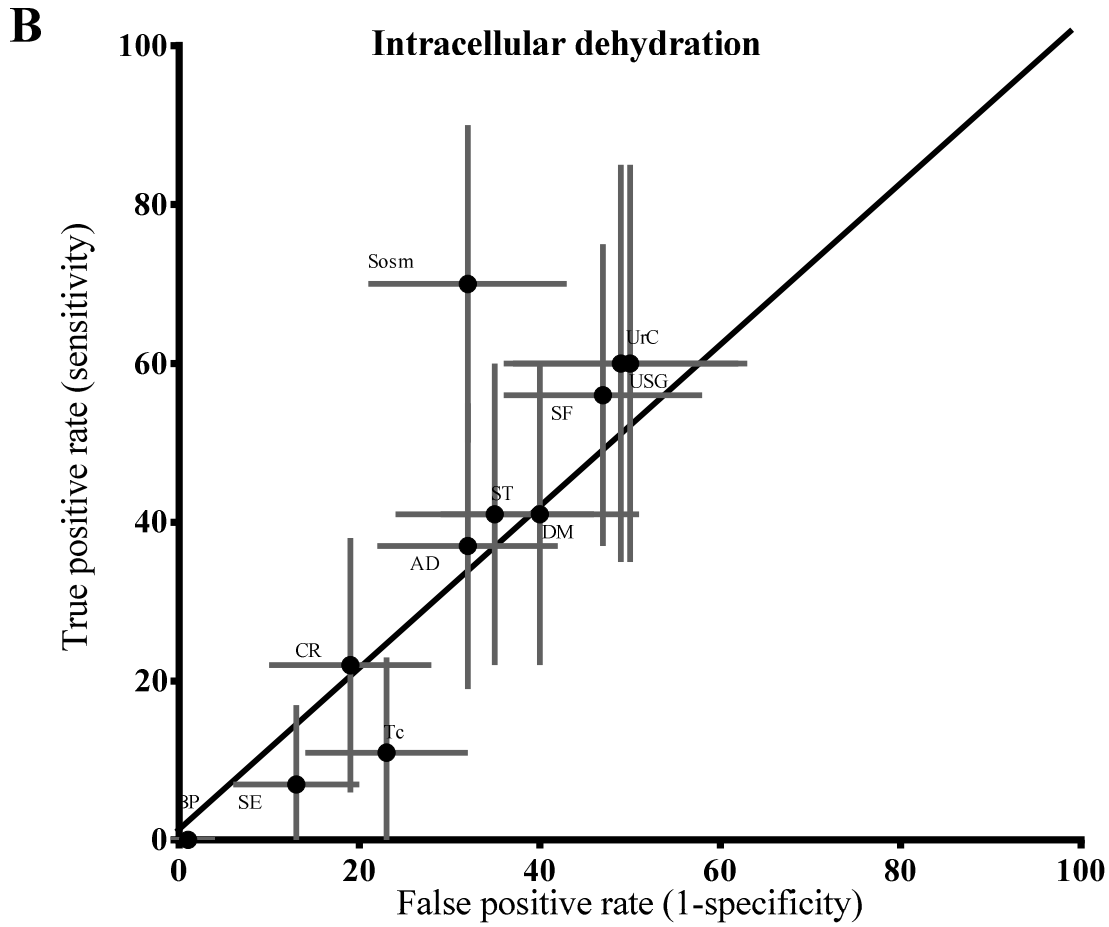
NOTE: Abbreviations: AUC_{ROC}, area under the ROC curve; USG, urine specific gravity; SFR, saliva flow rate; Sosm, saliva osmolality. *P* values represent the ability of the marker to discriminate between normal hydration and dehydration better than chance (i.e. AUCROC of 0.5).

Figure 4.2 A ROC curve comparison between clinical physical signs, saliva and urine indices for the assessment of both forms of dehydration combined.



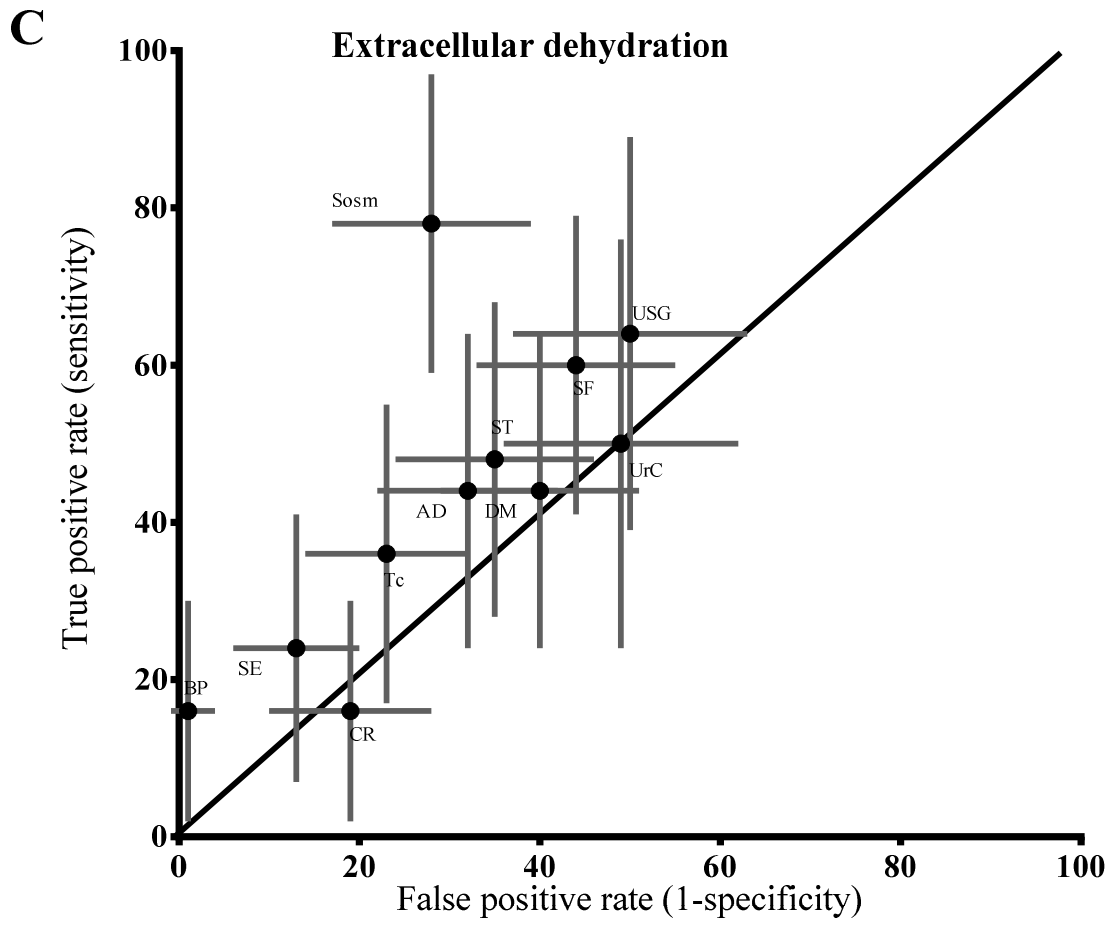
NOTE: Abbreviations: BP, low systolic blood pressure; SE, sunken eyes; CR, capillary refill time; Tc, Tachycardia; AD, axillary dryness, ST, skin turgor, DM, dry mucous membrane; Sosm, saliva osmolality; SF, saliva flow rate; UrC, urine colour; USG, urine specific gravity. Vertical error lines represent sensitivity 95% CI, horizontal error lines represent specificity 95% CI.

Figure 4.2 B ROC curve comparison between clinical physical signs, saliva and urine indices for the assessment of intracellular dehydration only.



NOTE: Abbreviations: BP, low systolic blood pressure; SE, sunken eyes; CR, capillary refill time; Tc, Tachycardia; AD, axillary dryness, ST, skin turgor, DM, dry mucous membrane; Sosm, saliva osmolality; SF, saliva flow rate; UrC, urine colour; USG, urine specific gravity. Vertical error lines represent sensitivity 95% CI, horizontal error lines represent specificity 95% CI.

Figure 4.2 C ROC curve comparison between clinical physical signs, saliva and urine indices for the assessment of extracellular dehydration only.



NOTE: Abbreviations: BP, low systolic blood pressure; SE, sunken eyes; CR, capillary refill time; Tc, Tachycardia; AD, axillary dryness, ST, skin turgor, DM, dry mucous membrane; Sosm, saliva osmolality; SF, saliva flow rate; UrC, urine colour; USG, urine specific gravity. Vertical error lines represent sensitivity 95% CI, horizontal error lines represent specificity 95% CI.

4.5 DISCUSSION

Dehydration in older adults is a leading cause of hospitalisations, contributing to increased morbidity and mortality during clinical care, and poorer functional status of the individual (O'Neill et al., 1990; Warren et al., 1994; Bhalla et al., 2000; Stookey, 2005; Rowat et al., 2012). As such, early identification of hydration status is paramount to prevent the development of further co-morbidities, and to reduce the burden on healthcare (Warren et al., 1994; Xiao et al., 2004). In an absence of NICE clinical guidelines to assess hydration status in older adults admitted to hospital, this prospective study sought to investigate the diagnostic accuracy of routinely used clinical physical signs and urine indices, and novel, simple, non-invasive saliva indices. The main finding was that currently used clinical physical signs were not able to discriminate between dehydration and normal hydration, and thus provide little help to the physician making an initial hydration assessment. Whilst showing promise in young healthy cohorts (Armstrong et al., 1994; Oppliger et al., 2005; Popowski et al., 2001), urine analysis demonstrated no utility in identifying dehydration in an older adult cohort admitted to hospital. However, the novel finding from the study was that saliva osmolality could discriminate between dehydration and normal hydration, and importantly, was sensitive to both intracellular and extracellular types of dehydration, demonstrating superior diagnostic accuracy than urinary parameters and currently used clinical physical signs. Saliva collection is non-invasive, compared to urine or blood, therefore this marker may have practical utility as an initial screening method for dehydration in older adults admitted to hospital. The clinical significance is that most markers identify one type of dehydration and therefore may misdiagnose dehydration *per se*. As an initial screening tool saliva osmolality could be used to inform further biochemistry to confirm the type of dehydration and treatment with appropriate rehydration solutions.

Despite a relative paucity of clear supporting evidence, and lack of clinical NICE guidelines, clinicians may rely on an array of simple physical screening tests to aid the hydration assessment of patients admitted to hospital. Whilst showing some clinical promise in young children (Gorelick, Shaw, & Murphy, 1997; Steiner, DeWalt, & Byerley, 2004), clinical physical signs often demonstrate poor diagnostic performance when applied to older adults, likely due to; a loss of skin elasticity with advancing age affecting skin turgor, smoking and cold environmental temperatures causing peripheral vasoconstriction which may result in false positives for capillary refill time, and anticholinergic medications and a reliance on mouth breathing in the elderly which can result in a dry oral mucosa (McGee et al., 1999; Sarhill et al., 2001; Weinberg & Minaker, 1995). Findings from previous studies investigating the utility of clinical physical signs should also be viewed with caution where they have adopted a non-criterion reference standard, e.g. difference in weight gain after rehydration, urinary measures, or relied on a clinicians overall diagnosis (Vivanti, 2010; Vivanti et al., 2008; Wakefield et al., 2002). A strength of the current study is the use of objective biochemical criterion measure such as plasma osmolality to distinguish between hydration states (Weinberg & Minaker, 1995; McGee et al., 1999; Thomas et al., 2004; Stookey, 2005; Thomas et al., 2008; Hooper et al., 2012). Furthermore, previous studies have been limited by failing to characterise the diagnostic accuracy of clinical physical signs in assessing both forms of dehydration commonly encountered in a clinical setting, i.e. intracellular, and extracellular dehydration (Thomas et al., 2008).

A particular strength of the current study was that both forms of dehydration were characterised simultaneously using valid biochemical assessments as reference standards, including the preferred direct measurement of plasma osmolality as opposed to calculated osmolality (Hooper et al., 2012). We observed that no clinical physical sign could

discriminate between either type of dehydration and normal hydration when assessed using AUC_{ROC}, and thus, should not be used in isolation to diagnose hydration status in older adults admitted to hospital.

Urinary markers have been reported as valid methods to assess acute changes in hydration status in young healthy people (Armstrong et al., 1994). In the current study, neither USG nor urine colour were able to discriminate between dehydration and normal hydration. This is likely due in part, to the decreased renal function that is characteristic of older age, and to a potential confounding effect on urine of the many types of medications that a hospitalised older adult cohort are likely to be prescribed. In support, previous studies have also shown that urine indices are poor markers of hydration status in elderly patients, in critically ill patients and in young children with gastroenteritis (Fletcher et al., 1999; Rowat et al., 2012; Steiner, Nager, & Wang, 2007; Vivanti et al., 2008). Urine collection is not always possible when required, and was only able to be collected in 65% of participants in the current study, and in only 79% of elderly patients in a recent clinical study (Vivanti et al., 2008). Taken together, we do not recommend the use of USG or urine colour as screening tools for dehydration in a hospitalised older adult cohort.

To the best of our knowledge, this is the first study that has investigated the diagnostic accuracy of saliva indices to assess dehydration in older adults admitted to hospital. Saliva sample collection is simple and non-invasive and has previously been shown to track modest intracellular dehydration in young healthy males (Walsh et al., 2004a; Walsh et al., 2004b; Oliver et al., 2008). Saliva flow rate did not have utility or the required diagnostic accuracy to discriminate between normal hydration and either type of dehydration, but the novel finding of the current study was that saliva osmolality was able to detect both forms of dehydration

with sensitivity >70%. Although an AUC_{ROC} of 0.7 may only be described as "fair to moderate", it is important to stress that any novel diagnostic marker should be compared against what is currently used in clinical practice (Obuchowski et al., 2004). In the case of the present study, a high saliva osmolality (>94mOsm/kg) was able to detect more cases of both types of dehydration than any single clinical physical sign or urinary marker without compromising specificity (**Figure 4.2**). Furthermore, the cohort in the current study reflects a representative, older adult clinical population, admitted with any primary diagnosis, and we did not remove participants taking medications (except for 4 patients taking glucocorticoid medications), thus the fact that a single marker is able to achieve a sensitivity > 70% for both types of dehydration at one-point in time is promising. Moreover, we set our reference standard cut-off at the lower end of the dehydration continuum to reflect impending, mild or pre-clinical dehydration (Stookey, 2005; Thomas et al., 2008; Hooper et al., 2012). Therefore, the measurement of saliva osmolality may have practical utility in identifying those individuals with mild dehydration, so that further biochemistry analysis can confirm the presence of, and type of dehydration, in order that specific, tailored rehydration is commenced to prevent the patient developing more severe dehydration along with its associated co-morbidities and poorer outcomes.

There are a few limitations of saliva sampling that we must acknowledge. Firstly, in the current study, saliva volume was sufficient for analysis in only 75% of the samples due to the requirement of a 25µl sample volume for analysis (albeit this is greater than the 65% of urine samples collected). However, point of care devices that utilise nano-technology for the assessment of saliva osmolality are under development (Miller et al., 2010; Woods & Mentes, 2011). For example, the osmolarity of tears can currently be assessed using the principle of impedance on as little as 50nl (Fortes et al., 2011b). Thus, this limitation should not be seen

to detract from the future application of saliva osmolality to assess hydration status in clinical care. Secondly, with saliva sampling in a clinical population, there may be certain patients who are unable to provide a saliva sample e.g. unconscious, stroke or mentally ill. However, we excluded only two participants with swallowing problems, further research should investigate the diagnostic utility of saliva indices in patients with these, and other oral-related problems (eg, oral trauma, recent dental surgery, salivary gland tumors etc). A further potential confounding effect could be anything which can affect saliva flow rate, e.g. anticholinergic medications, or recent food/fluid consumption (Ely et al., 2011; Perrier et al., 2013). This is potentially important since a decrease in saliva flow explained in part, the increase in saliva osmolality observed during acute dehydration in young healthy males (Walsh et al., 2004a; Walsh et al., 2004b; Oliver et al., 2008). However, in this series of studies the authors observed only a small association between saliva flow rate and osmolality (r = -0.40), suggesting that in the current study, saliva osmolality was largely independent of saliva flow rate. The physiological mechanisms responsible for an increase in saliva osmolality during dehydration are unclear, but may be due to an increase in water absorption in the saliva gland and/or neural factors (Walsh et al., 2004a; Walsh et al., 2004b; Oliver et al., 2008).

In conclusion, physical signs and urine markers show little utility to determine if an elderly patient is dehydrated. Saliva osmolality demonstrated superior diagnostic accuracy compared with physical signs and urine markers for the assessment of both intracellular and extracellular dehydration. The measurement of saliva osmolality has potential utility as a screening method to aid the diagnosis of dehydration in older adults admitted to hospital.

CHAPTER FIVE

Diagnostic accuracy and practicality of novel stimulated saliva hydration markers to identify mild to modest dehydration.

5.1 Summary

The aim of the study was to examine the effect of stimulating saliva on its utility and diagnostic accuracy as a marker of mild to moderate dehydration (1-3% of body mass) and if viable whether stimulating saliva would increase the practicality of saliva hydration markers by increasing saliva volume and decreasing collection time. On two randomized occasions, 14 participants (11 male, 3 female) exercised in the heat, either without fluids to evoke progressive body mass losses (BML) of 1%, 2%, and 3% (DEH) or with fluids to offset losses (CON). Markers of hydration status were measured before exercise and at each magnitude of dehydration. Stimulated whole saliva flow rate (STIM-SFR) was lower with dehydration than CON at 2% ($P = 0.03$) and 3% BML ($P = 0.02$). Stimulated whole saliva osmolality (STIM-Sosm) was not different to CON at any magnitude of dehydration. STIM-SFR had adequate diagnostic accuracy to discriminate between CON and dehydration at 3% BML ($AUC_{ROC} = 0.74$), but had less discriminatory power than unstimulated saliva parameters (SFR and Sosm), urine specific gravity and plasma osmolality at this magnitude of dehydration ($P < 0.05$). In addition, stimulating saliva improved the practicality of saliva hydration markers by increasing saliva flow rate by eight-fold and therefore reduced saliva collection time by 85%. In contrast to STIM-Sosm, STIM-SFR has some utility as a marker of dehydration at 3% BML and could provide a quick, non-invasive hydration marker in active populations in field-based settings.

5.2 Introduction

Many markers of hydration have been proposed such as dye or isotope-dilution techniques, haematological and urinary markers and techniques based on bioelectrical impedance. However, these assessment techniques are often expensive, invasive, are technically demanding or lack the required diagnostic accuracy to identify mild to modest dehydration (Armstrong, 2007). The use of saliva hydration parameters may provide an alternative practical solution to hydration assessment in clinical and active populations because saliva collection is simple, cost-effective and non-invasive. Unstimulated whole saliva osmolality and to a lesser degree saliva flow rate have been shown to have utility in identifying hydration status in a young healthy population and correlates strongly with the more widely accepted hydration indices of plasma osmolality (Posm) and urine specific gravity (USG) (Walsh et al., 2004a; Walsh et al., 2004b; Oliver et al., 2008). However, in some people with a low saliva flow rate, it can take several minutes (> 15 min) to collect sufficient saliva volume. Often, >200 μL of saliva is required to accurately determine saliva flow rate and provide enough volume for osmolality analysis and additional routine protein and immunological analyses, potentially compromising the practicality of saliva based hydration assessment (Oliver et al., 2008; Taylor et al., 2012) . This is particularly relevant to the older person as saliva flow rate decreases with age, and is pertinent to active populations because saliva flow rate decreases with progressive dehydration (Ben-Aryeh et al., 1984; Shirreffs et al., 2005; Maughan et al., 2005). Others have suggested that the inherent variability of saliva hydration parameters make their use as hydration markers uncertain (Ely et al., 2011; Taylor et al., 2012).

Stimulation of saliva with either a gustatory (e.g. citric acid) or masticatory (e.g. chewing) stimulus has been shown to increase saliva flow rate and therefore decrease saliva collection-

time by up to nine-fold compared with unstimulated saliva secretion (Flink et al., 2008). However, stimulation of saliva alters its composition resulting in an increase in pH and concentrations of total protein, sodium, chloride and bicarbonate and decreases in the concentration of other electrolytes such as magnesium and phosphate (Dawes, 1984). Despite a significant correlation between unstimulated and stimulated whole saliva flow rate (Sreebny & Schwartz, 1997), the effect of stimulating saliva on the utility of saliva osmolality and flow rate as a marker of hydration status is unknown. In addition, stimulation of saliva has been shown to decrease the variability in saliva flow rate by over 50%, which could improve the utility of these markers (Ben-Aryeh et al., 1984; Ljungberg et al., 1997). Therefore, the primary aim of this study was to examine the effect of stimulating saliva on the utility and diagnostic accuracy of saliva osmolality and flow rate to identify progressive acute intracellular dehydration evoked by exercise and heat-stress (1-3% BML). We hypothesised that stimulating saliva would improve the diagnostic accuracy of saliva osmolality and flow rate markers to identify mild to modest dehydration. In addition, we also hypothesised that stimulating saliva would improve the practicality of saliva based hydration markers by increasing saliva volume and therefore decreasing saliva collection time.

5.3 Methods

Participants

Fourteen healthy participants (eleven males and three females) volunteered to take part in the study (age, 23 ± 5 years (mean ± SD); height, 181 ± 8 cm; body mass, 78.4 ± 9.8 kg; maximal oxygen uptake ($\dot{V}O_{2max}$), 56.4 ± 7.4 $mL \cdot kg^{-1} \cdot min^{-1}$). Volunteers were all non-smokers and were not taking any prescription or non-prescription medications at the time of the study. No participant had known periodontal disease or recent oral trauma or dental surgery. Participants were required to abstain from caffeine, alcohol, and unaccustomed exercise for 24 h before the preliminary and experimental trials. Although trials were not scheduled to coincide with a certain phase of the menstrual cycle, baseline hydration status (Posm) of the three female participants was not significantly different between trials ($P = 0.1$). Additionally, it has been demonstrated that the change in Posm during dehydration and rehydration is not altered by menstrual cycle phase or oral contraceptive use (Stachenfeld, Silva, Keefe, Kokoszka, & Nadel, 1999).

Preliminary measures

Peak power output (PPO) and maximal oxygen consumption ($\dot{V}O_{2max}$) were estimated using the procedure described in **Chapter 3**. Following a 20-min rest, participants then cycled in an environmental chamber (Delta Environmental Systems, Chester, UK), maintained at a dry bulb temperature of 33 °C and 50% relative humidity, at 55% of PPO without fluids for 30 min to estimate whole-body sweating rate and exercise duration for the experimental protocol. A 3 min resting, unstimulated and stimulated saliva sample was collected using a Versi-SAL collection device (Oasis Diagnostics Corporation, Vancouver, Washington, USA) before and immediately after exercise to familiarize the participant with the collection

procedure (see section Saliva sample collection, handling, and analysis) and to ascertain the appropriate saliva collection time for the experimental trials. This was performed to avoid saturating the collection device in participants with high saliva flow rates and to collect sufficient saliva for osmolality analysis in participants with low saliva flow rates.

Experimental design

The effect of dehydration on stimulated saliva osmolality and flow rate was investigated using a within-subject repeated measures design. Participants reported to the laboratory on two occasions, separated by 7-14 days, at 0800 h following an overnight fast. Dietary intake was controlled during both trials by providing subjects with their estimated daily energy requirements multiplied by a physical activity factor (Harris & Benedict, 1918; World Health Organisation, 1985). Standardized meals were provided at 0815 h and 1200 h on the day of the trial. During the morning prior to exercise (08:00-14:00), plain water was provided proportional to 40 $mL \cdot kg^{-1} \cdot d^{-1}$ body mass to ensure subjects began exercise normally hydrated. Subjects were instructed to perform only light activity before returning to the laboratory at 13:45.

The intervention began at 1400 h after the participant had voided and a nude body mass (model 705, Seca, Hamburg, Germany) had been obtained. Pre-exercise unstimulated and stimulated saliva, blood, and urine samples were collected after the participant had been seated quietly for at least 5–10 min. The participant performed three consecutive exercise bouts with a 15-min rest between each bout to collect samples and facilitate completion of the dehydration protocol. The exercise consisted of cycling at a power output corresponding to 55% PPO in hot conditions (33 °C and 50% relative humidity) to evoke sweat loss. The exercise duration for each bout was predetermined via estimated sweat rates from the

preliminary measurements to equate to progressive approximate body mass losses (BML) of 1%, 2%, and 3% (mean sweat rate 1.3 $L \cdot h^{-1}$; 1.4 $L \cdot h^{-1}$ for males and 0.9 $L \cdot h^{-1}$ for females). Between each bout, participants left the chamber, removed their clothing, and dried with a towel and were weighed nude to determine BML. On one occasion, the participant received no fluid throughout the exercise (dehydration trial; DEH), and on the other occasion, the participant received three equal volumes of water to offset estimated sweat losses for each exercise bout (control trial; CON). These trials were completed in a randomized and counterbalanced order. Unstimulated and stimulated saliva, blood, and urine samples were collected at 1%, 2%, and 3% BML in the DEH trial and at the equivalent time points in the CON trial.

Assessment of hydration status – blood and urine

Blood and urine was collected and plasma osmolality (Posm) and urine specific gravity (USG) and urine colour were measured immediately as described in **Chapter 3**.

Saliva sample collection, handling, and analysis

Unstimulated and stimulated whole-saliva samples were collected using a pre-weighed Versi-SAL collection device. Unstimulated and stimulated saliva osmolality (Sosm and STIM-Sosm) and saliva flow rate (SFR and STIM-SFR) were measured as described in **Chapter 3**.

Statistical analysis

The required sample size for the study was estimated as 12 (G*Power, Version 3.1.2); using standard alpha (0.05) and beta levels (0.8), and mean and standard deviations of studies that have previously examined the effect of mild dehydration on endurance exercise performance (Armstrong et al., 1985). Data in the text and tables are presented as means ± SD, whereas for

clarity data in figures are presented as mean and standard errors of mean (± SEM). Significance was accepted at $P < 0.05$. One-way repeated measures analysis of variance (ANOVA) was performed on pre-experimental body mass, Posm and USG. To examine the effect of progressive dehydration on dehydration markers a two-way repeated measures ANOVA (2 trials x 4 time points) was performed on BML, Posm, USG, and both stimulated and unstimulated saliva flow rate and saliva osmolality (Prism version 6.0, GraphPad Software Inc., La Jolla, CA). Appropriate adjustments to the degrees of freedom were made in cases where the assumptions of sphericity and normality were violated. Post hoc Bonferroni adjusted t-tests were used where appropriate. To assess the diagnostic accuracy of stimulated saliva hydration parameters for assessment of hydration status, the dehydration trial, at each magnitude of dehydration, was compared with the normal hydration control trial. For all hydration markers including Posm, USG, urine colour and stimulated and unstimulated saliva osmolality and flow rate he area under the receiver operating characteristic curve (AUC_{ROC}) was used as a measure of global diagnostic accuracy (**see Chapter 3**). In addition, hydration marker cut-offs and the sensitivity and specificity of these cut-offs were calculated where appropriate (**Chapter 3**).

5.4 Results

Based on individual pre-established sweat rates (mean ± SD: 1.2 ± 0.4 $L \cdot h^{-1}$, range = 0.8–2.1 $L \cdot h^{-1}$), the total exercise duration on both the CON and DEH trials was 107 ± 18 min (range = 83–137 min), during which power output was maintained at 157 ± 21 W (range = 128–194 W).

5.4.1 Body mass and hydration status

To evaluate the success of the exercise and heat induced protocol to evoke progressive dehydration we used three indices of hydration status; BML, Posm and USG (Cheuvront et al., 2010). Before exercise there were no differences in body mass between trials, and values of Posm and USG were within normal hydration ranges (**Table 5.1**) (Casa et al., 2005). Fluid intake on CON maintained normal hydration during exercise as indicated by similar body mass, Posm and USG compared with pre-exercise values (**Table 5.2**). Fluid restriction on DEH was successful in achieving the target BMLs of 1%, 2%, and 3% (**Table 5.2**). In addition, Posm and USG increased at 2% and 3% BML compared with pre-exercise values ($P < 0.001$ for 2% and 3% BML) and were higher on DEH compared with CON at 1% BML for Posm ($P = 0.004$), and at 2% and 3% BML for Posm and USG ($P < 0.001$) (**Table 5.2**).

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Table 5.1 Hydration indices before each trial.

Dehydration marker	Threshold for mild to modest dehydration [a]	CON	DEH	*P-value*
Body mass (kg)	N/A	74.7 ± 9.3 (69.3 – 80.1)	74.7 ± 9.8 (69.1 – 80.4)	0.9
Posm (mOsm·kg^{-1})	≥ 290	287 ± 6 (283 – 291)	287 ± 4 (285 – 289)	0.9
Urine colour (1-8)	≥ 4	2 ± 1 (1 - 2)	2 ± 1 (1 - 2)	0.6
USG (g·ml^{-1})	≥ 1.020	1.007 ± 0.003 (1.006 – 1.009)	1.009 ± 0.006 (1.006 – 1.012)	0.2
Sosm (mOsm·kg^{-1})	> 61 [b]	48 ± 11 (42 – 55)	50 ± 11 (44 – 56)	0.5
SFR (µl·min^{-1})	N/A	313 ± 211 (191 – 435)	293 ± 205 (175 – 412)	0.6
STIM-Sosm (mOsm·kg^{-1})	N/A	78 ± 20 (67 – 89)	76 ± 25 (62 – 90)	0.7
STIM-SFR (µl·min^{-1})	N/A	1359 ± 956 (808 – 1911)	1335 ± 1232 (623 – 2046)	0.9

NOTE: Values represent means ± SD (95% CI). Abbreviations: CON, control trial; DEH, dehydration trial; AUC_{ROC}, area under the ROC curve; Posm, plasma osmolality; USG, urine specific gravity; Sosm, saliva osmolality; SFR, saliva flow rate; STIM, stimulated saliva.[a] normal hydration threshold values (Casa et al., 2005); [b] threshold derived from Walsh, et al. 2004.

Table 5.2 Actual BML, Posm and USG responses during progressive exercise and heat induced dehydration to 1%, 2% and 3% target during fluid restriction (DEH) and fluid intake to off-set fluid losses.

		Target % BML on DEH or equivalent time on CON			
		0%	**1%**	**2%**	**3%**
Actual body mass loss (%)	CON	N/A	0.1 (0.4) ##	0.1 (0.4) ##	0.2 (0.4) ##
	DEH	N/A	-1.1 (0.2) **	2.0 (0.2) **	2.9 (0.2) **
Posm (mOsmol·kg^{-1})	CON	287 ± 6	283 ± 7 ##	281 ± 6 ##	280 ± 7 ##
	DEH	287 ± 4	289 ± 4	292 ± 6 **	296 ± 5 **
USG (g·ml^{-1})	CON	1.007 ± 0.003	1.007 ± 0.004	1.010 ± 0.006 ##	1.009 ± 0.006 ##
	DEH	1.009 ± 0.006	1.010 ± 0.005	1.019 ± 0.006 **	1.022 ± 0.005 **

NOTE: Values are means ±SD (n = 14). Abbreviations: Posm, plasma osmolality; USG, urine specific gravity. Significant increase from 0% body mass loss (BML): **, $P < 0.01$. Significant between-trial differences: ##, $P < 0.01$

5.4.2 Utility of stimulated saliva hydration parameters

Before exercise there was no difference between trials for stimulated and unstimulated saliva hydration parameters and saliva osmolality was below the published threshold for mild to modest dehydration (**Table 5.1**). Stimulation of saliva did not decrease the variability in saliva flow rate in comparison to unstimulated saliva (CV = 67 and 70% for unstimulated and stimulated saliva flow rate). Dehydration resulted in a decrease in STIM-SFR at 2% and 3% BML compared with CON ($P = 0.03$ and $P = 0.02$ for 2% and 3% BML, respectively) and pre-exercise values ($P = 0.03$ and $P = 0.01$ for 2% and 3% BML, respectively). A similar decrease was also observed with SFR which was lower than CON and pre-exercise values at 1%, 2% and 3% BML ($P < 0.001$) (**Figure 5.1 A**). STIM-Sosm showed no utility as a marker of dehydration as values at each magnitude of dehydration were similar to pre-exercise values and CON ($P = 0.34$). Conversely, fluid restriction on DEH resulted in an increase in Sosm at 1%, 2% and 3% BML compared with pre-exercise values and CON ($P < 0.001$) (**Figure 5.1 B**).

5.4.3 Diagnostic accuracy of stimulated saliva as a marker of dehydration

Further analysis of the utility of STIM-SFR as a marker of dehydration showed that the diagnostic accuracy to discriminate between normal hydration and dehydration was classified between poor and fair at 1% BML ($AUC_{ROC} = 0.63$ (0.11)). The diagnostic accuracy remained between poor and fair at 2% BML, however, increased to between fair and moderate at 3% BML (**Table 5.2**). Comparison of ROC curves showed that SFR ($P = 0.01$), Sosm ($P = 0.04$), Posm ($P = 0.01$) and USG ($P = 0.03$) had superior diagnostic accuracy compared with STIM-SFR at this magnitude of dehydration (3% BML). However, there were no differences in diagnostic accuracy between, plasma, urine or unstimulated saliva hydration parameters with mild or modest dehydration (i.e. 2 and 3% BML) (**Table 5.2**).

A

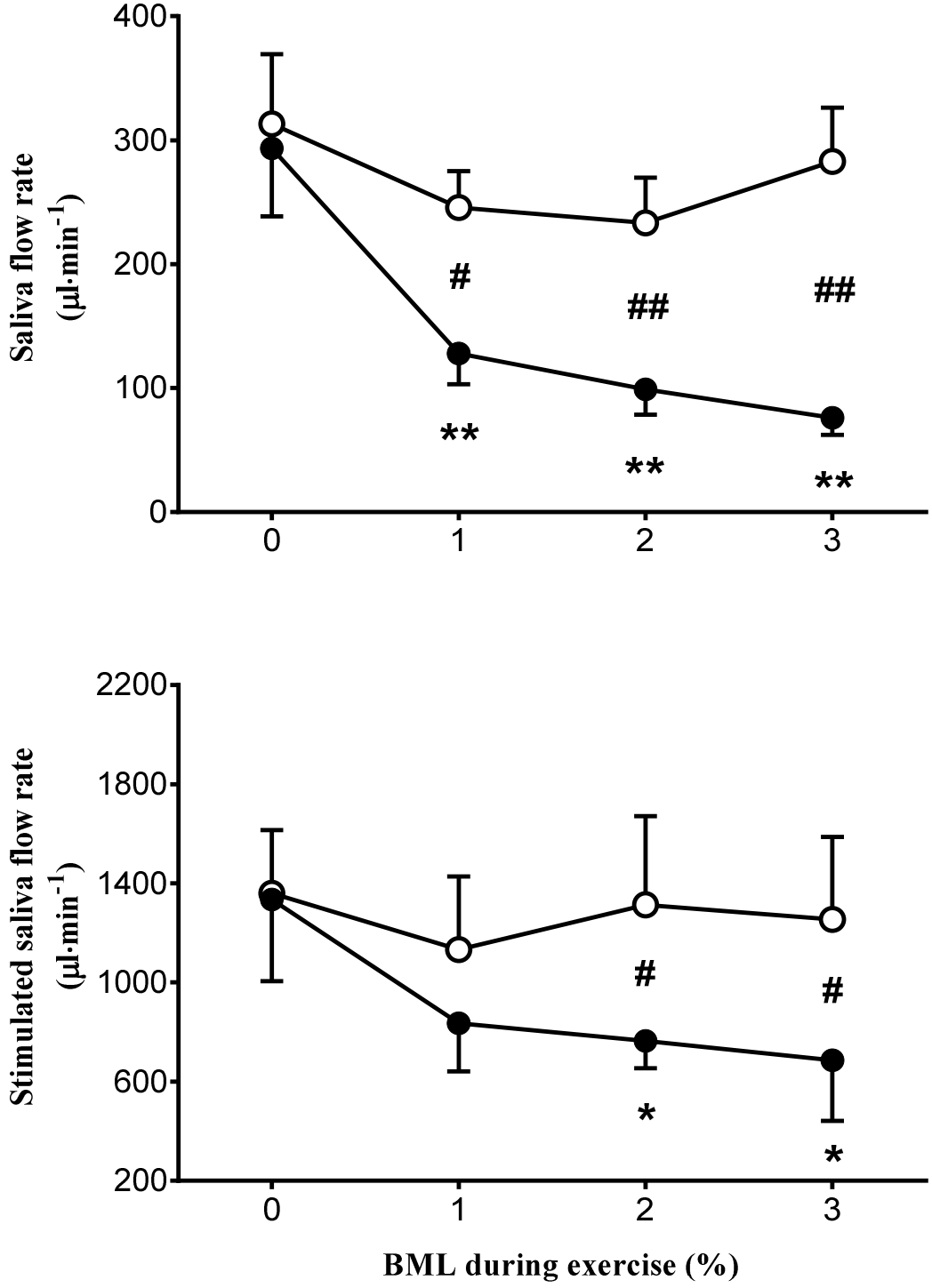

Figure 5.1 A Stimulated and unstimulated saliva flow rate responses during progressive exercise and heat induced dehydration to 1%, 2% and 3% target BML.

B

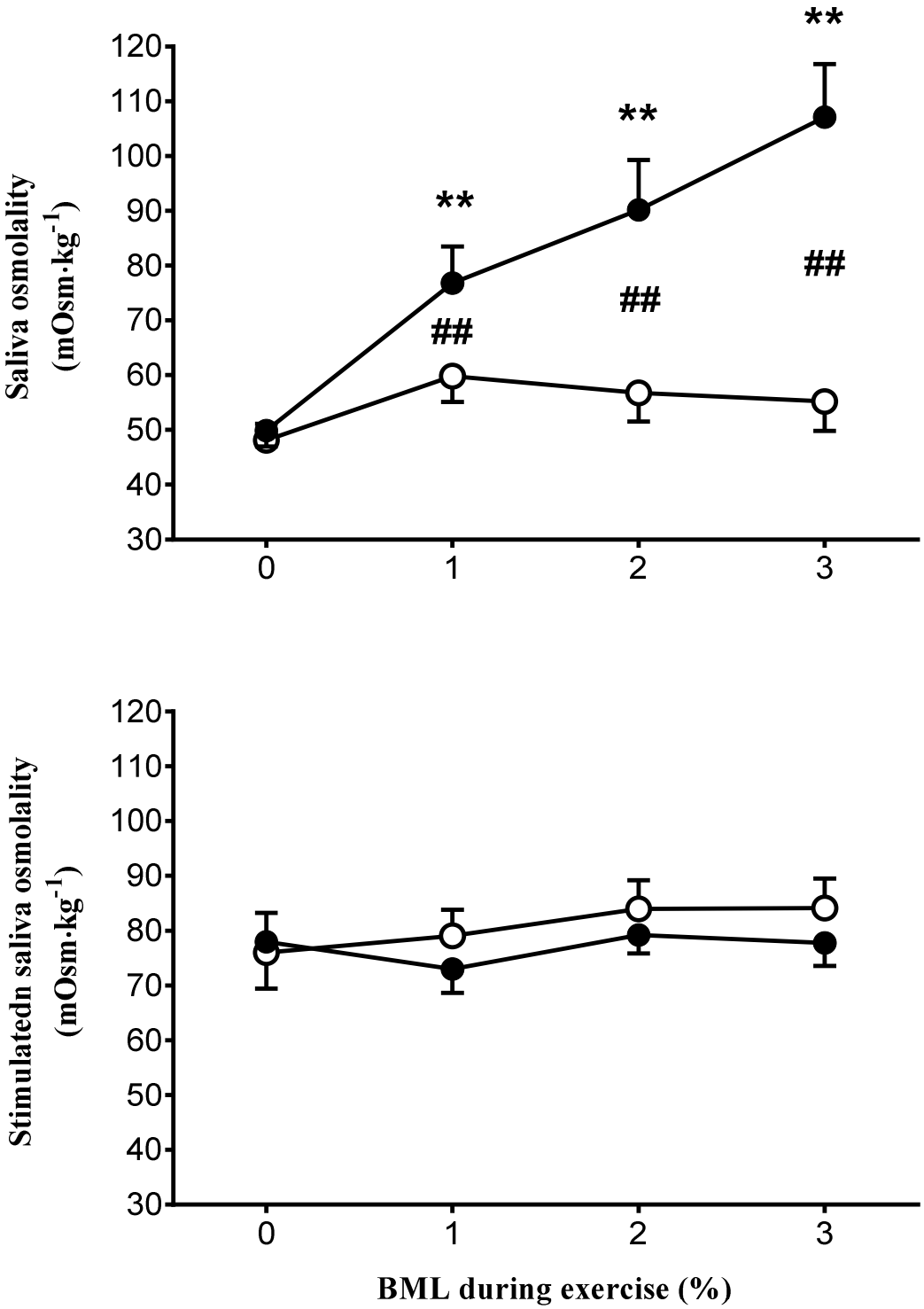

Figure 5.1 B Stimulated and unstimulated saliva osmolality responses during progressive exercise and heat induced dehydration to 1%, 2% and 3% target BML.

Table 5.3 Receiver operating characteristic (ROC) analysis of hydration markers in identifying dehydration equal to 2% and 3% BML.

	2% BML		**3% BML**	
	AUC_{ROC} (95% CI[a])	***P* value**	**AUC_{ROC} (95% CI[a])**	***P* value**
Sosm ($mOsm \cdot kg^{-1}$)	**0.82** (0.63 - 0.94)	**< 0.001**	**0.91** (0.73 – 0.98)	**< 0.001**
SFR ($\mu l \cdot min^{-1}$)	**0.83** (0.65 – 0.95)	**< 0.001**	**0.94** (0.78 – 0.99)	**< 0.001**
STIM-Sosm ($mOsm \cdot kg^{-1}$)	0.56 (0.36 – 0.75)	0.59	0.59 (0.39 – 0.77)	0.39
STIM-SFR ($\mu l \cdot min^{-1}$)	0.65 (0.45 – 0.82)	0.16	**0.74** (0.54 – 0.89)	**0.01**
Posm ($mosmol \cdot kg^{-1}$)	**0.93** (0.78 – 0.99)	**< 0.001**	**0.98** (0.85 – 1.00)	**< 0.001**
USG ($g \cdot ml^{-1}$)	**0.85** (0.67 – 0.96)	**< 0.001**	**0.93** (0.77 – 0.99)	**< 0.001**
Urine colour	0.69 (0.49 - 0.85)	0.06	**0.95** (0.79 – 0.99)	**< 0.001**

NOTE: n = 14. Abbreviations: AUC_{ROC}, area under ROC curve; Sosm, saliva osmolality; SFR, saliva flow rate; STIM, stimulated saliva; Posm, plasma osmolality; USG, urine specific gravity. [a] binomial exact 95% confidence interval. *P* values represent the ability of the marker to discriminate between normal hydration and dehydration better than chance (i.e. AUC_{ROC} of 0.5).

Table 5.4 Sensitivity and specificity of hydration markers to Youden's index derived thresholds at 2 and 3% BML.

	Threshold for mild dehydration (95% CI)	2% BML		3% BML	
		Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Sosm (mOsm·kg^{-1})	> 61 (43 – 103)	79 (49 – 95)	79 (49 – 95)	86 (49 – 95)	79 (57 – 98)
STIM-SFR (µl·min^{-1})	≤531 [a] (428 – 599)	N/A	N/A	71 (42 – 92)	71 (42 – 92)
Posm (mOsm·kg^{-1})	> 287 (280 – 287)	86 (57 – 98)	100 (77 – 100)	93 (66 – 100)	100 (77 – 100)
USG (g·ml^{-1})	> 1.015 (1.006 – 1.017)	79 (49 – 95)	79 (49 – 95)	79 (49 – 95)	86 (57 – 98)

NOTE: n=14. Abbreviations: Sosm, saliva osmolality, Posm, plasma osmolality, USG, urine specific gravity; STIM, stimulated saliva. 95% CI, 95% confidence interval. [a] threshold for modest dehydration (3% of body mass).

5.4.4 Practicality of stimulated saliva as a marker of dehydration

The sensitivity and specificity of the Youden index derived threshold for STIM-SFR to modest dehydration (3% of body mass) suggested that ten out of fourteen participants would have been correctly identified as either with normal hydration or modest dehydration (**Table 5.4**). Across hydration states stimulating saliva resulted in an eight fold increase in whole saliva flow rate. With normal hydration SFR was 293 (205) $\mu l \cdot min^{-1}$ and decreased progressively to 76 (52) $\mu l \cdot min^{-1}$ following dehydration to 3% of body mass. STIM-SFR also decreased progressively during dehydration from a high at normal hydration of 1335 (1232) $\mu l \cdot min^{-1}$ to 686 (916) $\mu l \cdot min^{-1}$ at 3% BML. From a practical perspective this increase in saliva flow rate with stimulation meant that the saliva collection time for a 200µl sample of saliva decreased by 85% across all hydration states (79%, 90%, 91% and 81% reduction in the collection time of 200 µl of saliva at 0, 1%, 2% and 3% respectively) (**Table 5.5**). At 3% BML mean collection time of 200 µl for unstimulated saliva was four and a half minutes with a range between one minute and fourteen minutes. At this magnitude of dehydration the mean collection time of the same volume of stimulated saliva was less than one minute with a range between three seconds and three minutes.

Table 5.5 Time taken to collect a 200µl sample of saliva when euhydrated and at 1%, 2% and 3% BML during stimulated and unstimualted collection procedures.

	Time to collect 200 µl saliva sample at each % BML on the DEH trial							
	0%		1%		2%		3%	
	Time (SD)	Range	Time (s)	Range (s)	Time (s)	Range (s)	Time (s)	Range (s)
Unstimulated collection (s)	81 (87)	18 - 343	188 (183)	56 - 551	245 (246)	45 - 546	268 (222)	61 - 857
Stimulated collection (s)	18 (16)	3 - 61	18 (7)	4 - 28	23 (17)	9 - 63	50 (48)	3 - 173

NOTE: Values are means ±SD (n = 14).

5.5 Discussion

The aim of the study was to test the hypotheses that stimulated saliva flow rate and saliva osmolality would; 1) have utility and adequate diagnostic accuracy to identify mild to modest dehydration evoked by exercise-heat stress and 2) improve the practicality of saliva dehydration markers by increasing saliva flow rate and therefore reducing saliva sample collection time. In agreement with our hypothesis STIM-SFR did have potential utility in identifying mild (2% BML) and modest (3% BML) dehydration following exercise heat-stress. However, further analysis showed that STIM-SFR had poor diagnostic accuracy at lower magnitudes of fluid deficit (1-2% BML) and fair to moderate diagnostic accuracy in identifying modest dehydration (AUC_{ROC} = 0.74 at 3% BML). In contrast to our hypothesis STIM-Sosm had no utility and poor diagnostic accuracy as a marker of dehydration. A further finding and in agreement with our hypothesis was that stimulating saliva reduced the time to collect a set volume of saliva (200 µl) by 85% across different hydration states. The findings from this study may be appealing to applied practitioners in the athletic setting as field-based hydration assessment with STIM-SFR is a rapid (< 1 min), low cost and non-invasive procedure. This may be particularly relevant in populations where multiple hydration assessments need to be completed and modest fluid deficits are common-place e.g. fire service, military, athletic populations and those venturing to austere environments.

To the author' knowledge this is the first study to demonstrate that stimulated saliva flow rate has some utility as a marker of dehydration. Probability statistics suggested that this novel dehydration marker had efficacy at both mild (2% BML) and modest magnitudes of intracellular dehydration (3%). However, diagnostic accuracy analysis suggests that STIM-SFR may only be useful at identifying dehydration at or above 3% BML. It is purported that exercise-induced dehydration that exceeds 2% of body weight will impair sport and exercise

performance (Cheuvront et al., 2003). Therefore, this may limit the practicality of stimulated saliva flow rate as a marker of dehydration in field-based settings. However, this marker could be useful as a basic measure of modest dehydration in athletic or occupational field-based settings due to the ease and speed of saliva collection allied to the non-invasive and non-technical nature of saliva collection. This is even more pertinent as there are currently no 'gold standard' hydration assessment techniques. Expensive isotope methodologies, such as deuterium or deuterium oxide, and haematological indices are considered the best methods to assess hydration status, but, these methods are time-consuming, analytically complex and invasive. Alternative non-invasive markers of dehydration include urinary, ocular and subjective ratings such as thirst. However, urinary markers identify dehydration with varied success, limited research is available on ocular measures such as tear osmolarity and thirst may be confounded by stressors such as exercise and exposure to cold, heat and high-altitude (Cheuvront & Sawka, 2005; Fortes et al., 2011b; Frayser et al., 1975; Popowski et al., 2001; Rogers et al., 1964; Rothstein et al., 1947). Future work to improve the diagnostic accuracy of this marker should focus on the method of stimulating saliva, as changes to the stimulus have been shown to have an effect on stimulated saliva flow rate (Dawes, 2004). Factor affecting stimulated saliva flow rate include; the nature of the stimulus which can be either gustatory or masticatory and the intensity and duration of the stimulus. In addition, the effect of rehydration or drinking on these markers should also be investigated, as has been previously carried out with unstimulated saliva hydration markers (Oliver et al., 2008).

Another finding from the current study and in contrast to our hypothesis was that stimulated saliva osmolality had no utility and poor diagnostic accuracy to identify acute intracellular dehydration. Stimulation of saliva has been reported to change the composition of saliva resulting in an increase in pH and concentrations of total protein, sodium, chloride and

bicarbonate and decreases in the concentration of other electrolytes such as magnesium and phosphate (Dawes, 1984). The reduction in utility and diagnostic accuracy of this marker can be explained in terms of the effect of stimulation on osmolality. As the osmolality of saliva is described as the concentration of electrolytes in saliva, any change in the electrolyte concentration will impact on the osmolality measured. Therefore, it is clear that the current method of stimulation resulted in an increase in the secretion of electrolytes and concomitant increase in saliva osmolality (~ 50% increase in osmolality at baseline). Clearly, this increase in osmolality observed with normal hydration and dehydration reduced the effect of dehydration on saliva osmolality.

A further important finding from the current study, which is supported by a recent publication, was that unstimulated saliva flow rate and osmolality had comparable diagnostic accuracy to plasma osmolality and urine hydration markers to identify both mild and modest acute intracellular dehydration (Muñoz et al., 2013). This is in contrast to a previous study that showed that saliva osmolality had less diagnostic accuracy than plasma and urine osmolality, urine colour and specific gravity to identify intracellular dehydration between 2-7% of body mass evoked by exercise in the heat (Cheuvront et al., 2010). This is an important finding as it shows that saliva hydration parameters are equally effective as more traditional hydration markers. From a practical perspective a mild intracellular dehydration threshold of 61 $mOsm \cdot kg^{-1}$ would have identified eleven out of fourteen participants with normal hydration or mild dehydration. Importantly, this Youden's index derived threshold for mild intracellular dehydration is the same as the threshold suggested in a previous study, and provides more support for a mild dehydration threshold for saliva osmolality, which is an important aspect of hydration marker development (Oliver et al., 2008).

In conclusion, stimulating saliva reduced the diagnostic accuracy of saliva hydration markers (osmolality and flow rate) to identify intracellular dehydration evoked by exercise and heat-stress. The results suggest that stimulated saliva osmolality has no utility and poor diagnostic accuracy to identify intracellular dehydration. However, stimulated saliva flow rate did have fair to moderate diagnostic accuracy to identify modest dehydration (3% BML). In addition, stimulating saliva reduced saliva collection time by 85% across hydration states. Therefore, stimulated saliva flow rate may be useful as a basic, non-invasive, non-technically demanding marker of modest intracellular dehydration in occupational or athletic field-based settings.

CHAPTER SIX

Diagnostic accuracy of hydration markers to identify mild dehydration in a young healthy cohort.

6.1 Summary

The aim of this study was to determine the diagnostic accuracy of hydration markers to mild intra- and extracellular dehydration. Fifteen males completed a hydration assessment after 48 h of normal hydration (CON), intracellular dehydration (ID), evoked by exercise and 48 h of fluid restriction (2 ml·kg·d^{-1}), and extracellular dehydration (ED), evoked by a 4 h diuresis (0.65 mg·kg^{-1} Furosemide). Area under the curve generated from a receiver-operating characteristic curve (AUC_{ROC}) was used to determine the global diagnostic accuracy of each hydration marker to discriminate between CON and each type of dehydration (ID and ED). Dehydration equaled 2% of body mass on ID and ED. Thirst and urine osmolality showed moderate to high overall diagnostic accuracy to discriminate between CON and mild dehydration *per se* (mean AUC_{ROC} = 0.87 and 0.90 for thirst and urine osmolality, respectively). Diagnostic accuracy to discriminate between CON and ID was perfect for urine colour and specific gravity (AUC_{ROC} = 1.00), near perfect for plasma osmolality (AUC_{ROC} = 0.95), and fair for LF-HF and saliva osmolality and flow rate (AUC_{ROC} = 0.70). Diagnostic accuracy to discriminate between CON and ED was generally poor for most markers (AUC_{ROC} < 0.70), with the exception of postural heart rate change (AUC_{ROC} = 0.80). The utility and diagnostic accuracy of hydration markers changed depending on the type of dehydration. A combination of thirst, urine colour and postural heart rate change improved dehydration diagnosis by discriminating between dehydration types, and is recommended as a non-invasive hydration assessment strategy.

6.2 Introduction

Numerous studies have examined the utility of hydration assessment markers in, blood, urine, saliva, tear and with the use of subjective sensations (Armstrong et al., 1994; Shirreffs & Maughan, 1998; Popowski et al., 2001; Walsh, et al., 2004; Oliver et al., 2008; Fortes et al., 2011; Maresh et al., 2014). Nevertheless, there is currently no consensus on the best method for assessing dehydration (Armstrong, 2007). This is partly true because dehydration is a complex condition. Indeed depending on the cause, dehydration can be due either to a water deficit, resulting in intracellular dehydration, or both a solute and water deficit, termed extracellular dehydration (Nadel et al., 1941). Most previous research has treated dehydration as a single condition and examined the utility of hydration markers to one type of dehydration (e.g. intracellular dehydration following exercise in the heat). An underappreciated factor is that the distinct physiological differences between "types" of dehydration may affect the choice of hydration assessment marker to be used and the treatment prescribed (Cheuvront & Kenefick, 2014). Currently, there is little known about the effect of dehydration type on the utility of hydration markers and whether combinations of markers can be used to improve diagnosis by identifying dehydration type.

To this end, we examined the diagnostic accuracy of a battery of hydration markers to both mild intra- and extracellular dehydration, evoked by exercise with prolonged fluid restriction and diuretics respectively. We hypothesized that, urine, saliva, indices of heart rate variability and thirst would identify both types of mild dehydration, that plasma and tear osmolarity would identify intracellular dehydration only and that cardiovascular marker including, indices of heart rate variability, postural change in heart rate and blood pressure would identify extracellular dehydration. It was further hypothesized that a combination of these markers would allow the identification of dehydration type.

6.3 Methods

Participants

Fifteen, physically active, males volunteered to complete the study (mean ±SD: age 22.8 (5.4) years, height 180.4 (5.0) cm, body mass 78.9 (8.6) kg, body mass index 24.2 (1.8) $kg \cdot m^2$, and maximal oxygen uptake 51.7 (6.7) $ml \cdot kg^{-1} \cdot min^{-1}$). Participants were excluded if they had a history of eye surgery or disease, wore contact lens, smoked, or were receiving any prescription or non-prescription medication or other treatment for eye or dental disease at the time of the study. Participants' blood urea, creatinine and electrolytes were also assessed to ensure they had normal blood chemistry and renal function.

Preliminary measures

Seven to ten days before the main experimental trials anthropometric measures of height and body mass were obtained before peak PPO and $\dot{V}O_{2max}$ were estimated using the procedure described in **Chapter 3** and a Lode Excalibur cycle ergometer (Medical Technology, Groningen, Netherlands). From the anthropometric measures individual resting metabolic rate was estimated using the formula of Harris and Benedict (Harris et al., 1918). Each participants required energy intake for the day before and each day of the experimental trial was then calculated as the product of their resting metabolic rate and an estimated physical activity factor. The physical activity factor was estimated to be 1.6, which was determined from a list of activities participants were permitted to complete on the experimental trials (8 h sleeping, 7 h sitting, 3 h light leisure activity, 2 h walking without load, 3 h cooking, personal care and general housework and 1 h eating).

On a separate day participants returned to the laboratory for habituation with the hydration assessment techniques and an exercise protocol they would complete on day one of each trial that involved cycling at 70% peak power output until exhaustion. During this exercise expired air was measured continuously by online gas analysis and energy expenditure calculated (caloric equivalent of oxygen x mean oxygen consumption x exercise time). The additional energy expended during this exercise was added to the estimated daily energy intake for day one of each trial.

Study Design

This was a prospective phase II challenge, defined as a study in which the accuracy of one or more tests is estimated for difficult cases, e.g. mild dehydration (Obuchowski et al., 2004), which followed standards for reporting diagnostic accuracy (STARD; Bossuyt et al., 2003). The study had a randomized, repeated measures, cross-over design. To ensure the allocation to trials was counter-balanced randomization of participants was completed in blocks of three participants using a pseudo random number generator (www.randomizer.org). Separated by seven days participants completed three intervention trials. The trials were a normal hydration control trial (CON), and two mild dehydration trials including; intracellular dehydration (ID), and extracellular dehydration (ED). Intracellular dehydration was evoked by exercise with 48 hours of fluid restriction whilst extracellular dehydration was evoked by a diuretic. These dehydration methods were known to be successful in inducing mild to modest dehydration and were developed from previous investigations in our laboratory (Oliver et al., 2007, 2008). Each trial consisted of a hydration assessment, followed by exercise and a 48 hour intervention period, and a second hydration assessment (Figure 1).

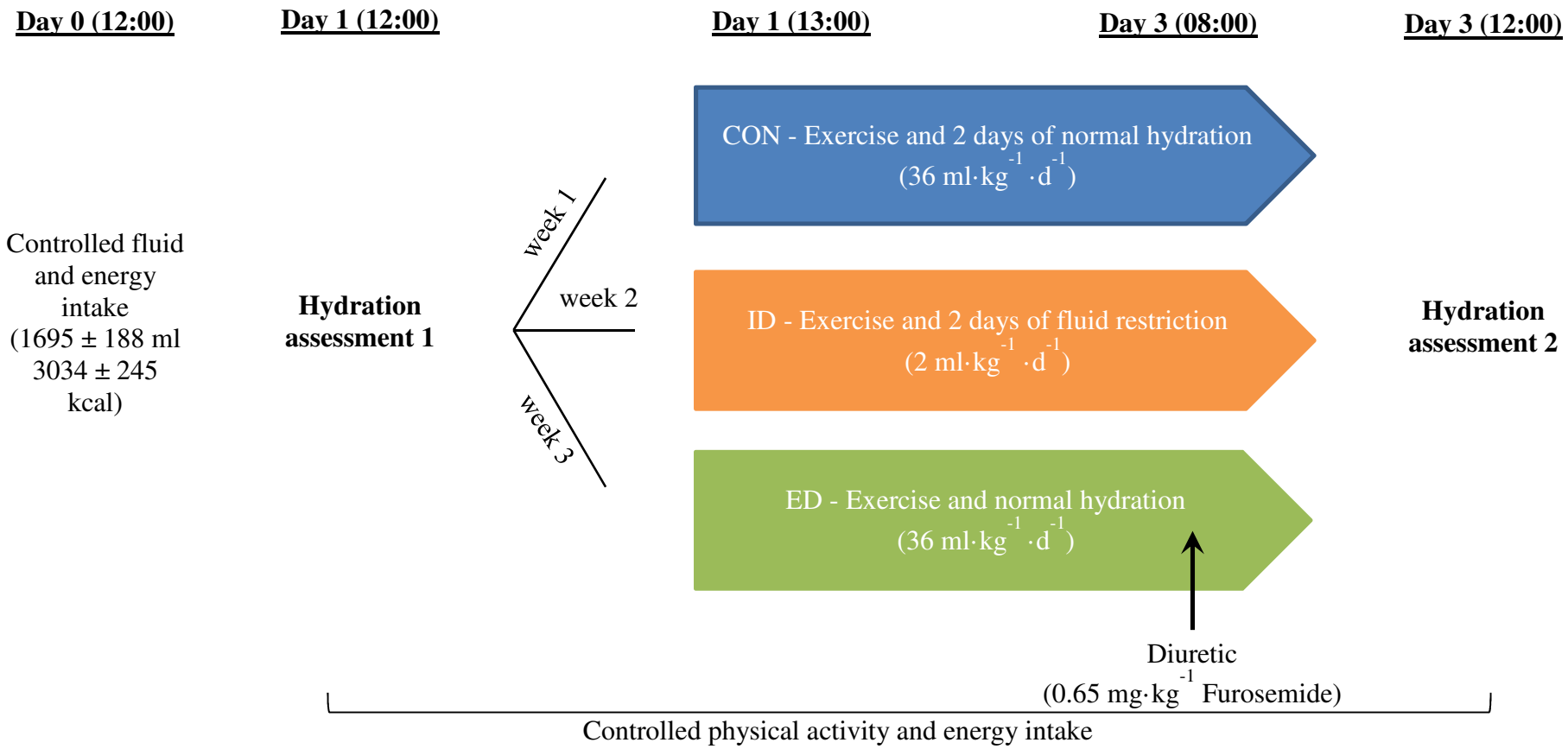

Figure 6.1 Schematic representation of each experimental trial.

Experimental procedures

The day before each experimental trial participants abstained from any dietary supplement, alcohol, caffeine or strenuous physical activity and consumed an individually prescribed diet (water to drink 1695 (188) ml and energy intake 3034 (245) kcal; 62, 25, 13% carbohydrate, fats and protein). The daily energy intake was the same for the duration of the trials except that on day one participants consumed additional food to replace energy expended during the exercise bout. This was calculated from the habituation session (391 (193) kcal). Participants were instructed to only consume what they were provided during trials. On day one of each experimental trial participants woke at 07:00 h voided and then drank water equal to 6 $ml \cdot kg^{-1}$ of body mass (471 (52) ml). On arrival to the laboratory at 08:00 h participants received a standardized breakfast (690 kcal; 62, 23 and 15% carbohydrate, fat and protein, respectively) and a further bolus of water equal to 6 $ml \cdot kg^{-1}$ of body mass. After, pedometers (Digi-Walker SW200, Yamax, Tokyo, Japan) were fitted to monitor participant physical activity and instructions were given to participants to standardize physical activity across trials. Participants returned to the laboratory at 12:00 h for the first hydration assessment. Immediately after they completed exercise, at 70% of peak power until exhaustion, and then began one of three 48-h trials. Sweat loss from the exercise was estimated as the body mass difference between measures before and after exercise. Care was taken to remove any sweat from the body with a towel before weighing. The calculated sweat loss was replaced with water on CON and ED but not on ID. Drinking water was then restricted for the 48 hour intervention on ID to 2 $ml \cdot kg^{-1}$ of body mass per day (total 314 (35) ml). In contrast, on CON and ED participants drank water equal to 36 $ml \cdot kg^{-1}$ of body mass per day (total 5728 (600) ml). These fluid intakes to maintain normal hydration or induce dehydration via fluid restriction were based on procedures developed from previous work from this research group (Oliver et al., 2008). On day two participants completed the same itinerary as day one, except

after breakfast they were free to leave the laboratory and did not complete the exercise. On day three participants reported to the laboratory at 08:00 h for body mass and urine measures. After breakfast on ED they consumed 0.65 $mg \cdot kg^{-1}$ (51 (6) mg) of liquid Furosemide (Frusol, Rosemount Pharma, Leeds, UK). At 12:00 h on all trials participants began the second hydration assessment.

Hydration Assessments

Hydration measures were obtained in the same order on each trial and at each hydration assessment. A urine sample was collected into a 24-h container for analysis of urine color, specific gravity and osmolality then nude body mass was determined (**Chapter 3**). Participants were then fitted with a heart rate monitor (Polar Electro RS800, Kempele, Finland) and after a 2-min customisation period, beat-to-beat heart rate was continuously recorded during seated rest over a 10-min period as described (Leicht & Allen, 2008; Marek, 1996). All R–R series were extracted on a Microsoft ® Windows compatible laptop computer with a processing program (Polar Precision Performance, Polar Electro, Kempele, Finland). Text files were then analysed in the time and frequency-domain after automatic removal of occasional ectopic beats (Kubios, BSAMIG, Kuopio, Finland). After, the participants sat quietly for 5-min before a tear fluid sample was collected from the right eye and analysed for tear osmolarity by a commercially available device (TearLab™ Osmolarity System, San Diego, USA), as previously described (Fortes et al., 2012). After 5-min supine rest blood pressure and heart rate were recorded (Tango, SunTech Medical Ltd, Morrisville, North Carolina). These measures were then repeated after 1-min on standing. The change between lying and standing measures was then calculated. Next, a 5-min unstimulated whole saliva sample was collected and flow rate and osmolality were measured (**Chapter 3**). Participants then completed subjective measure of thirst on a 0-9 thirst scale where 0 equals “not thirsty at

all" and 9 equals "very, very thirsty" (Engell et al., 1987). Finally, a venous blood sample was obtained and immediately used to determine plasma volume change and plasma osmolality (**Chapter 3**).

Statistical Analysis

To determine whether the magnitude and characterization of dehydration at the end of each trial were different change in body mass and plasma volume calculated between the first and second hydration assessments and all other hydration markers on the second assessment were compared by one-way within-groups analysis of variance. Post hoc Tukeys or Bonferroni correct *t*-tests were used where appropriate to follow up where analysis of variance was statistically significant. Diagnostic accuracy analysis was used to assess the agreement between each hydration marker (proposed index test), and percentage body mass loss (reference standard) for the ability to discriminate between normal hydration and mild dehydration. For all hydration markers the area under the receiver operating characteristic curve (AUC_{ROC}) was used as a measure of global diagnostic accuracy (**see Chapter 3**). In addition, hydration marker cut-offs and the sensitivity and specificity of these cut-offs were calculated where appropriate (**Chapter 3**). Fourteen participants were calculated as the required sample size to statistically distinguish between AUC_{ROC} of 0.5 and 0.8 using a study design with an equal amount of participants with and without dehydration (Obuchowski et al., 2004). However, to allow for potential dropout fifteen participants were recruited. Combined hydration marker diagnostic accuracy (sensitivity and specificity) was determined by the method proposed by Weinstein (Weinstein et al., 2005). In short, hydration markers were interpreted in series (one after another) and using the 'AND rule' that yields a positive diagnosis if both tests are positive and a negative diagnosis if either tests is negative. Unless stated all values are means ±SD.

6.4 Results

Before the interventions (hydration assessment 1) all 15 participants were normally hydrated, and body mass, plasma osmolality and urine specific gravity were similar before each trial (body mass, 78.3 (8.4), 78.3 (8.3), 78.4 (8.7) kg, $P = 0.91$; plasma osmolality, 287 (4), 289 (5), 287 (3) mOsm·kg^{-1}, $P = 1.00$; urine specific gravity, 1.009 (0.004), 1.009 (0.004), 1.007 (0.003) g·ml^{-1}, $P = 0.35$ for CON, ID and ED respectively). Immediately after the first hydration assessment exercise duration was also similar between trials (1274 (406) s; $P = 0.13$). Consequently, there was no difference in fluid replacement on CON and ED (0.54 (0.18) L; $P = 0.12$). Physical activity was also similar on the three trials (15766 (3967), 17016 (5285) and 17244 (3638) steps·trial^{-1}, CON, ID and ED respectively; $P = 0.30$).

6.4.1 Hydration status after the 48 h intervention

After the 48 hour intervention, body mass was maintained on CON but decreased on ID and ED ($P < 0.001$). As expected there was an increase in plasma osmolality on ID but no change on CON or ED ($P < 0.001$). Conversely, plasma volume decreased on ED but was unchanged on CON and ID ($P = 0.002$). A descriptive summary of the magnitude and type of dehydration and all hydration markers including the utility of hydration markers to identify the two different types of dehydration is included in **Table 6.1**.

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Table 6.1 Characterisation of hydration status and summary of hydration marker values after the 48 h intervention

	Normal hydration	**Intracellular dehydration**	**Extracellular dehydration**	***P* value**
Body mass change (kg)	0.0 ± 0.4 (-0.3 to 0.2)	-1.5 ± 0.5 ** (-1.3 to -1.8)	-1.6 ± 0.3 ** (-1.4 to -1.8)	**< 0.001**
Body mass change (%)	0.0 ± 0.6 (-0.3 to 0.3)	-1.9 ± 0.5 ** (-1.6 to 2.2)	-2.0 ± 0.3 ** (-1.8 to 2.2)	**< 0.001**
Plasma volume change (%)	2 ± 6 (-2 to 5)	0 ± 6 (-3 to 2)	-7 ± 4 ‡‡ (-9 to -4)	**0.002**
Posm (mOsm·kg^{-1})	287 ± 4 (285 to 289)	297 ± 7 †† (293 to 301)	286 ± 5 (284 to 289)	**< 0.001**
Urine colour (1-8)	2 ± 1 (1 to 3)	6 ± 1 †† (5 to 7)	2 ± 1 (1 to 3)	**< 0.001**
Uosm (mOsm·kg^{-1})	267 ± 138 (190 to 343)	1054 ± 126 †† (984 to 1124)	402 ± 110 * (341 to 462)	**< 0.001**
USG (g·ml^{-1})	1.008 ± 0.004 (1.006 to 1.010)	1.028 ± 0.005 †† (1.026 to 1.031)	1.010 ± 0.004 (1.008 to 1.013)	**< 0.001**
Sosm (mOsm·kg^{-1})	55 ± 13 (49 to 62)	65 ± 12 † (57 to 71)	56 ± 12 (48 to 62)	**0.002**
SFR (µl·min^{-1})	365 ± 241 (232 to 499)	196 ± 165 † (104 to 287)	425 ± 320 (248 to 603)	**0.002**
Tosm (mOsm·l^{-1})	296 ± 12 (289 to 303)	300 ± 10 (294 to 306)	292 ± 11 (286 to 298)	0.07

Thirst (0-9)	2.8 ± 1.1 (2.1 to 3.4)	6.2 ± 1.4 †† (5.4 to 7.0)	4.0 ± 1.3 ** (3.3 to 4.7)	**< 0.001**
△HR (b·min^{-1})	14 ± 8 (9 to 18)	19 ± 10 (13 to 25)	26 ± 12 ‡ (21 to 32)	**< 0.001**
△SBP (mmHg)	8 ± 12 (1 to 15)	4 ± 14 (-4 to 11)	0 ± 9 (-6 to 6)	0.16
RMSSD	83 ± 50 (54 to 112)	65 ± 39 (43 to 88)	84 ± 54 (53 to 116)	0.34
SDNN	118 ± 43 (93 to 143)	99 ± 40 (77 to 122)	125 ± 56 (93 to 157)	0.23
LF-HF	1.8 ± 1.1 (1.2 to 2.4)	3.4 ± 2.2 * (2.2 to 4.7)	2.9 ± 2.1 (1.6 to 4.1)	**0.03**

NOTE: Abbreviations: Posm, plasma osmolality; Uosm, urine osmolality; USG, urine specific gravity; Sosm, saliva osmolality; SFR, saliva flow rate; Tosm, tear osmolarity; △HR, change in heart rate from lying to standing; △SBP, change in systolic blood pressure from lying to standing, RMSSD, root mean square of successive N-N intervals; SDNN, standard deviation of successive N-N intervals, LF-HF, low frequency to high-frequency power ratio. Values represent means ± SD (95% CI). * $P < 0.05$ vs. CON, ** $P < 0.01$ vs. CON, †$P < 0.05$ vs. CON and ED, ††$P < 0.01$ vs. CON and ED, ‡ $P < 0.05$ vs. CON and ID, ‡‡ $P < 0.01$ vs. CON and ID.

Table 6.2 Receiver operating characteristic (ROC) analysis of hydration markers to mild intracellular and extracellular dehydration

Hydration marker	Intracellular dehydration			Extracellular dehydration		
	AUC_{ROC}	95% CI[a]	*P* value	AUC_{ROC}	95% CI[a]	*P* value
Uosm (mOsm·kg^{-1})	**1.00**	0.88 to 1.00	**< 0.001**	**0.81**	0.63 to 0.93	**< 0.001**
Thirst (0-9)	**0.97**	0.84 to 0.99	**< 0.001**	**0.77**	0.59 to 0.90	**0.002**
USG (g·ml^{-1})	**1.00**	0.88 to 1.00	**< 0.001**	0.68	0.48 to 0.83	0.08
Urine colour(1-8)	**1.00**	0.88 to 1.00	**< 0.001**	0.52	0.33 to 0.70	0.89
Posm (mOsm·kg^{-1})	**0.96**	0.82 to 0.99	**< 0.001**	0.53	0.34 to 0.71	0.79
LF-HF	**0.71**	0.51 to 0.86	**0.03**[1]	0.64	0.45 to 0.81	0.16
Sosm (mOsm·kg^{-1})	**0.70**	0.51 to 0.85	**0.04**[1]	0.55	0.36 to 0.73	0.67
SFR (µl·min^{-1})	**0.70**	0.51 to 0.85	**0.04**[1]	0.55	0.36 to 0.73	0.67
△HR (b·min^{-1})	0.66	0.47 to 0.82	0.12	**0.82**	0.63 to 0.93	**< 0.001**
RMSSD	0.63	0.44 to 0.79	0.20	0.50	0.32 to 0.69	0.97
SDNN	0.63	0.44 to 0.79	0.21	0.51	0.32 to 0.69	0.95
Tosm (mOsm·l^{-1})	0.61	0.41 to 0.78	0.31	0.61	0.42 to 0.82	0.29
△SBP (mmHg)	0.56	0.37 to 0.74	0.55	0.66	0.46 to 0.82	0.12

NOTE: Abbreviations: See **Table 6.1** for abbreviations relating to hydration markers; AUC_{ROC}, area under ROC curve; [1] Indicates significantly less diagnostic accuracy than urine parameters, thirst and plasma osmolality, [a] binomial exact 95% confidence interval.

6.4.2 Diagnostic accuracy of hydration markers

The diagnostic accuracy of hydration markers to mild intracellular and extracellular dehydration are presented in **Table 6.2**. Area under the curve analysis indicated that only thirst and urine osmolality could identify dehydration regardless of type with fair to moderate diagnostic accuracy. Diagnostic accuracy to identify ID was perfect for urinary parameters (colour, osmolality and specific gravity), near perfect for thirst and plasma osmolality and fair to moderate for LF-HF, saliva osmolality and flow rate. Comparison of individual AUC_{ROC} suggested that, urinary markers, thirst and plasma osmolality had greater diagnostic accuracy than LF-HF and saliva parameters (AUC_{ROC} for urinary parameters (color, osmolality, specific gravity), thirst and plasma osmolality >AUC_{ROC} for LF-HF, saliva osmolality and flow rate, $P < 0.01$). Postural heart rate change, urine osmolality and thirst showed moderate diagnostic accuracy to identify ED. Comparison of individual marker AUC_{ROC} showed that there was no statistical difference in the diagnostic accuracy of these hydration markers ($P > 0.15$).

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6.5 Discussion

The present findings highlight for the first time that the utility and diagnostic accuracy of hydration assessment markers is dependent on the dehydration type. In addition, even with mild fluid-deficits, simple, non-invasive hydration markers can identify more than one type of dehydration and when combined with other hydration markers can be used to identify dehydration type. In agreement with our hypothesis, thirst and urine osmolality had acceptable diagnostic accuracy (moderate or better) to identify both types of mild dehydration, and plasma and postural heart rate changes could only identify one type of dehydration (mild intracellular dehydration and extracellular dehydration respectively). In contrast to our hypothesis, saliva parameters and LF-HF (heart rate variability index) only identified mild intracellular dehydration and postural changes in blood pressure and tear osmolarity were ineffective in identifying extracellular dehydration and intracellular dehydration, respectively. These results have significant practical implication as they indicate which hydration markers are best for the early identification of dehydration irrespective of type. Early identification is fundamental if markers are to inform rehydration strategies and prevent the numerous negative effects of more severe dehydration on performance and health. In addition, the identification of dehydration type should enable more informed fluid replacement (e.g. water and solute replacement and the avoidance of hypotonic fluid replacement when extracellular dehydration is identified). Lastly, this combined-marker approach would enable simple routine assessment of hydration status in field-based settings due to the simple, rapid and non-invasive nature of the procedures.

As hypothesized the subjective rating of thirst identified both types of dehydration. Perhaps more surprising was that thirst, with the exception of urine osmolality, had the highest combined diagnostic accuracy to identify dehydration. Thirst might be considered the first

measure to obtain when screening an individual's hydration status, particularly as it is non-invasive, low cost and simple to perform. Although the diagnostic accuracy of urine parameters (colour, osmolality and specific gravity) was perfect for intracellular dehydration, only urine osmolality could identify both types of dehydration. These differences can be explained by the dehydration types evoked in this study and the methods used to determine urine concentration. Intracellular dehydration evoked by exercise and fluid restriction resulted in an intracellular dehydration causing the kidneys' to conserve water and hence the rise in the concentration of urine (ions and urea). In contrast, diuretic-evoked extracellular dehydration resulted in an extracellular dehydration (reduction in plasma volume), increasing urinary loses of both water and ions (in particularly potassium with the loop diuretic furosemide). This caused a mild increase in urine concentration that was not appreciable to the eye but was discernible by osmolality, which specifically assesses all dissolved particles such as ions. Similarly, differences between osmolality and specific gravity can be explained by the different sensitivity of the osmometer and refractometer methods (L. Armstrong, 2005). This highlights that careful consideration is required when selecting a urine marker. In contrast to thirst and urine osmolality, saliva parameters were not able to identify both types of mild dehydration, although did identify mild intracellular dehydration with fair diagnostic accuracy (AUC_{ROC} = 0.70). Previous research has shown that both saliva flow rate and in particular saliva osmolality can identify modest hypertonic and isotonic dehydration, equal to approximately 3% of body mass, evoked by prolonged fluid restriction and combined fluid and energy restriction, respectively (Oliver et al., 2008). The inability of saliva parameters to identify mild isotonic dehydration in the present study is likely due to the lower magnitude of fluid-deficit compared to previous work (i.e. 2 vs 3% of body mass). In support of this, a recent study has shown that saliva osmolality had fair diagnostic accuracy (AUC_{ROC} = 0.77-0.78) to identify modest extracellular dehydration (3.1% of body mass) evoked by diuretics

(Ely et al., 2014). Therefore, further research is required to examine the utility of saliva hydration markers to identify different types of dehydration. All other hydration markers failed to identify both hypertonic and isotonic dehydration with moderate diagnostic accuracy, and therefore should not be considered as suitable markers of mild dehydration *per se*.

In addition to thirst and urine we showed that postural heart rate change had moderate diagnostic accuracy to identify mild extracellular dehydration. This compliments a recent previous study that showed that changes in heart rate from sitting to standing identified moderate dehydration (3% BML) evoked by diuretics and an overnight fluid restriction (Cheuvront et al., 2012). Despite the smaller magnitude of fluid deficit in this study and using a similar threshold for mild dehydration, we observed a greater overall diagnostic accuracy for this marker (77 versus 69 %, determined as a weighted average of a test's sensitivity and specificity). A likely explanation for this improved diagnostic accuracy in the present study is the increased orthostatic stress incurred during a change in posture from lying to standing compared with sitting to standing in the previous study. Combined, these studies outline that this simple marker has utility to determine an individual's hydration status. In particular these studies indicate that this marker seems suitable as a measure of hypovolemia.

The findings of the present study also show that urinary hydration measures have the greatest diagnostic accuracy to identify mild intracellular dehydration after prolonged fluid-loss (AUC_{ROC} = 1.0). This is in agreement with a recent study that shows urinary hydration markers had better diagnostic accuracy to identify mild hypertonic dehydration than serum osmolality and saliva parameters when fluid loss was evoked by passive heat exposure (Muñoz et al., 2013). In contrast, in the same study, when fluid-loss occurred over a shorter

period of time, during active heat stress, serum osmolality and saliva osmolality exhibited greater diagnostic accuracy than urinary hydration markers. This highlights a potential limitation of the proposed hydration assessment procedure in the current study. Namely, if rapid hypotonic fluid loss occurs in the field e.g. through exercise sweat-loss in the heat, urine color may not have the required diagnostic accuracy to identify mild hypertonic dehydration. However, further research is required to examine the validity of the combined marker approach in this study. Although, saliva flow rate and osmolality could discriminate between normal hydration and mild intracellular dehydration, they had less diagnostic accuracy than urine, thirst and plasma osmolality. A novel finding of this study was that indices of heart rate variability were able to identify mild intracellular dehydration with fair diagnostic accuracy. These indices warrant further research as they are non-invasive and cost effective way of monitoring hydration status. In addition, despite previous reports which have shown that tear osmolality has utility to identify modest hypertonic-hypovolemia following exercise with heat stress, this marker could not identify mild intracellular dehydration (Fortes et al., 2011). The inability of tear hydration markers to identify mild intracellular dehydration may be due to differences in the magnitude of fluid deficit between this and previous studies (2 vs 3% BML).

This study enabled for the first time the determination of hydration marker diagnostic accuracy to hypertonic and isotonic dehydration. This contrasts previous studies that have examined hydration markers to only one type of dehydration (acute intracellular dehydration, or hyperosmotic-hypovolaemia), typically evoked by acute bouts of exercise in the heat with fluid restriction (Armstrong et al., 1994; Armstrong et al., 1998; Shirreffs & Maughan, 1998; Popowski et al., 2001; Bartok et al., 2004; Walsh et al., 2004; Walsh et al., 2004b; Shirreffs et al., 2004; Cheuvront et al., 2010; Fortes et al., 2011; Cheuvront et al., 2012). This enables

both a better understanding of the diagnostic accuracy of each hydration marker but also which dehydration characteristic, i.e. hypertonicity or hypovolemia, that influences each marker. However, a limitation of the study was that the well-controlled precise experimental procedures used to evoke distinct types of fluid-loss are unlikely to reflect fluid-loss in field-based settings. Indeed, it is unclear whether hydration marker response to extracellular dehydration following diuretics is the same as those which would be observed in the field. Further, it could be argued that fluid-loss in extreme environments (cold or altitude) results in a mixed dehydration from a combination of sweat-loss and diuresis. Conversely, the research-design has ultimately allowed the determination of hydration marker diagnostic accuracy specifically for each of the desired conditions, i.e. normal hydration, intracellular dehydration and extracellular dehydration. This was highlighted, as normal hydration was achieved on CON as the fluid and energy provided was adequate to maintain individual participants' body mass, from first to second hydration assessment, within the normal day-to-day variation previously reported (Cheuvront et al., 2004). Since energy intake and physical activity were similar on all trials the mild body mass loss on ID and ED most likely represents dehydration and not an energy deficit. A further strength of the study was the concurrent diagnostic accuracy assessment of hydration markers in a fully repeated measures design, where participants completed all trials. Consequently, hydration marker diagnostic accuracy to a specific dehydration type can be compared between markers and the diagnostic accuracy of an individual hydration marker can be compared between different types of dehydration.

We recommend a hydration assessment procedure where thirst is assessed first to establish the presence of dehydration. This can be followed by confirmation of dehydration type by urine color being used to identify hypertonicity and by orthostatic heart rate change being used to identify hypovolemia. In addition, acute intracellular dehydration (hyperosmotic-

hypovolaemia) following exercise with or without heat stress may also be identified using this algorithm. As this hydration strategy is non-invasive and simple to administer it can have significant application in field settings (e.g. during military maneuvers or athletic training camps) by improving identification of dehydration type and aiding the prescription of rehydration fluids. In conclusion thirst and urine osmolality were the only two hydration markers to identify both intracellular and extracellular dehydration with adequate diagnostic accuracy. A combination of thirst, urine color and postural heart rate change improved dehydration diagnosis by discriminating between dehydration types. This is therefore recommended as the optimal hydration assessment strategy.

CHAPTER SEVEN

Effect of mild intracellular and extracellular dehydration on endurance performance and well-being.

7.1 Summary

Endurance exercise performance and the mechanisms by which mild dehydration causes exercise fatigue may depend on the type of dehydration. To test this hypothesis we examined endurance performance determined by exercise time to exhaustion (TTE) at 70% peak power output in twelve males after they received adequate fluid and energy to maintain hydration status (CON), after intracellular dehydration (ID) caused by two days of restricted drinking fluid (2 ml·kg·d^{-1}), and after extracellular dehydration (ED) caused by a diuretic (0.65 mg·kg^{-1} of furosemide). Before TTE, dehydration was equal to 2% of body mass on ID and ED. As expected ID caused hyperosmolality (increased plasma osmolality, 9 mOsm.kg^{-1}, ID vs. CON and ED, $P < 0.01$) and ED caused hypovolemia (decreased plasma volume, ED vs. CON and ID, $P < 0.05$). Endurance performance was worse with dehydration (1471 (445), 1120 (383), 934 (323) s, on CON, ID and ED, respectively, $P < 0.01$) and poorer after ED than ID ($P = 0.04$). Mechanisms by which dehydration reduced endurance performance appear to be dependent on dehydration type. Mood and subjective feelings were unaltered after ED, but cardiovascular and ventilatory strain appeared higher during exercise (e.g. stroke volume, $P = 0.04$ and breathing frequency, $P < 0.001$). In contrast, ID caused poorer mood and subjective feelings, but did not alter cardiovascular and ventilator parameters (e.g. total disturbance in subjective feelings $P < 0.001$ and mood $P = 0.01$). In conclusion, dehydration type alters the magnitude and mechanism by which dehydration impairs endurance performance.

7.2 Introduction

Dehydration is associated with disruptions to performance and well-being, and is a significant risk for athletes, military personnel and those travelling to extreme environments. The effects of dehydration on performance and well-being are known to be moderated by the magnitude of dehydration and the environmental temperature (Coyle, 2004). Whether the physiological distinctions between dehydration types also moderate the effect of dehydration on performance and well-being is unclear. However it may explain the equivocal effects of mild to modest dehydration (equal to 2-3% of body mass) on endurance exercise performance previously reported in a temperate environment (Armstrong et al., 1985; Cheuvront et al., 2005, Daries et al., 2000; Oliver et al., 2007), and inconsistent effects on measures of affective well-being. This study therefore aimed to determine the effects of the two main types of dehydration (intracellular and extracellular) on endurance exercise performance measured by a time to exhaustion test and measures of affective well-being via self-reported measures of mood and subjective feelings. Furthermore, to determine the effect of dehydration type on the proposed mechanisms by which dehydration limits endurance performance we also measured selected psycho-physiological parameters before and during exercise. It was hypothesised that dehydration, irrespective of type, would reduce endurance performance and that endurance exercise performance would be poorest after extracellular compared to intracellular dehydration because the associated hypovolaemia would elevate cardiovascular and ventilatory strain. An additional hypothesis was that measures of affective well-being would be disrupted more by intracellular dehydration evoked by prolonged fluid restriction.

7.3 Methods

Participants

Twelve, physically active, males completed the study (mean ±SD) age 23 (6) years, height 180 (6) cm, body mass 79 (9) kg, body mass index 24.0 (1.6) $kg \cdot m^2$, peak power output 300 (41) W and maximal oxygen consumption ($\dot{V}O_{2max}$) 52 (7) $ml \cdot kg^{-1} \cdot min^{-1}$). Participants were excluded from the study if they smoked, or were receiving any prescription or non-prescription medication. Participants' blood urea, creatinine and electrolytes were also assessed to ensure they had normal blood chemistry and renal function. Informed consent was obtained in writing from each participant. The study was approved by the Institutional Ethics Committee and adhered to the Declaration of Helsinki. Originally fifteen participants were recruited however three participants were unable to complete all exercise time to exhaustions satisfactorily due illness or injury and they were therefore removed from all analyses.

Preliminary measures

Seven to ten days before the main experimental trials anthropometric measures of height and weight were recorded for each participant. From the anthropometric measures individual resting metabolic rate was estimated using the formula proposed by Harris and Benedict (Harris et al, 1918). Each participants required energy intake for the day before and each day of the experimental trial was then calculated as the product of their resting metabolic rate and an estimated physical activity factor of 1.6, determined from a list of activities participants' were permitted to complete on the experimental trials (8 h sleeping, 7 h sitting, 3 h light leisure activity, 2 h walking without load, 3 h cooking, personal care and general housework and 1 h eating). In addition, PPO and $\dot{V}O_{2max}$ were determined as outlined in **Chapter 3**. On a separate day participants returned to the laboratory for habituation with the exercise protocol

they completed on day one and three of each experimental trial. During this habituation exercise expired air was measured continuously by online gas analysis and energy expenditure calculated (caloric equivalent of oxygen multiplied by mean oxygen consumption multiplied by exercise time). The additional energy expended during this exercise was then added to the estimated daily energy intake for day one of each trial.

Study Design

The study had a randomised, repeated measures, cross-over design. To ensure the allocation to trials was counter-balanced randomisation of participants was completed in blocks of three using a pseudo random number generator (www.randomizer.org). The three trials completed were a control trial (CON), an intracellular dehydration trial (ID), and an extracellular dehydration trial (ED). Dehydration was evoked by exercise followed by 48 hours of fluid restriction (ID) and a diuretic (ED), respectively. These dehydration methods were adapted from previous investigations in our laboratory (Oliver et al., 2007). Each trial consisted of a pre-exercise assessment of hydration status, followed by exercise, a 48-hour intervention period, a second pre-exercise assessment of mood, subjective feelings and hydration status and finally a time to exhaustion (TTE) (**Figure 7.1**). Exercise on day one followed the same protocol as the TTE on day three.

Experimental procedures

The day before each experimental trial participants abstained from any dietary supplement, alcohol, caffeine or strenuous physical activity and consumed an individually prescribed diet (water to drink 1695 (188) ml and energy intake 3034 (245) kcal; 62, 25, 13% carbohydrate, fats and protein). The daily energy intake was the same for the duration of the trials except that on day one participants consumed additional food to replace energy expended during the

exercise bout, which was calculated from the habituation session (391 (193) kcal). Participants were instructed to only consume what they were provided during trials.

On day one of each experimental trial participants woke at 07:00 h voided and then drank water equal to 6 ml·kg^{-1}of body mass (471 (52) ml). On arrival to the laboratory at 08:00 h participants received a standardized breakfast (690 kcal; 62, 23 and 15% carbohydrate, fat and protein, respectively) and a further bolus of water equal to 6 ml·kg^{-1}of body mass. After, participants were fitted with a pedometer (Digi-Walker SW200, Yamax, Tokyo, Japan) to monitor their physical activity and they were provided with instructions as to the type of light activity they were permitted to perform (e.g. reading, watching TV, and writing). During each trial daily numbers of steps was monitored and instructions were given to participants to standardise physical activity on all trials. Participants returned to the laboratory at 12:00 h where hydration status was assessed to ensure hydration before the first bout of exercise (**Figure 7.1**). Sweat loss during this exercise was estimated as the difference in body mass before and after exercise. Care was taken to remove any sweat from the body with a towel before weighing. The calculated sweat loss was replaced with water on CON and ED trials but not on ID. Water to drink on the ID trial was then restricted for the 48-hour intervention to 2 ml·kg^{-1} of body mass per day (total water to drink, 314 (35) ml). In contrast, on CON and ED trials participants consumed water to drink equal to 36 ml·kg^{-1} of body mass per day (total water to drink, 5728 (600) ml). On day two participants completed the same itinerary as day one, except after breakfast they were free to leave the laboratory. On day three participants reported to the laboratory at 08:00 h. After breakfast on ED they consumed 0.65 mg·kg^{-1} (51 (6) mg) of liquid Furosemide (Frusol, Rosemount Pharma, Leeds, UK). At 12:00 h participants returned to the laboratory where mood, subjective feelings and hydration status were assessed before TTE (**Figure 7.1**).

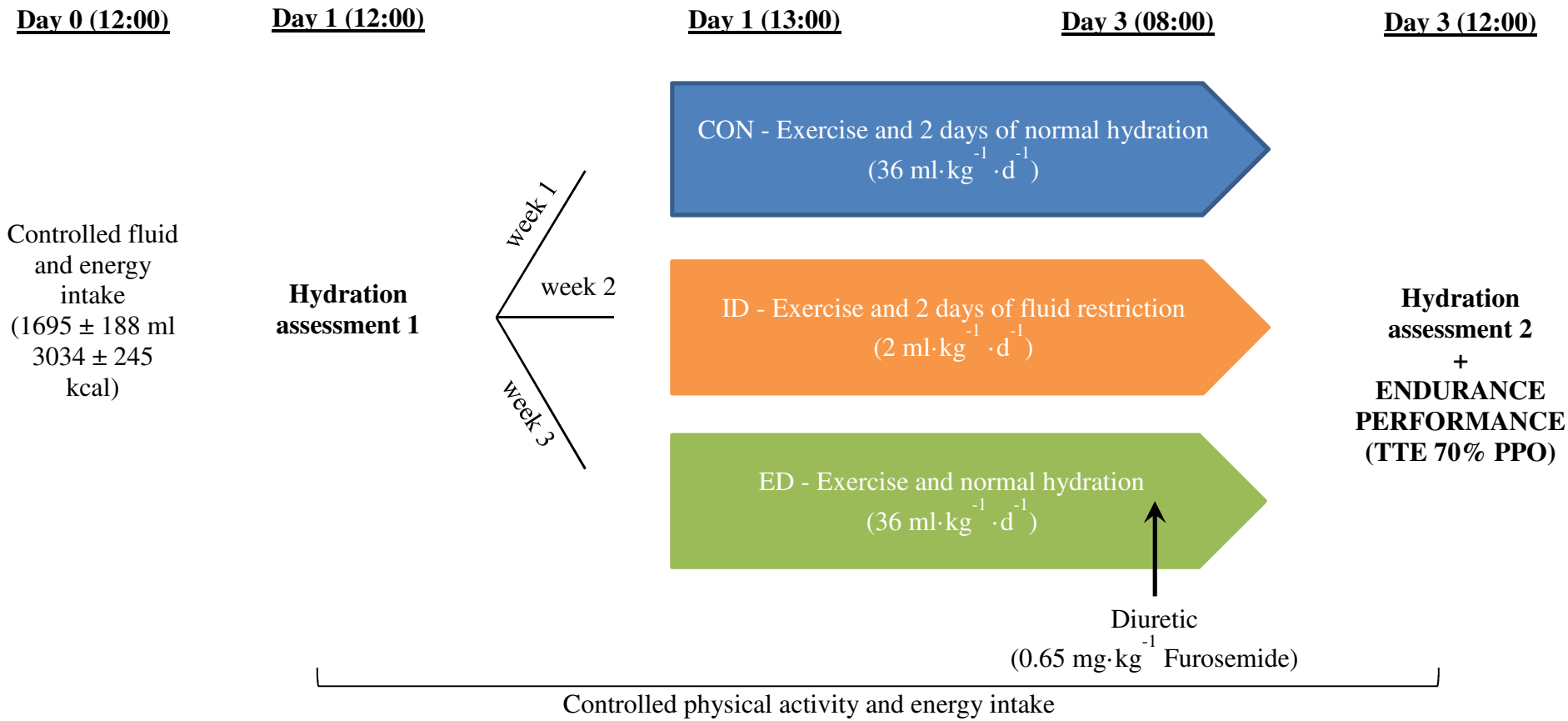

Figure 7.1. Schematic representation of experimental trials. **NOTE:** Abbreviations: TTE, time to exhaustion.

Hydration assessment

Nude body mass was determined to the nearest 50 g by a digital platform scale (Model 705 Seca, Hamburg, Germany). After the assessment of mood and subjective feelings, a venous blood sample was obtained and plasma volume change and plasma osmolality were measured as describe in **Chapter 3.**

Markers of affective well-being and motivation before exercise

Affective well-being was measured using questionnaires to assess mood and subjective feelings. Mood was assessed using the Brunel Mood Scale (Terry et al., 2003). This is a 24-item questionnaire sub-divided into six sub-scales including, anger, confusion, depression, fatigue, tension and vigour. Each question is answered on a 5-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely), with scores ranging from 0-16 for each sub-scale. Raw scores were then converted to standard scores (T-scores) using normative tables for adult students (aged >18 yr, Terry et al., 2003). Subjective feelings were recorded using an eight question 100mm visual analogue scale based on a previously described procedure (Shirreffs et al., 2004). Success and intrinsic motivation to perform the time to exhaustion exercise were measured using a 14-item questionnaire (Matthews et al., 2001). Each scale consists of seven questions answered on 5-point Likert scale, with scores ranging from 0-28.

Endurance performance

Endurance performance was assessed on a cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands), that involved cycling at 30% peak power output for 3 min (warm-up) followed by 70% peak power output until exhaustion (TTE). No motivation was provided during exercise and exhaustion was defined as a decrease in cadence below 60 revolutions

per minute for more than 5 seconds (Marcora et al., 2008). All exercise was performed in an air-conditioned laboratory (mean temperature and humidity 19.4 (1.0) ^{o}C and 42 (6) %).

Psycho-physiological responses during exercise

During TTE perceived exertion (Borg, 1998), and thermal comfort (Hollies, 1977) were recorded every 3 min. To prevent knowledge of time elapsed participants were also asked to rate perceived exertion and thermal comfort at random time-points during the time to exhaustion exercise. To measure heart rate (HR), stroke volume (SV) and cardiac output (CO) a transthoracic bioimpedance device (Physioflow PF05L1, Manatec, Petit-Ebersviller, France) was used continuously during TTE as described previously (Marcora et al., 2009). Oxygen uptake, respiratory exchange ratio (RER), minute ventilation, tidal volume and breathing frequency were measured breath-by-breath by gas analysis (MetaLyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany). Rectal temperature was measured with a thermistor inserted 10 cm beyond the anal sphincter and skin temperature was measured with thermistors attached at four skin sites (medial calf, anterior mid-thigh, anterior mid-bicep and chest, midway between the acromium process and the nipple). Mean skin temperature was calculated using the equation developed by Ramanathan (1964). Rectal and skin temperature were measured continuously during exercise and interfaced with a data logging system (Squirrel data logger, 2020 series, Grant Instruments, Cambridge, England). All respiratory, cardiovascular and thermoregulatory data were averaged over 1 min periods before statistical analysis.

Statistical Analysis

The required sample size for the study was estimated as 12 (G*Power, Version 3.1.2); using standard alpha (0.05) and beta levels (0.8), and mean and standard deviations of studies that

have previously examined the effect of mild dehydration on endurance exercise performance (Armstrong et al., 1985). To determine whether the magnitude and characterization of hydration state was different before TTE on each trial, change in body mass and plasma volume calculated between the first and second hydration assessments and plasma osmolality on the second assessment were compared by one-way within-groups analysis of variance. The effects of dehydration type on TTE, mood, subjective feelings and motivation before TTE, the mean perceptual and physiological parameters at isotime (first nine minutes of exercise after the end of warm-up) during TTE, and at exhaustion, were tested using a one-way within-group analysis of variance. An isotime of nine minutes was chosen to include all subjects in the analyses. Tukey's HSD were used with all analysis of variance where one-way within-group analysis of variance reported significant differences. For each trial an effect size was also calculated (Cohen, 1988). Effect sizes were interpreted as ≤ 0.2 trivial, > 0.2 small, > 0.6 moderate, > 1.2 large, > 2 very large and > 4 extremely large (Hopkins, 2004).The practical importance of the effects of dehydration on endurance performance was further analysed by calculating the percentage difference in TTE between CON and each dehydration trial. A paired t-test was then used to analyse differences between the two types of dehydration on performance. In addition, a one-sample t-test was carried out to compare these percentage differences for the dehydration trials with the normal variability of the performance test under normal hydration (11% see text). This affords the ability to examine whether the percentage change from CON was outside of this normal variability. Statistical significance was accepted at $P < 0.05$ and data in the text and tables are presented as mean ±SD.

7.4 Results

7.4.1 Hydration status and physical activity

Before each experimental trial participants had similar body mass and hydration status (body mass, 78.0 (8.3), 78.1 (8.3), 78.1 (8.7) kg, $P = 0.91$; plasma osmolality, 287 (4), 289 (5), 286 (3) mOsm·kg^{-1}, $P = 0.10$; CON, ID and ED trials, respectively). Following the 48-h interventions and before TTE, changes in body mass were suggestive of normal hydration on the CON trial and mild dehydration (2% of body mass) on the ID and ED trials (**Table 7.1**). Further there were no changes in plasma osmolality and plasma volume after the 48-hour CON trial. However, fluid restriction increased plasma osmolality and did not change plasma volume on the ID trial. Additionally, the diuretic decreased plasma volume without changing plasma osmolality on the ED trial (**Table 7.1**). Therefore, hydration status was successfully manipulated to evoke mild intracellular dehydration, mild extracellular dehydration and normal hydration. The research design was also effective in standardizing physical activity during the 48-hour trials (15905 (4319), 16531 (4169) and 18600 (5911) steps·trial^{-1}, CON, ID and ED trials, respectively; $P = 0.21$).

Table 7.1. Hydration status before TTE.

	Normal hydration	Intracellular dehydration	Extracellular dehydration
Body mass (kg)	78.0 (8.4)	76.6 (8.1) **	76.5 (8.5) **
Body mass change (kg)	0.0 (0.5)	-1.5 (0.5) **	-1.6 (0.3) **
Body mass change (%)	0.0 (0.6)	-1.9 (0.5) **	-2.1 (0.2) **
Plasma volume change (%)	2 (7)	0 (6)	-6 (4) ‡
Plasma osmolality (mOsm·kg^{-1})	286 (4)	297 (8) ††	286 (5)

NOTE: Values represent means ±SD. ** $P < 0.01$ vs. CON, †† $P < 0.01$ vs. CON and ED, ‡ $P < 0.05$ vs. CON and ID.

7.4.2 Markers of affective well-being and motivation before TTE

Before TTE, total mood disturbance was different between trials ($P = 0.02$) and was greater on ID compared to ED ($P = 0.008$). This was primarily due to differences in fatigue and vigour between trials ($P = 0.03$ for fatigue and vigour, respectively), more importantly fatigue was greater on ID than ED ($P = 0.02$; Figure 3), with no other differences between trials for the remaining mood states (anger, 46 (3), 47 (5), 45 (2), $P = 0.30$; confusion, 43 (3), 43 (3), 42 (1), $P = 0.34$; depression, 45 (5), 45 (4), 44 (2), $P = 0.48$; and tension, 46 (8), 49 (12), 48 (7), $P = 0.17$ for CON, ID and ED respectively). In addition, total disturbance in subjective feelings was greater on ID compared to the other two trials ($P < 0.001$ for ID vs. CON and ED). This primarily included a greater mouth dryness ($P < 0.001$ for ID vs. CON, $P = 0.008$ for ID vs. ED) and thirst on ID compared with the other trials ($P < 0.001$ for ID vs. CON, $P = 0.004$ for ID vs. ED; Figure 4). Other symptoms included a greater feeling of hunger ($P = 0.03$ for ID vs. CON) and unpleasant mouth taste ($P = 0.03$ for ID vs. CON, $P = 0.04$ for ID vs. ED), whilst other subjective feelings were not different between trials (head soreness, 15 (20), 24 (26), 20 (24), $P = 0.3$; concentration, 65 (23), 56 (22), 63 (19), $P = 0.2$; tiredness, 42 (21), 53 (24), 43 (25), $P = 0.2$ and alertness, 63 (22), 60 (18), 60 (21), $P = 0.7$ for CON, ID and ED respectively). Motivation to complete the TTE was similar on all trials (intrinsic motivation, 19.4 (4.9), 19.2 (4.2), 19.2 (4.7), $P = 0.89$; success motivation, 17.5 (6.9), 16.8 (6.5), 18.3 (7.1), $P = 0.20$ for CON, ID and ED respectively).

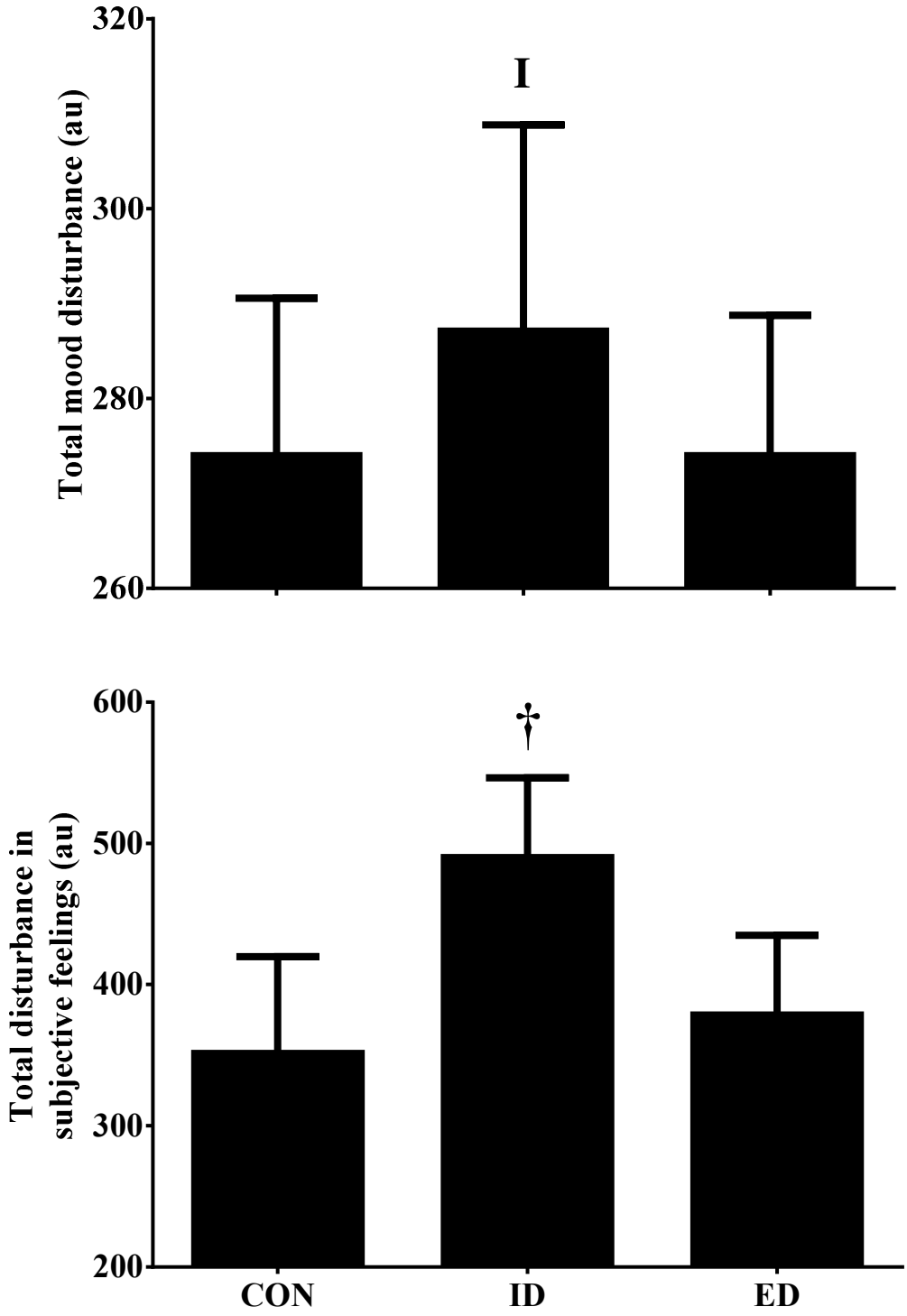

Figure 7.2. Mood (BRUMS) and subjective feelings (visual analogue scale, VAS) before TTE. **NOTE:** Abbreviations: CON, control trial; ID, intracellular dehydration trial; ED, extracellular dehydration trial. † $P < 0.05$ vs. CON and ED; I $P < 0.05$ vs. ED. n = 12.

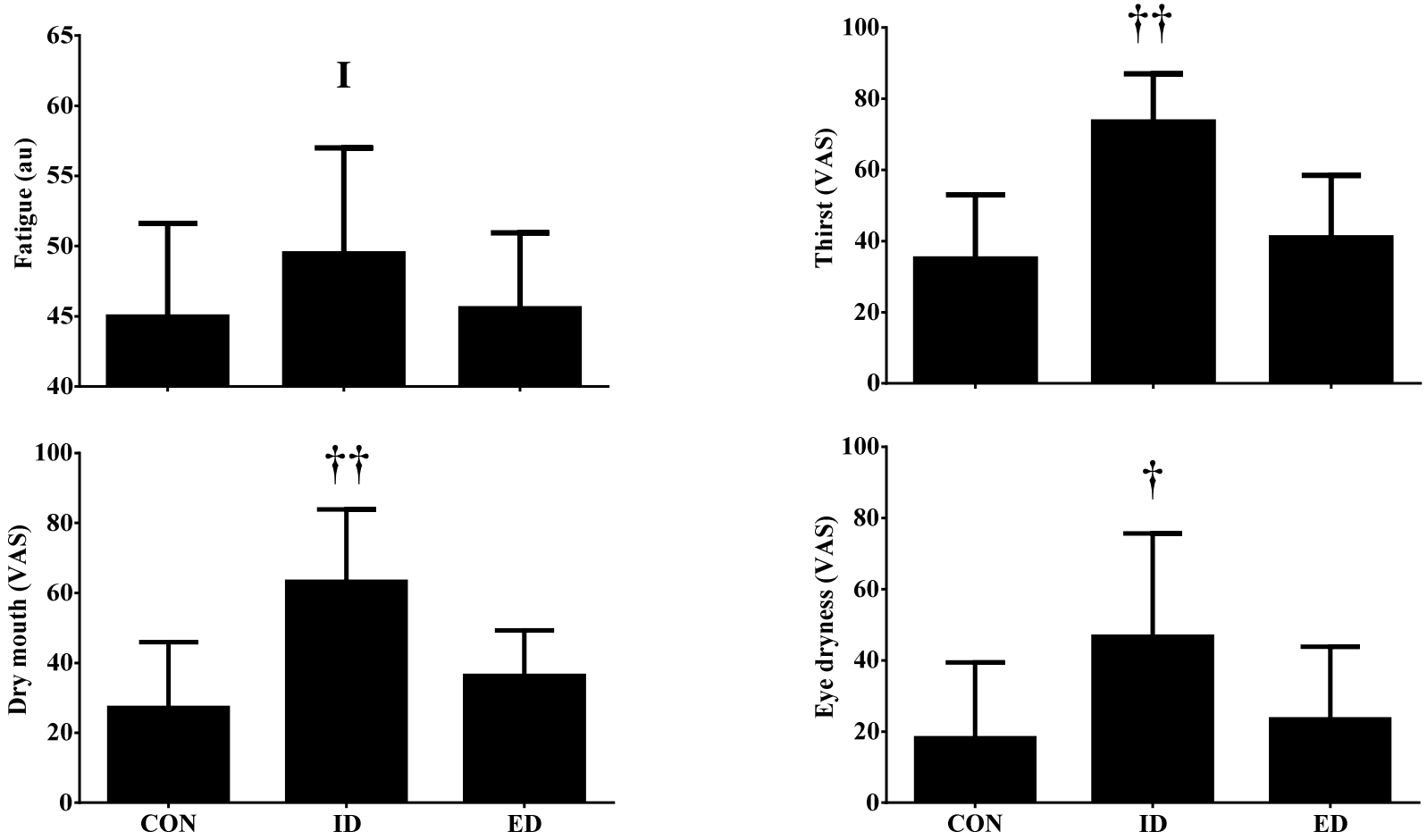

Figure 7.3 Selected mood and subjective feelings before TTE. **NOTE:** Abbreviations: CON, control trial; ID, intracellular dehydration trial; ED, extracellular dehydration trial. †$P < 0.05$ vs. CON, ††$P < 0.01$ vs. CON, I $P < 0.05$ vs. ED. n = 12

7.4.3 Endurance performance during TTE

Mild dehydration *per se* decreased endurance performance shown by a shorter time to exhaustion compared to CON (1471 (445), 1120 (383), 934 (323) s, for CON, ID and ED trials, respectively; $P < 0.001$, **Figure 7.4**). Notably, endurance performance was also poorer on ED compared to ID ($P = 0.04$). Effect size statistics also suggested that ED had a greater negative effect on endurance performance than ID ($d = 0.8$ for CON vs. ID, $d = 1.2$ for CON vs. ED). The percentage decrease in time to exhaustion compared to CON was greater on ED compared to ID (-35 ± 15, -24 ± 9 % for ED and ID respectively; $P = 0.04$). The decrease in performance in both ED and ID trials also fell outside the normal variability in performance based on time to exhaustion when normally hydrated (calculated from TTE 1 for each trial, 11% CV; **Figure 7.5**). Furthermore, every participants' TTE reduced by a larger percentage than this normal variability when dehydrated *per se*.

7.4.4 Perceptual and physiological responses during TTE

Mean perceived exertion during TTE was higher on the dehydration trials compared with CON (6 (2), 7 (2), 8 (1) for CON, ID and ED respectively; $P = 0.002$ for CON vs. ID; $P = 0.005$ for CON vs. ED). However, there was no difference in perceived exertion between dehydration type ($P = 0.45$). There were differences or trends between trials in mean cardiac output and stroke volume during exercise ($P = 0.07$, $P = 0.04$ for cardiac output and stroke volume respectively). Specifically, there was a reduced cardiac output ($P = 0.04$ for CON vs. ED) and stroke volume on ED compared to CON ($P = 0.03$ for CON vs. ED). There was also a trend for a reduced cardiac output and stroke volume on ED compared to ID ($P = 0.07$, for cardiac output and stroke volume). However, there was no difference in heart rate between trials ($P = 0.91$; **Figure 7.4A**).

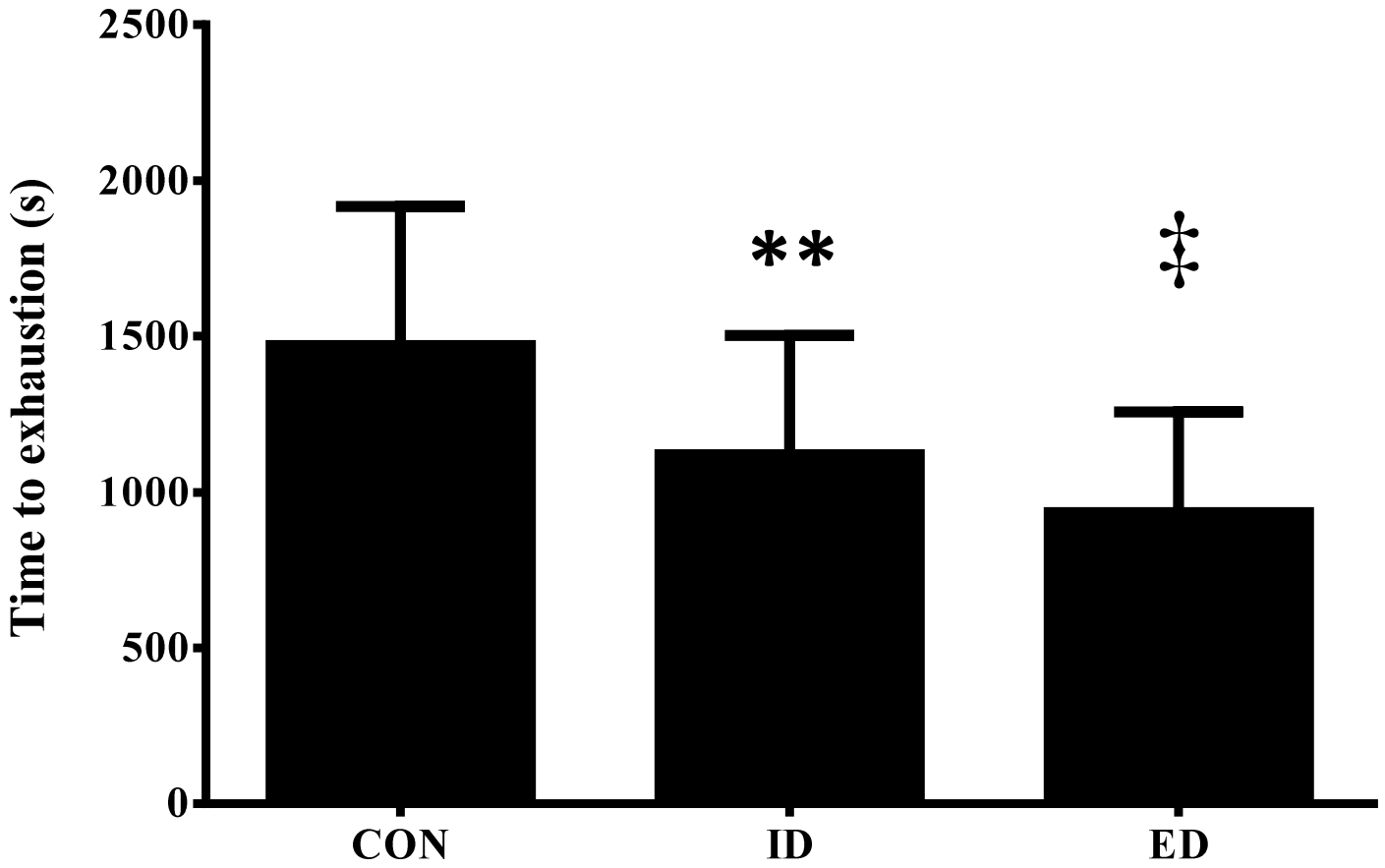

Figure 7.4 Mean endurance performance in the TTE following the 48 h intervention. **NOTE:** Abbreviations: CON, control trial; ID, intracellular dehydration trial; ED, extracellular dehydration trial. $^{**}P < 0.01$ vs. CON, ‡ $P < 0.05$ vs. CON and ID. n = 12.

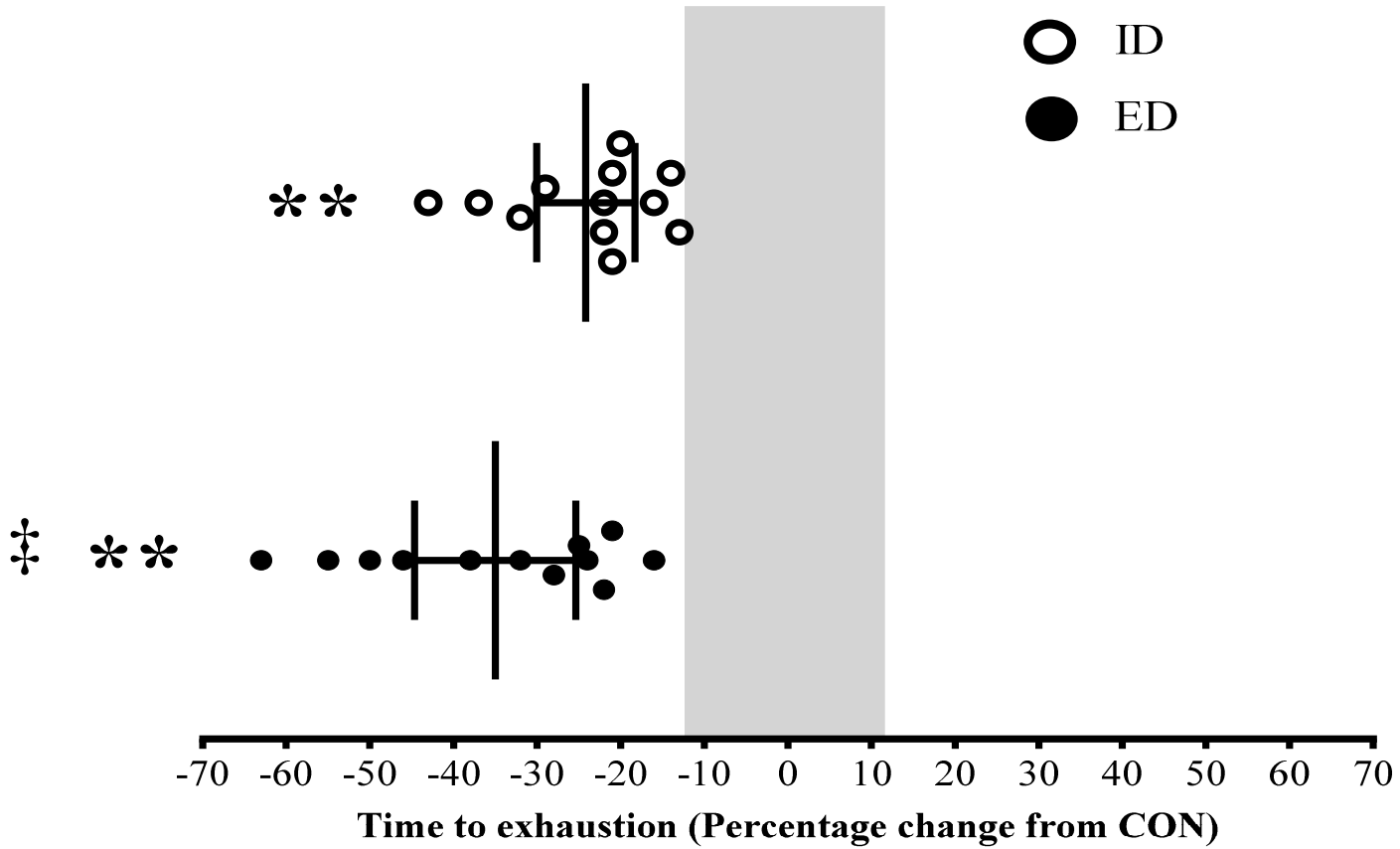

Figure 7.5 Percentage difference in TTE from control with mild intracellular and extracellular dehydration. **NOTE:** Abbreviations: CON, control trial; ID, intracellular dehydration trial; ED, extracellular dehydration trial. Shaded area represents variation based on the typical performance variability measured during practice sessions (± 11%). ‡ Significant difference between trials (paired t-test; $P < 0.05$) and **significant difference from natural variation (one-sample t-test; $P < 0.01$)

There were also differences between trials in ventilatory parameters during exercise, most notably minute ventilation ($P = 0.03$), tidal volume ($P = 0.01$) and breathing frequency ($P < 0.001$). More specifically, an increase in breathing frequency ($P < 0.001$ for CON vs. ED; $P = 0.004$ for ED vs. ID) and a lower tidal volume ($P = 0.02$ for CON vs. ED; $P = 0.03$ for ED vs. ID) resulted in a reduced minute ventilation on ED compared with CON ($P = 0.03$), and a similar trend between ED and ID ($P = 0.10$; **Figure 7.4B**). Conversely, hydration state did not appear to affect respiratory or metabolic parameters during the TTE ($P = 0.72$, $P = 0.69$, $P = 0.88$ oxygen consumption, percentage maximal oxygen consumption, respiratory exchange ratio respectively).

Mild dehydration *per se* also resulted in modest changes to thermoregulation which included a lower sweat rate (27.1 (4.1), 19.8 (3.9), 21.9 (3.9) $ml \cdot min^{-1}$ for CON, ID and ED respectively; $P < 0.001$ for CON vs. ID; $P = 0.01$ for CON vs. ED) and an increase in rectal temperature during exercise (37.3 (0.5), 37.4 (0.5), 37.5 (0.3) ^{o}C for CON, ID and ED respectively; $P = 0.02$ for CON vs. ID; $P = 0.03$ for CON vs. ED). However, the increase in rectal temperature did not result in differences in skin temperature between dehydration and control (31.3 (1.2), 31.3 (1.5), 31.0 (1.4) ^{o}C for CON, ID and ED respectively; $P = 0.35$). Despite similar a thermoregulatory response during exercise between dehydration types, thermal discomfort was only increased on ED compared with CON (8.9 (0.9), 9.2 (1.8), 9.7 (1.0) for CON, ID and ED respectively; $P = 0.008$ for CON vs ED).

At exhaustion there were no differences between trials in any of the perceptual or physiological parameters with the exception of a lower cardiac output on ED compared to CON (27.2 (4.0), 26.8 (4.1), 24.6 (2.1) $L \cdot min^{-1}$; $P = 0.04$ for ED vs. CON).

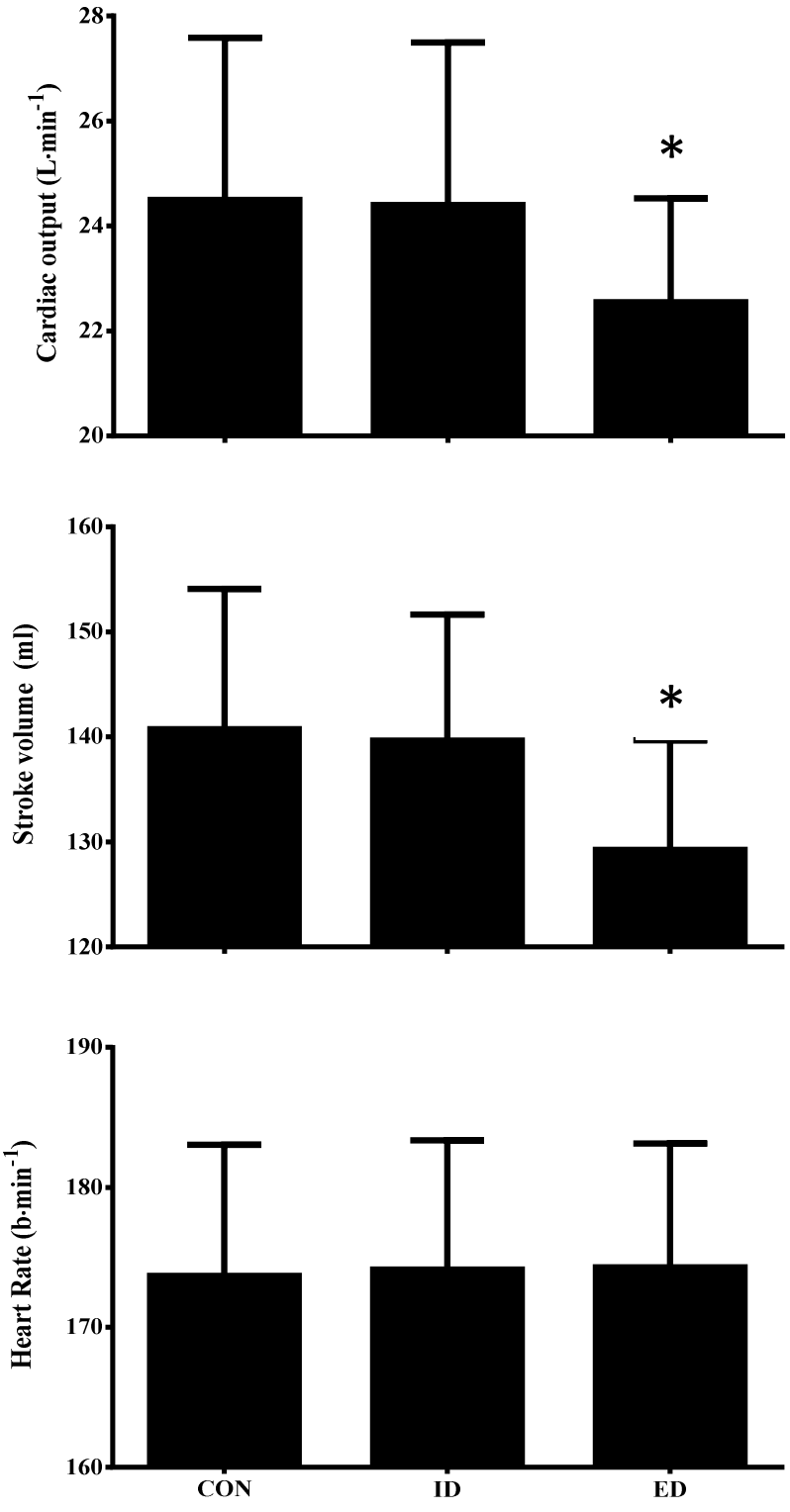

Figure 7.6 A. Cardiovascular response during TTE. **NOTE:** Abbreviations: CON, control trial; ID, intracellular dehydration trial; ED, extracellular dehydration trial. ‡ $P < 0.05$ vs. CON and ED; * $P < 0.05$ vs. CON. n = 10.

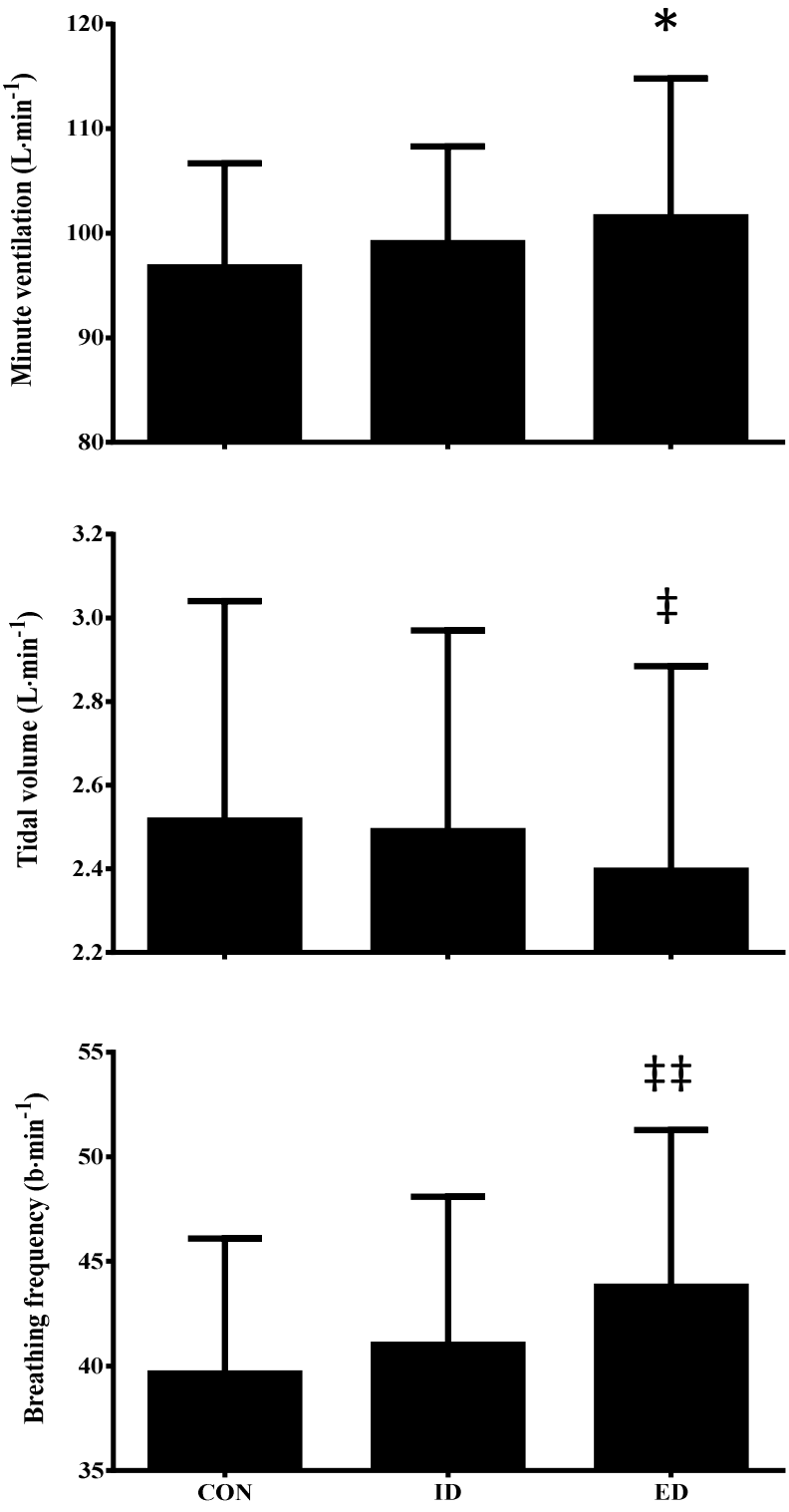

Figure 7.6 B. Ventilatory response during TTE. **NOTE:** Abbreviations: CON, control trial; ID, intracellular dehydration trial; ED, extracellular dehydration trial. ‡ $P < 0.05$ vs. CON and ED; * $P < 0.05$ vs. CON. n = 11.

7.5. Discussion

The purpose of this study was to examine the effect of two different types of mild dehydration (intracellular and extracellular) on endurance performance and to determine the effect of dehydration type on the proposed mechanisms by which dehydration limits endurance performance. A particular strength of the study was the dehydration model adopted. Prolonged fluid restriction over 48 h (ID) resulted in hyperosmolality (increase in blood solute concentration) with no change in blood volume. In contrast, diuretics (ED) resulted in an unchanged osmolality and hypovolemia. Many causes of dehydration result in a mixed dehydration where there are changes to both blood solute and volume (e.g. hyperosmolality and hypovolemia with exercise-heat stress). Therefore, for the first time this study allowed the comparison of two distinct types of dehydration at opposite ends of the blood solute and volume continuum. This has enabled a better understanding of the influence of hyperosmolality and hypovolemia on endurance performance and the mechanisms of fatigue during exercise with mild fluid deficits. In line with our hypothesis and previous research endurance performance was worse after mild dehydration than normal hydration (Armstrong et al., 1985; Cheuvront et al., 2005). The particularly novel finding was that this decrement in endurance performance was greater after mild extracellular dehydration than intracellular dehydration. The difference in endurance performance between dehydration types appeared to be due to different mechanisms of fatigue. Specifically, intracellular dehydration resulted in disrupted mood and subjective feelings in the absence of cardio-respiratory and musculo-energetic factors, whereas extracellular dehydration increased breathing frequency and decreased cardiac output. This suggests that dehydration type alters the magnitude and mechanism by which dehydration impairs endurance performance.

Mild dehydration *per se* likely reduced endurance performance due to a combination of psycho-physiological mechanisms that increased perceived exertion, which include; cardiovascular, ventilatory, thermoregulatory, disrupted mood and subjective feelings. However, the contribution of these mechanisms to fatigue during endurance performance was different and depended on dehydration type. In addition, it appears that dehydration associated with hypovolemia is more likely to have a negative effect on endurance exercise performance. In support of this, causes of dehydration known to result in a marked reduction in plasma volume also appear to reduce endurance performance, for example diuretics exercise in the heat and passive heat stress (Armstrong et al., 1985; Caldwell et al., 1984; Cheuvront et al., 2005; Montain et al., 1998). This would suggest that hypovolemia associated with some types of dehydration is likely to be a major factor in the development of fatigue during endurance performance in a fluid-depleted state. However, an exciting and novel finding from the current study was that intracellular dehydration evoked by prolonged fluid restriction (48 h) resulted in a decrease in endurance performance, in the absence of marked cardio-ventilatory or musculo-energetic factors.

Hypovolemia likely reduces endurance performance through a decrease in cardiac output via a reduced stroke volume during exercise (Fortney et al., 1983; Nadel et al., 1980; Roy et al., 2000). Dehydration-mediated decreases in cardiac output during exercise have been shown to result in a reduction in muscle blood flow at the termination of exercise (González-Alonso et al., 1998). Ultimately a reduction in muscle perfusion results in the development of fatigue due to inadequate oxygen delivery to the working muscle. An interesting observation in the present study was the effect of extracellular dehydration on ventilation (increased breathing frequency and minute ventilation and decrease in tidal volume; **Figure 7.4**). Increased ventilation during strenuous exercise ($\geq 85\%$ $\dot{V}O_{2max}$) has been shown to contribute to the

development of inspiratory muscle fatigue which has a negative effect on exercise performance (Amann, 2012a). In turn, this increases sympathetic vasoconstriction of vessels in the working muscle in order to redirect cardiac output to the respiratory muscles further compromising muscle oxygen delivery (Amann, 2012a). In addition, the increased ventilation during high-intensity exercise may further limit exercise performance by reducing central motor drive, due to the hypocapnia-associated cerebral vasoconstriction and subsequent decrease in cerebral perfusion and oxygen delivery to the brain (Seifert et al., 2009). In the present study, mean exercise intensity was ~85% of maximal oxygen consumption, and therefore it is possible that the increased ventilatory strain in the extracellular dehydration trial resulted in greater decrements in endurance performance possibly mediated by a redirection of cardiac output from the working muscle to the respiratory musculature and/or a reduction in cerebral perfusion.

An exciting and novel finding from the current study was that intracellular dehydration evoked by prolonged fluid restriction (48 h) resulted in a decrease in endurance performance, in the absence of cardio-respiratory or musculo-energetic factors. However, a particularly interesting finding in the present study was that disruptions to measures of affective well-being such as mood and subjective feelings before exercise were greater with mild intracellular dehydration and may have contributed to the decrease in endurance performance on this trial. The greater self-reported feelings of fatigue with mild intracellular dehydration observed in this study are in agreement with previous studies which have reported an increase in fatigue and tiredness in young healthy males and females following modest dehydration (2.7% BML) evoked by 37 h of fluid restriction (Shirreffs et al., 2004) and mild dehydration (≥ 1% BML) following 24 h of total fluid deprivation (Pross, et al., 2013) and a combination of exercise sweat loss and/or diuretic medication (Armstrong et al., 2012; Ganio et al., 2011).

Support that endurance performance can be reduced by increased feelings of fatigue, without altering physiological responses to exercise, is evident in studies that show mental fatigue induced by demanding cognitive tasks before exercise decreases time to exhaustion during both dynamic whole-body exercise (cycle ergometry) (Marcora et al., 2009) and submaximal isometric knee-extensor exercise (Pageaux et al., 2013). The authors have suggested that according to the psychobiological model of endurance performance (Marcora et al., 2008), exercise in constant–load tasks is terminated due to either a decrease in potential motivation or an increase in perception of effort. In the present study we did not observe any negative effect of intracellular dehydration on intrinsic or success motivation related to the endurance task. Accordingly a greater perceived exertion would have been expected with extracellular compared with intracellular dehydration as participants disengaged from the exercise task sooner. However, although perceived exertion was greater with mild dehydration *per se*, there were no differences between dehydration trials. Other potential mechanisms by which mild intracellular dehydration could have resulted in increases in perceived fatigue and subsequent decrement in endurance performance may lie in the effect of blood electrolyte disturbances on local brain regions. Electrolyte disturbances invoked by water deprivation have been shown to result in alterations to serotonergic function (Popova et al., 2001) and possibly hypothalamic signalling of cortical brain regions (Di & Tasker, 2004), both of which have been implicated in the regulation of subjective feelings (Ganio et al., 2011) and development of fatigue during endurance exercise (Meeusen et al., 2006).

To our knowledge this is the first study to investigate the effects of mild intracellular and extracellular dehydration on exercise performance in a well-controlled repeated measures design. In addition, the objective of the research design was effective as in contrast to the well maintain body mass on the control trial, the body mass losses in the dehydration trials

(ID and ED) were indicative of mild dehydration (2% BML). Further, prolonged fluid restriction over 48 h (ID) resulted in hyperosmolality (increase in blood solute concentration) with no change in blood volume. In contrast, diuretics (ED) resulted in an unchanged osmolality and hypovolemia. Categorization of dehydration type is primarily based on changes in blood solute concentration and blood volume. Many causes of dehydration result in a mixed dehydration where there are changes to both blood solute and volume (e.g. hyperosmolality and hypovolemia with exercise sweat loss). Therefore, for the first time this study allowed the comparison of two distinct types of dehydration at opposite ends of the blood solute and volume continuum. This has enabled a better understanding of the effect of mild dehydration on endurance performance and the mechanisms of fatigue during exercise with mild fluid deficits.

In conclusion, the results of this study show that mild intracellular and extracellular dehydration evoked by fluid restriction and diuretics respectively, result in decrements in endurance performance. An exciting new finding is that the decrease in performance following mild intracellular dehydration appears to be unrelated to disruptions in cardio-respiratory and musculo-energetic factors. Rather, an increase in negative subjective feelings (increased fatigue) appears to have contributed to the decrease in endurance performance. The greater decrease in endurance performance with mild extracellular dehydration coincided with an increased cardiovascular and ventilatory strain during exercise, likely due to the hypovolemia observed on this trial (6% decrease in plasma volume). Therefore, the results confirm that cardio-ventilatory factors, possibly caused by hypovolemia, appear to be an important mechanism in the decrement in endurance performance with dehydration.

CHAPTER EIGHT

General discussion.

8.1 Background

Mild to modest dehydration, or a fluid deficit equivalent to 2-3% of body mass, is common across populations and may be associated with negative consequences on human performance and well-being (Armstrong et al., 1985; Gopinathan et al., 1988; Ganio et al., 2011). A useful countermeasure involves the accurate identification of dehydration followed by simple and inexpensive oral rehydration. However, currently no consensus exists on the diagnostic superiority of hydration assessment markers (Armstrong, 2005). The lack of progress in the development of hydration markers may be due to the complexity of dehydration, which is poorly defined and often treated as a single condition (Crecelius, 2008). An underappreciated fact is that dehydration primarily manifests in one of two types, i.e. intracellular or extracellular dehydration (Nadel et al., 1941; Kozlowski & Saltin, 1964). An absence of standardised assessment techniques to identify dehydration according to type may lead to confusion, misdiagnosis and inappropriate treatment (Thomas et al., 2004). Further, the distinct physiological differences between these types of dehydration will likely determine the impact on human performance and well-being.

In light of this, the broad objectives of this thesis were to examine: 1. the diagnostic accuracy of dehydration markers to identify both intracellular and extracellular dehydration firstly, in an elderly hospitalised cohort and secondly, in a well-controlled laboratory study with healthy young participants; 2. whether stimulating saliva improved the diagnostic accuracy and practicality of hydration assessment with saliva parameters (saliva flow rate and saliva osmolality); 3. if mild intracellular and extracellular dehydration had a differential effect on endurance exercise performance and markers of well-being.

8.2 Summary of findings

One of the main findings of the thesis was that the diagnostic accuracy of hydration markers is dependent on dehydration type and the specific population being screened. In an elderly hospitalised cohort, saliva osmolality identified both intracellular and extracellular dehydration and demonstrated far superior diagnostic accuracy compared with routinely used urinary hydration parameters and clinical physical signs including blood pressure, which both had poor diagnostic accuracy to identify dehydration *per se* **(Chapter 4)**. In contrast, in a young healthy cohort thirst and urine osmolality identified mild intracellular and extracellular dehydration with moderate to high overall diagnostic accuracy. Postural heart rate change discriminated between normal hydration and mild extracellular dehydration, and urinary hydration parameters and plasma osmolality showed the best diagnostic performance to mild intracellular dehydration. An algorithm combining thirst, urine colour and postural heart rate change improved dehydration diagnosis by discriminating between dehydration types **(Chapter 6)**. When acute intracellular dehydration was evoked by exercising in the heat the diagnostic accuracy of saliva osmolality and saliva flow rate was comparable to both urinary markers and plasma osmolality. However, stimulating saliva resulted in a decrease in diagnostic accuracy, although stimulated saliva flow rate did have some utility as a marker of modest dehydration (fluid deficit equivalent to 3% of body mass) **(Chapter 5)**.

A further novel finding from the thesis was that mild intracellular and extracellular dehydration had differential effects on human performance and well-being. Specifically, mild extracellular dehydration resulted in a greater decrement in endurance exercise performance compared with mild intracellular dehydration. In addition, the mechanisms by which dehydration reduced endurance performance appear to be dependent on dehydration type. Mood and subjective feelings were unaltered with extracellular dehydration, but

physiological strain was higher during exercise. In contrast, intracellular dehydration caused poorer mood (lower vigour and higher fatigue), with less physiological strain. In conclusion, dehydration type alters the magnitude and mechanism by which dehydration impairs endurance performance **(Chapter 7)**.

8.3 Hydration markers

As previously discussed, hydration markers can be used as an effective countermeasure against the negative effects of dehydration on health and performance. However, markers of dehydration should accurately identify water deficits of 2% of body mass, as this threshold appears to be the point where human performance and health begins to suffer (Cheuvront & Kenefick, 2014). Currently, the diagnostic accuracy of traditional hydration markers to identify this magnitude of dehydration is unclear. Numerous hydration markers have been proposed but often they are not portable and require technical expertise (Armstrong, 2008). In addition, many hydration markers are invasive, expensive and time-consuming and therefore are not applicable to multiple assessments during the day, as may be required in clinical or athletic settings. The development of hydration markers has been further hindered by ignoring that the cause or type of dehydration as an important factor in the assessment process. Therefore the findings of this thesis provides further evidence on the prevalence of different types of dehydration in this population and reasons why there needs to be a paradigm shift in hydration marker research.

The prevalence of dehydration in elderly hospitalised patients is unclear. In **Chapter 4** the data suggested that almost 40% of patients above the age of 65 years admitted to hospital who were able to consent were dehydrated. Of these patients almost half presented with

intracellular dehydration (20%) and the other half with extracellular dehydration (19%). These figures are high compared with the previous literature which stated that dehydration on admission in this population and setting was between 5.3 and 6.7% from data derived from retrospective admission reports that included dehydration as a coded diagnosis (Vivanti, 2007; Warren et al., 1994). However dehydration is a difficult clinical diagnosis and can be under-reported due to a lack of definition (Thomas et al., 2008). When using physical signs as a method of assessing dehydration, 17% of elderly patients admitted to hospital were deemed dehydrated (Vivanti, 2007). Nevertheless, clinical and physical signs of dehydration are also confusing and have poor diagnostic accuracy to identify dehydration (Weinberg & Minaker, 1995). Therefore, a strength of the current study was the determination of dehydration and dehydration type by objective biochemical analysis using reference standards (i.e. Posm and BUN:Cr) (McGee et al., 1999; Stookey, 2005). The results suggest that dehydration is prevalent in this population and setting, and more importantly both intracellular and extracellular dehydration appear to be equally widespread. Suggesting that studies assessing the utility of hydration markers to identify dehydration type are warranted. However, there are potential limitations of using these reference standards to identify dehydration type. Although some believe that plasma osmolality is a 'gold standard' measure of hydration status, the marker has received criticisms from other authors (Armstrong et al., 2013). This is based on findings that plasma osmolality has an inconsistent response to dehydration, may be confounded by food or fluid intake and is a poor marker of hydration status when first morning fasted samples are analysed (Armstrong, 2007). Many of these criticisms centre around the fact that plasma osmolality is tightly regulated around a homeostatic set point, suggesting that any change in this marker may be transient. However, there is a body of literature that shows that this marker has utility and the diagnostic performance to identify intracellular dehydration (Bartok et al., 2004; Cheuvront et al., 2013; Oppliger et al., 2005;

Popowski et al., 2001). A limitation in the use of BUN:Cr ratio to aid in the identification of dehydration type is that BUN:Cr can be elevated due to other factors. These factors include renal failure, bleeding, congestive heart failure, sarcopenia, increased protein intake and the use of medications such as glucocorticoids (Thomas et al., 2008). However, in **Chapter 4** using a retrospective analysis of patient records, all participants with renal or cardiac failure or with a prescription for glucocorticoids were excluded from the analysis.

A general finding from the work on hydration assessment markers in this thesis is that the diagnostic accuracy of hydration markers changes dependent on the cause and therefore the type of dehydration (**Chapter 4-6**). Indeed an additional finding was that the diagnostic performance of markers is also dependent on the population being screened (**Chapter 4**). In **Chapter 4** saliva osmolality identified both intracellular and extracellular dehydration in an elderly hospitalised cohort, whereas saliva flow rate had no utility. This is partly in agreement with a previous study that showed that saliva osmolality was able to identify mild intracellular dehydration in a young healthy cohort when evoked by 48 h of fluid restriction and modest extracellular dehydration (3.2% of body mass) induced by fluid and energy restriction (Oliver et al., 2008). Further support for the utility of saliva osmolality to identify dehydration *per se*, is that this marker had utility in identifying modest mixed dehydration (3.1% of body mass) brought about by a combination of the diuretic furosemide and an overnight fluid restriction (Ely et al., 2014). This study showed that saliva osmolality had a moderate diagnostic accuracy to identify this type of dehydration measured by ROC analysis (AUC_{ROC} of 0.77 to 0.78). This suggests that saliva osmolality may be a useful screening tool to aid the initial diagnosis of dehydration in both elderly hospitalised patients and young healthy persons. However, in **Chapter 6,** although saliva flow rate and osmolality had fair to moderate diagnostic accuracy to identify mild intracellular dehydration following 48 h of

fluid restriction (AUC_{ROC} of 0.70), the diagnostic accuracy of these markers to identify mild extracellular dehydration was poor when evoked by a diuretic alone (AUC_{ROC} of 0.55). The inability of saliva osmolality or saliva flow rate to identify mild extracellular dehydration in a well-controlled laboratory study may be explained by the difference in the magnitude of dehydration between the studies (i.e. 2 % of body mass in **Chapter 6** vs. 3% of body mass in Oliver et al., 2008; Ely et al., 2014). This notion is supported by the fact that the diagnostic accuracy of saliva hydration markers increases with the magnitude of dehydration (**Chapter 5**). Therefore, saliva may have utility as a marker of extracellular dehydration at greater magnitudes of dehydration i.e. > 2% of body mass. As the magnitude of dehydration was not assessed in **Chapter 4**, it is feasible that the participants experienced an extracellular dehydration > 2% of body mass, thus explaining why saliva osmolality was able to identify extracellular dehydration in this population. One proposed mechanism for the utility of saliva as a hydration marker is that an increase in plasma osmolality during intracellular dehydration may account for the production of smaller amounts of more concentrated saliva and provide the diagnostic distinction for the utility of saliva flow rate and osmolality as markers of hydration (Ship & Fischer, 1997; Walsh et al., 2004). However, this does not account for the utility of saliva osmolality to identify extracellular dehydration in some settings. An alternative mechanism may be that an increase in sympathetic tone and increases in circulating AVP during dehydration *per se*, results in a decrease in saliva flow rate and increases in saliva osmolality (Dawes, 2004). AVP is a potent vasoconstrictor and may reduce the blood supplying the salivary glands or increase water and electrolyte reabsorption. In support of this theory, the threshold for AVP response is different depending on the type of dehydration. The intracellular dehydration threshold for AVP is thought to occur with a 2% increase in plasma osmolality. This corresponds to an intracellular dehydration of approximately 2% of body mass. Although AVP was not measured in **Chapter 6**, this

threshold was likely exceeded as indicated by a plasma osmolality increase of 2.1% and a body mass loss of 2% between baseline and 48 h following fluid restriction. In contrast, the extracellular dehydration threshold for AVP is thought to occur with blood volume losses > 10% or body mass loss > 4%. This threshold was not exceeded as blood volume decrease was 3.5% and the body mass loss was 2%. Therefore, in **Chapter 6**, it is unlikely that this extracellular dehydration threshold for AVP had been reached. Therefore, the potential differences in magnitude of dehydration and likely effect on AVP may account for contradictory diagnostic performance of the marker to this type of dehydration. Although this neruroendocrine mechanism may explain some of the findings in this thesis with regard to the utility of saliva hydration parameters, the inability of saliva flow rate to identify dehydration in **Chapter 4** is unclear, but may be associated with the xerostomia associated with some medication (Ben-Aryeh et al., 1984).

Another finding was that the diagnostic accuracy of saliva osmolality was comparable to the more widely establish markers in urine and plasma when mild to modest intracellular dehydration was evoked rapidly with exercise and heat-stress (**Chapter 5**). However, the diagnostic performance of the marker was worse than urine and plasma when intracellular dehydration was induced over 48 h (**Chapter 6**). Saliva gland function and subsequent composition of saliva may also be governed by autonomic regulation. Specifically, saliva flow rate decreases and saliva osmolality increases with parasympathetic withdrawal and an increase in sympathetic nerve activity (Chicharro, Lucía, Pérez, Vaquero, & Ureña, 1998). It is likely that the addition of exercise in the dehydration protocol in **Chapter 5** may have resulted in an increase in sympathetic nerve activity above that seen with fluid restriction alone and could have improved the diagnostic performance of saliva hydration parameters. Indeed, differences in dehydration methods and concurrent differences in neuroendocrine

response may also explain why saliva osmolality is a better marker of acute versus chronic dehydration. Tthe findings of this thesis are in agreement with a recent study which showed that saliva osmolality has comparable diagnostic accuracy to plasma osmolality and urine markers with acute dehydration during active heat stress (Muñoz et al., 2013). However, the diagnostic accuracy of saliva osmolality is lower than these other markers when dehydration occurs over a more prolonged period of time (passive heat stress). This is highlighted in **Chapters 5 and 6** which show the diagnostic accuracy of saliva osmolality assessed by AUC_{ROC} to be 0.7 with prolonged dehydration (48 h fluid restriction, **Chapter 6**) and 0.8 when dehydration is acute (exercise heat stress, **Chapter 5**).

The neuroendocrine response to dehydration may also explain the varied diagnostic performance of urine hydration markers. Urine markers (specific gravity and colour) had poor to moderate diagnostic accuracy to identify mild intracellular dehydration evoked by exercise and heat-stress (**Chapter 5**). However, when intracellular dehydration was evoked over a prolonged period of time by restriction of fluid the diagnostic performance of urine hydration markers was near perfect to perfect (**Chapter 6**). This is in agreement with a recent study which showed that urine hydration markers performed better when dehydration was evoked via passive compared to active heat exposure (Muñoz et al., 2013). As discussed in **Chapter 2**, this might be explained by the impact of an increase in sympatho-adrenal system activity on urine production during exercise compared with at rest (Zambraski, 1996; Melin et al., 2001). Specifically, an increase in sympathetic nervous system (SNS) activity has been suggested to cause vasoconstriction of the glomerular afferent arterioles which decrease renal blood flow which in turn decreases urine flow (Wade, 1996; Zambraski, 1996). The reduction in urine flow is accompanied by an impaired renal concentrating ability which may be responsible for the inferior sensitivity of urine indices during exercise. However, this does

not explain why urine hydration parameters were unable to identify dehydration in an elderly hospitalised cohort. This is more likely explain by a decrease in renal function with ageing or the influence of certain drugs on urine output (Rowe et al., 1976).

Heart rate variability may also prove to be a useful marker of dehydration. In **Chapter 6**, LF-HF, which is a proxy measure of the ratio between sympathetic to parasympathetic tone, was able to identify mild intracellular dehydration. It is likely that a greater magnitude of extracellular dehydration above the AVP threshold would have been coupled with an increase in sympathetic activity. Therefore this potential marker may have been useful in identifying both types of dehydration.

In summary, the findings of this thesis have shown that the cause and type of dehydration as well as the population being assessed affect the diagnostic performance of hydration markers. Future studies should focus on hydration marker diagnostic performance in different populations, such as children and other clinical populations. In addition, markers that aim to assess extracellular dehydration should be focused upon. Graded magnitudes of this type of dehydration may elucidate potential new marker such as indices of heart rate variability, saliva hydration parameters, thirst and some urine hydration markers. Further, studies should examine the validity of the multiple marker method proposed in **Chapter 6**, or other non-invasive alternatives, as these may prove useful in identifying the type of dehydration present and therefore the optimum rehydration solution can be administered. Thirst has been shown to be blunted with some external stressors e.g. heat, cold, exercise, therefore studies investigating the utility of thirst alone or in combination with other markers is needed. In addition to the limitations already mentioned the use of two saliva collection techniques may have affected the utility of saliva hydration parameters (**Chapters 4, 5 and 6**). Saliva

hydration parameters have clear utility as markers of hydration and further examination of collection procedures needs to be carried out to achieve a true picture of its use in different settings.

8.4 Effect of mild dehydration on endurance performance

The findings in **Chapter 7** showed for the first time that dehydration type had an effect on the magnitude of the dehydration-mediated decrease in endurance exercise performance. This is an important finding and may explain some of the previous equivocal findings regarding the effect of dehydration on endurance performance. Notably, this differential decrease in endurance performance appears to be due to different mechanisms of fatigue. These mechanisms of fatigue are likely to be numerous and have a cumulative effect on endurance performance. Of these mechanisms, the findings of **Chapter 7**, suggest that hypovolaemia is the main limiting factor in dehydration-mediated fatigue during endurance exercise. Hypovolaemia has been shown to decrease cardiac output and stroke volume during exercise resulting in a reduction in muscle, cerebral and respiratory blood flow (Fortney et al., 1983; Nadel et al., 1980; Roy et al., 2000; González-Alonso et al., 1998). This in turn results in a reduction in muscle perfusion and the development of fatigue due to inadequate oxygen delivery. In addition, a reduction in stroke volume during high-intensity endurance performance may lead to respiratory fatigue due to competition between working muscle and ventilatory systems (Amann, 2012a)

An interesting observation in **Chapter 7** was the absence of cardiovascular and ventilatory strain during endurance exercise with chronic intracellular dehydration evoked by fluid restriction. This suggested that there were different mechanism of fatigue responsible for the

decrease in performance. The increased disruption of markers of affective well-being may have contributed to this fatigue as previously postulated (Marcora et al., 2009). An interesting theory could be that the contribution of central and peripheral mediated fatigue was different depending on the type of dehydration. Indeed, it is likely that afferent feedback from the working muscles was greater during exercise following extracellular dehydration. Further, the discomfort associated with disruptions in mood (particularly an increase in fatigue) and subjective feelings may be due to an increase in central fatigue with intracellular dehydration (Meeusen et al., 2006). Alternatively, the effects of chronic intracellular dehydration may increase the mental effort required to maintain endurance performance as postulated in the psychobiological theory of fatigue (Marcora, 2008). The experimental design in **Chapter 7** certainly provides an interesting model to study aspects of fatigue during endurance performance. For example, future work could examine the effect of these types of dehydration on maximal muscle activation following fatiguing tasks which may elucidate the contribution of central fatigue to endurance decrements following both types of dehydration. In addition, different magnitudes of extracellular dehydration achieved by using different concentrations of diuretic medication coupled with more sensitive monitoring systems to assess respiratory fatigue and muscle blood flow may explain the contribution of these systems to fatigue during endurance exercise in the presence of hypovolaemia. A practical application of this information is that hydration strategies should aim to minimise hypovolaemia during or before exercise.

8.5 Effect of mild dehydration on affective well-being

Markers of affective well-being such as subjective feelings and mood have been shown to be disrupted by even mild dehydration. However, for the first time we have been able to show

that the type of dehydration will effect disruptions to subjective feelings and mood. This has important implications, as dehydration *per se* is thought to be widespread and many people are prescribed diuretic type medication for prolonged periods of time (Roy et al., 2000). In addition, the findings of **Chapter 7**, suggest that subtle increases in blood solute concentration are more likely to affect well-being than small changes in blood volume. Further work in this area should examine the effect of these types of dehydration and possibly mixed dehydration on a more thorough battery of markers of affective well-being.

The results of this thesis add to the existing literature with respect to the further refinement and development of hydration markers including; the use of diagnostic accuracy statistics which has been limited in hydration marker research; the examination of novel hydration markers such as indices of heart rate variability, tear and stimulated saliva hydration parameters; examination of the diagnostic accuracy of traditional hydration markers to identify two different types of mild dehydration at opposite ends of the blood-solute and volume continuum; examination of saliva parameters in an elderly clinical population. In addition, the thesis highlights the effect of two types of commonly experienced dehydration on human performance and well-being. Recent evidence suggests that humans may also acclimatize to the effects of dehydration and that this protective adaptation can lessen the negative effects on exercise performance. The mechanisms of dehydration-mediated decrements in endurance performance discussed in Chapter 7 may also add light to this recent development in the field (Fleming & James, 2014)

8.8 Conclusions

The studies reported in this thesis extend previous knowledge regarding the development of hydration markers and the effects of mild dehydration types on human performance and well-being. The main conclusions from this work can be summarized as follows:

1. Using haematological reference standards dehydration was present in 40% of patients over the age of 65 years who were able to consent. Notably, of these patients 20% presented with intracellular dehydration and 19% with extracellular dehydration. This suggests that both intracellular and extracellular dehydration exists in the population and setting (**Chapter 4**).

2. Saliva osmolality has fair to moderate diagnostic accuracy to identify both intracellular and extracellular dehydration in an elderly hospitalised cohort and has superior diagnostic performance to currently used routine physical signs and urine hydration parameters. On the contrary, saliva flow rate has poor diagnostic accuracy to identify either type of dehydration in an elderly hospitalised cohort (**Chapter 4**).

4. Stimulating saliva reduced the diagnostic accuracy of saliva hydration markers to identify acute intracellular dehydration evoked by exercise and heat-stress. Stimulated saliva osmolality has poor diagnostic accuracy to identify mild and modest acute intracellular dehydration. However, stimulated saliva flow rate did have fair to moderate diagnostic accuracy to identify modest dehydration (3% of body mass). In addition, stimulating saliva increased saliva flow rate and markedly reduced collection time, meaning that this marker could be used as a rapid, non-invasive marker in field-based settings (**Chapter 5**).

5. Saliva hydration markers had comparable diagnostic accuracy to identify both mild and modest intracellular dehydration (2 and 3% of body mass) when evoked by exercise and heat-stress (**Chapter 5**).

6. Thirst and urine osmolality had moderate to high diagnostic accuracy to identify mild dehydration *per se*. Urine hydration markers and plasma osmolality were the best markers of mild intracellular dehydration evoked by prolonged fluid restriction. Postural heart rate change had moderate diagnostic accuracy to identify mild extracellular dehydration evoked by diuretics (**Chapter 6**).

7. A non-invasive multiple-marker system utilising thirst, urine colour and postural heart rate change allowed the identification of mild dehydration, and dehydration type (**Chapter 6**).

8. Saliva hydration parameters had fair to moderate diagnostic accuracy to identify mild intracellular dehydration evoked by fluid restriction, but had less diagnostic accuracy than urine hydration parameters and plasma osmolality to this type of mild dehydration (**Chapter 6**).

9. Mild dehydration per se, reduced endurance exercise performance measured by time to exhaustion. However, endurance performance was worse following mild extracellular compared with intracellular dehydration. Increased physiological strain including ventilator and cardiovascular appeared to contribute to fatigue with mild extracellular dehydration (**Chapter 7**).

10. Markers of affective well-being such as mood and subjective feelings were disrupted to a greater degree with mild intracellular compared with extracellular dehydration. It is postulated that these disruptions contributed to the development of fatigue with this type of dehydration (**Chapter 7**).

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APPENDIX A

School of Sport, Health and Exercise Sciences,
Bangor University,
George Building,
Bangor,
Gwynedd, LL57 2PZ

PARTICIPANT INFORMATION SHEET
Version number: 1
Date: 7/2/2011

1. Study Title

The utility of novel hydration markers and clinical assessment methods in detecting dehydration in the elderly.

2. Invitation paragraph

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

3. What is the purpose of the study?

The measurement of a person's hydration status is extremely important since a reduction in the amount of water in your body (dehydration) is a leading cause of hospitalisations of the elderly, and continued dehydration increases the risk of further complications in a number of illnesses. The current clinical methods used to assess a person's hydration status are not well defined, and are very subjective. We have recently identified that simple measurements on a saliva and/or tear fluid sample may provide an accurate measure of hydration status. It is the aim of this study to assess the accuracy of currently used clinical hydration assessment methods, and to investigate the usefulness of new saliva and tear fluid-based hydration methods.

4. Why have I been chosen?

You have been chosen because you are over 60 years of age and have been admitted to Ysbyty Gwynedd Hospital. We believe that you do not have any other medical condition that excludes you from taking part in this study. One hundred and eighteen other persons will be recruited from this hospital.

5. Do I have to take part?

Your taking part in this study is entirely voluntary. It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be

asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. Your decision on whether you take part, or not, will not affect the standard of care you receive now or in the future.

6. What will happen to me if I take part?

All of the hydration assessment measures that we collect would normally be taken as routine upon admission to hospital with the exception of a saliva and tear fluid sample. We are not changing any of your usual care.

Upon admission to hospital, a clinician will make an assessment of your hydration status. To do this they will take a blood pressure measurement, measure your pulse, and observe the dryness of your armpit and mouth. They will then take a blood sample from a vein in your forearm, and if possible, they will ask you to provide a urine sample. You will then be asked to provide a saliva sample. To do this, you will be asked to place a swab under your tongue, and leave it there for 4-8 minutes whilst saliva is collected. This swab is similar in size and shape to a lolly pop, and the collection procedure is sterile and painless. You will then be asked to provide a tear fluid sample. This will be performed by placing a sterile pen-like device on your lower eye-lid for no more than 5 seconds. Again, this procedure is painless. Finally, we will ask you to rate the dryness of your mouth and eyes on a simple line scale. This will complete all the hydration assessment measures. If you are assessed as hydrated (normal hydration) by the care team, this will conclude your involvement in the study and we will not collect any more samples.

If the care team's initial assessment indicates you are dehydrated, then you will be admitted to hospital to undergo fluid replacement. This is standard hospital protocol. We will collect another set of samples (clinician's assessment, blood, urine, saliva, tear fluid, and dryness scales) once you have been fully hydrated. It is anticipated that this measurement will occur 1-2 days after your first assessment. Once you have been fully hydrated and all samples have been collected, your participation in the study will cease.

It is important to re-affirm that except for the collection of the saliva and tear fluid samples, and rating your mouth and eye dryness, all procedures that happen to you would occur as routine to anyone who is admitted to hospital. We are not changing any of your usual care.

7. What do I have to do?

You will not have to do anything outside of normal care throughout the study except provide 1-2 saliva and tear fluid samples and rate your mouth and eye dryness.

8. What is the procedure that is being tested?

We are determining whether the currently used hydration assessment methods used at Ysbyty Gwynedd are accurate, and whether new saliva or tear fluid-based sampling methods offers additional accuracy to the assessment of hydration.

9. What are the alternatives for diagnosis or treatment?

Currently used clinical methods to assess hydration may not be very accurate, and vary depending on who takes the measurement. Whilst other, more accurate methods can be used

to assess hydration, they are often time consuming, expensive and invasive, thus may not be practical in a clinical setting. Our aim is to assess the accuracy of quick, simple, inexpensive and relatively non-invasive hydration markers in saliva and tear fluid: these methods might present viable alternatives for diagnosing dehydration.

10. What are the side effects of taking part?

Since we are not providing an intervention, or asking you to perform any treatment outside of normal care, it is not anticipated that you will experience any side effects from participating in this study.

11. What are the possible disadvantages and risks of taking part?

We do not anticipate any disadvantages or risks to you by taking part in this study. You may experience slight discomfort by the saliva sampling and tear fluid sampling procedures, but any discomfort you experience will be short lived.

You can contact the following person if you are worried about anything:
Dr Claire Bishop on (07534 850557)

12. What are the possible benefits of taking part?

Whilst taking part in this study will have no immediate benefit to you, data gleaned from this study will be valuable in terms of making assessments on the accuracy and usefulness of currently used clinical methods to assess hydration. In addition, if saliva and/or tear fluid sampling is shown to be accurate in determining hydration status, these may provide rapid methods by which clinicians can assess hydration in patients admitted to hospital, thereby accelerating the correct course of treatment.

13. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about our testing protocol. If this happens, we will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information, the research team might consider it to be in your best interests to withdraw you from the study. He / she will explain the reasons and arrange for your normal care to continue.

14. What if something goes wrong?

If taking part in this research project harms you, there are no special compensation arrangements. However, if you are harmed due to our negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanisms may be available to you.

15. Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital and University will have your name and address removed so that you cannot be identified from it. In addition, only the following people will have access to your medical records: Dr. Claire Bishop (Clinical Research Fellow), Dr. Neil Walsh (Principal Investigator) and Dr. Matthew Fortes (Research Investigator).

16. What will happen to the samples and results of the research study?

The overall results of this study may be published in international medical journals. We can provide you with copies of these if you wish. You will not be identified in any publication. By signing our consent form please note that you are also agreeing to us storing your data for five years and using your data in similar future studies. The data will be handled in the same manner as in the present study and again you will not be identified. Should you withdraw from the study for any reason, your data will be destroyed.

18. Who is organizing and funding the research?

This research is being organised jointly by the School of Sport, Health and Exercise Sciences, Bangor University and The Department of Elderly and General Medicine of Ysbyty Gwynedd. It is being funded by a US based company, Hydra DX. Neither the Doctors nor researchers are being paid extra for conducting or including you in this study. None of the researchers have a conflict of interest.

19. Who has reviewed the study?

This study was reviewed and approved by the North West Wales Research Ethics Committee.

20. Contact for further information

If you need further information please contact: Dr. Claire Bishop (07534 850557) cm.bishop@yahoo.co.uk of Ysbyty Gwynedd, or Dr. Matthew Fortes (01248 383687) (m.fortes@bangor.ac.uk) of Sport, Health and Exercise Sciences, Bangor University, George Building, Bangor, Gwynedd, LL57 2PZ.

Thank you very much for taking the time to read this information sheet.

If you choose to participate in this study you will be given a copy of the information sheet and a signed consent form to keep.

School of Sport, Health and Exercise Sciences,
Bangor University,
George Building,
Bangor,
Gwynedd, LL57 2PZ

The usefulness of stimulated saliva indices to track dehydration during exercise heat stress and subsequent fluid restriction.

Participant Information sheet

Project Investigators

Dr Matthew Fortes
Dr Neil Walsh
Dr Sam Oliver
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Invitation to take part

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, family and your GP if you wish. If there is anything that is not clear or if you would like more information, please do not hesitate to ask us. Take time to decide whether or not you wish to take part.

What is the purpose of this study?

We have previously shown that simple measurements of saliva (e.g. the total amount of protein within saliva) can be used to detect dehydration. Measurement of a person's hydration status is very important since research shows that even modest dehydration (as little as 2% of body mass loss) can impair physical and mental performance. The aim of this study is to assess the usefulness of specific saliva proteins in tracking changes in hydration status. These novel saliva markers will be compared to traditional gold standard markers of dehydration at rest (whilst hydrated) and at various levels of dehydration. Dehydration will be induced by exercising in a hot, humid environment.

Why have I been chosen?

You have been chosen because you are recreationally active, aged between 18 and 40 years of age, free from infection and dental disease, and not currently taking medication.

Do I have to take part?

This is entirely your decision. If you decide to take part you will be asked to sign a consent form. You will be given a copy of this form to keep for your information. You will be free to withdraw at any time without giving a reason. If you decide not to participate, it will not affect your relationship with the School of Sport, Health and Exercise Sciences or any of the researchers. All the information collected will be treated confidentially.

What will happen to me if I take part?

The study will involve you making five visits to the School of Sport, Health and Exercise Sciences. The purpose and time commitment of each visit are outlined below:

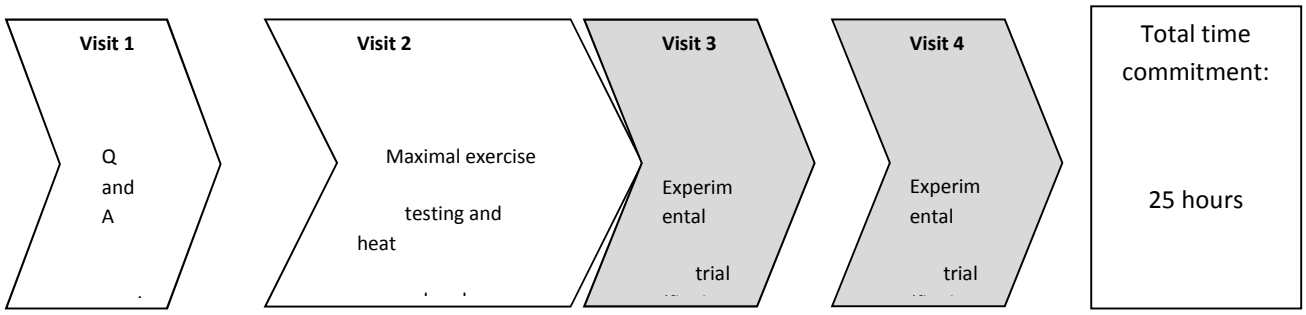
Visit 1) You will be talked through the subject information sheet by an investigator and given the opportunity to ask any questions. If you decide to take part, you will be asked to sign a consent form (visit length ~30 mins).

Visit 2) During this visit you will be asked to perform an incremental exercise test on an exercise cycle which will last approximately 20 minutes. This test starts off easy and gets progressively harder until you cannot continue. This will allow us to calculate your maximal oxygen uptake (a good indicator of your overall fitness) and to calculate the intensity at which you will exercise during the experimental trial (visit 5). Following this test, you will be asked to cycle in a hot, humid environment at a moderate intensity for about 30 minutes. By measuring your nude body mass before and after this exercise session, we will be able to calculate your sweat rate (visit length ~ 2 hours). We will also ask you to provide two saliva samples prior to and immediately after exercise.

Visit 3). This is the main experimental trial lasting ~11 hours. You will be asked to report to the department at 8am to provide baseline urine, saliva and nude body mass measures. You will then have a 6 hour period in the department when you are free to do as you wish as long as it only involves light activity (e.g. reading, working). We will provide you with a standardized breakfast and lunch during this period, and will provide you with fluids in order that you start exercise in a hydrated state. The exercise trial will begin at 2pm and will consist of you performing cycle exercise in a hot and humid environment. Before you begin exercising, we will ask you to provide urine, saliva and blood samples, and to weigh yourself nude. The blood sample will be taken by venepuncture of a forearm vein. You will also be asked to provide these measures again when you are 1%, 2% and 3% dehydrated. In order for us to ascertain these levels of dehydration, you will stop exercising and weigh yourself nude at regular intervals (i.e. weight loss will indicate degree of dehydration). In total, you will be exercising for approximately 90 minutes during which time you will be given fluid equivalent to your estimated sweat losses. After exercise, you will be asked to remain in the department for 1 more hour. A final blood, saliva, urine and nude body mass measure will be collected upon completion of the 1 h period, after which you are free to leave.

Visit 4). This is the second experimental trial lasting ~11 hours. You will be asked to report to the department at 8am to provide baseline urine, saliva and nude body mass measures. You will then have a 6 hour period in the department when you are free to do as you wish as long as it only involves light activity (e.g. reading, working). We will provide you with a standardized breakfast and lunch during this period, and will provide you with fluids in order that you start exercise in a hydrated state. The exercise trial will begin at 2pm and will consist of you performing cycle exercise in a hot and humid environment. Before you begin exercising, we will ask you to provide urine, saliva and blood samples, and to weigh yourself nude. The blood sample will be taken by venepuncture of a forearm vein. You will also be asked to provide these measures again at three separate time points during exercise. At these time points, you will stop exercising and weigh yourself nude (i.e. maintaining weight will indicate that you are hydrated). In total, you will be exercising for approximately 90 minutes during which time you will not be allowed to drink any fluid. After exercise, you will be asked to remain in the department for 1 more hour during which time we will fully rehydrate you. A final blood, saliva, urine and nude body mass measure will be collected upon completion of the rehydration period, after which you are free to leave.

Summary of visits:



What do I have to do before each visit?

We ask that in the 24 hours prior to visit 3 and 4 you do not perform any unaccustomed exercise, drink alcohol or consume any form of diuretics or tobacco. Additionally, we ask you to consume a meal high in carbohydrates (e.g pasta, rice or potatoes) the night before the visits 3 and 4.

What are the possible disadvantages and risks of taking part?

The disadvantages of taking part in this study are the discomfort associated with the use of temperature thermistors, blood sampling, and exercising in a hot environment whilst dehydrated, and the embarrassment associated with nude body mass measurement. For your safety your core temperature will be monitored by a rectal temperature probe inserted 10 cm beyond the anal sphincter. These probes are commonly used in thermoregulation research and most people do not notice them once they have started exercising. Blood sampling will be performed by qualified phlebotomists and all procedures will be put in place to ensure sterility of the blood letting procedure. However, you may experience bruising of the area around the venepuncture site. Any discomfort you experience will be short lived. For the measurement of nude body mass you will step behind a screen, remove all your clothes and step onto a platform scale. You will read your own body mass out loud to the experimenter and will be completely hidden from any view to maintain your privacy. There are standard risks associated with performing intense exercise in hot conditions. Accordingly, all experimenters are trained in recognizing and treating signs and symptoms of exertional heat illness. Additionally, persons trained in CPR and AED will be readily available or present at all exercise trials. However, it should be noted that measures are in place to minimize the risk of exertional heat illness, and accordingly that risk of this occurring is very small.

What are the possible benefits of taking part?

Taking part in this study will enable you to receive comprehensive feedback, with full explanations of your fitness level. The feedback you will receive regarding your fitness level is similar to that which many fitness testing centres provide as a fee-paying service. Advantages for undergraduates from within SSHES are that you will gain a valuable insight into the procedures and work involved in a PhD project. Additionally participation in this project can cover both the open and personal performance skills units of the undergraduate portfolio.

What if something goes wrong?

If you are harmed whilst taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study you may contact the Head of the School of Sport, Health and Exercise Sciences, Dr Tim Woodman.

Will my taking part in this study be kept confidential?
All information collected about you during the course of the study will be kept strictly confidential. It will be anonymized so that only the following people will have access to your records:

Dr Neil Walsh (University lecturer), Dr Sam Oliver (University lecturer), Julian Owen (PhD student)

Your data will be stored on a password protected computer and in locked filing cabinets. This data will be destroyed 10 years after the conclusion of the study.

What will happen to the results of the research study?
The results from this study may be published in a leading international physiology journal. Your blood samples will be stored for a maximum period of six years and may be used in future investigations in this area of research. Again, you will not be able to be identified from this. A written report will be given to any participants who are interested in the results of the study.

Who is organising and funding the research?
This research is organized by the named investigators at Bangor University and is funded by HydraDx.

Who has reviewed the study?
The study has been approved by the School of Sport, Health and Exercise Sciences ethics committee of Bangor University.

Contact for further information
If you require any further information about the study please contact any of the project investigators whose contact details are displayed below:

Julian Owen pepc26@bangor.ac.uk 01248 38 8273

Thank you very much for taking the time to read this information sheet.

If you choose to participate in this study you will be given a copy of the information sheet and a signed consent form to keep.

PRIFYSGOL BANGOR UNIVERSITY

School of Sport, Health and Exercise Sciences

Bangor University,
George Building,
Bangor,
Gwynedd,
LL57 2PZ

Research Title: Exercise capacity and utility of novel hydration markers after dehydration evoked by exercise and fluid restriction or diuretics.

Research Investigators: Dr Sam Oliver, Julian Owen.

Research Co-ordinator: Dr Sam Oliver
Telephone: 01248 383965
Email: s.j.oliver@bangor.ac.uk

Invitation to take part
You are being invited as an athletic male to take part in a research investigation. Before you decide to take part it is important for you to understand why the research is being conducted and what will be required of you should you agree to be involved. Please take time to read the following information carefully and discuss it with the investigators. Ask us if there is anything that is not clear or if you would like more information.

Do I have to take part?
This is entirely your decision. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form**. If you decide to take part you are still free to withdraw at any time without giving a reason.** If you decide not to participate or withdraw during the study, your decision will not affect your relationship with the School of Sport, Health, and Exercise Sciences, or any of the investigators involved in the study. **All information collected during the study will be treated confidentially**.

Background
Modest levels of dehydration (2-3% body mass loss) are typically experienced by athletes and military personnel in daily life as result of training and competition. From previous investigations it is unclear if modest dehydration negatively affects people's capacity to complete exercise. These differences may be due to the method by which dehydration is caused. Exercise capacity has been shown to be significantly reduced after diuretic induced modest dehydration. In contrast, a similar magnitude of dehydration evoked by prolonged fluid restriction has recently been shown to have no significant negative effect on exercise capacity. These differences in exercise capacity are perhaps related to diuretics inducing greater cardiovascular and thermoregulatory strain compared with fluid restriction. This hypothesis has yet to be investigated, therefore the primary aim of this study is to compare the effects of different methods of dehydration on:

- Exercise capacity
- Cardiovascular and thermoregulatory strain prior to and during exercise.

We have also previously shown that simple measurements of saliva (e.g. the total amount of protein within saliva) and concentration of tear fluid can be used to detect dehydration. However, it is unclear whether different method of evoking dehydration will alter the effectiveness of these hydration markers. Therefore, the secondary aim of this study is to compare the effects of different methods of dehydration on the:

- Effectiveness of saliva and tear hydration markers in tracking dehydration

These novel markers will be compared to traditional gold standard markers of dehydration at rest (whilst hydrated) and at various levels of dehydration.

What will be expected of you?

Before starting experimental trials you will be required to:

- Perform a maximal aerobic exercise test on a cycle ergometer
- Attend an initial health screen at Ysbyty Gwynedd

Prior to experimental trials you will be required to:

- Consume only the food and drink provided
- Refrain from exercise 24 h before trials

During experimental trials you will be required to

- Attend the lab on 3 separate occasions per trial of (there are 3 trials to complete)
- Have 4 venous blood samples (8 ml per sample) taken per trial.
- Consume only the food and drink provided
- Perform 2 exercise capacity tests per trial.
- To be weighed nude (behind the privacy of screens) and provide urine, saliva and tear samples

Summary of visits

Visit 1: Project Briefing (~30 minutes)
At this meeting, you will be fully briefed about the requirements of the project. You will be talked through the subject information sheet by an investigator and given the opportunity to ask any questions. You will then leave with the information sheet so as to allow you time to discuss your possible involvement in the study with significant others. This will also allow you additional time to think of questions.

Visit 2: Health Screen (~1 h)
As this study involves the use of diuretics which work by inhibiting sodium and water reabsorption in the kidney, you will have to have an initial health screen to check for normal blood electrolyte balance and renal function. This will take place at Ysbyty Gwynedd where results will be interpreted by medical doctor.

Visit 3: Aerobic fitness & familiarisation session (~3 h)
On an agreed day between 12:00-14:00 hours you will be required to perform a 10-15 minute cycle ergometer test that will be used to measure your aerobic fitness (maximal oxygen uptake) and establish your peak power output on the bike (PPO). This test will require you to cycle at your maximal aerobic capacity for approximately one minute.

After a recovery period, this test will be followed by a familiarisation of the exercise capacity test that will be used in the main experimental trials. This test will require you to cycle for as long as possible at 75% of your peak power output until exhaustion. Prior to and during this test measurements that will be recorded during the experimental trials will be taken to familiarise you with equipment. These include cardiovascular (Heart rate, stroke volume, cardiac output), thermoregulatory (core temperature and skin temperature), perceptual (perceived exhaustion, thermal sensation, thirst) and

cognitive (memory recall and Stroop test), measures. The details of these measures are described in further detail below.

Familiarisation of other techniques will also take place during this visit including: unstimulated and stimulated saliva collection, tear collection and other ocular measures, venous blood, urine, and nude body mass measures. A blood sample will be collected by a qualified member of staff from a forearm vein using a small needle (~ 8 ml of blood). Urine will be collected in privacy by passing urine into a plastic container and nude body mass will also be measured in privacy behind a screen.

Cardiovascular, respiratory, thermoregulatory, perceptual and cognitive measures
These measurements will take place prior to and during exercise in both the familiarisation session and each experimental trial.

1. Cardiovascular measures refer to heart rate, stroke volume, blood pressure and cardiac output. These will be measured via a machine that requires electrodes to be attached to your chest, neck and back.

2. Respiratory measures refer to the analysis of expired air which will involve wearing a face mask.

3. Thermoregulation refers to skin temperature which will be measured via electrodes placed on skin sites and core temperature, which will require the use of a rectal thermometer.

4. Perceptual measures refer to thirst, perceived exhaustion, thermal sensation and motivation. These will be measured by asking you to indicate a rating on a scale or complete a questionnaire.

5. Cognitive measures refer to memory recall and reaction time. These will be measured by asking you to complete 2 different tests on a laptop computer.

Experimental trials (~14 h each)

In addition to the health screen and familiarisation session you will be required to take part in three 48 hour experimental trials in a randomised order, separated by 7 days. Each trial will include a pre exercise capacity test (0 h), an intervention period, a post exercise capacity test (48 h) and a rehydration period.

The three experimental trials are a euhydrated control trial, a prolonged fluid restriction trial and a diuretic trial . On fluid restriction and diuretic trials the target dehydration prior to starting the 2nd exercise capacity test is a ~2.5% body mass loss from the body mass at the start of the trial.

Protocol
(A schematic flow chart of the study protocol is attached at the back of this form)

On the day prior to each trial you will be provided with food and water to meet your nutritional requirements and asked to refrain from exercise and consuming alcohol.

Day 1

7:30 h. You will report to the lab where a first morning urine sample will be collected and body mass will be recorded

8:00 h. An unstimulated and stimulated saliva sample, tear, urine and blood sample will be collected and you will be asked to complete a questionnaire to rate your thirst level. Following this you will be

provided with breakfast and be free to carry out your normal daily activities with the exception of only eating and drinking what is provided by the investigators and not performing any formal exercise (i.e. go running or to the gym)

11:30 h. You will be asked to return to the lab where you will be provided with lunch after which you can return to your normal activities.

13:30 h. You will report back to the lab and be prepared for cardiovascular, respiratory and thermoregulation measures. Blood (venous and earlobe blood sample), saliva, tear and urine samples will be taken prior to exercise. You will also be asked to complete two cognitive function tests and record perceptual measures prior to exercise.

14:00 h. You will begin the exercise capacity test which requires you to cycle at 75% peak power output to exhaustion. During this exercise, cardiovascular, respiratory and thermoregulation and perceptual measures will be measured. Post exercise a venous blood sample will be taken.

On completion of this exercise, sweat losses that have occurred will be replaced for control and diuretic trials, however, on the fluid restriction trial sweat loses will not be replaced and your fluid intake will be restricted (~200 ml to drink per day) from this point onwards, this is in order to achieve a dehydration equal to 2.5% of your body weight prior to completing the exercise test on day 3.

Day 2

7:30 h. You will report to the lab where a first morning urine sample will be collected and body mass will be recorded

You will be provided with a prescribed food and fluid on all trials to meet your estimated daily requirements, however fluid will be restricted on the fluid restriction trial (~ 200ml a day). You will be asked to refrain from exercise and to record your perception of thirst at 8:00 h and 14:00 h.

Day 3

7:30 h. You will report to the lab where a first morning urine sample will be collected and body mass will be recorded

8:00 h. An unstimulated and stimulated saliva sample, tear, urine and blood sample will be collected and you will be asked to complete a perception of thirst questionnaire before consuming breakfast. Body mass will then be recorded and on the diuretic trial you will ingest 40 mg of a diuretic (Furosemide) in order to induce a ~ 2.5% body mass loss. Body mass will then be recorded at 1 h intervals from this point to ensure the target dehydration is achieved. During this period you will have access to television, internet and computer consoles for entertainment.

11:30 h. You will be provided with lunch

13:30 h. You will be prepared for cardiovascular, respiratory and thermoregulation measures. An unstimulated and stimulated saliva sample, tear fluid sample, a blood (venous and earlobe) and urine sample will be taken prior to exercise. You will again be asked to complete two cognitive function tests and record perceptual measures prior to exercise.

14:00 h. You will begin the exercise capacity test which requires you to cycle at 75% of your maximum effort until exhaustion. During this exercise, cardiovascular, respiratory and thermoregulation and perceptual measures will be measured and blood samples will be taken from the earlobe. Post exercise, an unstimulated and stimulated saliva sample, tear fluid sample venous and earlobe blood samples will be taken. On fluid restriction and diuretic trials this exercise will occur when you are dehydrated by ~ 2.5% of your body mass at the start of the trial. Before leavening the

lab you will be rehydrated with a carbohydrate drink (e.g. flavoured drink that contains sugar - Lucozade sport) in order to replace fluid losses that have occurred during the trial.

What are the possible disadvantages and risks of taking part?

The disadvantages of taking part in this study, which you will probably be most concerned about are: blood samples, diuretics, fluid restriction and exercising when dehydrated.

1. Venous blood samples

All venous blood sample collections will be conducted by fully qualified phlebotomists (certified by Ysbyty Gwynedd). Blood samples will be taken with a very small needle and will require only 15 ml per sample of which there are 4 per trial. Therefore the amount of blood per trial is only ~ 60 ml, a total of ~ 180 ml for the whole study.

2. Tear fluid collection

A very small amount (~50 nL) of tear fluid will be collected using a commercially available device used primarily in the diagnosis of dry-eye disease (Tearlab osmolarity system, USA). This device will be place on your lower eyelid for a few seconds which is enough time to collect a tear sample. We have conducted a full study using this device to collect tear fluid and the procedure is both painless and non-invasive and causes only minor irritation to the eye (i.e. a slight watering). The test cards used to collect the device are sterile and only used once. The test cards used to collect the device are sterile and one use only. Non-invasive tear break-up time (NITBUT) measurement will be made using a Keeler Tearscope® which is a handheld instrument used for routine dry eye examination. Tear secretion rate will be assessed using the phenol red cotton thread test. This is a simple, routinely used test in dry eye examinations whereby a single-use and sterile thread is placed on the lower tear meniscus and the length of wet thread is recorded after a standardized 15 second period.

3. Diuretics

You may also be concerned about the other method of dehydration, diuretics. Other than dehydration, a side effect of furosemide is hypokalaemia (reduction in plasma potassium). The risk of this occurring will be minimised by ensuring you have normal blood chemistry and renal function prior to starting the experimental trials. In addition, only a small dose of diuretics will be administered (which will be prescribed by medical doctor: Dr Saeed Rahman, a consultant nephrologist at Ysbyty Gwynedd) therefore the incidence of hypokalaemia extremely unlikely. It is also noteworthy that other studies have administered diuretics prior to exercise and reported no unexpected or harmful side effects

4. Fluid Restriction

A disadvantage of this study is that your fluid will be restricted on the fluid restriction trial for a 48 h period. It is important to remember that you will still be provided with some fluid (~ 200 ml a day) and your food will also provide a source of fluid. Other studies have used fluid restriction as a method of dehydration and found no side effects.

5. Exercising when dehydrated

Lastly, exercising when dehydrated may be seen as a disadvantage to this study. Side effects may include increased cardiovascular strain (e.g. raised heart rate and decrease blood pressure), elevated core body temperature during exercise and general symptoms such as fatigue, headaches, thirst and muscle cramps may also occur. It is important to note that ~ 2.5% is a mild level of dehydration. Numerous studies have exercised individuals following dehydration of this level and at greater levels

(e.g. 7%) and have reported no side effects in athletes who are of similar age and fitness to you. In terms of the exercise itself, the exercise capacity test intensity is similar to what you would often experience in training and competition and the termination of the test is under your control.

It is also important to remember you have to visit the lab on 7 separate occasions and the total time of the study is 46 h. If you have any further questions these will be happily answered by Sam Oliver or any of the other investigators.

What are the possible benefits of taking part?

A benefit of taking part in this study is that you will receive comprehensive feedback and explanation of your aerobic fitness. This feedback will provide you with information of your current fitness helping you plan and monitor your athletic training program. In addition, the initial screen assessing kidney function and blood electrolyte balance will provide information about your renal function health.

APPENDIX B

INFORMED CONSENT FORM

School of Sport, Health and Exercise Sciences
Bangor University

Betsi Cadwaladr University Health Board
Ysbyty Gwynedd

1	Title of project	The utility of novel hydration markers and clinical assessment methods in detecting dehydration in the elderly
2	Name and e-mail address(es) of all researcher(s)	Dr. Neil Walsh (n.walsh@bangor.ac.uk) Dr. Matthew Fortes (m.fortes@bangor.ac.uk) Dr. Salah Elghenzai (Salah.Elghenzai@wales.nhs.uk) Dr. Claire Bishop (cm.bishop@yahoo.co.uk)

Please tick boxes

1 I confirm that I have read and understand the Information Sheet dated 7/2/2011 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the standard of my medical care now or at any time in the future, nor will my legal rights be affected. ☐

3 I understand that I may register any complaint I might have about this experiment with the Head of the School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

4 I agree to take part in the above study. ☐

Name of Participant …….

Signature ……. Date ……..

Name of Person taking consent…….

Signature ……. Date ……..

WHEN COMPLETED – ONE COPY TO PARTICIPANT, ONE COPY TO RESEARCHER FILE

INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT OR EXPERIMENT

Title of Research Project: The usefulness of stimulated saliva indices to track dehydration during exercise heat stress and subsequent fluid restriction.

The researcher conducting this project subscribes to the ethics conduct of research and to the protection at all times of the interests, comfort, and safety of participants. This form and the information sheet have been given to you for your own protection and full understanding of the procedures**.** Your signature on this form will signify that you have received information which describes the procedures, possible risks, and benefits of this research project, that you have received an adequate opportunity to consider the information, and that you voluntarily agree to participate in the project.

Having been asked by Julian Owen of the School of Sport, Health and Exercise Sciences at Bangor University to participate in a research project experiment, I have received information regarding the procedures of the experiment.

I understand the procedures to be used in this experiment and any possible personal risks to me in taking part.

I understand that I may withdraw my participation in this experiment at any time.

I also understand that I may register any complaint I might have about this experiment to Professor Michael Khan, Head of the School of Sport Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using standard report forms.

I may obtain copies of the results of this study, upon its completion, by contacting:

Julian Owen **pepc26@bangor.ac.uk**

_____

I confirm that I have been given adequate opportunity to ask any questions and that these have been answered to my satisfaction. ☐

I have been informed that the research material will be held confidential by the researcher. ☐

I agree to participate in the study ☐

NAME (please type or print legibly): _____

ADDRESS: (Optional)_____

_____

PARTICIPANT'S SIGNATURE: _____ **DATE**: _____

RESEARCHER'S SIGNATURE: _____ **DATE**: _____

Two sheets should be completed - one for the participant and one for the researcher

Bangor University

SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

1	Title of project	Exercise capacity and utility of novel hydration markers after dehydration evoked by exercise and fluid restriction or diuretics.
2	Name and e-mail address(es) of all researcher(s)	Julian Owen (pepc26@bangor.ac.uk) Samuel Oliver (s.j.oliver@bangor.ac.uk)

Please tick boxes

5 I confirm that I have read and understand the Information Sheet dated ……. for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

6 I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected. ☐

7 I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the marks I receive, the outcome of my period of study, or my standing with my supervisor, other staff members of with the School. ☐

8 I understand that I may register any complaint I might have about this experiment with the Head of the School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

9 I agree to take part in the above study. ☐

Name of Participant …….

Signature ……. Date ……..

Name of Person taking consent…….

Signature ……. Date ……..

WHEN COMPLETED – ONE COPY TO PARTICIPANT, ONE COPY TO RESEARCHER FILE

APPENDIX C

Bangor University
SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

Name of Participant ……..……..

Age ……

Are you in good health? ☐ YES ☐ NO

If no, please explain

How would you describe your present level of activity?
Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Low intensity	

Duration (minutes)…….

How often?

< Once per month		2-3 times per week	
Once per month		4-5 times per week	
Once per week		> 5 times per week	

Have you suffered from a serious illness or accident? ☐ YES ☐ NO

If yes, please give particulars:

Do you suffer from allergies? ☐ YES ☐ NO

If yes, please give particulars:

Do you suffer, or have you ever suffered from:

	YES	NO		YES	NO
Asthma			Epilepsy		
Diabetes			High blood pressure		
Bronchitis					

Are you currently taking medication? ☐ YES ☐ NO

If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? YES ☐ NO ☐

If yes, please give particulars:

Have you, or are you presently taking part in any other laboratory experiment?

☐ YES ☐ NO

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, cough or cold, or suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

PLEASE COMPLETE AND SIGN THE DECLARATION BELOW

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of ……20……

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature *(participant)* …….. Date ……..

Print name …….

Signature *(experimenter)* ……. Date ……..

Print name ……..…..