Examining hydration status and the physiological and behavioural influences on voluntary water intake

by

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A Doctoral Thesis

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CERTIFICATE OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this thesis, that the original work is my own except as specified in acknowledgments or in footnotes, and that neither the thesis nor the original work contained therein has been submitted to this or any other institution for a degree.

…… (Signed)

…… (Date)

Abstract

Understanding the physiological and behavioural reasons that result in voluntary water intake and the volume subsequently consumed in both the work place and during and following an exercise setting can provide further information on water balance and the necessity and requirements of water intake.

The first study (Chapter 3) aimed to assess hydration status in the adult population at the start and end of a working day and the amount of water from beverages that was consumed. Urine osmolality and urine specific gravity (USG) suggested a large proportion of subjects arrived (osmolality: 54%; USG: 53%) and left (osmolality: 35%; USG: 33%) work in a hypohydrated state, with variation between subjects in the same and different places of work. Reported water intake varied between groups with males consuming more than females. To further examine hydration status it was proposed to assess the use of capillary blood sampling as an alternative to more restrictive venous blood sampling (Chapter 4), however, despite tracking changes in blood parameters in a similar capacity, the inconsistencies of results suggested capillary blood sampling could not be used reliably.

The remaining chapters in the thesis examined voluntary water intake. In Chapter 5 this was during and following exercise in the cold. Less water was consumed compared to exercise in a warm environment and there was an indication of a blunted thirst response in the cold. Following high intensity intermittent exercise, more water was voluntarily consumed during a one hour recovery period compared to when continuous exercise of the same average power output was performed (Chapter 6). Following exercise there was increased serum osmolality, serum sodium concentration, plasma vasopressin concentration and blood lactate concentration compared to baseline values. The relative contribution that decreasing blood lactate concentrations and water intake during the recovery period had on serum osmolality could not be determined, so the study in Chapter 7 was carried out. The time period during which voluntary water intake was allowed was manipulated during a recovery period following a period of high intensity intermittent exercise. Allowing water intake for the full hour, the final 30 minutes or not at all, resulted in similar decreases in serum osmolality throughout the duration of the recovery period. A combination of finishing the period of exercise allowing plasma volume restoration, reduction in blood lactate concentration, reduction in serum sodium

concentration, a restoration of blood lactate concentration and water intake appeared to contribute to decreased serum osmolality. Sensations of thirst were the main stimulants of voluntary water intake (Chapters 3, 5, 6 and 7), however, following exercise, sensations of thirst resulted in water consumption despite the majority of subjects not losing enough water (>2% body mass loss) to require additional rehydration.

In this thesis, it can be concluded that voluntary water intake differs between individuals, between work environments, during and following exercise in different environments and following different exercise intensities. Water intake is generally initiated by sensations of thirst arising from physiological and behavioural mechanisms even in the absence of significant hypohydration and will reduce once satiated.

Key words: Voluntary water intake, hydration status, thirst, osmolality, cold, capillary, high intensity, work place

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Published abstracts and conference communications

Some of the results from Chapter 5 of this thesis have been presented in the following abstract:

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Some of the results from Chapters 3, 5 and 6 have been presented at the following conferences and meetings as oral communications:

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>
> Voluntary fluid intake in the cold. 3rd International Sports Science and Sports Medicine Conference, Newcastle-upon-Tyne, 2011.
>
> The effect of high intensity intermittent exercise compared to continuous exercise on voluntary water ingestion. Progress report, European Hydration Institute Network Meeting, London, 2011.

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List of abbreviations

%	percent
°C	degrees celcius
ACSM	American College of Sports Medicine
ANOVA	Analysis of Variance
AVP	arginine vasopressin
beats.min^{-1}	beats per minute
CHO	carbohydrate
CHO-E	carbohydrate electrolyte
CV	coefficient of variation
d	days
EFSA	European Food Safety Authority
EU	euhydrated
FR	fluid restriction
g	grams
h	hours
Hb	haemoglobin
Hct	haematocrit
Hg	mercury
HI	high intensity trial
HIIE	high intensity intermittent exercise
HR	heart rate
HY	hypohydrated
K^+	potassium
K_2EDTA	potassium ethylenediamine tetra acetic acid
kg	kilogram
km	kilometre
l	litres
l.min^{-1}	litres per minute
LO	low intensity (continuous) trial
m	metre
mg	milligram
min	minutes

ml	millilitre
mm	millimetre
mmol	millimole
mOsmol	milliosmole
n	number of subjects
Na^+	sodium
NaCl	sodium chloride
NW	no water
pb	personal best
PCA	perchloric acid
pg	pictogram
pO_2	partial pressure of oxygen
pOsm	plasma osmolality
r	effect size
RH	relative humidity
RPE	rating of perceived exertion
s	seconds
SD	standard deviation
SFQ	subjective feelings questionnaire
T	temperature
TBW	total body water
TT	time trial
U_{osm}	urine osmolality
USG	urine specific gravity
$\dot{V}O_{2max}$	maximum oxygen uptake
$\dot{V}O_{2peak}$	peak oxygen uptake
W	watts
WA	warm trial
y	years
µl	microlitre

CHAPTER 1

General introduction

1.1 Introduction

Understanding the physiological and behavioural reasons that result in voluntary water intake and the volume subsequently consumed in both the work place and during and following an exercise setting can provide further information on water balance and the necessity and requirements of water intake.

Through the understanding of the physiological and behavioural mechanisms that can impact on voluntary water intake, a clearer understanding of the reason people drink may be found.

1.2 Water balance

Water constitutes approximately 60% of the average human body. It is stored in intracellular (approximately 65%) and extracellular (approximately 35%) compartments and can move freely between compartments. Maintenance of body water in humans is tightly controlled under normal physiological conditions (Greenleaf, 1992). Water balance occurs when water input is equal to water output. Water is usually obtained through consumption of beverages, but is also obtained through water in food and through metabolic production (McArdle *et al.*, 2009). Approximately 250-350 ml of water is produced a day in sedentary individuals, rising to 500-600 $ml.d^{-1}$ in more active individuals. Water loss can occur through respiration, sweat loss, urine output and faecal losses. Maintenance of water balance classes individuals as being in a state of euhydration. Greenleaf (1992) suggested that a sinosuidal wave can represent euhydration when normal daily water content fluctuates around a mean value. In normal temperate conditions, body water can be regulated to ±0.22% (±165 ml), in normal healthy humans, whilst in hot conditions and during exercise, body water can be maintained to ±0.48% (±382 ml) (Greenleaf, 1992). When there is reduced total body water, a state of hypohydration is apparent. When water is lost, often due to exposure to the heat or from exercise, the process is known as dehydration. In this thesis the terms dehydration and hypohydration will be used interchangeably.

1.2.1 Maintaining water balance - Thirst

To help maintain water balance, a behavioural mechanism to stimulate water intake is thirst. Sensations of thirst usually occur during a state of hypohydration or when dehydration causes total body water to decrease and an individual moves from a euhydrated to a hypohydrated state. Greenleaf (1992) defined thirst as a, "desire to drink resulting from a deficit of water" and suggested that under resting conditions the feeling of thirst is enough to stimulate the correct water intake, however under some stressful physiological and psychological conditions, thirst may often be an inadequate stimulus. The physiological and behavioural aspects of thirst are described in greater detail later in this chapter.

1.3 Measurement of hydration status

Various techniques can be employed to measure hydration status and a wide variety have been commonly used in the literature including body mass changes and blood and urinary indices. In addition, hydration status has been assessed using total body water turnover, salivary indices and subjective responses to sensations of thirst.

When assessing hydration status, various methods of measurement can be used to monitor both static (measurement at one particular time point) and/or dynamic dehydration (monitoring over a period of time or a change in hydration status); however change in body mass, for example, may only be used to measure dynamic dehydration whilst plasma osmolality, has been shown to assess dynamic dehydration and has shown statistical promise when measuring static dehydration (Cheuvront *et al.*, 2010).

In terms of measuring dynamic dehydration, perhaps the most common measure, and one of the simplest, is to measure changes in body mass over a short period of time. Assuming that 1 ml of water is equal to 1 g of mass (Lentner, 1981), change in body mass from pre- to post-exercise can be calculated. Sweat losses can be calculated once correction for water intake and urine output has been taken into account. A body mass loss is indicative of dehydration, whilst an increase in body mass can be related to possible hyperhydration. Body mass change has been shown to provide an accurate estimate of changes in hydration status, due to changes in total body water (Sawka *et al.*, 2007). Cheuvront *et al.* (2010) examined body mass over three consecutive days

and found it to be a strong measure of dynamic dehydration particularly if two or more variables, including plasma osmolality and urine specific gravity, were used in conjunction. However, when using body mass change, discrepancies can occur when food and unaccounted water intake, body composition changes and metabolic changes can affect values.

Blood sampling can allow assessment of hydration status. Haemoglobin concentrations in combination with haematocrit, and also plasma and serum osmolality have all been used. Using haemoglobin concentrations and haematocrit to calculate blood and plasma volume changes, requires a baseline value to be established to allow assessment of dynamic dehydration, as long as postural changes are also accounted for (Harrison, 1985). As mentioned previously, plasma osmolality has been described as the best potential measure of static dehydration assessment when compared to standard reference values and along with urine specific gravity and body mass change, a valid marker when dynamic dehydration is assessed (Cheuvront *et al.*, 2010) but further investigation into this statement is required. When dehydration is hypotonic and increases with water loss, plasma osmolality increases (Popowoski *et al.*, 2001). Popowoski *et al.* (2001) measured plasma osmolality, urine specific gravity and urine osmolality at 1, 3 and 5% of body mass loss following dehydration to 5%. They found that plasma osmolality progressively increased with increased body mass loss, however, urine osmolality and urine specific gravity did not differ until 5% and 3% body mass loss had occurred respectively. In contrast, Francesconi *et al.* (1987) found that urinary markers tracked changes in hydration status better than blood parameters. They found that when body mass losses were greater than 3%, urine specific gravity values were significantly different to baseline, whereas serum osmolality values were not.

A less invasive method to examine hydration status is to analyse urine parameters. Urine osmolality (U_{osm}), urine specific gravity (USG) and urine colour have all been widely used particularly as a simple measure or when blood sampling is unsuitable. Shirreffs & Maughan (1998) have effectively assessed urine osmolality as measure of hydration status in athletes training in a hot environment. Values of greater than 900 $mOsmol.kg^{-1}$ were observed in athletes who had lost 1.9% of body mass due to water losses. Armstrong *et al.* (1998) found, in a study of 34 healthy males, urine specific gravity could be used interchangeably with urine osmolality (r^2=0.96). For morning

urine samples the following equation could be used: $y(USG) = 0.00002(U_{osm}) + 1.0046$ (r^2=0.81, p<0.001) and for 24 hour urine collections: $y(USG) = 0.00002(U_{osm}) + 1.0026$ (r^2=0.91, p<0.001) (Armstrong *et al.*, 2010). In addition, urine colour has also shown to correlate well with urine specific gravity (Ormerod *et al.*, 2003). Over a period of six weeks of physical training and heat acclimation in women, correlation values (r^2) of between 0.77 and 0.96 were found. However, caution must be taken when a large bolus of water is ingested as this can dilute urine over a range of hydration states (Armstrong *et al.*, 1998).

1.4 Water intake

As mentioned previously, water intake mainly occurs from water and other beverages, food and metabolic production. Many agencies and authorities have provided adequate intake values for water consumption for males and females. The European Food Safety Authority (EFSA) suggest adequate intake values of 2.5 $l.d^{-1}$ for males and 2.0 $l.d^{-1}$ for females from food and beverages in moderate environmental temperature for those with moderate physical activity levels (EFSA, 2010). In the United Kingdom, it has been suggested that 1.2 l of water from beverages per day will help maintain health and water balance (Food Standards Agency, 2010), whilst the British Dietetic Association (2007) have intake values of between 1.5-2.5 $l.d^{-1}$ of water from beverages (http://www.bda.uk.com/foodfacts/water.pdf). In contrast, the Institute of Medicine have adequate intake values of approximately 3.7 $l.d^{-1}$ of water for males from food and beverages, and approximately 2.7 $l.d^{-1}$ for women (http://www.iom.edu/Reports/2004/Dietary-Reference-Intakes-Water-Potassium-Sodium-Chloride-and-Sulfate.aspx).
These adequate intakes were for sedentary individuals in temperate environmental conditions. For more active individuals and for those in warmer climates both the Institute of Medicine and EFSA outline greater water intake depending on sweat losses. It is apparent that, even between these sources, there is variation in adequate intake values.

Although the adequate intake values are often based on population studies, some values for water intake are not always validated with scientific research. In a review by Valtin (2002), the recommendation of drinking eight standard (~200-250 ml) glasses of water per day was questioned and it was found that there was no scientific evidence to base

the volume of water intake on. Problems arising from promoting a standardised intake are that excessive water intake or too little water intake may occur, particularly when body mass is considered and in those who ignore, or are uneducated about, many of the physiological symptoms relating to the desire to drink and water regulatory behaviour.

Prescribing a single set of water intake guidelines will always prove challenging, given the many questions that arise: does everyone require the same amount of water, do all types of beverages have the same effect, or is only pure water beneficial, how does body composition affect water requirements, what is the impact of various lifestyles including work and leisure activities? Due to these suggestions, the reasoning for EFSA and the Institute of Medicine setting adequate intake guidelines can be made, however these values may or may not be too little of too much water for an individual. Depending on the water intake values, there is the possibility that hypohydration and/or hyperhydration can arise, with both, if severe enough, resulting in possible health consequences.

1.5 Water replacement during and following exercise

1.5.1 How much should be replaced?

Determining and quantifying the amount of water that should be replaced during and following exercise has been much debated and has changed as more research into the area has been conducted.

Drinking guidelines have progressively changed as the importance of water intake during exercise has been assessed. One of the earliest study advocating the necessity of water intake examined marathon runners. Wyndham & Strydom (1969) assessed water intake during a marathon and rectal temperatures upon completion. Water intake varied between individuals with 13 of 31 subjects consuming no water or less than 300 ml, whilst seven consumed between 1150 ml and 4100 ml. One runner had water intake values that matched sweat loss. They found that when body mass loss was greater than 3%, rectal temperature increased linearly with further body weight losses.

Since this early study, knowledge on the importance of water intake during exercise has developed to the point where water intake guidelines before, during and following exercise have been continually published and, in turn, altered to follow recently published scientific literature. Recent water replacement guidelines, published by the American College of Sports Medicine (ACSM) (Sawka *et al.*, 2007), recommended consuming enough water during exercise to prevent excessive dehydration levels (greater than 2% body mass loss) from occurring and to not drink so much that body mass increases.

Despite the majority of the literature focussing on water intake during exercise, water intake following exercise should not be overlooked. The amount of water recommended to be consumed following exercise depends on the requirements of the individual and how much water has been lost during exercise. ACSM guidelines suggested that following exercise, normal water and food intake would eventually restore a state of euhydration, however, if rapid replacement was required and/or there was excessive dehydration then water intake should equal approximately 1.5 l per kilogram of body weight lost. The previous ACSM guidelines (Convertino *et al.*, 1996) contained no recommendations for water intake following exercise.

1.5.2 Drinking to thirst?

Prior to the current guidelines, the previous statement by ACSM (Covertino *et al.*, 1996) recommended consuming as much water as tolerable during exercise to prevent dehydration levels from occurring up to a rate equal to that of sweat lost and that thirst should not be completely relied on to dictate water replacement as complete restoration does not always occur. In response to these guidelines, Noakes (2003, 2007, 2010) has been widely supportive of the notion of drinking to thirst: consuming enough water to satisfy the demands of thirst. He has suggested that this is often sufficient to prevent dehydration levels reaching body mass losses greater than 2%. In addition, Noakes (2010) has propositioned, in his review of the literature examining water replacement, that no studies reporting water intake during exercise greater than *ad libitum* intake found any advantageous effect and that the increased water intake only served to increase the possible risk of exercise-associated hyponatraemia occurring.

Maughan & Shirreffs (2010) have suggested that drinking only to thirst can result in inappropriate drinking behaviours, and may not be sufficient to replace enough water to prevent dehydration becoming greater. In addition, when drinking to thirst, water intake may be delayed and prove insufficient to match high sweat losses. For example, in many running races, water stations are positioned at set intervals and when adopting the drinking to thirst strategy, the water station may be passed when thirst is not experienced, therefore resulting in no water consumed, whilst thirst may be then experienced when water stations are not close. Alternatively, consuming too much water due to inappropriate thirst signals, resulting in hyperhydration can potentially lead to increased urine output and in extreme cases, hyponatraemia, which can become a very serious concern to health (Almond *et al.*, 2005). An early study from Pitts *et al.* (1944) showed that *ad libitum* drinking compared to matching water intake with sweat loss resulted in greater increases in rectal temperatures and reduced performance. Examining recent studies, it has been shown that *ad libitum* water intake resulted in low levels of water being replaced (Table 1.1). From Table 1.1, it appeared that the majority of studies assessing voluntary water intake, reported water replacement values of approximately 50-90%. However, O'Hara *et al.* (2010) have shown water replacement values of 32% in rugby league players, whilst Broad *et al.* (1996) found male footballers replaced only 35% during competition in the winter months.

It is apparent that in most cases, *ad libitum* water intake is sufficient to prevent levels of dehydration from reaching greater than 2% body mass loss, however in some situations this has not been the case due to inappropriate drinking behaviours and therefore caution should be taken if prescribing this approach. Amongst others, Maughan & Noakes (1991) have suggested that an individualised drinking prescription, factoring in both the individual and the situation, should be employed and that there is no single prescription that can be generalised to all individuals and exercise situations.

1.6 Physiology of thirst

To understand the rationale behind water intake guidelines and recommendations requires knowledge of the physiological mechanisms that underpin the desire to drink, with these often resulting from increased sensations of thirst. In regards to increased water intake and thirst, Stricker & Verbalis (1988) outlined two main mechanisms

involving hyperosmolality and hypovolaemia, containing single and double feedback loops respectively, whilst the notion of mouth dryness increasing sensations of thirst has also been proposed (Brunstrom *et al.*, 2000; Figaro & Mack, 1997; Seckl *et al.*, 1986).

Hyperosmolality of the interstitial water is sensed by osmoreceptors in the brain resulting in thirst and subsequent water intake. As osmolality increases there is a threshold at which the anti-diuretic hormone, arginine vasopressin (AVP), is released. This has been suggested at 285 mosmol.kg^{-1} (Thompson *et al.*, 1986, while the threshold for the sensation of thirst has been shown to be 290 mosmol.kg^{-1} (Phillips *et al.*, 1985). The organum vasculosum of the lamina terminalis (OVLT) and the subfornical region (SFO) within the brain have been suggested as the most likely sites for the sensitive osmoreceptors (McKinley & Johnson, 2004). Both of these circumventricular organs lack a blood-brain barrier, therefore allowing hormonal responses and osmotic stimuli to act (McKinley & Johnson, 2004). When high osmolality is detected, a sensation of thirst is often experienced, thereby creating a desire to drink.

Arginine vasopressin plays an important role in the regulation of water intake and conservation. It is a peptide hormone synthesised in the supraoptic nuclei and paraventricular nuclei of the hypothalamus, which is then stored and released from the posterior pituitary gland. Acting on the V2R receptor in the kidney, vasopressin increases the permeability to water of the collecting ducts of the nephron, thus allowing reabsorption of up to 10% of the filtered water (Bankir, 2001). It has been reported that plasma vasopressin levels increase by 0.4 to 0.8 pg.ml^{-1} for every 1 mOsmol.kg^{-1} increase in plasma osmolality above resting levels (Verbalis, 2007). The release of vasopressin appeared to be mainly osmotically controlled, but was also affected by changes in plasma volume and neurogenic factors (Convertino *et al.*, 1981). Sodium (Nose *et al.*, 1991) and lactate (Sjøgaard *et al.*, 1985) have both been shown to be effective osmoles in the release of vasopressin.

The second mechanism proposed involving hypovolaemia was a negative double feedback loop (Stricker & Verbalis, 1988). Reductions in extracellular water volume through haemorrhaging, vomiting, diarrhoea, sweating and diuresis can all stimulate

thirst (Greenleaf, 1992). Baroreceptors detect changes in plasma volume and therefore stimulate water intake whilst the renin-aldosterone system plays an important role in the second loop of the model (Stricker & Verbalis, 1988). Activation of the renin-aldosterone system releases the hormone aldosterone to conserve sodium through retention in the ducts of the kidney. When hypovolaemia is detected, the body aims to conserve sodium and or replace sodium that has been lost. This increases the sodium appetite because intake of sodium is desired. In addition, this helps prevent rapid reductions in osmolality through ingestion of plain water alone.

A third mechanism that has been suggested is mouth dryness and oropharyngeal responses (Brunstrom, 2002; Brunstrom *et al.*, 2000), however this mechanism appears to be less widely accepted and is difficult to establish causality. Examining the oropharyngeal response, Figaro & Mack (1997), monitored thirst feelings and vasopressin concentrations during three rehydration conditions following dehydration; *ad libitum* drinking, infusion of a similar volume of liquid in to the stomach during the first 25 minutes followed by *ad libitum* drinking and *ad libitum* drinking with extraction of water from the stomach via a nasogastric tube. Through bypassing the mouth and osmoreceptors in the oesophagus, they were able to examine stimulation by water intake and thus assess the effect of mouth and oesophageal sensations on *ad libitum* water intake. Thirst was measured using a 100mm visual analog scale. Subjects were asked to mark a 100 mm line and the distance from the end was measured (0 mm = 'not at all thirsty', 100 mm = 'very thirsty'. In all three trials, thirst and vasopressin concentration increased on average by 76 ± 13 mm and 3.1 ± 0.4 $pg.ml^{-1}$ respectively. It was observed that there was a reflex inhibition of vasopressin production and thirst during the first 5 minutes of rehydration in both the control and extraction trials ($p<0.05$). This supported their hypothesis that thirst and vasopressin release was osmotically modulated by oropharyngeal reflexes.

Specifically examining mouth dryness, Brunstrom *et al.* (2000) observed water intake to satiety whilst placing one or two cotton wool balls in the mouth to act as a control or reduce salivary output respectively. In the dry mouth condition water intake after 20 min of self-selected exercise was significantly greater (428 ± 20 ml v 300 ± 148 ml) than the control trial ($p<0.001$), as was length of drinking episode (93.8 ± 73 v 69.3

± 54 seconds; $p<0.015$) and drinking bouts (7 ± 4 v 5 ± 3; $p<0.001$). It was suggested that mouth dryness helped govern the termination of drinking behaviour.

The studies examining the effect of mouth dryness on *ad libitum* water intake have dehydrated subjects to some extent, through either exercise or water restriction and therefore it is difficult to isolate the effect of mouth dryness without the interactive effect of dehydration. Guest *et al.* (2006) manipulated mouth dryness using air in hydrated subjects but then assessed perceived pleasantness of a fixed water volume (0.75 or 1.5 ml) of different temperatures (8, 16 and 25°C) rather than the effect on voluntary water intake.

All three mechanisms play a role in both initiating and terminating the drinking behaviour. It is important to understand how this reflects in applicable situations in sporting and everyday lifestyle environments and how manipulation of beverages in terms of quantities, flavour and the environment under which they are ingested can affect thirst in a variety of populations. Therefore, it is paramount to understand the behavioural response associated with thirst in addition to the physiological mechanisms.

1.7 Behavioural responses

1.7.1 Voluntary water intake

The feeling of thirst stimulates water intake, often termed voluntary water intake (Greenleaf, 1992). When the amount of water ingested is insufficient to replace water lost, the term involuntary dehydration is used. Several studies have examined the extent of involuntary dehydration in differing climatic environments, sporting contexts, populations and following varying rehydration strategies. Table 1.1 summarises a portion of the literature pertaining to voluntary water intake and involuntary dehydration. It appears from the literature that very few studies examine the role of vasopressin response during the monitoring of voluntary water intake, maybe in part due to the difficulty and accessibility of blood sampling. The studies in Table 1.1 only provide an indication of water replacement at one particular moment and do not describe water balance over a longer period of time, therefore determining the extent of involuntary dehydration in each study, particularly if the maintenance of total body water can fluctuate in a sinosuidal wave, may be difficult. Understanding water is not

always completely replaced is important but is difficult to gauge from self reported questionnaires and other subjective responses. As mentioned previously water intake is controlled and monitored by physiological mechanisms and so assessing these in conjunction with the results of these studies would help provide a clearer understanding of the behaviour of thirst.

1.7.2 Sensations of thirst

Thirst has been shown to vary in intensity during the day (Mattes *et al.*, 2007). Over a seven day recorded dietary intake of 50 American, free-living adults, the authors found that peak thirst sensations generally occurred at meal times, however, there was large intra-individual variability. Thirst was measured using 100mm visual analogue scales recorded every waking hour. Measurement of thirst fluctuations during the course of a day has not been measured through hormonal markers due to the invasive nature of the sampling procedure (venepunctures) and the tendency for the sample to impact on "normal" life. Visual scales of thirst have proven to provide a consistent method of monitoring thirst (Engell *et al.* 1987), however, they are subjective and so can be easily influenced by small intakes in water especially when sensations of mouth dryness and mouth pleasantness are involved. In order to overcome the problems associated with questionnaires and venepuncture blood samples, capillary blood sampling could be used. Godfrey *et al.* (2004) validated human growth hormone analysis and found strong correlation ($r=0.986$, $p<0.01$) between venous samples from a forearm vein and capillary samples; however, there appears to be a lack of research pertaining to vasopressin analysis using capillary samples.

Sensitivity to thirst has been demonstrated to vary within populations, specifically with age (Takamata *et al.*, 1999). As age increases, it appears that thirst sensitivity decreases and therefore water intake is potentially reduced. As a result, elderly individuals tend to experience a greater involuntary dehydration. Mack *et al.* (1994) compared the osmotic control of thirst in healthy older (65-78 years) and younger subjects (18-28 years). The authors found that at similar levels of dehydration, the plasma osmolality threshold for the stimulation of thirst was higher suggesting that for control of water volume and composition there is shift in the operating point. However, hormonal response was not measured, with thirst ratings based on visual analogue scales, therefore potentially limiting the study.

Table 1.1. A summary of studies examining voluntary water intake. *denotes differences between trials ($p<0.05$), †denotes differences between groups ($p<0.05$), ^denotes seasonal difference ($p<0.05$).

Author	Subjects	Exercise/Activity	Environment	Water/beverage ingested	% Dehydration (body mass loss)	Voluntary water intake
Baker *et al.* (2005)	13 male and 14 female (62±1 y)	4x15 min cycling @65% $\dot{V}O_{2peak}$	30°C 50%RH	Beverage during rest. CHO-E (6% CHO and 19.0 $mmol.l^{-1}$ NaCl) and water		None when beverage available. CHO-E >water*. female>male*.
Bergeron *et al.* (2006)	9 male and 5 female tennis players (15.1±1.4 y)	2x120 min intermittent tennis practice drills	26.4°C	CES and water	CHO-E: 0.5 ± 0.7%, W: 0.9 ± 0.6%*	CHO-E: 85%, W: 74%
Broad *et al.* (1996)	22 female netballers (18-21 y)	Training (T) and competition (C)	T: Winter (W): 19.1±1.1°C, 30.0±3.1% RH, summer (S): 28.2±2.2°C, 35.8±4.6% RH	Own choice of beverage	T: W: 0.4 ± 0.5% S: 0.7 ± 0.5%	T: W: ~70% S: ~61%^
			C: W: 16.5±2.6°C, 42.5±2.7% RH, S: 22.1±0.1°C, 66.1±1.7% RH		C: W: 0.3 ± 0.6% S: 0.9 ± 0.5%	C: W: ~75% S: ~53%
	19 male basketballers (16-18y)		T: W: 19.9±1.4°C, 24.1±3.3% RH, S: 27.4±2.5°C, 33.7±6.3% RH		T: W: 1.2 ± 0.4% S: 1.0 ± 0.5%	T: W: ~47% S: ~58%
			C: W: 18.9±0.9°C, 36.3±5.8% RH, S: 23.3±2.6°C, 41.4±10.6% RH		C: W: 1.0 ± 0.6% S: 0.9 ± 0.7%	C: W: ~58% S: ~67%
	12female basketballers (16-18y)		T:W: 17.2±1.9°C, 56.2±11.8% RH, S: 25.1±0.9°C, 42.8±6.8% RH		T: W: 1.0 ± 0.4% S: 0.7 ± 0.4%	T: W: ~48% S: ~61%^
			C: W: 17.0±1.3°C, 58.1±15.6% RH, S: 25.6±1.5°C, 59.6±7.5% RH		C: W: 0.7 ± 0.5% S: 0.7 ± 0.5%	C: W: ~62% S: ~65%
	32 male footballers (16-18y)		T: W: 8.6±1.5°C, 61.1±15.6% RH, S: 24.8±3.9°C, 41.1±14.8% RH		T: W: 0.8 ± 0.5% S: 1.2 ± 0.7%	T: W: ~42% S: ~44%^
			C: W: 9.6±0.0°C, 56.2±0.0% RH, S: 24.6±2.1°C, 41.4±15.1% RH		C: W: 1.4 ± 0.7% S: 1.4 ± 0.9%	C: W: ~35% S: ~43%
	17 female footballers (16-28y)		T: S: 30.2±0.6°C, 35.2±3.4% RH		T: S: 0.9 ± 0.5%	T: S: ~49%
			C: S: 25.5±0.4°C, 78.4±0.1% RH		C: S: 1.2 ± 0.9%	C: S: ~54%

Author	Subjects	Exercise/Activity	Environment	Water/beverage ingested	% Dehydration (body mass loss)	Voluntary water intake
Cheuvront & Haymes (2001)	8 female marathon runners (37 ± 4 y)	30 km treadmill time trial at marathon PB pace ~ 71% $\dot{V}O_{2max}$	Hot 29.9°C, 55%RH, moderate 20.4°C, 54%RH, cool 14.3°C, 64%RH. (Wet temp 25, 17, 12°C)	Water every 5 km (5-10°C)	Hot (2.8 ± 1.14%), moderate (2.4 ± 0.89%), cool (2.5 ± 1.09%)	Hot (62.9 ± 24.2%), moderate (68.2 ± 27.1%), cool (72.8 ± 30.9%). Hot>cool*
Cox *et al.* (2002)	23 male national water polo players, 20 female and 21 male national swimmers (16-32 y)	Polo: 5 training sessions (99min) and 3 matches (47min). Swim: 13 training sessions (3.9km)	Polo training: 23.9°C (inside), 69.5%RH, 27.2°C (water) Polo competition:24.1°C (inside), 54.3%RH, 27.3°C (water) Swim: 30.6°C (inside), 62.8% RH, 29.0°C (water)	Usual water intake practices	Polo training: 0.26%, Polo competition: 0.35% Swim M: +0.11%, Swim F: 0.14%	Polo training: ~50%, Polo competition: ~48% Swim male: ~113% Swim female: ~89%
Daries *et al.* (2000)	8 male endurance runners (pb<2h50min) (31 ± 4 y)	90 min running @65% $\dot{V}O_{2peak}$ then as far as possible in 30 min	25°C, 55% RH, wind speed 13-15 $km.h^{-1}$	CHO-E (6.9% CHO 16 mEq $Na^{+}.l^{-1}$: either *ad libitum*, 150 or 350 $ml.70kg^{-1}$	*Ad libitum*: 1.95 ± 0.62%, 150: 1.59 ± 74%, 350: 0.74 ± 0.44%*	~59%
Fudge *et al.* (2008)	14 male elite Kenyan endurance runners (22 ± 3 y)	5 d training camp, multiple sessions per day	Morning 10.7 ± 1.6°C, 75 ± 3% RH, interval 17.9 ± 1.1°C, 68 ± 4% RH, afternoon 21.1 ± 2.1°C, 43 ± 11% RH	Water, milky tea, soft drinks and milk	0.8 ± 0.5%, 1.5 ± 0.5%, 2.0 ± 0.7%, 1.3 ± 0.5% and 1.0 ± 0.6% short, medium, long morning runs, interval and afternoon sessions respectively	
Godek *et al.* (2005)	10 male American football players (AF) (21.2 ± 1.1 y) and 5 cross country runners (CC) (22.8 ± 2.8 y)	AF: 2d x 2 x 2 ¼ hours. CC: 2d x 2 x 60 min	Morning: 28.4°C, 64.9% RH, Afternoon: 34.5°C, 43%RH	Water	AF: am – 1.5%, pm – 1.5% CC: am – 1.9%, pm – 1.7%	AF: am ~65%, pm ~65% CC: am ~20%, pm ~40%

Author	Subjects	Exercise/Activity	Environment	Water/beverage ingested	% Dehydration (body mass loss)	Voluntary water intake
Horswill *et al.* (2005)	19 male and 15 female (25-46 y), 9 male and 6 female (17-18 y). Triathletes and high school athletes	1 h (3x20 min) @ 80-85% HR_{max}	26.5°C, 27.3% RH	CHO-E (6.4% CHO, 22 $mmol.l^{-1}$ Na^{+}) (4-6°C)	Adolescents: +0.01 ± 0.40% (male), +0.04 ± 0.45% (female). Adults: 0.19 ± 0.56% (M)†, 0.22 ± 0.30% (F)	Adolescents: 102 ± 37% (male), 106 ± 50% (female). Adults: 89 ± 32% (male)†, 82 ± 23% (female)†
Logan-Sprenger *et al.* (2011)	24 male elite junior ice hockey players (18.3 ± 0.3 y)	1 match, 6/24 players 2 matches	10.8 ± 0.2°C, 30 ± 2% RH	Water and CES	1.3 ± 0.3%†	~66 ± 5%
Mack *et al.* (1994)	10 male (65-78 y), 6 male (18-28 y)	105 min cycling @60% HR_{max}	36°C, <30% RH	Water (~15°C) for 180min after 30min recovery period	O ld: 2.2%, Young: 2.5%	Old: ~46%, Young: ~65%†
Maresh *et al.* (2004)	10 male (21 ± 1 y)	4x90 min, 5.6$km.h^{-1}$, 5% gradient. 2x hypohydrated (-3.5 ± 0.2%) (1x water, 1x no water), 2x euhydrated (1x water, 1x no water)	33°C, 56% RH	Water	EU+NW: 1.4±0.1%, EU+W: 1.0±0.2%, HY+NW: 1.6±0.1%, HY+W: +0.9±0.2%	HY+W: 172% (44% of total body mass loss from dehydration protocol) EU+W: 31%*
Maughan *et al.* (2004)	24 male elite soccer players (27 ± 4 y)	90 min pre season training session	24-29°C, 64-46 % RH	CHO-E (6.4%CHO, 22 $mmol.l^{-1}$ Na^{+})	1.37 ± 0.43 %	~47.8%
Maughan *et al.* (2007a)	20 male footballers. Team A (*n*=9) (24 ± 6 y), team B (*n*=11) (18 ± 1 y)	Competitive game	~6-8°C, ~50-60% RH	Team A: Water and CHO-E (6.4% CHO and 20 $mmol.l^{-1}$ Na^{+}). Team B: Water and CHO-E (5.2% CHO, 1.3% protein and 23 $mmol.l^{-1}$ Na^{+})	Team A: 0.85 ± 0.70%. Team B: 1.25 ± 0.54%.	Team A: ~62%. Team B: ~42%.

Author	Subjects	Exercise/Activity	Environment	Water/beverage ingested	% Dehydration (body mass loss)	Voluntary water intake
O'Hara *et al.* (2010)	14 male professional rugby league players. Club A (*n*=7): 26.0 ± 4.1 y, club B (*n*=7): 26.0 ± 4.1 years	72 observations of match play	12.1 ± 5.3°C, 70.5 ± 11.4% RH	Various energy drinks and water	1.31 ± 0.66%	~32%
Ormerod *et al.* (2003)	5 female untrained (23 ± 1y)	6 week training. Exercise heat acclimatisation for 90min.d^{-1}, 3 d.$week^{-1}$ and outdoor training 3 d.$week^{-1}$	Heat acclimatisation: 36°C, RH 50-70%	Water (11-17°C)		Water intake decreased as subjects became more heat acclimatised, number of drinks per week decreased, time to first drink increased
Osterberg *et al.* (2009)	29 male elite basketball players	2 indoor basketball games	20-22°C, 18-22% RH	Water and CHO-E (6% CHO and 18 mmol.l^{-1} Na^{+})	1.4 ± 0.6%	~45.5%
Passe *et al.* (2004)	34 male (40.6 ± 1.7 y and 16female (36.4 ± 2.0 y) triathletes and runners	75min 80-85% HR_{max} running		60 s access to drink at 30 and 60 min. Diluted orange juice, homemade 6% CHO drink, Commercial 6% CHO (Gatorade), water (4-7°C)	Water>orange juice>homemade >commercial*	Commercial CHO drinks were voluntarily consumed in greater concentrations than water. Commercial >homemade >orange juice/water*
Passe *et al.* (2007)	15 male (40.5 ± 2.5 y), 3 female (42.0 ± 2.3 y) marathon runners	Outdoor 10 mile race	20.5 ± 0.7°C, 76.6 ± 1.7% RH	6% CHO-E drink at miles 2,4,6,8	1.9 ± 0.8%	30.5 ± 18.1% (range 5-67%)

Author	Subjects	Exercise/Activity	Environment	Water/beverage ingested	% Dehydration (body mass loss)	Voluntary water intake
Peacock *et al.* (2011)	31 male and 21 female fitness centre members (36 ± 12 y)	Freely chosen gym session	21.6 ± 0.8°C, 56.8 ± 2.5% RH	Own choice of beverages (92% consumed water)	0.6 ± 0.2%	~49%
Rivera-Brown *et al.* (1999)	12 male (11-14 y), variety of sports	Two 3-hr sessions (4x 20-min cycling bouts @60% $\dot{V}O_{2max}$ with 25-min rest)	30.4±1.0°C	Water or CHO-E (6% CHO and 18 mmol.l^{-1} Na^+) (both 10-17°C)	Water: 0.94%, CHO-E: +0.18%*	Water: ~75%, CHO-E: ~106%
Shirreffs *et al.* (2005)	9 male and 6 female (30 ± 12 y)	37 h of water restriction or euhydration		Choice of drinks	FR: 2.7 ± 0.6%, EU: 0.4 ± 0.2%*	FR: ~37%, EU: ~87%
Takamata *et al.* (1999)	9 male (70 ± 3 y) and 6 male (25 ± 3 y)	6 days heat acclimation. 4x20 min bouts @ HR=40% $\dot{V}O_{2\ peak}$ separated by 10 min intervals	36°C, 40% RH	CHO-E during recovery (~14°C)		Young: 80 ± 9%, Old: 34 ± 5%†
Wilk & Bar-Or (1996)	12 male recreationally active (10.4 ± 0.8 y)	3x 3 h sessions (4x 25 min cycling bouts at 50% $\dot{V}O_{2max}$ followed by 25 min rest)	35±1°C, 40-45% RH	Water, grape flavoured water and grape flavoured CHO-E (6% CHO and 18.0 mmol.l^{-1} NaCl)	Water: 0.65%, Grape flavoured water: 0.32%, Grape CHO-E: +0.47%	Water: ~70%, Grape flavoured water: ~85%, Grape CHO-E: 115%
Wilk *et al.* (1998)	12 male competitive and recreationally active (11.2 ± 0.7 y).	6x 70 min intermittent exercise sessions (3x20 min cycling @50% $\dot{V}O_{2max}$ with 5 min rest in between) within 2 weeks	35 ± 1°C, 50/60% RH	CES (6% CHO and 18 mmol.l^{-1} NaCl) (8-10°C)	No change in body mass (range: +0.75% (visit 5) to +1.07% (visit 1))	Voluntary dehydration prevented and is consistent when exposed to the heat over 3 weeks

Author	Subjects	Exercise/Activity	Environment	Water/beverage ingested	% Dehydration (body mass loss)	Voluntary water intake
Wilk *et al.* (2010)	8 male cross-country runners (13.7 ± 1.1 y)	5x15 min running @65% $\dot{V}O_{2peak}$, run to exhaustion @95% $\dot{V}O_{2peak}$	30.0 ± 0.5°C, 60-65% RH	Water, grape flavoured water and grape flavoured CHO-E (6% CHO and 18.0mmol.l^{-1} NaCl) (8-10°C)	Water: 0.45±0.68%, Grape flavoured water: 0.66±0.50%, Grape CHO-E: 0.13±0.71%	Water: ~77%, Grape flavoured water: ~74%, Grape CHO-E: ~93%
Zetou *et al.* (2008)	47 male beach volleyball players (21 elite and 26 non-elite) (26.2 ± 5.1 y)	Official tournament. Matches of 42.2 ± 9.8 min	33.6 ± 2.8°C, 56.0 ± 8.7% RH	Own choice, water or CHO-E	Match (42.2min): 0.8±0.68% Per hour: 1.14±1.19%	~52% possibly due to limited opportunities to take on drink

Notes: RH=relative humidity, CHO-E =carbohydrate electrolyte solution.

One problem that may arise from drinking in response to sensations of thirst is hyponatraemia. If sensations of thirst are misunderstood and individuals drink beyond this, the resulting hyperhydration, if severe enough, may cause hyponatraemia. Hyponatraemia is a reduction in serum sodium levels below 135 mmol.l^{-1}. Incidences of hyponatraemia tend to occur in prolonged endurance events and in extreme cases can lead to death (Almond *et al.*, 2005).

1.7.3 Water intake behaviours in the general population

To date it appears that very little research has been conducted on general water intake behaviours. There appears to be a plethora of studies examining water intake, before, during and after exercise and in the general population (EFSA, 2010; Institute of Medicine, 2004) but in more specific areas of population lifestyles, has been largely ignored. This appears particularly evident in the work place, with hydration studies focussing on work in hot and/or humid environments (Bates & Schneider, 2008; Brake & Bates, 2003). Reviews of the literature indicated that dehydration can have a negative effect on cognitive function when body mass loss is greater than 2% (Gandjean & Grandjean, 2007). Assessing this possible effect in a working environment appears to be an important area of investigation, particularly when decreased cognitive function may have an impact on work productivity.

When assessing hydration in the general population and specifically the workplace, it would be ideal to have an accessible method of testing to provide blood samples and thus provide information on plasma volume changes and plasma and serum osmolality. Although the most commonly used methods of assessing hydration status through urinary measures (urine specific gravity, osmolality and colour) have demonstrated good correlation with hydration status (Armstrong, 2005, Shirreffs & Maughan, 1998), they can often be insensitive to rapid change in hydration status (Popowski *et al.*, 2001). Scaling down techniques often employed in laboratory settings to take blood samples, would reduce the invasive nature of venepuncture and antecubital vein cannulations, and potentially provide a more informative overview of hydration status compared to urinary measures.

1.8 Water intake and exercise in the cold

Exercise in the cold, and particularly water intake during exercise in the cold, has been less extensively studied compared to exercise in the heat. Cold is often described as less than 10°C with many studies using temperatures of 0-7°C (Cheuvront *et al.*, 2005; Kenefick *et al.*, 2004ab; Kenefick *et al.*, 2008; O'Brien *et al.*, 1998). Perhaps to a lesser extent than in the heat, dehydration is still apparent in the cold with water losses through sweating, cold induced diuresis and respiration (Freund & Sawka, 1995; Kenefick *et al.*, 2004ab). Sweat rates have been shown to be similar in the heat (32 ± 3°C, 20 ± 5% relative humidity) (Shirreffs *et al.*, 2005) and the cold (5.1 ± 0.7°C, 81 ± 6% relative humidity) (Maughan *et al.*, 2005) in elite footballers during a 90 minute training session, however water intake was reduced in the cold leading to voluntary dehydration. Sweat rates were 1.46 ± 0.24 $l.h^{-1}$ and 1.13 ± 0.30 $l.h^{-1}$ in the hot and cold conditions, respectively. However, a possible explanation of this may be due to the clothing worn. Typically, athletes wear more clothes in the cold and will, therefore create a warm microenvironment for them to exercise in. When assessing studies of exercise and water intake in the cold, careful consideration should be directed towards the type and amount of clothing worn.

Galloway & Maughan (1997) examined the effect of ambient temperature on cycling to exhaustion at 70% $\dot{V}O_{2max}$. They found that exercise performance was reduced in the cold (4°C) (81.4 ± 9.6 min) compared to 11°C (93.5 ± 6.2 min), however the time to exhaustion was similar to 21°C (81.2 ± 5.7 min) and greater than 31°C (51.6 ± 3.7 min). Sweat rate was significantly less in 4°C (0.55 $l.h^{-1}$) compared to the other conditions ($p<0.01$). Sweat rate was almost half of that experienced in 31°C (1.15 $l.h^{-1}$). Despite large differences in sweat rates, water was still lost (approximately 0.7 l in 4°C to approximately 1.7 l in 31°C) and would need to be replaced to ensure adequate recovery from exercise.

It has also been suggested that there is a blunted thirst response in the cold (Kenefick *et al.*, 2008). They found that following 45 minutes of exposure to 4°C and 50% RH, sensations of thirst were attenuated to a threshold of approximately 304 $mOsmol.kg^{-1}$.

Dehydration, water intake and hormonal responses in the cold have been examined in a variety of conditions (Table 1.2), although research concerning voluntary dehydration combined with hormonal responses is limited with many papers focussing on fixed volumes of water intake (Kenefick *et al.*, 2008; O'Brien *et al.*, 2005). The work by Maughan *et al.* (2005), conducted on footballers in the cold was able to examine sweat rates, voluntary water intake and changes in hydration status during a 90 minute training session, however, conducting field work is not always conducive to allow venous blood sampling, especially when a large sample size is tested. Therefore, there is reduced data concerning blood markers of hydration status and hormonal data, which may assist with understanding the water intake behaviour in the cold. In controlled laboratory settings, in which blood samples have been taken, exercise is often, either not performed (i.e. subjects resting or sitting in a cold environment) (O'Brien *et al.*, 1998; O'Brien *et al.*, 2005), or exercise is not of sufficient intensity, either to elicit large dehydration effects or to mirror similar conditions of water balance experiments conducted in the heat (Kenefick *et al.*, 2004ab; Kenefick *et al.*, 2008).

1.9 Water intake and high intensity intermittent exercise

The majority of literature examining voluntary water intake has focussed on prolonged continuous exercise at moderate percentages of $\dot{V}O_{2max}$ (~50-70%) (Table 1.1). Little work has examined the effect of high intensity bouts of exercise particularly when they are performed for repeated, intermittent periods, on subsequent water intake. Hew-Butler *et al.* (2008) found that following a $\dot{V}O_{2max}$ test on the treadmill, AVP concentrations, were significantly elevated compared to a continuous period of steady state running (60% of $\dot{V}O_{2max}$). AVP release was closely related to rises in plasma osmolality (Baylis, 1987) and Nose *et al.* (1991) have theorised that an increase in blood lactate concentrations, typically associated with high intensity exercise, prevents plasma sodium release from the vascular space, thus increasing plasma sodium and therefore, plasma osmolality levels. Nose and colleagues increased exercise intensity until voluntary exhaustion on a cycle ergometer. A significant rise in plasma sodium concentration was observed when exercise increased to 70 and 95% of $\dot{V}O_{2max}$. Changes in plasma sodium were significantly correlated with changes in plasma lactate concentrations ($r=0.99$). At 90% $\dot{V}O_{2max}$, the increase in plasma sodium concentration accounted for 60% of the increase in plasma osmolality.

Table 1.2. A summary of dehydration effects and hormonal responses to exercise in the cold.

Author	Subjects	Methods	Environment	Exercise/Rest	Blood sampling	Results	Conclusions
Adam *et al.* (2008)	8 soldiers (6 males, 2 females)	4 trials: EU/HY @2/20°C	2°C, 20°C	Cognitive tests and 60 min cycling (~550kcal)		Hydration did not affect performance variables	HY did not have an effect on any measures of cognitive performance
Cheuvront *et al.* (2005)	8 soldiers (6 males, 2 females)	4 trials: EU/HY @2/20°C. 3h in the heat (am), 60 min sat in cold/temperate room, 30 min cycling @50% $\dot{V}O_{2max}$, 30 min time trial (TT)	2°C, 20°C (both 50% RH)	30min cycling @50% $\dot{V}O_{2max}$ then 30 min TT		Cycling TT performance was impaired due to HY by 3% body mass in temperate but not cold environment. Difference in CV and O_2 uptake kinetics possible explanation for maintenance of performance in cold when HY	In temperate air but not cold, HY impairs endurance performance
Kenefick *et al.*(2004a)	8 males ~35 y	Dehydration trial the previous day. 4 trials: HY-Cold, EU-Cold, HY-Temperate, EU-Temperate	4°C, 74% RH, 27°C, 38.5% RH	60 min walk @60% $\dot{V}O_{2max}$ in 4/27°C		During exercise and rest in the cold when both EU and HY, thirst sensations were attenuated. When EU or HY during cold exposure, AVP responses were reduced. Reduction in AVP in cold occurred during a hyperosmotic state – increased central volume	Up to 40% attenuation of thirst in the cold
	9 males ~21 y	Dehydration trial the previous day. 4 trials: HY-Cold, EU-Cold, HY-Temperate, EU-Temperate	4°C, 74% RH, 27°C, 38.5% RH	30 min rest @27°C, 30 min rest in 4/27°C, 30 min walking @50% $\dot{V}O_{2max}$ in 4/27°C	0, 30, 60, 90 min.		

Author	Subjects	Methods	Environment	Exercise/Rest	Blood sampling	Results	Conclusions
Kenefick *et al.* (2004b)	8 males ~35 y	Dehydration trial the previous day. 4 trials: HY-Cold, EU-Cold, HY-Temperate, EU-Temperate. Hypertonic hypohydration – usually experienced in cold exposure due to sweat loss	4°C 74% RH, 25°C 38.5% RH	60 min walk @60% $\dot{V}O_{2max}$ in 4/25°C		4% HY had no effect on thermoregulatory or cardiovascular responses during exercise in the cold. No difference in core temp between EU and HY in cold – cold-induced vasoconstriction	Moderate intensity exercise in the cold while HY does not incur a similar physiological penalty usually seen in a warm environment.
Kenefick *et al.* (2008)	8 males ~24 y	Dehydration trial the previous day. 3 Trials DH+Placebo, DH+NaCl, EU+Placebo. 45 min exposure to 4°C following ingestion of 500ml water +Placebo/NaCl	Dehydration: 37°C, day 2 – 30 min in 24°C, 45 min in 4°C 60%RH	90 min walking (dehydration), day 2: 75 min rest	0, 30, 75 min	Decrease in thirst following cold exposure. Thirst sensations increased in response to increased plasma osmolality.	Increasing plasma osmolality can override attenuation of thirst due to cold exposure.
Maughan *et al.* (2005)	17 male footballers	90 min training session	5.1 ± 0.7°C, 81 ± 6% RH	90 min training session		Sweat losses similar to those in warmer environments. Salt losses and sweat electrolyte contents similar. 6/17 had pre-training urine osmolality greater than 900 mOsmol.kg^{-1} (mild dehydration). Water intake was 25% of sweat loss	Sweat losses in footballers in the cold are substantial. Water intake low and large individual variation.

Author	Subjects	Methods	Environment	Exercise/Rest	Blood sampling	Results	Conclusions
O'Brien *et al.* (1998)	9 males	10d prelim period, 3 trials – EU, hypertonic HY (HH), isotonic HY (IH). HH: exercise sweating (hypertonicity, limited hypovolaemia), IH: diuretic (furosemide) (hypovolaemia, min change in tonicity)	30 min in 25°C and 120 min in 7°C	Total 150 min rest	@25, 60 and 120min.	Hypohydration level averaged 6-7% reduction in Total body water. HH: Plasma osmolality increased by 12 $mOsm.kg^{-1}$ but no change in plasma volume. IH: Plasma volume decreased by 18% whilst plasma osmolality increased by 5 $mOsm.kg^{-1}$	Hypohydration may impair the vasoconstrictor response to cold
O'Brien *et al.* (2005)	7 males	3 trials: 2x Hyperhydration (water (WI) and glycerol and water (GI)). Ingested a total of 37ml $water.l^{-1}$ TBW prior to entering chamber	45-60 min control period in 20-24°C then 4h in 15°C 40% RH	4 h rest	Every 60 min	Plasma anti-diuretic hormone activity was lower during cold exposure and was lower with both GI and WI. With cold air exposure nearly 2x more water was retained with GI compared to WI. Effectiveness of glycerol for hyperhydration results from its action on the kidney. Hyperhydration not effective at preserving plasma volume, GI may better preserve the extravascular water volume accounting for the improved total body water	Glycerol more effective than water at preserving total body water. Hyperhydration has no effect on haemoconcentration during cold air exposure. Thermoregulation not affected by hyperhydration.

Author	Subjects	Methods	Environment	Exercise/Rest	Blood sampling	Results	Conclusions
Seifert *et al.* (2006)	14 male skiers	2 trials: Back Mounted Hydration system (BMHS) or no water (NW).	-10°C and -8°C at start of day rising to 6°C and 9°C. 2438-3200 m above sea level	2.75 h skiing, then *ad libitum* water at lunch for 1.5 h. 2.5 h skiing in afternoon (~18 min per hour skiing)		1. During 2.75h of skiing dehydration occurred, 2. When given free access to water at lunch they were unable to recover lost water volume, 3. In NW trial urine output exceeded intake (1.01 v 0.78l), 4. Using BMHS dehydration was prevented. NW replaced 71% of water lost at midday	In BMHS, skiers could maintain hydration status, felt physically better and perceptions of thirst were lower.
Sun *et al.* (2003)	36 rats	6 groups – 3 in 5 ± 2°C, 3 in 25 ± 2°C for 1,3 and 5 weeks of exposure	5 ± 2°C and 25 ± 2°C		AVP and serum osmolality	Serum osmolality was increased in cold exposed rats. Water loss from excretion increased by prolonged cold exposure. Acute cold exposure → dehydration → plasma levels of AVP decreased, chronic cold exposure → dehydration → V_2 receptors inhibited without plasma levels of AVP reducing. Plasma volume increased during first 3 weeks of exposure to cold and returns to control levels by 5 weeks of cold exposure	Dehydration induced by chronic cold exposure. This may increase excreted water loss.

Author	Subjects	Methods	Environment	Exercise/Rest	Blood sampling	Results	Conclusions
Sun (2006)	12 Long-Evans (LE) rats and 12 AVP deficient rats (VD)	5 weeks of cold exposure	5 ± 2°C		Plasma osmolality and AVP	Blood pressure increased by week 2, increased food intake, AVP deficiency did not affect food intake. VD- increased water intake and urine output. Water intake and urine output in LE rats but not VD was increased due to cold exposure. Diuresis not affected by prevention of cold-induced hypertension.	Cold induced elevation of blood pressure was not attenuated by genetic AVP deficiency

Notes: EU=euhydration, HY=hypohydration, RH=relative humidity.

Following a period of high intensity exercise there is shift in water from the vascular to the interstitial and intracellular spaces (Convertino *et al.*, 1981; Nose *et al.*, 1991; Sjøgaard *et al.*, 1985). The movement of hypotonic water out of the vascular space causes a rise in plasma osmolality. Sjøgaard *et al*, (1985) analysed extra- and intracellular muscle water shifts following one-legged dynamic knee-extensions in six males. They attributed the movement of water to the interstitial space due to an increase in blood pressure and to an increase in perfused capillaries, whilst an osmotic gradient caused by an increase in lactate concentration was believed to cause water to move into the intracellular space.

1.10 Summary

From the literature it is apparent that the sensation of thirst plays an important role in maintaining hydration status. Numerous studies have been conducted involving a variety of subjects, conditions and type of beverages ingested with analysis through questionnaires, hormonal analysis and voluntary water intake. However, there are still areas in which further research can be conducted specifically pertaining to examining hydration status in the general population in order to examine over and under drinking, and to examining voluntary water intake in the cold and following high intensity intermittent exercise by combining the knowledge gained from previous laboratory and field work.

1.11 Aims

The general aim of the thesis is to assess and understand what causes voluntary water consumption in both general and exercise populations. Within the thesis, further aims include to:

- Assess hydration status and water intake in the general population.
- Assess the validity of using capillary blood samples to monitor hydration status and track changes in markers of hydration status.
- Examine voluntary water intake in different environmental conditions and in response to different bouts of exercise.

CHAPTER 2

General methods

This chapter provides an overview of the common methods used in this thesis. Generic protocols are outlined, with more specific details included in the methods section of each experimental trial. The chapter in which each method is used is outlined in each subsection.

2.1 Ethical Approval

Ethical approval was granted by the Loughborough University Ethical Advisory Committee prior to the commencement of the data collection for each experimental trial. Human volunteers were used in all experimental trials included in this thesis. Subjects had the trial explained to them both verbally and in writing and were informed of their right to withdraw from the investigation at any point. Following this, signed informed consent was then obtained.

2.2 Subjects

Subjects were healthy volunteers mainly recruited from Loughborough and the surrounding area. Subjects were recruited by word of mouth, email and posters. In the studies presented in Chapters 4, 5, 6 and 7, subjects were aged between 18 and 36. In Chapter 3, participants were aged between 18 and 63. Descriptive characteristics are presented in the methods section of each specific chapter.

2.3 Pre-trial standardisation

In all experimental studies except of that presented in Chapter 3 subjects were asked to replicate dietary intake and refrain from exercise in the 24 hours preceding the trials. The trials in Chapters 5, 6, and 7 commenced in the morning and subjects were asked to arrive following an overnight fast. Subjects were instructed to consume 500 ml of water two hours before arrival at the laboratory to ensure they arrived in a state of euhydration. In the study presented in Chapter 3, pre-trial standardisation did not occur. In Chapters 4, 5, 6 and 7 subjects performed a familiarisation trial prior to the experimental trials. Exact details of the familiarisation trials are presented in the methods section of the corresponding chapter.

2.4 Body mass

Body mass was measured to the nearest 10 g in each study (Chapters 4, 5, 6 and 7, Adam AFW-120K, Milton Keynes, UK; Chapter 3, Adam CFW-150, Milton Keynes, UK). Specific details regarding timing, frequency and clothing worn are described in each chapter. In Chapters 4, 5, 6 and 7, combining body mass data with urine output and water intake allowed net body water balance to be calculated, assuming that subjects arrived at the beginning of the trial in a euhydrated state based on urine and serum osmolality values.

2.5 Blood sampling – collection and storage

Blood samples were collected in the experimental studies in Chapters 4, 5, 6 and 7. Specific details concerning method of collection, volume of blood, frequency and timing of the samples are included in each chapter in the methods section.

2.6 Blood analysis

2.6.1 Haemoglobin concentration, haematocrit, blood glucose and blood lactate concentration

In Chapters 4, 5, 6 and 7 after collection into a dry syringe (BD Plastipak, Becton Dickinson S.A., Madrid, Spain), whole blood was dispensed into a 1 ml tube containing anticoagulant (K_2EDTA; 1.5 $mg.ml^{-1}$, Teklab, Sacriston, UK). The cyanmethaemoglobin method was used to determine haemoglobin concentration and haematocrit values were measured following microcentrifugation (Hawksley micro-haematocrit centrifuge, UK). To determine blood glucose and lactate concentrations, 100 µl of whole blood was added to 1000 µl of ice cold 0.3M perchloric acid (PCA) to deproteinise the sample. This was carried out in duplicate. Blood glucose concentration was then determined by the GOD-PAP method (Randox Laboratories Ltd., Crumbin, UK) whilst blood lactate concentrations were determined by method outlined by Maughan (1982) using fluorimetry. Haemoglobin concentration, blood lactate concentration and blood glucose concentration measurements were made in duplicate whilst haematocrit analysis was carried out in triplicate. In Chapter 4, the blood glucose analysis was scaled down for capillary and 20 µl of whole blood was

added to 200 µl of 0.3M PCA. Using the method of Dill & Costill (1974), haemoglobin concentrations and haematocrit values were used to calculate percent changes in plasma, blood and cell volume from baseline values.

2.6.2 Serum osmolality and serum electrolyte concentrations

A sample of whole blood was aliquotted into a plain tube (Teklab, Sacriston, UK) and centrifuged at 1500 x *g* (3000 rpm) for 15 minutes at 4°C (ALC multispeed refrigerated centrifuge, UK). Serum was removed and stored in a refrigerator until analysis was performed. Serum samples were analysed for osmolality by freezing point depression (Chapter 4, Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany; Chapters 5, 6 and 7, Gonotec Osmomat auto Cryoscopic Osmometer; Gonotec, Berlin, Germany), sodium and potassium concentrations in Chapters 4, 5, 6 and 7 by flame photometry (Corning Clinical Flame Photometer 410C; Corning Ltd., Halstead, Essex, UK) and chloride concentration in Chapter 4 by columetric titration (Jenway Chloride Meter; Jenway Ltd., Dunmow, Essex, UK). Samples were analysed in duplicate.

2.6.3 Vasopressin, aldosterone and plasma osmolality

A sample of whole blood was aliquotted into 2.5 ml tubes containing anticoagulant (K_2EDTA; 1.5 mg.ml^{-1}, Teklab, Sacriston, UK) and centrifuged at 1500 x *g* (3000 rpm) for 15 minutes at 4°C (ALC multispeed refrigerated centrifuge, UK). In Chapter 4 a sample of plasma was analysed in duplicate for osmolality by freezing point depression (Chapter 4, Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany). The remaining plasma and the plasma in the other studies involving blood sampling was frozen and stored at -80°C for later analysis of vasopressin and aldosterone concentration. Vasopressin analysis was carried out by enzyme immunoassay (Enzyme Immunoassay; Assay Designs (Enzo Life Sciences), Ann Arbor, MI, USA). Analysis was carried out in singular (Chapters 4 and 5) and in duplicate (Chapters 6 and 7). In Chapter 4 a sample size of 150 µl was extracted using the acetone-ether method and reconstituted with 150 µl of assay buffer, with a 100 µl sample then used for analysis of venous blood samples. For capillary samples a 100 µl sample was extracted and 75 µl of the reconstitution was used for analysis. In Chapter 5 a volume of 600 µl was extracted and reconstituted with 150 µl. In Chapters 6 and 7 a volume of 900 µl was extracted and reconstituted with 300 µl. Aldosterone

concentration was measured in duplicate by enzyme immunoassay (Enzyme Immunoassay; Enzo Life Sciences, Ann Arbor, MI, USA) in Chapters 6 and 7. A plasma volume of 100 µl was diluted with 300 µl of assay buffer and the resultant concentration following analysis was divided by four.

2.7 Urine collection and analysis

Urine sample collection was similar in all trials except in the study presented in Chapter 3, when volume was not measured. In Chapters 4, 5, 6 and 7 subjects completely emptied their bladders into plastic containers, volume was measured and for each study a 5 ml sample was retained for analysis. Specific timings and frequencies of urine sampling are outlined in each chapter. Urine samples were analysed for osmolality by freezing point depression (Chapter 4, Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany; Chapters 3, 5, 6 and 7, Gonotec Osmomat auto Cryoscopic Osmometer; Gonotec, Berlin, Germany), specific gravity by refractometry (Chapters 3 and 4; Digit-012, Ceti, Belgium) and colour using the scale outlined by Armstrong *et al.* (1994) (Chapter 3). Urine sodium and potassium concentrations were measured by flame photometry (Corning Clinical Flame Photometer 410C; Corning Ltd., Halstead, Essex, UK) in all experimental chapters and urine chloride concentrations were measured in Chapter 4 by columetric titration (Jenway Chloride Meter; Jenway Ltd., Dunmow, Essex, UK). Analysis for all samples was completed in duplicate.

2.8 Peak oxygen uptake ($\dot{V}O_{2peak}$)

Peak oxygen uptake was determined using a discontinuous, incremental protocol in Chapters 5, 6 and 7 to allow determination of subsequent exercise workloads. The tests were performed on an electronically braked cycle ergometer (Lode Corival; Lode BV, Groningen, Netherlands) consisting of 4 minute stages. The first stage commenced at a workload of 100 watts (W) and increased in the subsequent stages depending on heart rate and rate of perceived exertion data collected during the final 30 seconds of the previous stage. Workrate was increased until volitional exhaustion occurred. During the final minute of each stage, expired air was collected into a Douglas bag and analysed for gas content (oxygen and carbon dioxide) (Servomex 1400, Crawley, East

Sussex, United Kingdom), gas temperature (Edale digital thermometer) and gas volume (Harvard Dry Gas Meter, Harvard Apparatus Ltd., Kent, United Kingdom).

2.9 Core and Skin Temperature

Core temperature was measured using a rectal thermometer. Subjects were asked to insert a rectal thermometer 10cm past the anal sphincter. Skin thermistors were attached to the right-hand side of the body at four sites: the chest, tricep, thigh and the calf. Core and skin thermistors were connected to a Biopac System (BIOPAC MP100 System; BIOPAC, Santa Barbara, CA, USA) to allow continuous measurement of data. Mean weighted skin temperatures were calculated using the formula outlined by Ramanathan (1964):

Mean weighted skin temperature = 0.3(chest) + 0.3(tricep) + 0.2(thigh) + 0.2(calf)

The mean values for each minute were calculated and data for every fifth minute is presented in the results section of the experimental chapters. Core and skin temperatures were measured in Chapters 5, 6 and 7.

2.10 Exercise protocols

Specific details of exercise protocols can be found in each chapter. In general, exercise was conducted on either a friction-braked cycle ergometer (Chapter 4; Monark Exercise AB, Vansbro, Sweden) or an electronically-braked cycle ergometer (Chapters 5, 6 and 7; Lode Corival; Lode BV, Groningen, Netherlands) in the environmental chamber (air circulation speed $1 m.s^{-1}$) and laboratories with in the Sport, Exercise and Health Science department at Loughborough University. The standard fitted pedals of the cycle ergometer were used in each experiment.

2.11 Subjective feelings questionnaires

Subjective feelings were assessed using 100 mm visual analog scales in each experimental study. The specific questions assessed on each scale and the respective timings and frequencies are outlined in the methods section of each chapter. Subjects marked a vertical line along the 100 mm horizontal line. In general 0 mm referred to

'not at all [feeling]' and 100 mm referred to 'very [feeling]'. The specific questionnaires used in each chapter are presented in the appendices (C, D and E).

2.12 Heart rate, rate of perceived exertion and thermal sensation

Heart rate was measured in Chapters 5, 6 and 7 using a Polar Electro heart rate monitoring system (Polar Electro Oy, Kempele, Finland). Rate of perceived exertion was measure during the exercise period in Chapters 5, 6 and 7 using the scale by Borg (1970) (Appendix A). A modified scale by Hardy (1970) was used to assess thermal sensation (Chapters 5, 6 and 7) (Appendix B).

2.13 Coefficient of variation of analytical procedures

Coefficients of variation (CV) were calculated for commonly used analytical techniques and presented in Table 2.1. CV was calculated for each analytical measure, using a random sample of 30 measures. The following calculation was used:

CV (%) = (Standard deviation of the difference between duplicates / mean) x 100

2.14 Statistical analysis

The statistical analysis used in each investigation is described in the methods section of the corresponding experimental chapter. In general data were assessed for normal distribution using Kolmogorov-Smirnoff ($n \geq 30$) and Shapiro-Wilks ($n < 30$) tests and then analysed using the test described in each chapter. When a significant main effect was observed, post-hoc tests were used to identify where the difference occurred. Post-hoc tests were carried out on significant and non-significant interaction effects. Post-hoc analysis was conducted when an interaction effect was not present as it is possible, due to many time points being measured and compared, that despite a lack of an interaction effect there may have been statistical differences between time points. Correlation analysis was calculated between variables deemed to be closely related in terms of the physiological and behavioural mechanisms related to water balance. The variables used in correlation analysis have all been shown in the literature to influence or contribute to at least one other variable measured. Normal data is expressed as mean ± standard deviation, whilst non-parametric data is expressed as median (range). An

alpha level of $p<0.05$ was used in all experiments unless otherwise stated. When post-hoc tests were conducted, p values presented were multiplied to correct for repeated samples. Statistical analysis was conducted using Statistical Package for the Social Sciences for Windows, version 18.0 (SPSS *inc*, Chicago, IL, USA).

Table 2.1. Coefficient of variation for analytical measurements commonly used in experimental studies. SD = standard deviation, CV = coefficient of variation.

Measure	*n*	Mean	SD	CV (%)
Urine osmolality ($mOsmol.kg^{-1}$).	30	650	256	0.22
Urine specific gravity	30	1.018	0.007	0.05
Urine sodium concentration ($mmol.l^{-1}$)	30	84	29	0.89
Urine potassium concentration ($mmol.l^{-}$	30	83	34	0.83
Urine chloride concentration ($mmol.l^{-1}$)	30	90	55	1.52
Serum osmolality ($mOsmol.kg^{-1}$)	30	289	5	0.24
Serum sodium concentration ($mmol.l^{-1}$)	30	142	2	0.65
Serum potassium concentration	30	4.5	0.4	1.27
Serum chloride concentration ($mmol.l^{-1}$)	30	104	3	1.04
Plasma osmolality ($mOsmol.kg^{-1}$)	30	293	7	0.40
Haemoglobin concentration ($g.l^{-1}$)	30	163	12	0.66
Haematocrit (%)	30	46.8	2.8	0.46
Blood glucose concentration ($mmol.l^{-1}$)	30	4.15	0.30	2.40
Blood lactate concentration ($mmol.l^{-1}$)	30	4.9	3.4	5.96
AVP concentration ($pg.ml^{-1}$)	30	5.4	3.2	8.87
Aldosterone concentration ($pg.ml^{-1}$)	30	493	475	14.96

CHAPTER 3

Assessing hydration status in the work place

3.1 Abstract

Hydration status of the general adult population has not been assessed in many areas of employment. When undertaken, previous research has focussed on work in hot and humid environments (Bates & Schneider, 2008; Brake & Bates, 2003) or on the effects of wearing personal protective equipment (Cheung & McLellan, 1998; McLellan *et al.*, 1999). The primary aim of this study was to examine the hydration status of adults working in different jobs at the beginning and end of a shift. A secondary aim was to examine the self-reported water intake in the groups monitored and examine whether this could affect or influence observed hydration status. 156 subjects (89 males, 67 females: age 32 (19-63) years, height 1.74 ± 0.10 m, mass 77.6 ± 15.3 kg) were recruited from work places within the local area (Start of shift indoors: 19.6 ± 1.6°C, end: 20.5 ± 1.0°C). Groups were research students (n=33), masters students (n=24), secondary-school teachers (n=31), security staff (n=15), firefighters (n=22), office workers (n=15) and catering staff (chefs and kitchen assistants) (n=16). Subjects were asked to provide a urine sample and complete a 100 mm visual analogue scale subjective feelings questionnaire comprising of six questions relating to thirst, mouth dryness, hunger, tiredness, concentration and energy at the start and end of their shift. Body mass, in one layer of loose fitting clothing without shoes, was measured at the start of the shift. At the end of the shift subjects were asked if they experienced a sensation of thirst during the shift and to report all water intake from beverages from the shift. Urine was analysed for osmolality (U_{osm}), specific gravity (USG) and sodium and potassium concentrations. Euhydration was considered U_{osm} <700 mOsmol.kg^{-1} or USG <1.020 (Sawka *et al.*, 2007). Significance level was $p<0.05$. Females had lower U_{osm} values than males at the start (656 (85-970) v 738 (164-1090) mOsmol.kg^{-1}) and end (461 (105-1014) v 642 (130-1056) mOsmol.kg^{-1}; $p<0.05$) of their working day. U_{osm} values suggested that 64% of males compared to 42% of females arrived at work in a hypohydrated state. This reduced to 40 and 28% at the end of the shift for males and females respectively. 52% of individuals who appeared hypohydrated at the start of the shift were hypohydrated at the end of the shift. Reported water intake from beverages was greater in males compared to females (1.2 (0.0-3.3) v 0.7(0.0-2.0) l respectively; $p<0.0001$). Firefighters reported consuming more water (2.1 (0.5-3.0) l) compared to all other groups ($p<0.05$). 117 workers reported experiencing a feeling of thirst at some point during the shift with 85% alleviating the sensation by consuming a

drink. In conclusion a large proportion of subjects exhibited urine values indicating hypohydration with many remaining in a state of hypohydration at the end of the shift. Access to water and other beverages at work helped alleviate sensations of thirst. Further investigation is required to gain insight into the causes and significance of these findings.

3.2 Introduction

The effect of dehydration has been studied in a variety of situations relating to cognitive performance, however the concluding effect is often varied (Gandjean & Grandjean, 2007). In their review, Grandjean & Grandjean (2007) concluded that a body mass loss of greater than 2% caused by dehydration can have a negative impact on cognitive performance. In the workplace, a reduction in cognitive performance may reduce quality of work, productivity and decision making, thereby making workers ineffectual. Gopinathan *et al.* (1988) reported decreases in short-term memory, visual motor tracking, arithmetic efficiency and attention when dehydration was induced to 2, 3 and 4% of body mass loss, whilst Cian *et al.* (2000) found a decrease in perceptive discrimination, psycho-motor skills and short-term memory following dehydration up to a body weight loss of 2.8%. However, these studies used exercise and heat or exercise respectively to induce dehydration and therefore direct application to the assessment of dehydration in the work place may not always be appropriate.

Previous studies examining hydration status in the workplace have focussed on workers in hot and humid conditions performing physical activity (Bates & Schneider, 2008; Brake & Bates, 2003) and those wearing personal protective equipment (Cheung & McLellan, 1998; McLellan *et al.*, 1999), however this may not be applicable to those who work in temperate conditions performing less strenuous activity without protective equipment. In many work places, environmental conditions are often controlled by air conditioning and heating systems, and many workers may remain seated at a desk for a large portion of the shift.

When hydration status has been examined in the workplace, many workers have arrived already dehydrated (Brake & Bates, 2003). Brake & Bates (2003) found that 60% of underground miners reported to work in a dehydrated state and hydration status did not improve over the course of the 10-12 hour shift. Examining 39 workers in average wet bulb temperatures of 28.4 ± 2.2°C, they measured urine specific gravity at the start, middle and end of the shift and water intake every 60-90 minutes. Urine specific gravity (USG) values were 1.025 ± 0.005, 1.025 ± 0.005 and 1.025 ± 0.007 respectively, whilst water intake was 0.8 ± 0.3 $l.h^{-1}$. With the majority of workers arriving already dehydrated, extra water would be required on top of what would

normally be consumed in order to return to a euhydrated state. Monitoring hydration status at the end of the shift may provide an indication of whether the arrival hydration status was maintained or changed throughout the day.

When examining hydration status, consideration and assessment of drinking influences and access to beverages is important. Understanding why individuals chose to drink or not and in what scenarios this is apparent may help prevent hypohydration and possible subsequent impairment of performance. For example, Kenefick & Sawka (2007) reported anecdotal evidence from questionnaires that individuals restricted water intake if toilet facilities were not available. Limited access to toilet facilities may have had an impact on the amount of subsequent water consumed during the shift, and may have contributed to any dehydration that may have occurred.

In working environments, where water intake exceeds water losses, hyperhydration may become apparent, however, it is often difficult to quantify hyperhydration and make the distinction between an individual being euhydrated or hyperhydrated. Armstrong *et al.* (2010) analysed morning and 24h urines samples in 59 males over 5 out of 12 days and assigned urine and serum osmolality and specific gravity values to hydration categories (extremely hyperhydrated, slightly hyperhydrated, well hydrated, euhydrated, slightly dehydrated, very dehydrated and extremely dehydrated) based on percentiles, however, the authors stressed that the categories should only assist clinicians with objective assessments of hydration status. In order to further examine this, water intake behaviour in association with urine markers of hydration status and frequency of urination may provide indication of hyperhydration but further investigation is warranted. In extreme cases of hyperhydration, consuming too much water can lead to hyponatraemia, with potentially serious health consequences (Adrogué, 2005) especially if combined with exercise (Beltrami *et al.*, 2008). However, in most cases of hyperhydration, hyponatraemia is not apparent as increased water intake usually results in diuresis occurring (Kovacs *et al.*, 2002) and therefore health problems are usually not an issue.

Assessing water intake behaviours during a shift and hydration status of workers at the start and end of their shift may help identify those who are dehydrated and hyperhydrated whilst also identifying ways to prevent it from occurring. Therefore, the primary aim of this study was to examine the hydration status of different work groups

at the beginning and end of a shift. A secondary aim was to examine the water intake influences and behaviours between the groups monitored and examine whether this could affect or influence observed hydration status.

3.3 Methods

3.3.1 Subjects

156 subjects (age 32 (19-63) years, height 1.74 ± 0.10 m, body mass 77.6 ± 15.3 kg) comprising of 89 males and 67 females were recruited from the local area. Subjects were research students (n=33), masters students (n=24), teachers (n=31), security staff (n=15), firefighters (n=22), office workers (n=15) and catering staff (chefs and kitchen assistants) (n=16). Subjective characteristics for each group are displayed in Table 3.1. All subjects had the experimental protocol explained to them verbally and in writing. Subjects provided written consent and the experiment was approved by the Loughborough University Ethics Committee (R09-P188).

Table 3.1. Subjective characteristics for each group.

Group	n	Age (years)	Body mass (kg)	Height (m)
Research	33	26 ± 4	72.0 ± 11.6	1.75 ± 0.11
Masters	24	23 ± 1	71.8 ± 11.2	1.74 ± 0.08
Teachers	31	47 ± 10	72.4 ± 12.0	1.68 ± 0.08
Security	15	44 ± 9	97.1 ± 11.3	1.83 ± 0.09
Firefighters	22	38 ± 8	85.8 ± 7.9	1.80 ± 0.05
Office	15	32 ± 9	74.1 ± 17.8	1.73 ± 0.11
Catering	16	50 ± 13	81.8 ± 21.5	1.64 ± 0.10

Groups

Each group is described below with a brief description of a typical working day and any breaks that were allowed for each group of subjects. Any major barriers to water intake are also noted.

Research – University PhD and research students primarily based in an office environment but with visits to laboratory for short periods of experimental work. No restriction on frequency and duration of break times and were able to eat and drink freely as they worked.

Masters – University MSc students who participated in laboratory classes all day. Food and water intake banned in the laboratory so subjects had to leave the laboratory to eat and drink. One hour break at lunch.

Teachers – Taught classes for at least five hours per day with a small break of approximately 5 minutes after each one hour lesson. One hour for lunch break and a 20 minute break at around 10am. Unable to leave the classroom and use toilet facilities whilst teaching classes. Able to consume own drinks during class but not eat.

Security – University security staff working a variety of shift patterns including night shifts. A comparison was not made between day and night shifts as they were deemed to be similar in behaviour. 15 minute breaks in the period before and after a 30 minute lunch/dinner break. Staff patrolled the university on foot, bike and in motorised vehicles and were able to drink freely during the shift when time permitted.

Firefighters – Day and night shifts observed. Staff performed maintenance and practice drills throughout the day as well as having a physical activity session involving strength and aerobic activity in the onsite gym. Average number of call outs was three per day. Were able to eat and drink freely when not performing drills or on call outs, when there was limited access to water.

Office – Staff were sat at computers throughout the duration of the day, two small 15 minute breaks in the morning and afternoon and a 30 minute lunch break. Were able to eat and drink freely whilst working.

Catering – Kitchen staff and chefs at university canteen. On feet throughout the majority of the shift, with a large portion of work time (exact time unknown) spent in the kitchen preparing food. Two 15 minute breaks and a 30 minute break for lunch. Were able to drink, outside of scheduled break times, if time permitted.

3.3.2 Procedure

Subjects arrived at their place of work immediately prior to their shift and were asked to sign a consent form, complete a 100 mm visual analogue subjective feelings questionnaire comprising of six questions relating to thirst (0= not at all thirsty, 100= very thirsty), mouth dryness (0= not at all dry, 100= very dry), hunger (0= not at all hungry, 100= very hungry), tiredness (0= not at all tired, 100= very tired), concentration (0= not very well, 100= very well) and energy (0= no energy, 100= lots of energy) (Appendix C) and a small questionnaire relating to their water intake patterns during a typical shift (Appendix F). The questionnaire asked about access to drinks, any influences on drinking, typical water consumption and whether they experienced thirst and changes in concentration during a shift. They then provided a urine sample, before height and body mass were measured to the nearest 10 g (Adam CFW-150, Milton Keynes, UK) whilst wearing loose fitting clothing (one layer) and without shoes. Subjects were then asked to complete their work shift as normal. On completion of the shift subjects provided a urine sample and had body mass measured in the same clothing worn during the previous measurement. They were asked to fill in the same

subjective feelings questionnaire and a small questionnaire relating to their water intake during the shift (Appendix F). Questions related to access to drinks during the shift, how much they consumed, whether they experience a feeling of thirst and if so did they drink to alleviate this? They were asked to rate their concentration at the start, middle and end of the shift using a 100 mm visual analogue scale and whether they felt they remained hydrated throughout the duration of the shift. Subjects were then free to leave. Ambient temperature and relative humidity was measured at the start and end of each shift both inside and outside the place of work (RH85 Digital Thermo-Hygrometer; Omega, Manchester, UK). The duration of each shift was based on a typical eight hour working day. To participate, all subjects must have completed a shift of at least seven hours. All subjects completed this and none were excluded. If physical activity (e.g. running at lunch) was performed, and unless mentioned, was considered part of a typical shift and was therefore not taken into account during analysis.

3.3.3 Sample analysis.

Urine was analysed for osmolality, potassium and sodium concentration, specific gravity and colour (see Chapter 2 for details).

3.3.4 Statistical analysis

All data were checked for normality using the Kolmogorov-Smirnov test if the data set was large ($n>30$) and the Shapiro-Wilk test if the data were less than $n=30$. One-way ANOVA and Kruskal-Wallis tests were used for parametric and non-parametric data respectively to identify differences between groups. A two-way ANOVA was not performed as start and end values were deemed to be individual time points because it was not possible to accurately measure water input and output during the shift. Independent sample t-tests and Mann-Whitney tests were subsequently performed as post-hoc analysis when significant differences were observed and also to compare between start and end values within each population. Linear regression was used to identify relationships. A significance value of $p<0.05$ was used. When post-hoc tests were conducted, p values presented were multiplied to correct for repeated samples. Parametric data is expressed as mean ± SD and non parametric data expressed as median (range).

3.4 Results

3.4.1 Environmental conditions

Inside the places of work at the start of the shift, environmental conditions were 19.6 ± 1.6°C and 41.9% (27.8-55.5%) relative humidity. At the end of the shift, temperature was 20.5 ± 1.0°C and relative humidity was 41.7% (17.0-49.5%). Outside conditions were 8.7 ± 3.6°C and 60.1 ± 14.7% at the start and 9.5 ± 4.1°C and 56.0 ± 14.3% at the end of the shift. Environmental data recorded at each place of work is presented in Table 3.2.

3.4.2 Pre-shift questionnaire

98% (n=153) of subjects had access to drinks during the course of their shift. When asked about barriers to drinking during their shift, 67% reported perceived influences on drinking behaviour including sensations of thirst and mouth dryness, a lack of toilet facilities, timings of breaks, remembering to drink and access to drinks in particular environments (e.g. on call or in a laboratory). During a normal shift males reported (through cups and volumes) consuming more water than females (1.0 (0.2-4.2) l v 0.9 (0.1-2.0) l) ($p<0.0001$). Typical reported water intake by masters (0.6 (0.1-1.5) l), teachers (0.6 (0.2-3.0) l), security (1.0 (0.4-1.5) l), catering (1.0 (0.5-2.0) l) and office groups (1.0 (0.3-2.5) l) was similar ($p>0.05$), whilst greater water intake was typically reported to be consumed in the research group (1.0 (0.4-3.0) l) compared to the teachers group ($p<0.0001$). The firefighters (2.5 (1.0-4.2) l) reported normally consuming more water than all other groups during a typical shift ($p<0.0001$). During a typical shift 56% of subjects reported normally experienced a sensation of thirst and 45% felt, during a normal shift, their concentration was affected if they did not drink enough water.

Table 3.2. Environmental conditions inside and outside the place of work. Shifts column denotes number of different shifts required to collect all subject group data. Data expressed as mean ± SD.

		Environmental Conditions							
		Inside				Outside			
Group	Shifts	Start Temp (°C)	Start RH (%)	End Temp (°C)	End RH (%)	Start Temp (°C)	Start RH (%)	End Temp (°C)	End RH (%)
Research	5	19.3 ± 1.4	30.9 ± 4.0	20.7 ± 0.9	27.4 ± 9.7	5.4 ± 2.0	74.0 ± 7.9	5.4 ± 3.6	66.5 ± 11.1
Masters	2	20.7 ± 0.4	35.1 ± 8.8	21.8 ± 2.1	34.0 ± 12.0	5.1 ± 0.1	83.4 ± 8.2	8.1 ± 1.6	79.9 ± 3.3
Teachers	2	18.7 ± 2.2	45.1 ± 2.5	21.4 ± 1.7	39.0 ± 4.2	5.1 ± 0.1	83.4 ± 8.2	8.1 ± 1.6	79.9 ± 3.3
Security	15	20.0 ± 0.3	41.9 ± 0.5	20.0 ± 0.4	41.9 ± 0.4	9.5 ± 3.0	50.9 ± 8.4	9.1 ± 2.4	50.0 ± 8.8
Firefighters	3	19.7 ± 1.1	49.8 ± 5.1	20.4 ± 1.7	47.9 ± 1.7	12.8 ± 5.2	56.5 ± 4.8	13.1 ± 5.2	54.3 ± 0.1
Office	2	20.6 ± 3.0	48.4 ± 6.8	22.0 ± 0.8	41.4 ± 0.7	12.5 ± 1.5	66.3 ± 13.4	17.8 ± 3.4	52.2 ± 9.6
Catering	2	15.5 ± 1.9	41.8 ± 0.9	20.5 ± 0.1	25.9 ± 0.1	7.4 ± 0.1	46.5 ± 5.9	12.5 ± 2.1	34.0 ± 9.5

Table 3.3. Start and end values of urine parameters and subjective feelings questionnaires for all subjects and male and females separately. * Different to start value ($p<0.05$). ^ Different to males ($p<0.05$). Data expressed as median (range).

	All		Males		Females	
	Start	End	Start	End	Start	End
Urine Specific Gravity	1.021 (1.002-1.034)	1.016 (1.002-1.033)*	1.022 (1.004-1.034)	1.018 (1.004-1.033)*	1.019 (1.002-1.029)^	1.013 (1.002-1.030)^
Osmolality (mOsmol.kg^{-1})	717 (85-1090)	571 (105-1056)*	738 (164-1090)	642 (130-1056)*	656 (85-970)^	461 (105-1014)^
Colour	4 (1-7)	3 (1-7)*	4 (1-7)	3 (1-7)*	3 (1-7)^	3 (1-6)
Sodium conc. (mmol.l^{-1})	95 (13-203)	90 (14-205)	101 (32-203)	108 (16-205)	86 (13-182)^	76 (14-177)*^
Potassium conc. (mmol.l^{-1})	95 (11-169)	86 (16-179)*	96 (21-157)	85 (17-179)*	93 (11-169)	87 (16-159)
Thirst	49 (0-100)	49 (2-100)	52 (0-100)	48 (2-89)	38 (0-100)^	57 (2-100)*
Mouth Dryness	46 (0-100)	50 (0-93)	50 (0-100)	50 (2-86)	40 (0-84)	48 (0-93)
Tiredness	49 (0-100)	63 (0-100)*	50 (0-100)	63 (7-100)*	47 (0-98)	62 (0-100)*
Hunger	19 (0-96)	30 (0-94)*	24 (0-85)	35 (0-80)*	13 (0-96)^	20 (0-94)*^
Concentration	70 (2-100)	62 (5-98)*	68 (7-100)	63 (6-95)*	70 (2-95)	61 (8-98)
Energy	63 (0-100)	53 (0-92)*	63 (0-100)	55 (0-91)*	63 (1-100)	55 (11-92)*

3.4.3 General results

Start and end values for urine parameters and subjective feeling questionnaires are presented in Table 3.3 for the population as a whole, and for males and females separately. Individual values for urine osmolality and specific gravity are presented in Figure 3.1. There was large variation in start and end values of urine osmolality and USG for males and females with no clear patterns or trends emerging from the data. Subjects were classed as euhydrated if urine osmolality was less than 700 mOsmol.kg^{-1} or urine specific gravity was less than 1.020 (Sawka *et al.*, 2007). Hypohydration was classed as urine values above these values. The percentage of individuals who arrived and left the place of work in a dehydrated state are presented in Table 3.4. Body mass at the end of the shift was measured but not reported. Food intake and urine output were not measured and water intake was not accurately measured through weighing of drinks, therefore making assumptions as to why body mass may have changed difficult.

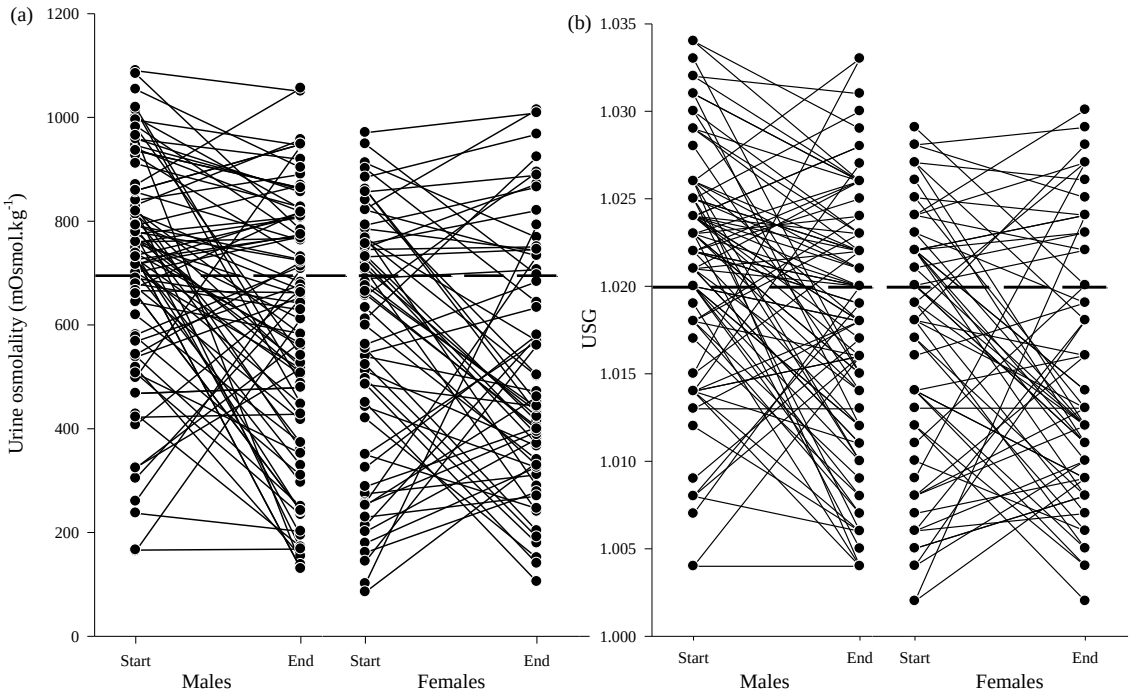

Figure 3.1. Start and end (a) osmolality (mOsmol.kg^{-1}) and (b) urine specific gravity for males and females. - - - represents euhydration values of less than 1.020 and 700 mOsmol.kg^{-1} outlined by Sawka *et al.* (2007).

3.4.4 Group comparison

Between groups

Values for USG, osmolality, colour, sodium and potassium concentration at the start and end of the shift are presented in Figure 3.2. USG values at the start of the shift in the research and firefighter group were greater than the office, teachers and catering groups and were greater than end of shift values ($p<0.05$). Urine osmolality values showed a similar pattern except start values for the research and firefighters group were also greater than the masters group ($p<0.05$) and the firefighters group were not greater than the start values in the catering group ($p>0.05$). Urine sodium concentrations were greater in the security group at the start of the shift compared to the masters and teachers group and at the end of the shift compared to the research, masters, teachers, firefighters and office groups ($p<0.05$). Urine potassium concentrations were lower at the end of the shift compared to the start in the research group ($p<0.05$).

Table 3.4. Percentage of subjects in each group who were hypohydrated at the start and end of the shift using the values outlined by Sawka *et al.* (2007).

			Subjects hypohydrated (%)					
			Osmolality (mOsmol.kg^{-1})			Urine Specific Gravity		
Group		*n*	Start	End	Both	Start	End	Both
All		156	54	35	26	53	33	29
	Males	89	64	40	33	63	37	35
	Females	67	42	28	19	40	27	21
Research		33	73	33	27	70	27	30
	Males	22	77	36	32	73	32	36
	Females	11	64	27	18	64	18	18
MSc		24	46	54	33	42	50	38
	Males	12	50	58	33	42	50	42
	Females	12	42	50	33	42	50	33
Teachers		31	39	23	16	39	23	13
	Males	11	45	27	18	33	17	18
	Females	20	35	20	10	30	20	10
Security		15	67	53	40	60	53	47
	Males	11	73	55	45	73	55	45
	Females	4	50	50	25	25	50	50
Fire		22	73	36	23	73	36	32
	Males	22	73	36	23	73	36	32
	Females	0	-	-	-	-	-	-
Office		15	40	13	13	47	20	13
	Males	8	38	25	25	38	38	25
	Females	7	43	0	0	57	0	0
Catering		16	38	38	38	38	38	38
	Males	3	67	67	67	67	67	67
	Females	13	31	31	31	31	31	31

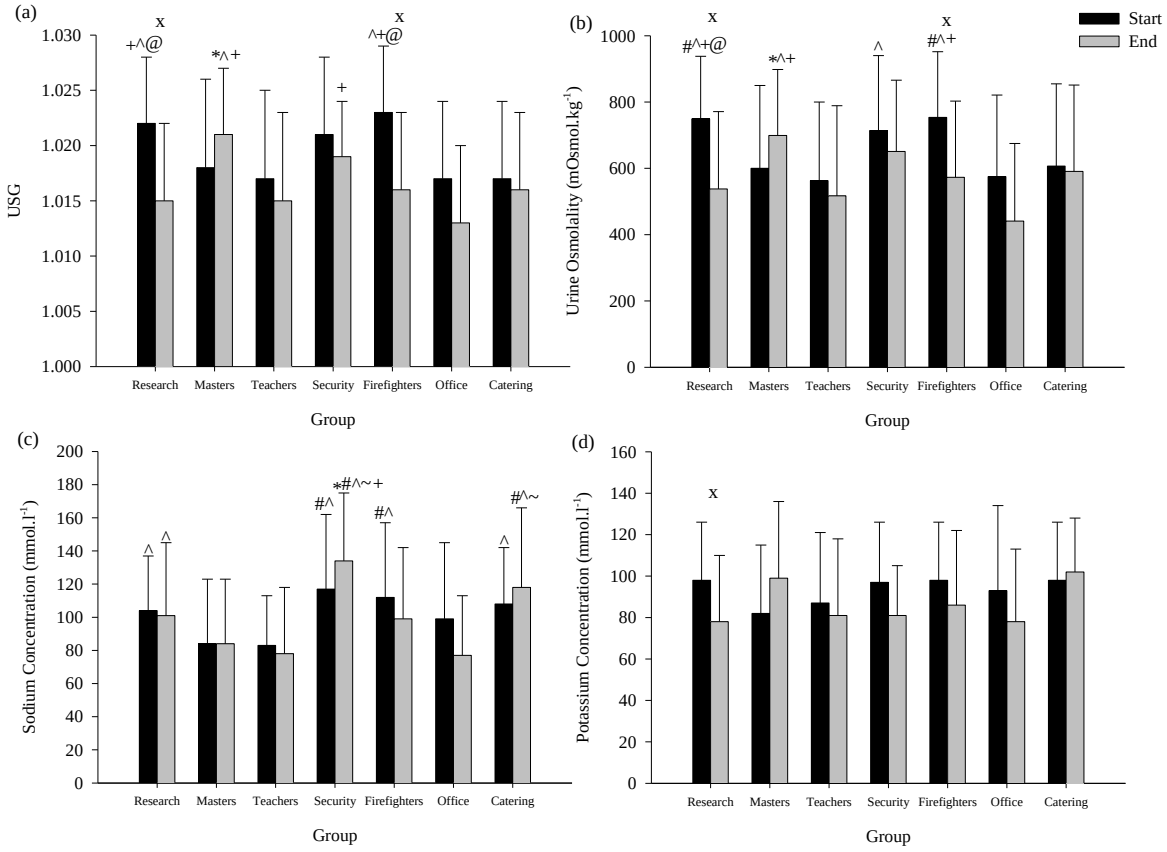

Figure 3.2. USG (a), osmolality (mOsmol.kg^{-1}) (b), urine sodium (c) and potassium concentrations (mmol.l^{-1}) (d) at the start and end of the shift (mean ± SD). * greater than research group, # greater than masters, ^ greater than teachers, ~ greater than firefighters, + greater than office and @ greater than catering ($p<0.05$). x difference between start and end values ($p<0.05$).

Urine colour for males at the end of the shift was lower in the research group (2 (1-6)) compared to the masters group (4 (3-7)), security group (4 (1-6)) and the catering group (5 (3-6)) ($p<0.05$). Masters students had greater values of urine colour compared to teachers (2 (1-5)) but lower values than catering staff ($p<0.05$).

Urine sodium concentrations at the end of the shift for males were higher in the security group (145 ± 39 mmol.l^{-1}) compared to the researchers (105 ± 43 mmol.l^{-1}), masters students (93 ± 45 mmol.l^{-1}), teachers (91 ± 47 mmol.l^{-1}), firefighters (99 ± 43 mmol.l^{-1}) and office staff (83 ± 41 mmol.l^{-1}) ($p<0.05$). Catering staff (151 ± 30 mmol.l^{-1}) had greater sodium concentrations at the end of the shift compared to masters, teachers and firefighters ($p<0.05$).

Females in the masters group had higher end of shift concentrations for urine potassium concentrations (110 ± 33 mmol.l^{-1}) compared to researchers (73 ± 34 mmol.l^{-1}), teachers (79 ± 40 mmol.l^{-1}) and security guards (58 ± 17 mmol.l^{-1}) ($p<0.05$). Catering staff females had higher urine potassium concentrations at the end of the shift (100 ± 24 mmol.l^{-1}) compared to the researchers and security guards ($p<0.05$).

Within groups

In the research group a reduction from the start to the end of shift values for USG, osmolality and potassium concentrations occurred for the whole group and within males and females ($p<0.05$). Urine colour was less at the end of the shift in the whole research group and for male researchers (both 4 (1-6) v 2 (1-6)) whilst comparing the research group as a whole revealed a reduction in energy levels at the end of the shift (63 ± 16 v 54 ± 21) ($p<0.05$).

Females in the masters group had an increase in potassium concentrations at the end of the shift (80 ± 47 v 110 ± 33 mmol.l^{-1}) ($p<0.05$). Reported feelings of hunger were greater at the end of the shift for all the masters population (22 ± 19 v 50 ± 26), male masters students (22 ± 21 v 52 ± 26) and female masters students (21 ± 17 v 48 ± 27) ($p<0.05$).

All reported subjective feelings in the teacher group were different between the start and end of the shift ($p<0.05$). Thirst (37 ± 24 v 56 ± 26), mouth dryness (39 ± 25 v 56 ± 27), tiredness (51 ± 23 v 69 ± 22) and hunger (15 ± 21 v 32 ± 23) were significantly higher at the end of the shift ($p<0.05$). Concentration (69 ± 22 v 51 ± 23) and energy (63 ± 20 v 50 ± 20) levels declined throughout the shift ($p<0.05$). In male teachers mouth dryness (30 ± 24 v 47 ± 28) and hunger (9 (0-49) v 35 (5-65)) increased whilst concentration (82 (13-98) v 50 (10-80)) decreased throughout the shift ($p<0.05$). In female teachers thirst (23 (5-100) v 59 (13-100)), mouth dryness (47 (1-82) v 74 (11-93)) and tiredness (44 ± 25 v 69 ± 19) increased throughout the shift whilst concentration significantly decreased (66 ± 21 v 51 ± 22) ($p<0.05$).

In all security guards, concentration levels decreased throughout the shift (63 ± 20 v 50 ± 20) ($p<0.05$). Urine specific gravity (1.023 ± 0.006 v 1.016 ± 0.007) and urine osmolality (754 ± 198 v 573 ± 230 mOsmol.kg^{-1}) were lower at the end of the shift in

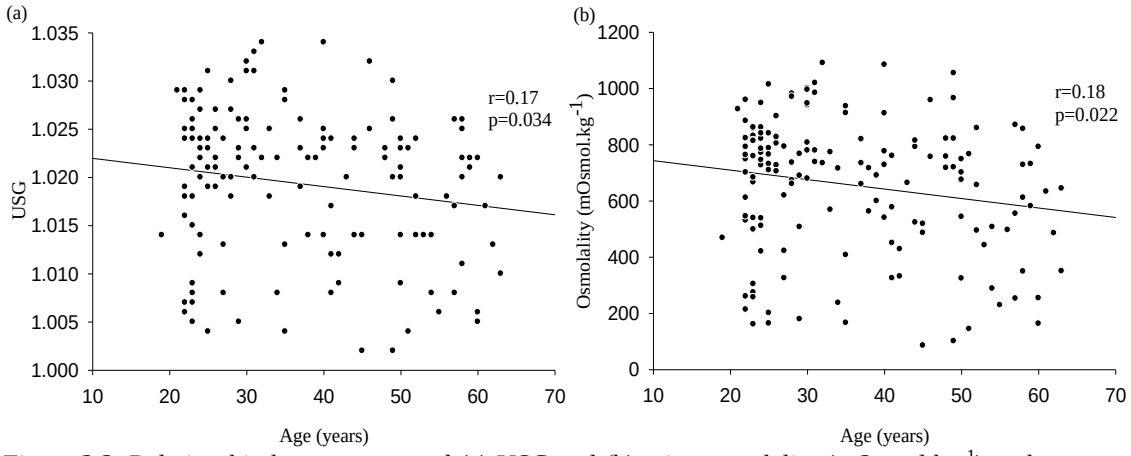

Figure 3.3. Relationship between age and (a) USG and (b) urine osmolality (mOsmol.kg^{-1}) at the start of the shift. Regression line and r and p values displayed.

the firefighters group ($p<0.05$). Concentration levels in all office workers (70 ± 18 v 49 ± 20) and in only male office workers (73 ± 18 v 46 ± 21) were lower at the end of the shift ($p<0.05$). Catering staff reported greater levels of tiredness at the end of the shift (29 ± 23 v 45 ± 25). Male catering staff experienced greater feelings of thirst (63 ± 20 v 50 ± 20) and mouth dryness (58 ± 8 v 19 ± 9) at the start of the shift ($p<0.05$).

3.4.5 Age

A significant negative relationship was found between age and start values of both osmolality and specific gravity (Figure 3.3) ($p<0.05$): older individuals had lower starting values, however there was no relationship between end values of urine osmolality and specific gravity and age. There was no relationship between age and change in osmolality ($r=0.108$, $p=0.179$). Age was negatively related to start values for feelings of thirst ($r=0.326$, $p<0.0001$), mouth dryness ($r=0.268$, $p=0.001$), tiredness ($r=0.228$, $p=0.004$) and hunger ($r=0.227$, $p=0.004$) and positively related to concentration ($r=0.221$, $p=0.005$) and energy ($r=0.217$, $p=0.007$). Age was negatively related to post values of hunger ($r=0.190$, $p=0.018$). No relationship was found between age and start values of urine colour, sodium, potassium and end values of urine colour, sodium concentrations, potassium concentrations, feelings of thirst, mouth dryness, tiredness, concentration and energy (all $p>0.05$). Age was not significantly related to reported water intake ($p>0.05$).

3.4.6 Reported water intake

Males reported more water consumption compared with females during the monitored shifts ($p<0.0001$). Males reported consuming 1.2 l (0.0-3.3 l) compared with 0.7 l (0.0-2.0 l) for females. This was equivalent to 14 (0-47) $ml.kg^{-1}$ and 10 (0-32) $ml.kg^{-1}$ for males and females respectively ($p=0.004$). Reported water intake values for the population and for males and females separately between groups are presented in Figure 3.4. Within each group there was no difference between the reported water consumed by males and females ($p>0.05$). Reported water intake was weakly related to feelings of thirst at the start of the shift (positively) ($r=0.161$, $p=0.044$) but not at the end of the shift. At the end of the shift USG values were negatively related to reported water intake for the whole population ($r=0.226$, $p=0.005$), males ($r=0.356$, $p=0.001$) and females ($r=0.253$, $p=0.039$). A similar pattern occurred for osmolality values (whole population ($r=0.230$, $p=0.004$), males ($r=0.349$, $p=0.001$) and females ($r=0.272$, $p=0.026$)). USG and osmolality values at the start of the shift were not related to reported water intake values.

3.4.7 Sensations of thirst and concentration levels

117 workers experienced a sensation of thirst at some point throughout the duration of the shift. 85% (n=99) alleviated thirst by consuming a drink. The average amount of water reported that was used to satiate sensations of thirst was 0.2 (0.05-1.4) l. 92% of males who experienced thirst alleviated the sensation by consuming water compared with 75% of females. Concentration levels at the start and end of the shift were not related to the corresponding values for osmolality and specific gravity ($p>0.05$). 65% (n=101) of the workers felt that they kept themselves hydrated throughout the duration of the shift. Of those that thought they were hydrated at the end of the shift 70% (n=71) and 68% (n=69) had urine osmolality and urine specific gravity values respectively that were below 700 $mOsmol.kg^{-1}$ and 1.020, whilst of those that did not feel like they kept themselves hydrated 41% (n=22) and 43% (n=23) had osmolality and USG values respectively, not classed as euhydrated.

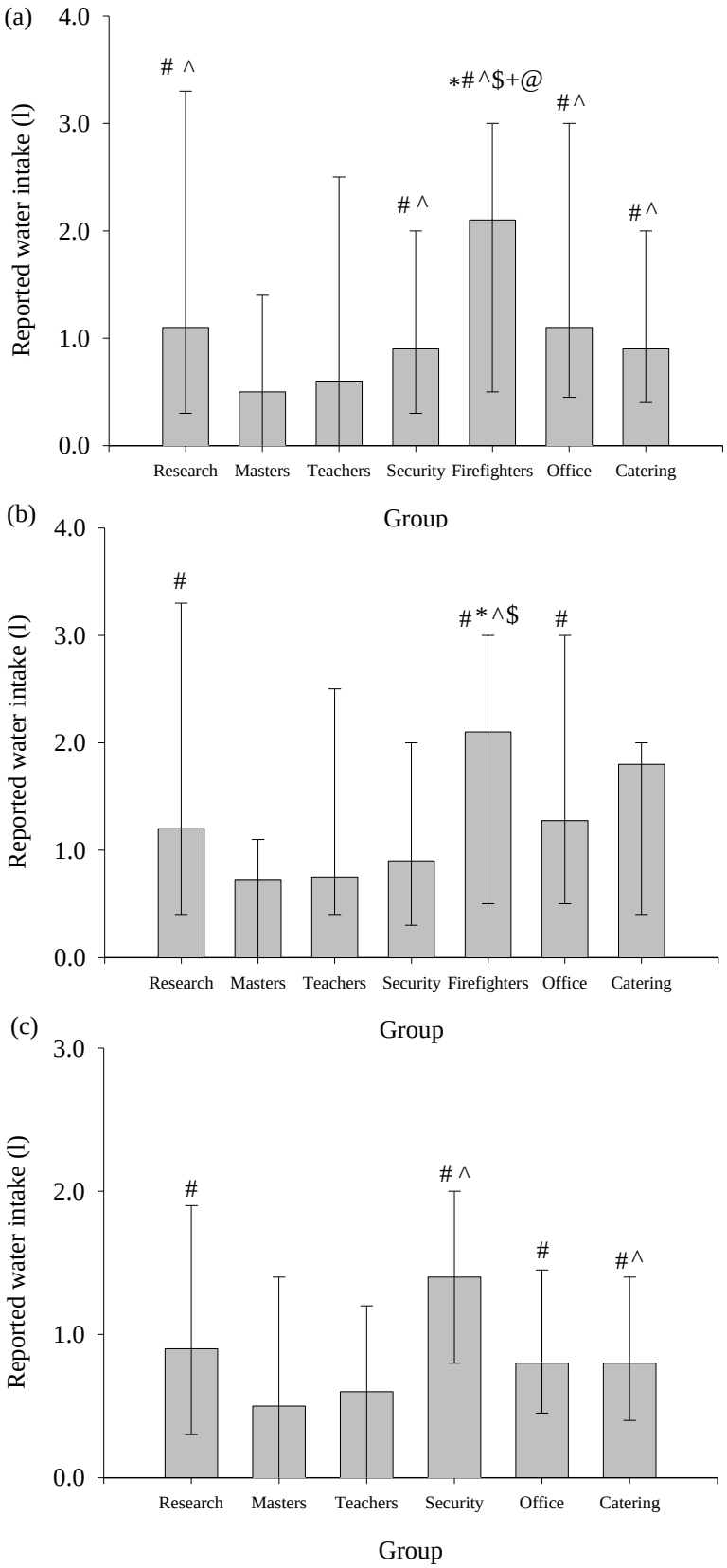

Figure 3.4. Reported water intakes for each group in (a) all subjects, (b) males and (c) females during the shift (median (range)). * greater than research group, # greater than masters, ^ greater than teachers, $ greater than security, + greater than office and @ greater than catering ($p<0.05$).

3.5 Discussion

The purpose of the study was to examine hydration status in different working groups at the start and end of a shift and examine water intake during the shift. Overall there was very little difference in the hydration status parameters and reported water intake values between the groups observed. Reported water intakes between groups were very similar with slight differences between males and females, with males consuming more water.

Individuals in the masters group reported that the observed shift was not typical of a normal day. This was because they were in laboratory classes where drinking was prohibited unless they left the laboratory. Although not typical of a normal day but typical of one out of five working days, the group was chosen based on the laboratory classes to allow for a comparison to similar subjects in the University research/studying environment.

In general, subjects had higher values of urine specific gravity and osmolality at the start of the shift. A large proportion of subjects (54% at the start and 35% at the end) exhibited urine values indicating hypohydration with many (52%) remaining in a state of hypohydration at the end of the shift. Data used as markers of hydration status (USG and urine osmolality) were lower at the end the shift; however, from a physiological perspective it was difficult to determine if the difference in hydration values corresponded to a change in hydration status. Based on the ACSM guidelines outlined by Sawka *et al.* (2007) urine osmolality values less than 700 mOsmol.kg^{-1} and urine specific gravity values less than 1.020 are classed as euhydrated. A question that arose from these results was whether subjects with slightly lower values in this range could be categorised into different levels of hydration status (Armstrong *et al.*, 2010). However, the study by Armstrong *et al.* (2010) applied to active, healthy, free-living men, not exercising in a hot environment or performing strenuous training and therefore, more research into the application of these categories to the general population is required before conclusions regarding changes in hydration status can be made. When categorising hydration status, Armstrong and colleagues produced euhydration values for morning samples (818-924 mOsmol.kg^{-1} and 1.024-1.026) and for 24 hr urine samples (587-766 mOsmol.kg^{-1} and 1.018-1.020). In the current study, urine samples were more indicative of 24 hr samples as the sample at the start of the shift may not

always have been the first void of the day, in some cases may not have been in the morning and at the end of the shift, urine sample were at least the duration of the shift ($\geq$8 hours) from waking.

Female subjects started and ended their shift with lower values of USG and urine osmolality compared with males. Males had lower values of USG and urine osmolality at the end of the shift ($p<0.05$), whilst females exhibited similar values ($p>0.05$). Females have been shown to consume less water (Kant *et al.*, 2009; Mueller *et al.*, 2005; Raman *et al.*, 2004), confirming reported water intake values reported in this study. Kant *et al.* (2009) examined 4112 individuals in North America and found no difference in plain water intake between males and females (1044 ± 48 v 1079 ± 67 g for males and females respectively; $p=0.5$) but females consumed significantly less water from other beverages (1783 ± 55 v 1298 ± 35 g for males and females, respectively; $p<0.0001$). All three studies examined water intake over 24 hours so direct comparisons may not be used but the general trends were similar. The lower reported water intake in the present study may be attributed to the lower values of USG and urine osmolality at the start of the shift. If males and females had both begun the shift in a similar state of hydration, reported water intake values in females may have been greater.

As age increased, USG and urine osmolality values at the start of the shift decreased, however there was no relationship between age and the end values of urine parameters. Concurrent with this finding, start sensations of thirst were lower with increasing age. It has been shown that the thirst response is blunted in the elderly (age 64-76 years) compared to a young group (age 20-32 years) (Phillips *et al.*, 1993) but changes in thirst sensations as age increases have not been reported in the literature. From the study, it would appear that sensations of thirst decreased with age. In the current study the decreases in USG and urine osmolality values with age and the accompanying decreases in sensations of thirst as age increased make it difficult to determine if hydration status was responsible for reduced thirst levels or if there was a blunted thirst response and hydration levels were coincidental.

The firefighting group reported greater water intake during the observed shift compared with all the other groups ($p<0.05$). The firefighters are generally encouraged to drink

during the shift by management and through urine colour charts in the toilets and have been previously made aware of the necessity to drink and maintain hydration status to prevent declines in cognitive and physical performance through initiatives and regular health testing. Brake & Bates (2003) found that educating workers about dehydration, assessing hydration state and using a water replacement program increased the ability to maintain euhydration. The structure of their general day was dependent on emergency calls (average of three per day) and so it appeared that they would drink in anticipation of this and the possibility of wearing personal protective equipment which can often cause heat dress due to the uncompensable environment they create (Cheung & McLellan, 1998). In contrast, the masters group reported drinking very little water. During the laboratory classes they were restricted on where they could drink and thus was reflected in the reported volume consumed and the subsequent urine parameters.

Typical water intake values that have been reported in the general population from beverages are approximately 1.3 $l.d^{-1}$ from The National Diet and Food Survey (Ruston *et al.*, 2004). This value was an average per day over a seven day observation period and included alcohol consumption (approximately 0.3 $l.d^{-1}$). In the present study, water intake was only reported during the working day and so it was difficult to make direct comparison. In 2010, the European Food Safety Authority outlined an adequate intake of 2.5 $l.d^{-1}$ for males and 2.0 $l.d^{-1}$ for females from also sources of water including food (EFSA, 2010) whilst the Institute of Medicine had an adequate intake of approximately 3.7 l of water per day for males from food and beverages and 2.7 $l.d^{-1}$ for females (http://www.iom.edu/Reports/2004/Dietary-Reference-Intakes-Water-Potassium-Sodium-Chloride-and-Sulfate.aspx). The Food Standards Agency suggested a value of 1.2 l from beverages to prevent dehydration occurring. The recommendations vary in suggested water intake, but if the lowest value is taken, only five groups reported intake close to, or above this value in the monitored shift alone whilst the remaining two groups (masters and teachers) had the greatest barriers to water intake due to availability of water, restrictions on when and where they could drink and access to toilet facilities. Again, it must be stressed that the adequate water intakes for a day cannot be compared to the water intake during the shift as subjects were only at work for a relatively small portion of the day, and were likely to not include 1 or 2 main meals, where large amounts of water, through food and accompanying drinks, would probably be consumed.

Several subjects reported water intake over 2 litres per day with one subject in the research group and two in the firefighters group reporting a water intake value of 3.3, 3.0 and 3.0 litres respectively throughout the shift. The firefighters group appeared most at risk from overdrinking with 14 out of 22 subjects reporting water intake over 2 litres during the shift. Despite this, urine osmolality values were, on average, above 700 $mOsmol.kg^{-1}$ at the start of the shift and this decreased slightly at the end of the shift, therefore suggesting that their reported water intake was adequate or that the actual volumes reported were inaccurate. When asking individuals to self report food and drink intake, often errors can occur particularly with underreporting of energy intake (Mertz *et al.*, 1991).

Of the workers monitored, 117 (75%) experienced a sensation of thirst at some point during the duration of the shift with 85% alleviating the sensation through a drink. During day to day activity thirst is an adequate stimulus to promote water replacement and maintain hydration status (Greenleaf, 1992). The results of this study would therefore agree, with sensations of thirst being very similar at the start and end of the shift.

The frequency of testing could have potentially been increased. A start and end sample provided information regarding these time points but little information regarding hydration status was gathered throughout the duration of the shifts. It may not be appropriate to assume that end values of urine parameters arose directly from start values as euhydration has been shown to follow a sinosuidal wave and fluctuate around an average value over a period of time (Greenleaf, 1992). Therefore, to determine the pattern throughout the shift it would have been advantageous to increase the frequency of sampling to a fixed number or a collection of all samples produced. The major problem with this would have been the interference with the "typical" day of the subject creating a deviation from normality and thus potentially affecting urine output and normal water intake patterns. A solution to improve this would be to test over a number of days with greater frequency of sampling, thereby allowing the subject to adjust to the method of testing.

Testing was conducted in the winter months with relatively low to moderate ambient temperatures. *Ad libitum* water intake has been shown to be less in cool temperatures

compared to warmer conditions (Cheuvront & Haymes, 2001), whilst hypohydration is more prevalent in warmer conditions predominantly due to increased water loss through sweating (Galloway & Maughan, 1997). Examining groups during shifts in different climatic periods would have provided an interesting comparison and may have amplified the differences within and between groups due to greater water loss and resulting water intake.

3.6 Conclusion

In conclusion a large proportion of subjects exhibited urine values indicating hypohydration with many remaining in a state of hypohydration at the end of the shift. A large proportion of workers (75%) experienced a sensation of thirst throughout the shift. Access to water and other beverages at work helped alleviate sensations of thirst. Increasing awareness of drinking and hydration status, helped increase water consumption during the observed shift, whilst males reported consuming more water per kg of body mass compared to females. Further investigation is required to gain insight into the causes and significance of these findings through blood indices and hormone analysis.

CHAPTER 4

A comparison of venous and capillary blood samples to track changes in hydration status and to measure vasopressin concentration

4.1 Abstract

Changes in hydration status can be tracked through blood indices following venous blood collection. Capillary blood sampling provides a less invasive method of blood collection than venous sampling. The aim of the study was to assess the use of capillary sampling to track changes in hydration status, measure vasopressin concentration and to assess the impact of hand-warming on blood parameters. Eight healthy males (age 23 ± 5 years, mass 72.7 ± 9.2 kg, height 1.79 ± 0.06 m) completed two trials (euhydrated–EU; hypohydrated–HY) following familiarisation to the sampling procedure. In each trial, four samples were collected: evening baseline, the next morning following controlled water intake, post-exercise (60 min cycling at 112 ± 15 W in 18.0 ± 1.0°C (EU) or 32.8 ± 1.0°C (HY) and 60.1 ± 1.3% RH) and following 60 min recovery during which 1 litre (2 x 0.5 l) of a 6% CHO electrolyte drink was consumed. At each sample time body mass was measured and 5.5 ml of venous and 0.6 ml of capillary blood was collected from each arm and hand respectively following 15 min immersion of one hand in warm water (40-43°C). Blood samples were analysed for plasma osmolality (pOsm), haematocrit (Hct), haemoglobin concentration (Hb) and vasopressin concentration. Plasma volume change from baseline was calculated. Greater body mass loss occurred in HY in the morning (0.93 ± 0.34 v 0.32 ± 0.45%), post-exercise (2.15 ± 0.36 v 1.06 ± 0.43%) and after recovery (1.09 ± 0.49 v 0.29 ± 0.44%) ($p<0.05$). Differences between sampling technique were found at baseline and morning samples for Hb, all samples for Hct, morning, post-exercise and recovery samples for pOsm and morning samples for vasopressin concentration ($p<0.05$). Tracked changes between sampling points were similar between sampling techniques for Hb, pOsm and vasopressin concentrations except for pOsm from post-exercise to recovery. Hct did not track similarly between sampling techniques ($p<0.05$). In general warming did not affect Hb, Hct, pOsm, vasopressin concentrations and plasma volume changes. The use of capillary samples, due to the difficulty in obtaining a free flowing sample, to track changes in markers of hydration status varied. Vasopressin concentration results can be inconsistent between techniques, therefore further research is required.

4.2 Introduction

Hydration status can be tracked and assessed using a variety of methods (Shirreffs *et al.*, 2003). In some situations, particularly in a field environment, it is often quicker and easier to assess hydration status through urinary indices, however the use of urinary compared to blood markers is mixed (Francesconi *et al.*, 1987; Popowoski *et al.*, 2001). Ideally, a blood sample would be collected in addition to the urine assessment, but this may not always be suitable to the situation. In order to allow adequate analysis of plasma/serum osmolality, plasma volume changes and hormonal concentrations to track changes in hydration status, a relatively large sample (~5-10 ml) needs to be collected. This normally requires a cannulation or venepuncture blood sample, usually taken from a forearm or antecubital vein. A possible alternative to venous blood sampling would be a capillary blood sample from the fingertip. This less invasive method can be conducted in a wider range of environments. However, the use of capillary sampling as a comparable method is limited and thus difficult to examine the potential use. In terms of tracking changes in hydration status using capillary samples, dynamic changes have not been assessed. In addition measures between capillary and venous blood samples for vasopressin concentration have not been examined. Previous research involving hormone analysis at capillary and venous sample sites has focussed on human growth hormone (Godfrey *et al.,* 2004). In that study, capillary samples immediately followed venous blood samples during an intermittent bout of cycling. The aim for the authors was to allow accurate sampling in a less invasive environment whilst still monitoring hormonal changes. They suggested that the invasive nature of venous sampling prevented analysis in certain groups including children, pregnant women and elite athletes. A correlation coefficient of $r=0.986$ ($p<0.01$) between capillary and venous blood sampling methods for human growth hormone was found, however it was noted that sample sites should not be used interchangeably.

Typically sensations of thirst are measured through subjective feeling questionnaires (SFQ) (Engell *et al.,* 1987), or inferred from hormonal analysis, specifically vasopressin, and plasma/serum osmolality or a combination of the two techniques. Vasopressin analysis has been undertaken in a variety of studies including running in the heat (Melin *et al.,* 1997; Montain *et al.,* 1997), following water intake after dehydration (Phillips *et al.,* 1993) and exposure to, and exercise in the cold, whilst SFQ

have been shown to correlate well with thirst based on graded increases in hypohydration (Engell *et al.*, 1987).

In laboratory settings, to improve ease of blood sampling, the hand and/or forearm are often immersed in warm water to increase blood flow (Galloway & Maughan, 1997: Wood *et al.*, 2009) and arterialise the venous blood to create a representation of arterial blood, which is often unnecessarily invasive. In the field setting warming the hand/arm is not always possible due to access to warm water and the logistics of using venous blood sampling away from the laboratory. Although vasopressin concentration has not been examined following hand warming, blood gas measurements have (Linderman *et al.*, 1990). Linderman and colleagues found similar values of partial pressure of oxygen (pO_2) in arterialised blood following warming of the hand for 10 minutes to 37°C using a heating pad and to 42°C using a hydrocolator pack. Examination of haemoglobin concentration, haematocrit, plasma and serum osmolality and vasopressin concentrations are required in order to quantify the effect hand warming has on measured variables and whether this may then impact on assessment of hydration status.

Therefore there were three main aims to the study. The first aim was to assess the difference between capillary and venous blood samples to track changes in hydration status. The second aim was to assess the comparability of venous and capillary blood samples for analysis of vasopressin concentration. In addition a further aim was to assess the impact of hand warming on blood parameters.

4.3 Methods

4.3.1 Subjects

Eight healthy male subjects were recruited (age 23 ± 5 years, mass 72.7 ± 9.2 kg, height 1.79 ± 0.06 m) to take part in two trials, undertaken in a randomised order. All subjects had the experimental protocol explained to them verbally and in writing. Subjects provided written consent and the experiment was approved by the Loughborough University Ethics Committee (R09-P74).

4.3.2 Experimental protocol

Subjects were required to attend the laboratory for one familiarisation session and two experimental trials; euhydrated (EU) and hypohydrated (HY). During the familiarisation session they provided a urine sample and had nude body mass measured. They also had a venous (~5 ml venepuncture) and capillary sample taken and completed the subjective feelings questionnaire.

Pre-trial standardisation

Each trial started in an evening between 1500 and 1700 h and each subject arrived at the lab at the same time for each trial, 4h after their last meal and 2 h following the ingestion of 500 ml of water. Food intake portion size was recorded in the 24 h before the first trial and repeated for the second trial. Subjects were asked to refrain from strenuous exercise and alcohol consumption during this 24 h period. All subjects had been fully familiarised with blood sampling, urine collection procedures and body mass measurements prior to the first trial.

On arrival at the laboratory subjects voided their bladder and had nude body mass measured. Total urine volume was recorded and a 5 ml sample was retained for analysis. Subjects were seated and rested for 15 minutes with one hand immersed in warm water (40-43°C). Whilst resting, subjects completed a 100 mm visual analogue subjective feelings questionnaire comprising of 12 questions relating to thirst, stomach fullness, pleasant mouth taste, bloatedness, hunger, tiredness, dry mouth, scratchy throat, alertness, sore head, concentration and energy (Appendix D). A baseline blood sample (5.5 ml) was collected without stasis from an antecubital vein in the warmed

arm (WA), before a capillary sample was taken from the finger (Accu-Chek Safe-T-Pro Plus, Roche Diagnostics Ltd: UK), into two 0.3 ml Microvette CB 300 tubes (Sarstedt Ltd, Leicester, UK). The procedure of venepuncture and fingerprick blood sample was repeated in the non-warmed arm (NA). Subjects were allowed to return home to consume an evening meal, which they replicated for the second trial. They received either 500 ml (EU) or 100 ml (HY) of water to consume during the evening. Subjects were instructed to eat only the evening meal and refrain from eating foods containing a large amount of water (e.g. soup and fruit and vegetables containing a large amount of water).

The following morning subjects performing the EU trial consumed 500 ml of water 2 h before arriving at the laboratory. During the HY trial subjects consumed no water before arriving at the laboratory. On arrival at the laboratory, 15 h following the evening sample, subjects voided their bladder, had nude body mass measured and then rested for 15 minutes with one forearm immersed in warm water. During the period of rest, 21g cannulas (Surflo, Terumo, Leuven, Belgium) were inserted into a superficial vein on each forearm to allow venous blood sampling. The line was flushed with 2-3 ml of heparinised saline. A venous and fingerprick capillary sample was taken following the same order of blood sampling as in the previous evening.

Following sampling, subjects cycled for 60 minutes at a workrate of 1.5 $W.kg^{-1}$ (112 ± 15 W) in the environmental heat chamber. Conditions were 18.0 ± 1.0°C during the EU trial and 32.8 ± 1.0°C during the HY trial with relative humidity in both trials of 59.4 ± 1.2% and 60.8 ± 1.0% respectively. Subjects then voided, had nude body mass measured and the series of blood samples were repeated. Subjects then had 30 minutes to consume two 500 ml volumes of a commercially available 6% carbohydrate-electrolyte sports drink (CHO-E) (Gatorade, Chicago, IL, USA), allowing 15 minutes for consumption of each beverage. Subjects rested for 30 minutes before having a final series of blood, urine and nude body mass taken. The subjective feelings questionnaire was administered each time the forearm was immersed in warm water. After completion of the final sample, subjects were allowed to leave the laboratory. For each blood sample, skin temperature was measured at the sampling site in duplicate (Thermometer LS; Micro-Epsilon, Ortenburg, Germany). Ambient temperature and

relative humidity was measured during each sample (RH85 Digital Thermo-Hygrometer; Omega, Manchester, UK).

Experimental trial order was performed by incomplete Latin square design and subjects did not know which trial they were participating in when arriving at the laboratory for the first experimental trial. Each trial was separated by a period of seven days and performed on the same day of the week at the same time of day.

4.3.3 Sample analysis

Venous blood was analysed for haemoglobin concentration, haematocrit, blood glucose concentration, serum sodium, potassium and chloride concentrations, vasopressin concentration, plasma volume, blood volume and red blood cell volume changes and plasma and serum osmolality, whilst capillary blood was analysed for haemoglobin concentration, haematocrit, blood glucose concentration, vasopressin concentration, plasma osmolality and plasma volume, blood volume and red blood cell volume changes (see Chapter 2 for details). A 0.5 ml sample was mixed with heparin and analysed for *p*H, pCO_2, pO_2 and oxygen saturation using a blood-gas analyser (Radiometer, UK).

The total volume of each urine sample was measured and a 5 ml sample was retained and later analysed for osmolality, sodium, potassium and chloride concentration, specific gravity (see Chapter 2 for details) and *p*H (Corning pH Meter 140; Corning Ltd., Halstead, Essex, UK).

4.3.4 Statistical Analysis

Data were checked for normality of distribution using Kolmogorov-Smirnov ($n>30$) and Shapiro-Wilks tests ($n\leq30$). When normally distributed paired sample t-tests or repeated measures ANOVA were performed and when a significant ANOVA was found paired sample t-tests with Bonferroni correction were performed to identify where statistical differences occurred. Non-parametric data were examined using Friedman's ANOVA and Wilcoxon signed-rank tests. Post-hoc tests were carried out on significant and non-significant interaction effects. Linear regression values, Pearson's

product moment correlation coefficients and Spearmon's ranked correlation coefficients were calculated when appropriate. Bland-Altman plots were conducted when mean difference was required (plasma osmolality, haemoglobin concentration, haematocrit, blood glucose, plasma volume change and vasopressin concentration). Statistical significance was accepted when $p<0.05$. When post-hoc tests were conducted, p values presented were multiplied to correct for repeated samples. Parametric data is expressed as mean ± SD and non parametric data expressed as median (range).

4.4 Results

4.4.1 Baseline values

Baseline serum osmolality was not different between trials (284 ± 4 v 284 ± 4 mOsmol.kg^{-1}; p=0.964, for trials EU and HY respectively). Hydration status assessed by urine specific gravity was not different between trials (1.015 ± 0.006 v 1.014 ± 0.008; EU v HY) (p=0.651). Similar baseline values were observed between EU and HY trials for body mass (72.74 ± 9.24 v 72.64 ± 9.22 kg; p=0.584), serum sodium concentration (142 ± 1 v 142 ± 1 mmol.l^{-1}; p=0.843), serum potassium concentration (4.3 ± 0.3 v 4.2 ± 0.2 mmol.l^{-1}; p=0.337), serum chloride concentration (103 ± 2 v 103 ± 2 mmol.l^{-1}; p=0.105), urine osmolality (493 ± 213 v 513 ± 289 mOsmol.kg^{-1}; p=0.794), haemoglobin concentration (161 ± 5 v 161 ± 6 g.l^{-1}; p=0.728) and haematocrit (45.7 ± 2.1 v 45.7 ± 1.7%; p=0.776). Values measured would indicate subjects arrived in a state of euhydration for baseline measurements (Sawka *et al.*, 2007).

4.4.2 Body mass

Body mass decreased to a maximum loss of 1.06 ± 0.43% and 2.15 ± 0.36% in the EU and HY trials respectively (Figure 4.1). Both maximum losses occurred following water restriction and/or exercise. Following recovery, body mass returned to near baseline value (72.74 ± 9.24 v 72.51 ± 9.10 kg; p=0.072) in the EU trial but not in the HY trial (72.64 ± 9.22 v 71.82 ± 8.90 kg; p<0.0001). There was a difference between trials in the morning (72.49 ± 9.07 v 71.96 ± 9.06 kg for EU and HY trials respectively), post-exercise (71.95 ± 9.01 v 71.07 ± 8.92 kg for EU and HY trials respectively) and following recovery (72.51 ± 9.10 v 71.82 ± 8.90 kg for EU and HY trials respectively) (p<0.05). In the EU and HY trials, body mass following exercise was lower compared to baseline, morning and recovery values (p<0.0001).

4.4.3 Tracking changes

Changes in vasopressin concentrations from previous samples were similar between venous and capillary samples. Changes from baseline to morning were 0.3 ± 1.9 v 0.8 ± 1.4% (p=0.440), from morning to post-exercise were 0.5 ± 1.2 v 1.0 ± 2.6%

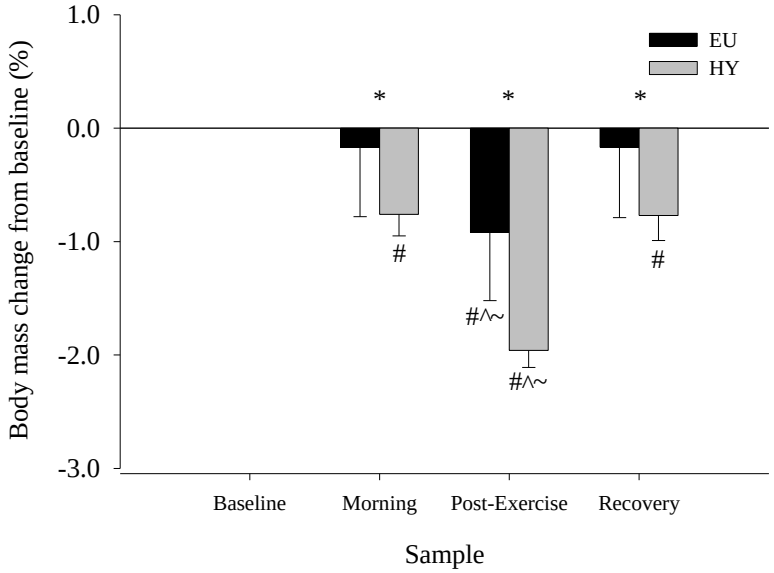

Figure 4.1. Body mass change from baseline over the duration of the trials (%) (mean ± SD). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning and ~ denotes different from recovery ($p<0.05$).

($p=0.472$) and from post-exercise to after recovery were -0.1 ± 1.6 v -1.3 ± 2.7% ($p=0.134$) for venous and capillary samples respectively. A change in plasma osmolality values from post-exercise to after recovery was found between venous (-1.5 ± 3.3%) and capillary values (-3.5 ± 5.7%) ($p=0.043$). No difference was found in changes between baseline and morning samples (2.1 ± 5.9 v 2.1 ± 6.2% ($p=0.982$)) and morning to post-exercise samples (1.6 ± 3.3 v 1.7 ± 4.2% ($p=0.887$)) for venous and capillary samples respectively. Haemoglobin concentration changes were similar between venous and capillary samples (baseline to morning: 0.8 ± 3.2 v 3.4 ± 7.4% ($p=0.064$); morning to post-exercise: 1.7 ± 3.8 v 2.4 ± 6.1% ($p=0.543$); post-exercise to after recovery: -4.4 ± 3.8 v -3.1 ± 4.1% ($p=0.219$) for venous and capillary samples respectively). Haematocrit changes were different between each sample point ($p<0.05$). Changes from baseline to morning were -0.1 ± 0.6 v 0.9 ± 1.0% ($p<0.0001$), from morning to post-exercise were 0.3 ± 0.5 v 0.0 ± 0.7% ($p=0.045$) and from post-exercise to after recovery were -0.8 ± 0.6 v -0.4 ± 0.7% ($p=0.014$) for venous and capillary samples respectively.

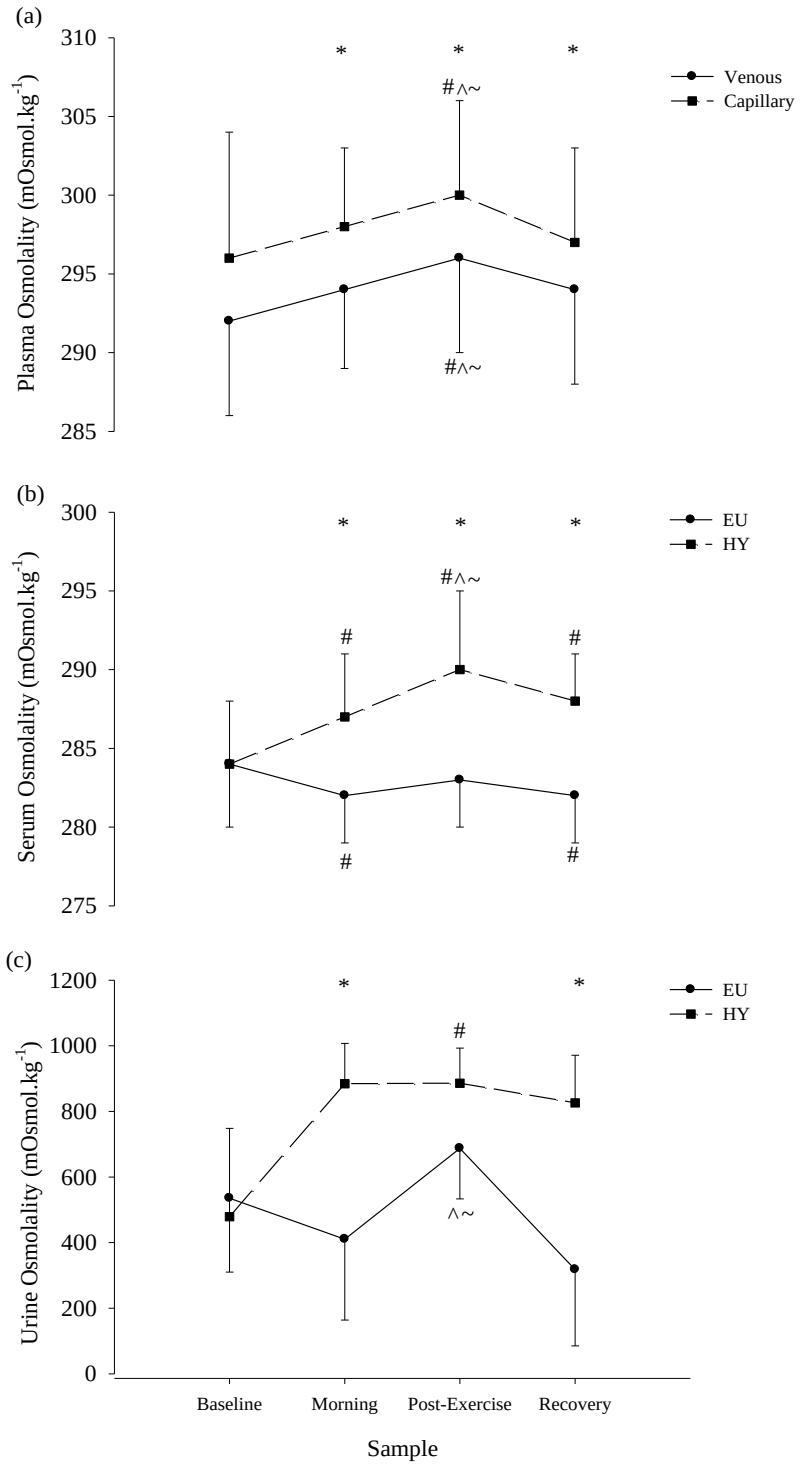

Figure 4.2. Plasma (a), serum (b) and urine (c) osmolality over the duration of each trial. Plasma osmolality is compared between sampling technique. Serum and urine osmolality are compared between trials (mean ± SD). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning and ~ denotes different from recovery ($p<0.05$).

4.4.4 Plasma, serum and urine osmolality

Plasma osmolality comparisons between venous and capillary samples are presented in Figure 4.2a. Higher plasma osmolality was found in capillary samples in the morning,

post-exercise and after recovery ($p<0.05$). No difference was observed between warmed and non-warmed samples ($p>0.05$).

Higher serum osmolality was found in the EU trial at each sample point except baseline ($p<0.05$; Figure 4.2b). Serum osmolality did not return to baseline values following recovery in either trial. Warming a hand resulted in a difference between post exercise values in the EU trial only (282 ± 2 v 284 ± 3 $mmol.l^{-1}$ for warmed and non-warmed respectively; $p=0.027$).

Urine osmolality was higher in the HY trial in the morning ($p=0.002$) and following recovery ($p=0.009$) (Figure 4.2c). In the EU trial urine osmolality values were higher post-exercise compared to the morning ($p=0.003$) and following recovery ($p=0.004$).

4.4.5 Blood analysis

A greater vasopressin concentration was found between venous and capillary samples in the morning sample ($p=0.009$; Figure 4.3a) but not in the other samples. However, analysis was successfully completed on only 64 out of a possible 128 samples due to a detection error with a vasopressin enzyme immunoassay plate and therefore venous and capillary comparisons are for four subjects only. In only the capillary samples there was an increase in vasopressin concentration post-exercise compared to the baseline

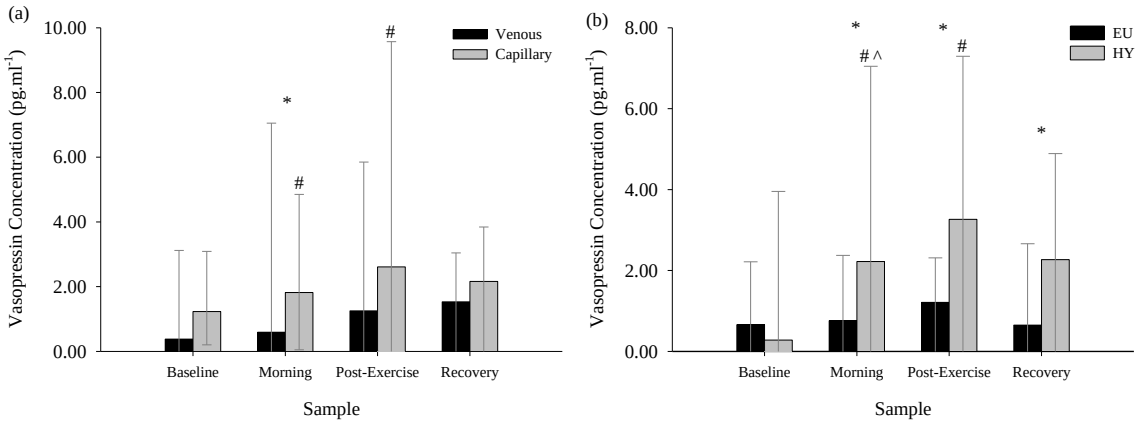

Figure 4.3. Vasopressin concentration over the duration of the trials. Compared between sampling technique (a) and trial (b) (median and range). * denotes different between technique or trial, # denotes different from baseline and ^ denotes different from morning ($p<0.05$).

(p=0.003) and morning samples (p=0.033). In venous samples, hand warming resulted in differences in vasopressin concentration at baseline in the EU trial (0.0 (0.0-1.3) v 1.2 (0.0-2.2) $pg.ml^{-1}$ for warmed and non-warmed respectively; p=0.018) and in the HY trial (0.4 (0.0-1.6) v 1.6 (1.1-2.3) $pg.ml^{-1}$ for warmed and non-warmed respectively; p=0.012) and post-exercise in the EU trial (0.0 (0.0-0.6) v 1.6 (0.0-4.0) $pg.ml^{-1}$ for warmed and non-warmed respectively; p=0.028) (Figure 4.3b). Increased venous concentrations were observed in the HY trial compared to the EU trial in the morning (p=0.017), post-exercise (p=0.002) and following recovery (p=0.05) in venous samples (Figure 4.3b). In the HY trial venous vasopressin concentrations were greater post-exercise compared with baseline (p=0.030) and in the morning (p=0.001).

Haemoglobin concentration was higher in venous samples at all sample points ($p<0.05$; Figure 4.4a). In both venous and capillary samples post-exercise concentrations were higher than all other sample points ($p<0.05$). In venous blood the haemoglobin concentrations for the recovery sample were lower than all other samples ($p<0.05$), whilst in capillary samples baseline concentrations were lower than the morning (p=0.012) and post-exercise concentrations ($p<0.0001$). Warming the hand caused a difference in venous concentrations following recovery in the EU (157 ± 7 v 160 ± 7 $g.l^{-1}$ for warmed and non-warmed respectively; $p<0.0001$) and HY (159 ± 4 v 162 ± 5 $g.l^{-1}$ for warmed and non-warmed respectively; p=0.009) trials and at baseline in the HY trial (161 ± 76 v 163 ± 6 $g.l^{-1}$ for warmed and non-warmed respectively; p=0.028). Capillary concentrations were affected by warming a hand in the HY trial in the morning sample (160 ± 8 v 165 ± 8 $g.l^{-1}$ for warmed and non-warmed respectively; p=0.032).

Haematocrit values were different between venous and capillary samples at all sample points (Figure 4.4b; $p<0.05$). Recovery values for venous samples were lower compared to all other samples, whilst post-exercise values were higher than morning values ($p<0.05$). Baseline haematocrit values were lower compared to the three other sample points ($p<0.05$), whilst following recovery haematocrit was lower than samples taken in the morning (p=0.001) and post-exercise (p=0.007).

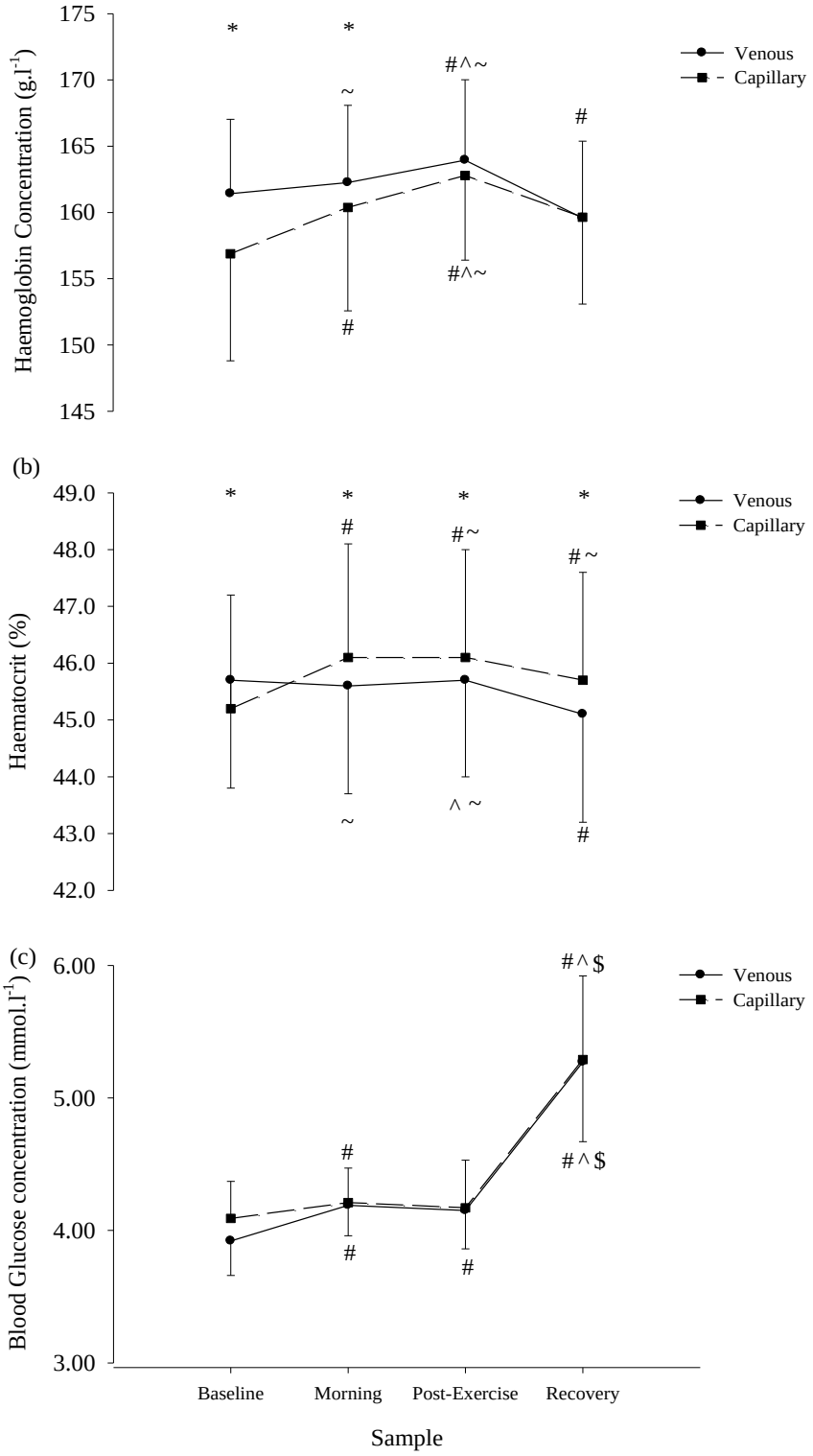

Figure 4.4. Comparison between sampling techniques for (a) haemoglobin concentration ($g.l^{-1}$), (b) haematocrit (%) and (c) blood glucose concentration ($mmol.l^{-1}$) (mean ± SD). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning, $ denotes different from post-exercise and ~ denotes different from recovery ($p<0.05$).

Plasma volume changes from baseline using venous sampling were different following warming in the EU trial after the recovery period (5.1 ± 3.5%) and in the HY trial post-

exercise following warming (-4.1 ± 3.4%) and non-warming (-2.8 ± 2.3%). Plasma volume changes from baseline using capillary sampling were different post-exercise in the EU trial without hand-warming (-3.9 ± 2.4%) and post-exercise in the HY trial following warming (-7.2 ± 4.6%) and without warming (-7.2 ± 6.1). Between sampling techniques differences occurred in the EU trial after the recovery period without warming (1.7 ± 3.3 v -2.0 ± 2.4% for venous v capillary sampling) and in the HY trial after the recovery period with warming (1.2 ± 2.8 v -4.9 ± 5.0% for venous v capillary sampling) ($p<0.05$). All other sampling technique comparisons were similar ($p>0.05$). Blood volume changes from baseline using venous blood sampling were different in the EU trial after recovery and warming (3.0 ± 2.4%), and in the HY trial post-exercise following warming (-3.2 ± 2.0%) and non-warming (-2.2 ± 1.5%). Blood volume changes measured using capillary sampling were similar to baseline except post exercise in the EU trial without warming (-0.8 ± 2.5%) and in the HY trial post-exercise with warming (-5.6 ± 3.5%). Changes compared between venous and capillary sampling were similar ($p>0.05$), whilst there was a difference between trials post-exercise when venous sampling was used following warming (0.3 ± 2.3 v -2.3 ± 4.0% for EU and HY trials respectively) ($p<0.05$). Using venous blood sampling to measure red blood cell volume changes compared to baseline resulted in differences in the HY trial in the morning (-1.0 ± 0.8%) and post-exercise (-2.0 ±1.3%) after warming ($p<0.05$). Using capillary sampling, a difference occurred in the HY trial post-exercise after warming (-3.4 ± 3.0%) ($p<0.05$). No differences occurred between sampling technique or between trials ($p>0.05$). Hand warming did not result in differences in plasma volume, blood volume, and red blood cell volume changes ($p>0.05$).

Baseline blood glucose concentrations were similar between venous and capillary samples (Figure 4.4c; $p<0.0001$) and recovery concentrations were higher than all sample points ($p<0.0001$). Baseline concentrations were lower than morning samples for capillary samples and lower than morning and post-exercise values for venous samples ($p<0.05$). Venous and capillary samples were affected by warming in the final recovery sample in both trials. In the EU trial venous glucose concentrations of 5.50 ± 0.47 v 4.81 ± 0.39 $mmol.l^{-1}$ for warmed and non-warmed respectively were found ($p=0.001$), whilst in the HY trial warmed concentrations were 5.76 ± 0.47 $mmol.l^{-1}$ compared to non-warmed concentrations of 5.02 ± 0.59 $mmol.l^{-1}$ ($p=0.003$). In the EU trial capillary glucose concentrations of 5.43 ± 0.58 v 5.02 ± 0.42 $mmol.l^{-1}$ for warmed

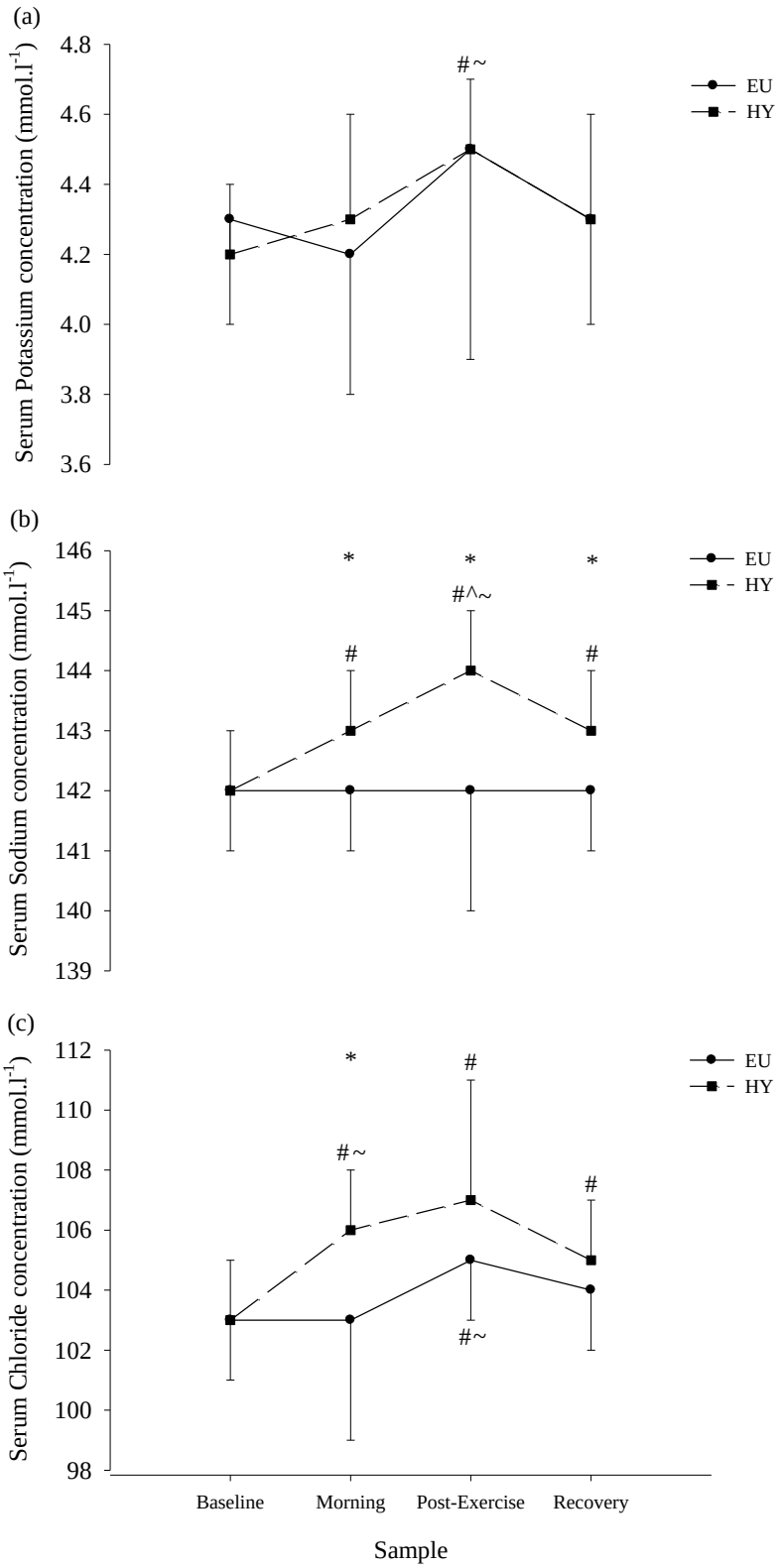

Figure 4.5. Comparison of serum (a) potassium, (b) sodium and (c) chloride concentrations between trials (mean ± SD). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning and ~ denotes different from recovery ($p<0.05$).

and non-warmed respectively were found ($p=0.003$), whilst in the HY trial warmed concentrations were 5.74 ± 0.60 mmol.l^{-1} compared to non-warmed concentrations of

4.96 ± 0.65 mmol.l^{-1} (p=0.001). Non-warmed capillary concentrations were higher in the HY trial in the morning (4.21 ± 0.25 v 4.31 ± 0.21 mmol.l^{-1} for warmed and non-warmed samples respectively; p=0.046).

No differences were found between trials for serum potassium concentrations (p>0.05; Figure 4.5a). In the HY trial serum potassium concentrations were higher post-exercise compared to baseline (p=0.009) and following recovery (p=0.023). Warming had no effect on results apart from in the EU trial at baseline (4.4 ± 0.3 v 4.2 ± 0.3 mmol.l^{-1} for warmed and non-warmed respectively; p=0.021).

Serum sodium concentrations were higher in the HY trial in the morning (p=0.016), post-exercise (p=0.003) and following recovery (p=0.001) (Figure 4.5b). In the HY trial post-exercise values for serum sodium concentrations were higher than all sample points (p<0.05), whilst the morning concentrations were higher than baseline (p=0.001). Hand warming did not affect serum sodium concentrations (p<0.05).

Serum chloride concentrations were higher in the HY trial in the morning compared to the EU trial (p=0.044; Figure 4.5c). In the HY trial baseline concentrations were lower than morning (p<0.0001), post-exercise (p=0.001), and recovery concentrations (p=0.003), whilst morning concentrations were also higher than following recovery (p=0.017). In the EU trial, post-exercise concentrations were higher than recovery (p=0.016) and baseline concentrations (p=0.015). Hand warming did not affect serum chloride concentrations (p<0.05).

Oxygen blood gas partial pressures were higher in the HY trial post-exercise compared to the EU trial (75 ± 11 v 66 ± 14 mmHg respectively; p=0.035). Samples in the morning (EU: 67 ± 18, HY: 70 ± 17 mmHg) and post-exercise (EU: 66 ± 14, HY: 75 ± 11 mmHg) were both higher than both baseline (EU: 43 ± 11, HY: 44 ± 12 mmHg) and recovery samples (EU: 49 ± 12, HY: 45 ± 10 mmHg) (p<0.05). Hand-warming resulted in an increase compared to non-warming post-exercise in the HY trial (79 ± 9 v 71 ± 11 mmHg respectively; p=0.042).

4.4.6 Urine analysis

No difference was found between trials and samples for values of urine pH ($p>0.05$). There was no difference between urine sodium concentrations between trials ($p>0.05$). In the EU trial urine sodium concentrations were lower after recovery (40 ± 24 $mmol.l^{-1}$) compared to baseline (89 ± 50 $mmol.l^{-1}$; $p=0.048$) and post-exercise concentrations (88 ± 23 $mmol.l^{-1}$; $p<0.0001$). In the HY trial concentrations were lower following recovery (50 ± 24 $mmol.l^{-1}$) compared to the morning (103 ± 43 $mmol.l^{-1}$; $p=0.009$) and post-exercise concentrations (85 ± 22 $mmol.l^{-1}$; $p=0.002$). Urine potassium concentrations were higher in the HY trial compared to the EU trial in the morning (102 ± 20 v 33 ± 14 $mmol.l^{-1}$; $p=0.007$), post-exercise (149 ± 27 v 112 ± 27 $mmol.l^{-1}$; $p=0.019$) and following recovery (141 ± 29 v 61 ± 29 $mmol.l^{-1}$; $p=0.009$). In the EU trial post-exercise urine potassium concentrations were higher than baseline (70 ± 43 $mmol.l^{-1}$; $p=0.018$), morning ($p=0.005$) and recovery samples ($p=0.001$). In the HY trial post-exercise concentrations were higher than baseline (75 ± 42 $mmol.l^{-1}$; $p=0.005$), morning ($p=0.002$) and following recovery ($p=0.003$), whilst following recovery, urine potassium concentrations were greater than in the morning ($p=0.029$). Urine chloride concentrations were lower in the morning in the EU trial (50 ± 31 v 129 ± 63 $mmol.l^{-1}$; $p=0.023$). In the EU trial post-exercise concentrations (114 ± 55 $mmol.l^{-1}$) were higher than in the morning ($p=0.015$) and following recovery (52 ± 36 $mmol.l^{-1}$; $p=0.009$). In the HY trial post-exercise concentrations (155 ± 40 $mmol.l^{-1}$) were greater than baseline (86 ± 58 $mmol.l^{-1}$; $p=0.017$) and following recovery (80 ± 53 $mmol.l^{-1}$; $p=0.001$), whilst morning concentrations were also higher than following recovery ($p=0.044$).

4.4.7 Subjective feelings questionnaires

Subjective feeling responses for thirst and mouth dryness are presented in Figure 4.6. Sensations of thirst were higher in the HY trial at post-exercise and after recovery ($p<0.05$). Sensations of mouth dryness were higher in the HY trial in the morning, post-exercise and after exercise ($p<0.05$). Subjects reported a more pleasant taste in the mouth in the HY trial following recovery (62 (35-81) compared to post-exercise (17 (0-57); $p=0.041$). Subjects felt more bloated following recovery (19 (1-67)) compared to in the morning following an overnight fast (6 (1-51); $p=0.036$) in the HY trial. Hunger was greater in the HY trial in the morning (78 (56-90)) compared to baseline (68 (23-

81); p=0.021). Subjects felt more tired in the EU trial in the morning (68 (36-81)) compared to the same sample point in the HY trial (46 (18-67); p=0.008) and to baseline (32 (11-71); p=0.013) and following recovery (35 (19-100); p=0.021) in the EU trial. Greater feelings of a scratchy throat were experienced in the EU trial post-exercise (16 (0-69)) compared to baseline (5 (0-53); p=0.008) and following recovery (9 (0-68); p=0.021), whilst in the HY trial feelings were higher in the morning (22 (0-78)) compared to baseline (13 (0-46); p=0.017) and following recovery (10 (0-61); p=0.047) and also from post-exercise (23 (0-89)) compared to baseline (p=0.019). Subjects reported greater feelings of a sore head following recovery in the HY trial (8 (0-52)) compared to the EU trial (8 (0-52); p=0.047). In the EU trial subjects reported greater feelings of a sore head in the morning (36 (0-73)) compared to baseline (10 (0-57); p=0.028) whilst feelings were lower following recovery (6 (0-30)) compared to both morning (p=0.008) and post-exercise samples (16 (0-69); p=0.015). Post-exercise, in the HY trial, greater feelings of a sore head were reported (21 (0-66)) compared to baseline ((6 (0-58); p=0.037), in the morning (13(0-64); p=0.037) and following recovery (p=0.027). No difference was found between subjective feelings of stomach fullness, alertness, concentration levels and energy levels (p>0.05).

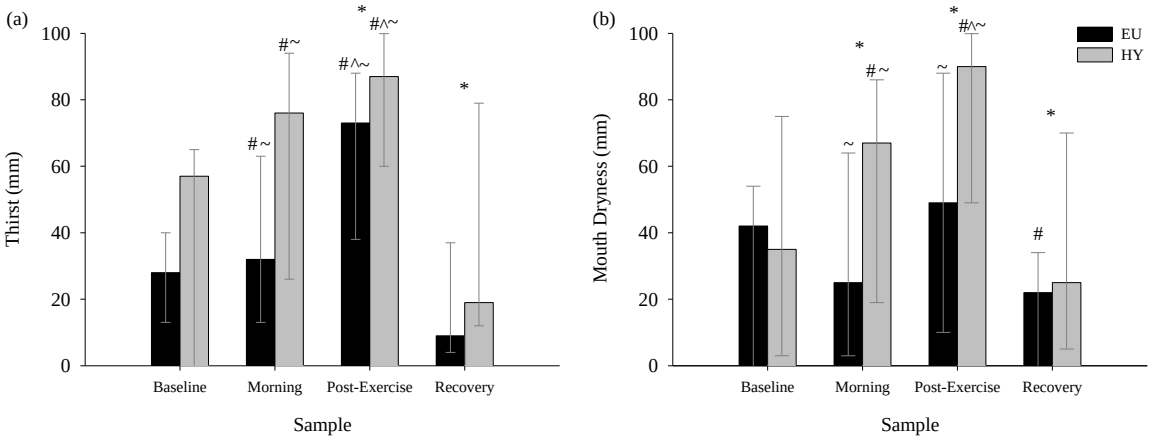

Figure 4.6. Subjective feelings responses for (a) thirst and (b) mouth dryness over the duration of the trials (median and range). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning and ~ denotes different from recovery (p<0.05).

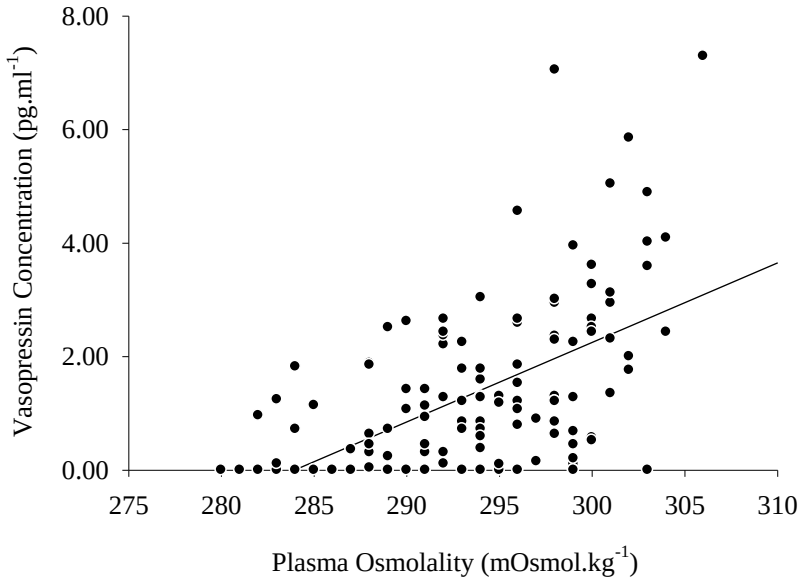

Figure 4.7. Correlation of plasma osmolality (mOsmol.kg^{-1}) and vasopressin concentrations (pg.ml^{-1}) for venous blood samples. (r_s=0.503, p<0.0001).

4.4.8 Correlations

Significant positive Spearman rank correlations were found between vasopressin concentration and perceived mouth dryness for venous (r_s=0.355, p<0.0001) and capillary samples (r_s=0.359. p=0.004), plasma osmolality and vasopressin concentration for venous samples (r_s=0.503, p<0.0001) (Figure 4.7) and between vasopressin concentration and perceived thirst for venous (r_s=0.232, p=0.008) and capillary samples (r_s=0.272, p=0.030). Significant positive Pearson correlations were found between venous and capillary samples for plasma osmolality (r=0.560, p<0.0001), haemoglobin concentration (r=0.764. p<0.0001), haematocrit (r=0.931, p<0.0001) and vasopressin (r=0.550, p<0.0001).

4.4.9 Skin temperature

Skin temperature at capillary sample sites were all lower than the equivalent venous blood sampling site in both trials at all sample points(p<0.05) (Table 4.1). Skin temperature for venous sampling were higher in the warmed condition (34.0 ± 1.1°C) compared to non-warmed (32.2 ± 1.0°C) (p<0.0001). For capillary samples, skin temperature in the warmed condition was higher (31.3 ± 1.4°C) compared to the non-warmed condition (30.7 ± 2.1°C) (p=0.002).

4.4.10 Bland- Altman plots

Bland-Altman plots comparing mean differences between venous and capillary samples for (a) plasma osmolality, (b) blood glucose concentration, (c) haemoglobin concentration, (d) haematocrit, (e) plasma volume change and (f) vasopressin concentration, are presented in Figure 4.8. Mean bias was higher for capillary samples when measuring plasma osmolality, haematocrit, blood glucose concentration and vasopressin concentration differences. Mean bias was higher for venous samples when measuring haemoglobin concentration and plasma volume change differences.

Table 4.1. Skin temperature values at sample sites over the duration of the trials (mean ± SD). * denotes different to EU trial ($p<0.05$). # denotes different to venous sample site in the same trial ($p<0.05$).

	Venous sample site temperature (°C)		Capillary sample site temperature (°C)	
Sample	EU	HY	EU	HY
Baseline	33.7 ± 1.1	31.9 ± 0.8	31.1 ± 1.2#	30.4 ± 2.0#
Morning	33.5 ± 1.0	32.1 ± 1.0	31.7 ± 1.0#	31.4 ± 1.3#
Post-exercise	34.8 ± 0.9	33.2 ± 0.9	32.3 ± 1.2#	31.9 ± 1.6*#
Recovery	33.9 ± 0.8	31.6 ± 0.9	30.1 ± 1.4#	29.1 ± 2.3#

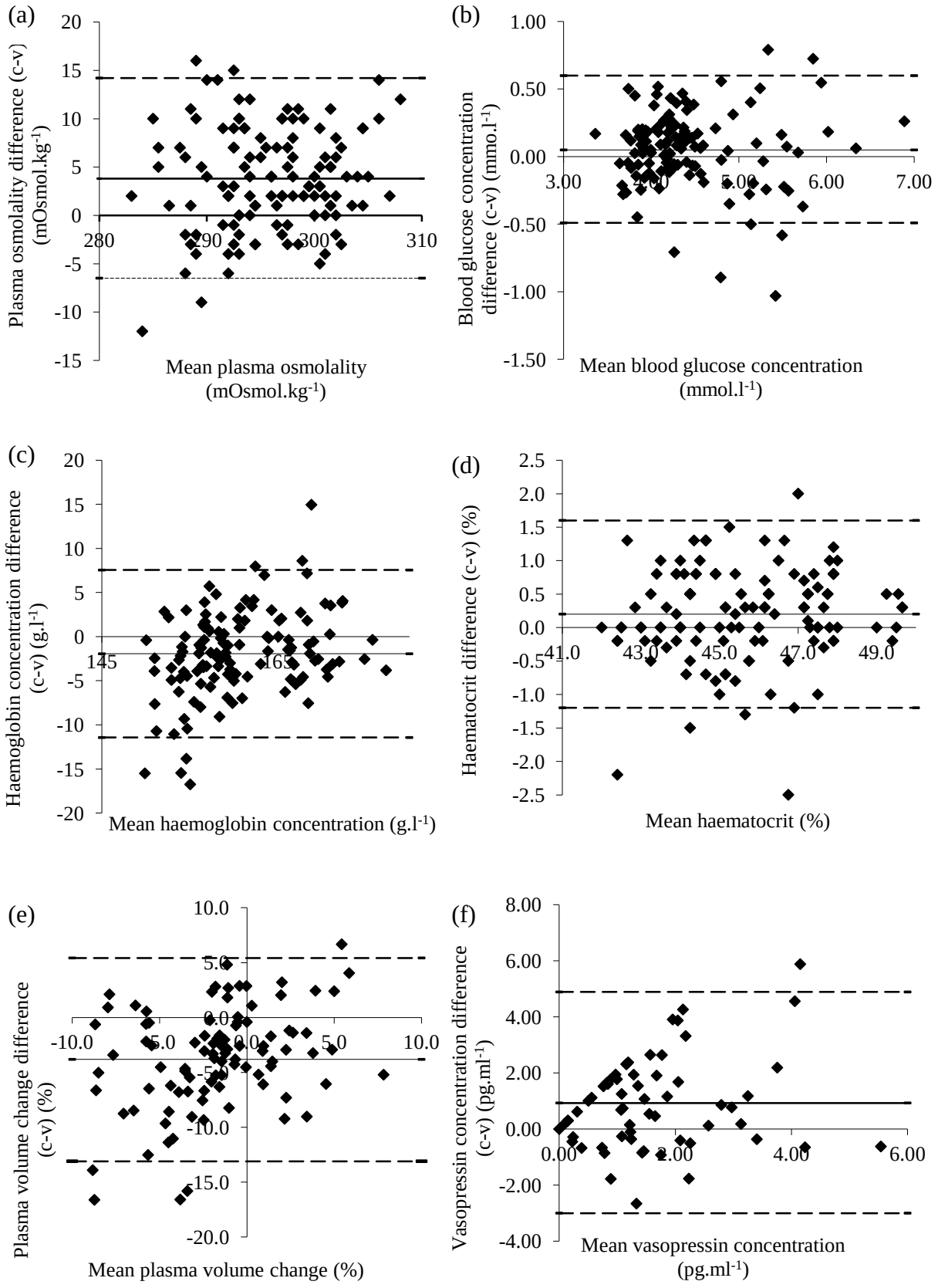

Figure 4.8. Bland-Altman plots showing the (a) plasma osmolality, (b) blood glucose concentration, (c) haemoglobin concentration, (d) haematocrit, (e) plasma volume change and (f) vasopressin concentration differences between venous and capillary blood samples against the mean of the two sampling techniques. ___ = mean of the difference; --- = mean of the difference ± 2SD.

4.5 Discussion

The results of this study suggested that when using capillary samples to analyse vasopressin concentrations and blood markers of hydration status compared to venous samples, less consistent results were obtained. Venous and capillary samples tracked changes in hydration similarly for some parameters and hand warming in general did not produce different results.

The aim of comparing venous and capillary blood samples when analysing vasopressin concentrations revealed no significant difference between samples. No literature exists pertaining to vasopressin concentrations, however, previous research analysing human growth hormone (Godfrey *et al.*, 2004) and blood glucose concentrations (Martin *et al.*, 2005) have found acceptable agreement between venous and capillary samples.

In this study, problems with the analysis of capillary vasopressin arose, resulting in a comparison between only 64 samples out of a possible 128. When using the ELISA kit several of the capillary samples were unable to be read. The reason for this was unclear but may be attributed to the plate used in the kit as all unreadable samples arose from the same plate. Whilst a relatively large number of samples were obtained, therefore providing sufficient statistical power, the inconsistency in obtaining results cast doubt over the reliability of using capillary blood samples. Combined with large variation at each sample point, further doubt is raised when using capillary samples. The range of concentrations found in this study during the EU and HY trials was larger than has previously been found (Montain *et al,*. 1997). Explanations concerning the inconsistency experienced during analysis were unclear.

Within the literature several explanations and sources of error are offered concerning the difficulty and validity of capillary blood sampling. Problems relating to clotting, contamination on the surface of the skin, insufficient sample size and "milking" of the finger have all been suggested as areas of concern (Godfrey *et al.*, 2004). In the current study, the sample area was thoroughly cleaned with an alcohol swab prior to sampling and nearly all blood was collected directly from the lancing site before it collected on the skin, while "milking" of the finger was limited. As the same examiner collected the blood, using the same technique for each sample, the agreement between the majority

of vasopressin concentrations would suggest that the sampling technique employed did was not responsible for the vasopressin concentrations.

Previous pilot work conducted in this laboratory has indicated that plasma samples of smaller volumes could be used to measure vasopressin concentrations. Sample values of plasma used in the EIA kits were reduced from 100 µl to 75 µl, with satisfactory results being obtained, therefore suggesting that this method could be used with confidence. Obtaining results from 50% of the capillary samples in the current study provided further indication that smaller sample sizes can be used in the analysis kits. However it may be that when also trying to measure haemoglobin concentration, haematocrit and plasma osmolality in addition to vasopressin concentrations from a relatively small sample, the volume was not sufficient.

In this study a moderate but significant correlation value of $r=0.55$ ($p<0.0001$) between venous and capillary samples for vasopressin concentration was found. Previous studies examining the validity of capillary and venous blood samples have compared blood glucose and haemoglobin concentration (Martin *et al.*, 2005) and human growth hormone (HGH) (Godfrey *et al.*, 2004), while Morris *et al.* (1999) examined the reliability of capillary samples during haemoglobin concentration assessment. Martin *et al.* (2005) found a correlation value of 0.98 and 0.99 for glucose and haemoglobin concentrations respectively, while Godfrey *et al.* (2004) found a correlation value of 0.986 for HGH concentrations. However, despite strong correlation, the authors expressed caution relating to their variance in sample responses as high standard deviations were found at most time points.

The rise in vasopressin concentration (in both venous and capillary samples) with increased plasma and serum osmolality was consistent with previous research (Maresh *et al.*, 2004; Rolls *et al.*, 1980). Vasopressin release has been shown to occur when a plasma osmolality of 285 $mOsmol.kg^{-1}$ is reached (Thompson *et al.*, 1986). In the current study, low levels of vasopressin release occurred at moderately high levels of plasma osmolality (~285-295 $mOsmol.kg^{-1}$) before a greater increase in vasopressin concentrations as plasma osmolality reached approximately 300 $mOsmol.kg^{-1}$. The moderate correlation of plasma osmolality and vasopressin may be explained by the

theory of Verbalis (2007), who suggested that this relationship was unique to an individual and therefore the rise in vasopressin will vary amongst subjects.

As expected with a greater degree of dehydration in the HY trial, there was increased plasma and serum osmolality resulting in higher vasopressin concentrations, with peak concentrations observed post-exercise. At similar levels of dehydration (~2-3% of body mass loss), researchers have found rises in vasopressin concentrations comparable with this study (Figaro & Mack, 1997). The authors found that vasopressin concentrations rose from 1.3 ± 0.2 to 4.4 ± 0.4 $pg.ml^{-1}$ following two hours of low intensity cycling in the heat to induce 2-3% dehydration levels.

Plasma volume remained relatively unchanged during each trial, between sampling techniques and was unaffected by hand-warming. Based on the method by Dill & Costill (1974) plasma volume was calculated from haemoglobin concentrations and haematocrit values. In the current study, there were significant differences between venous and capillary samples in all haematocrit samples and in baseline and morning haemoglobin concentrations. Tracking the changes in venous and capillary samples over the trials resulted in no difference between haemoglobin concentrations but significant differences in haematocrit values. The difference in haematocrit values and the inability to track changes using capillary samples, questions the accuracy of calculating plasma volume changes to provide an indication of changes in hydration status. As mentioned previously "milking" of the finger when extracting the capillary sample was attempted to be minimised, however even a slight increase in pressure may have caused more cells, as shown by the increased haematocrit values, to enter the sample and therefore influence haematocrit values. Unless this can be prevented or minimised further then doubt is cast over the ability to use capillary haematocrit values to calculate plasma volume changes.

There was an increase in plasma osmolality post-exercise, in both venous and capillary blood samples. Values ranged from 280 to 306 $mOsmol.kg^{-1}$ (median 294 $mOsmol.kg^{-1}$) and were similar to the results found by Maresh *et al.* (2004) in their examination of low intensity exercise in the heat. In this study venous and capillary samples tracked the small changes in plasma osmolality similarly but when there was a larger reduction in plasma osmolality following CHO-E consumption, the tracked changes were

different. Plasma osmolality values were also consistently higher than serum osmolality values. This may have been attributed to the anticoagulant used. The K^+ EDTA may have caused a rise in osmolality in the plasma samples. This should be taken into account during future studies when plasma osmolality is required.

Apart from when there was a rapid reduction in plasma osmolality following ingestion of CHO-E, venous and capillary blood samples tracked changes similarly, however venous and capillary samples were different during the final three samples. It would appear that capillary samples could be used to track changes in hydration status through changes in plasma osmolality but using plasma osmolality may not prove reliable in identifying hydration status at a particular sample point (static dehydration).

Results from the Bland-Altman plots suggest that venous and capillary samples could be used interchangeably to measure blood glucose concentration and haematocrit, however statistical analysis showed haematocrit to be different between venous and capillary samples. 95% confidence intervals were relatively small for these two variables. Large confidence intervals were found when measuring plasma osmolality, haemoglobin concentration, plasma volume changes and vasopressin concentrations indicating caution must be taken if attempting to use venous and capillary blood sampling interchangeably. The large confidence intervals found, expressed as a proportion of expected values could lead to discrepancy in interpretation of results. A 95% confidence interval of approximately ±10 $mOsmol.kg^{-1}$ was observed for plasma osmolality values. Plasma osmolality values were expected to range from approximately 280-310 $mOsmol.kg^{-1}$, whilst increases in plasma osmolality of 1 $mOsmol.kg^{-1}$ have been correlated to rises in vasopressin concentrations of 0.41 $pg.ml^{-1}$ (Thompson *et al.*, 1986). This might explain the relatively large 95% confidence interval also experienced for vasopressin concentrations. With such narrow changes, accepting a confidence interval of approximately ±10 $mOsmol.kg^{-1}$ does not seem appropriate.

Weak correlation values were observed between venous and capillary vasopressin concentrations and thirst sensations. This may be attributed to the subjects being unaccustomed to reporting feelings of thirst. Subjects were familiarised with questionnaires prior to testing, whilst Engell *et al.*, (1987) have reported strong

correlation when using subjective feelings questionnaires to monitor thirst responses during progressive levels of hypohydration. The conditions within the trials may not have been severe enough to elicit strong feelings of thirst. The low vasopressin concentrations contribute to this theory, however, similarly low levels of vasopressin concentrations have been found during greater periods of water restriction (Rolls *et al.*, 1980) or more intense periods of exercise (Montain *et al.*, 1997). Therefore, it would appear that amongst the subjects in this study, thirst was not reported reliably or did not respond in the manner expected.

Following CHO-E ingestion in the HY trial, thirst sensation returned to baseline level whilst serum osmolality values had decreased from post-exercise but were still significantly raised from baseline. The change in vasopressin concentrations following CHO-E consumption was similar between venous and capillary samples. When water is allowed through *ad libitum* intake to a dehydrated subject, it has been shown that drinking is terminated when thirst is satiated (Brunstrom *et al.*, 2000). This often occurs before vasopressin and serum osmolality levels have returned to baseline and is termed voluntary dehydration (Adolph *et al.*, 1954). Despite a prescribed set volume of CHO-E ingested in the current study, the premise of voluntary dehydration, with thirst being quenched before vasopressin and plasma osmolality returned to baseline, was apparent. In the EU and HY trials, consumption of 1 litre of CHO-E, replaced 127 and 64% of the water lost, respectively. This volume of water was sufficient enough to rehydrate, according to body mass values, back to baseline values in the EU condition only.

In response to the third aim, warming of one hand did not appear to have a marked affect on general results. Some sample points exhibited significant differences but in general these were contrary to the norm and were deemed insufficient to warrant concern that hand warming affected samples. Although not focussing on hormonal responses, previous work has centred on blood gases and acid-base measurements (Linderman *et al.*, 1990). They found no significant difference between arterialised venous and venous bloods when analysing pO_2. However, differences at rest did occur and this was credited to shunting of arterial blood through the arterio-venous anastomoses. Despite significant results in the current study, differences in pO_2 values between the two studies were similar.

4.6 Conclusion

In conclusion, using capillary samples to analyse vasopressin concentration results can be inconsistent. Further research is required to develop reliability of the sampling procedure. The use of capillary samples to track changes in markers of hydration status varied, therefore caution is required. Hand warming did not have a significant effect on most analytical parameters relating to the measurement of hydration status.

CHAPTER 5

Voluntary water intake in the cold

5.1 Abstract

When exercising in a cold environment water loss can occur via sweat, cold induced diuresis and respiration but failure to replace lost water is common in the cold due to a blunted thirst response (Kenefick *et al.*, 2004a). This study assessed voluntary water intake during and following moderate intensity exercise in the cold. Ten healthy males (age 22 ± 2 years, mass 67.8 ± 7.0 kg, height 1.77 ± 0.06 m, $\dot{V}O_{2peak}$ 60.5 ± 8.9 ml.kg^{-1}.min^{-1}) completed two trials following familiarisation separated by 7-14 days. In each trial subjects sat for 30 min before cycling at 70% $\dot{V}O_{2peak}$ for 60 min in either 25.0 ± 0.1°C, 50.8 ± 1.5% RH (warm) or 0.4 ± 1.0°C, 68.8 ± 7.5% RH (cold). Subjects then sat for 120 min at 22.2±1.2°C, 50.5±8.0% RH. *Ad libitum* drinking was allowed during the exercise and recovery periods. Urine volume, body mass, serum osmolality and sodium and potassium concentrations and sensations of thirst were measured at baseline, post-exercise and after 60 and 120 min of the recovery period. Sweat loss was lower in the cold trial (0.48 ± 0.15 l v 0.96 ± 0.18 l) ($p<0.0001$) but body mass losses over the trials were similar (1.15 ± 0.34% v 1.03 ± 0.26% for cold and warm trials respectively). More water was consumed in the whole duration of the warm trial (0.81 ± 0.42 l) compared to the cold (0.50 ± 0.49 l) ($p=0.001$) replacing 51 ± 17 and 33 ± 27% of the total water lost respectively ($p=0.013$). Cumulative urine output was greater in the cold trial (0.81 ± 0.46 v 0.54 ± 0.31 l) ($p=0.036$). Serum osmolality, sodium and potassium concentrations and plasma volume changes were not different between trials ($p>0.05$). Post-exercise serum osmolality was higher compared to baseline in the cold (292 ± 2 v 287 ± 3 mOsm.kg^{-1}, $p<0.0001$) and warm trials (288 ± 5 v 285 ± 4 mOsm.kg^{-1}; $p=0.048$). Thirst sensations were similar between trials ($p>0.05$). Voluntary water intake was less in the cold environment, however in both the warm and cold environment, *ad libitum* water intake combined with water losses resulted in similar decreases in body mass.

5.2 Introduction

It is well documented in the literature that dehydration during and resulting from endurance exercise can impair performance, particularly when exercise is conducted in temperate or hot conditions (Cheuvront *et al.*, 2003; Murray, 1995; Wendt *et al.*, 2007). Dehydration resulting in body mass losses of greater than 2% body mass loss have been shown to have a negative impact on performance both physically (Sawka *et al.*, 2007) and cognitively (Grandjean & Grandjean, 2007). One of the main mechanisms of dehydration is sweat loss which is increased by exercise in the heat (Galloway & Maughan, 1997). Although often to a lesser extent than in warm and humid conditions, dehydration is still apparent in the cold, in part due to many athletes wearing several layers of clothing and creating a warm microenvironment for them to exercise in. In the cold, water losses occur through sweating, cold induced diuresis, and respiratory losses and in addition to this there is a reduction in voluntary water intake (Freund & Sawka, 1995). Cold is often described as less than 10°C with many studies using temperatures of 0-7°C (Cheuvront *et al.*, 2005; Kenefick *et al.*, 2004ab; Kenefick *et al.*, 2008; O'Brien *et al.*, 1998).

Despite the sweat losses in cold environments water intake is often reduced and is often insufficient to replace the water losses that have occurred (Maughan *et al.*, 2005). Sweat losses during 90 minute football training sessions have been shown to be similar in a cold (Maughan *et al.*, 2005) and hot (Shirreffs *et al.*, 2005) environment, however this could be attributed to greater amounts of clothing worn in the cold therefore creating a warm microclimate.

With many researchers concentrating on exercise performance and water intake in the heat, literature examining thirst and voluntary dehydration in the cold is sparse and has focussed on low to moderate intensity exercise (50% $\dot{V}O_{2max}$) (Kenefick *et al.*, 2004ab), temperatures that have not been very cold (~7-10°C) (O'Brien *et al.*, 1998) and work in field environments where hormonal responses have not been analysed and a warm microclimate is often created by clothing worn (Maughan *et al.*, 2005; Seifert *et al.*, 2006). Therefore the aim of this study was to assess voluntary water intake and the response to thirst following moderate intensity exercise in the cold in a controlled laboratory setting.

5.3 Methods

5.3.1 Subjects

Ten healthy male subjects (age 22 ± 2 years, mass 67.8 ± 7.0 kg, height 1.77 ± 0.06 m, $\dot{V}O_{2peak}$ 60.5 ± 8.9 ml.kg^{-1}.min^{-1}) were recruited to take part in two trials, undertaken in a randomised order. All subjects had the experimental protocol explained to them verbally and in writing. Subjects were not acclimatised to the heat or cold (i.e. had not visited hot or cold climates in the month preceding the first trial and throughout the duration of the trial). Subjects provided written informed consent and the experiment was approved by the Loughborough University Ethical Advisory Committee (R10-P62).

5.3.2 Experimental protocol

Subjects were asked to visit the laboratory on four separate occasions for a $\dot{V}O_{2peak}$ test, a familiarisation trial and two experimental trials; warm and cold (schematic of the trial is presented in Figure 5.1). During the first visit $\dot{V}O_{2peak}$ was measured (details in Chapter 2). Subjects visited the lab a further three times for the familiarisation trial and two experimental trials. The familiarisation trial was identical to the warm trial. Prior to each experimental trial subjects were asked to perform the pre-trial standardisation outlined in Chapter 2.

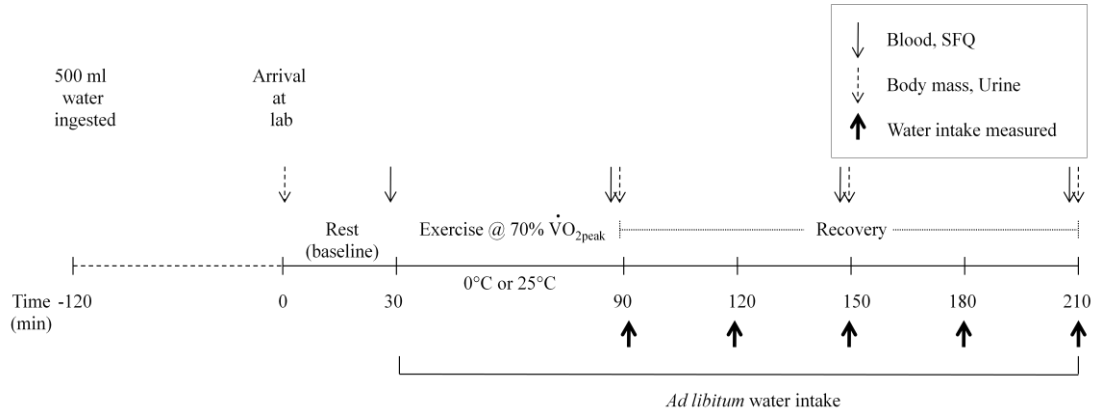

Figure 5.1. Schematic diagram indicating the testing protocol. Arrows represent sampling points.

The experimental trials were separated by a period of seven or eight days and began in the morning at the same time. Trials were identical apart from the environmental conditions exercise was performed in. Experimental trial order was created by incomplete Latin square design and subjects did not know which trial they were participating in when arriving at the laboratory for the first trial. Exercise in the warm trial was performed at 25°C, whilst the cold trial was performed at 0°C. In each trial, on arrival, subjects voided and the whole volume measured and a 5ml sample retained and had nude body mass measured. Subjects were asked to insert a rectal thermistor 10 cm past the anal sphincter to measure core temperature and skin thermistors and a heart rate monitor were positioned (see Chapter 2 for details). Subjects sat for 30 minutes at 21.4 ± 1.0°C and 52.4 ± 7.6% relative humidity (RH). Baseline heart rate values every 10 minutes were recorded and a 100 mm visual analogue subjective feelings questionnaire comprising of thirst and dry mouth scales was administered at the completion of the 30 minutes seated rest (0 mm = not all thirsty/mouth not at all dry, 100 mm = very thirsty/mouth very dry) (Appendix E). A baseline blood sample (5.5 ml) was collected without stasis from an antecubital vein in the arm. Subjects then cycled at 70% $\dot{V}O_{2max}$ for 60 minutes in either 25.0 ± 0.1°C and 50.8 ± 1.5% RH (warm) or 0.4 ± 1.0°C and 68.8 ± 7.5% RH (cold). Every 10 minutes heart rate was recorded and subjects were asked to provide a rating of their perceived exertion and thermal sensation. Subjects had free access to tap water maintained at a temperature of 11 ± 3°C throughout the duration of the exercise. The amount of water consumed was measured but the subject was not made aware of the volume or that the volume was being measured. They were provided with no external cues to drink and informed at the start that they could drink as they wanted and that the bottle would be refilled if necessary. During the familiarisation trial expired gas was collected between 14-15 minutes and 29-30 minutes to confirm the correct workload was being performed. Immediately following completion of exercise a blood sample (5.5 ml) was collected without stasis from an antecubital vein and thirst and dry mouth subjective feelings questionnaires were completed. Subjects voided, the volume was measured and a 5ml sample was retained for later analysis and had body mass measured. Mass was measured in clothing (trainers, socks and shorts) and with thermistors still attached and therefore the mass of the thermistors and clothing were subtracted from the body mass recorded. Subjects rested for 120 minutes in 22.2 ± 1.2°C and 50.5 ± 8.0% RH with

water (11 ± 3°C) intake measured during each 30 minute period. Subjects were unaware of this. HR and thermal sensation were measured every 10 minutes. At 60 and 120 minutes a blood sample (5.5 ml) was collected without stasis from an antecubital vein in the arm and thirst and dry mouth subjective feelings questionnaires were completed. Subjects voided, the volume was measured and a 5 ml sample was retained for later analysis and they then had body mass measured. After completion of the body mass measurement, subjects were allowed to leave the laboratory. Ambient temperature and relative humidity was measured at 10 minute intervals (RH85 Digital Thermo-Hygrometer; Omega, Manchester, UK). During each trial subjects wore only shorts, socks and trainers.

5.3.3 Sample analysis

Blood was analysed for haemoglobin concentration, haematocrit, blood glucose concentration, plasma volume change, blood volume change, red blood cell volume change, serum sodium and potassium concentration, serum osmolality and vasopressin concentration (see Chapter 2 for details).

The total volume of each urine sample was measured and a 5 ml sample was retained and analysed for osmolality and sodium and potassium concentration (see Chapter 2 for details).

5.3.4 Statistical analysis

Data were checked for normality of distribution using Shapiro-Wilks tests. All samples were normally distributed and subsequently either paired samples t-tests or repeated measures ANOVA was performed and if a significant ANOVA result was found, a paired samples t-tests with Bonferroni correction were performed to identify where the statistical differences occurred and also used on significant and non-significant interaction effects. Linear regression values and Pearson's product moment correlation coefficients were calculated when appropriate. Statistical significance was accepted when $p<0.05$. When post-hoc tests were conducted, p values presented were multiplied to correct for repeated samples. Data is expressed as mean ± SD.

5.4 Results

5.4.1 Baseline measures

Baseline measures of body mass (67.96 ± 6.33 v 67.69 ± 6.42 kg), serum osmolality (287 ± 3 v 285 ± 4 mOsmol.kg^{-1}), urine osmolality (320 ± 205 v 432 ± 228 mOsmol.kg^{-1}) and sensations of thirst (39 ± 23 v 42 ± 18) and mouth dryness (36 ± 23 v 38 ± 23) were similar between cold and warm trials respectively ($p>0.05$). The results indicate that subjects arrived with similar hydration statuses, which may be interpreted as a similar state of euhydration.

5.4.2 Body mass

Body mass decreased from start to end in both trials from 67.96 ± 6.33 to 67.17 ± 6.17 kg and from 67.69 ± 6.42 to 66.99 ± 6.38 kg in the cold and warm trials respectively. Body mass losses expressed as a percentage of baseline body mass are shown in Figure 5.2. Body mass was not significantly different between trials at any sample point ($p>0.05$). In both trials body mass was significantly lower compared to baseline after one and two hours of recovery and was lower following one and two hours of recovery compared to post exercise values ($p<0.05$). After exercise one subject in the cold trial and two in the warm trial had drunk more water than they had lost and thus, had gained

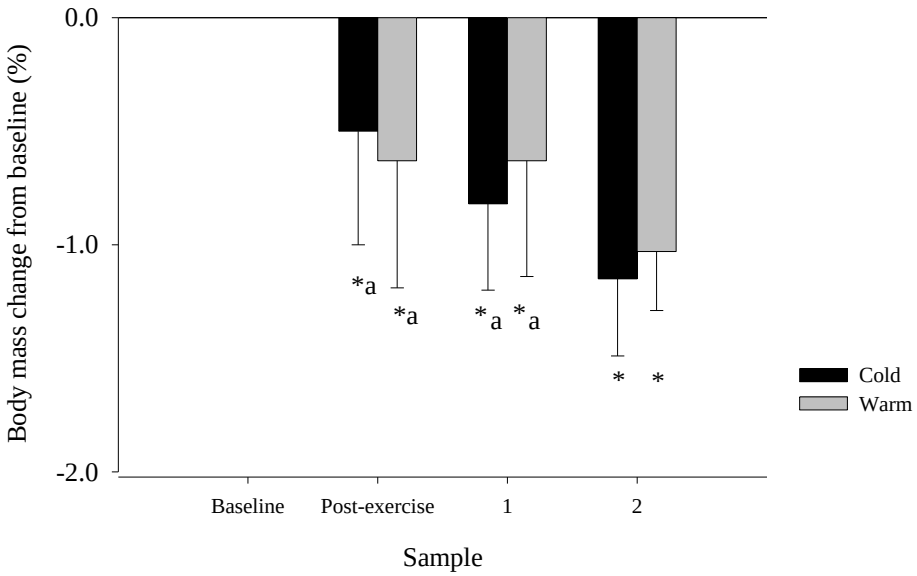

Figure 5.2. Body mass change from baseline on each trial (%). * different to baseline ($p<0.05$). 'a' different to two hours of recovery ($p<0.05$). Mean ±SD.

body mass. Negating water intake, body mass losses over the whole trial would have been 1.9 ± 0.7% and 2.2 ± 0.5% for the cold and warm trials respectively (p=0.023) with 4 subjects in the cold and 6 in the warm trial experiencing a body mass loss of greater than 2%. During the exercise period alone, without water intake, body mass losses would have been approximately 0.9 ± 0.3% and 1.4 ± 0.3% (p=0.001), with no subjects losing more than 2% body mass loss.

5.4.3 Water balance and subjective feeling questionnaires

Sweat rates during exercise were lower in the cold trial (0.39 ± 0.13 $l.h^{-1}$) compared to the warm trial (0.80 ± 0.17 $l.h^{-1}$) (p<0.0001). Cumulative urine output was greater by the end of the trial in the cold condition (p=0.036) (Figure 5.3).

More water was consumed in the warm trial (0.81 ± 0.42 l) compared to the cold (0.50 ± 0.49 l) (p=0.001). Water intake values during each period of the trial are presented in Figure 5.4. During the exercise period 44 ± 57% and 57 ± 39% of water loss was replaced in the cold and warm trials respectively (p=0.259). Following one hour of recovery total water replacement was 37 ± 32% and 62 ± 31% for the cold and warm trial respectively (p=0.003). After two hours total water replacement had decreased to 33 ± 27 and 51 ± 17% due to urine losses for the cold and warm trial respectively (p=0.013).

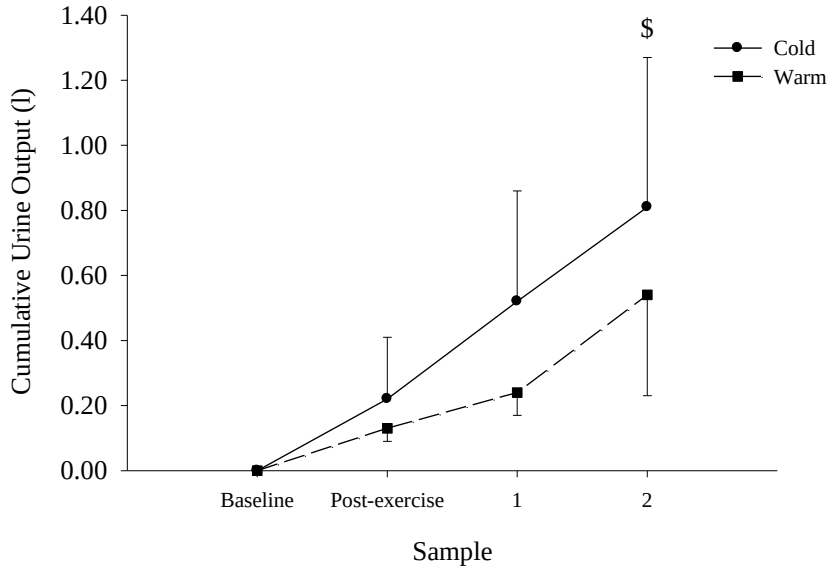

Figure 5.3. Cumulative urine output (l) over the duration of each trial. $ different between trials (p<0.05).

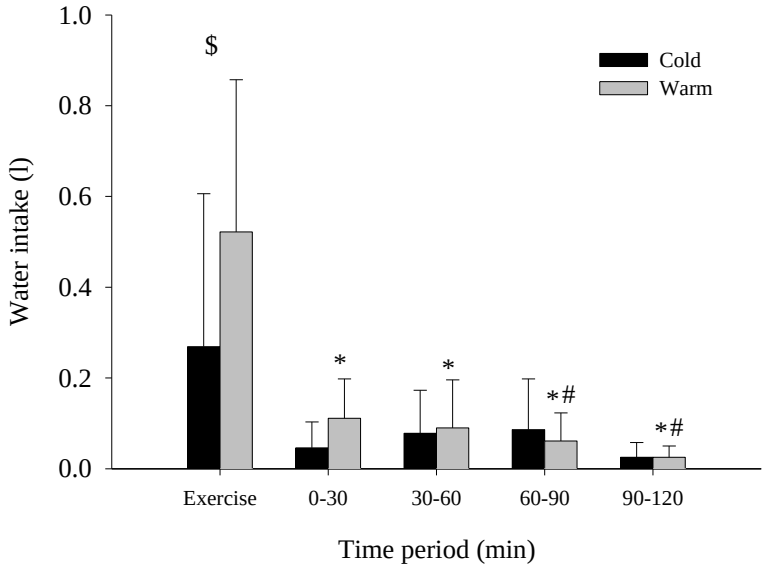

Figure 5.4. Water intake (l) during each trial. * different to exercise period ($p<0.05$). # different to 0-30 min ($p<0.05$). $ different between trials ($p<0.05$). Mean ± SD.

In the cold trial serum osmolality was higher post-exercise compared to baseline ($p<0.0001$) and there was a tendency to be higher compared to one ($p=0.054$) and two hours of recovery ($p=0.054$) (Figure 5.5). In the warm trial post-exercise values were higher than baseline values ($p=0.048$) but returned to baseline values during the recovery period.

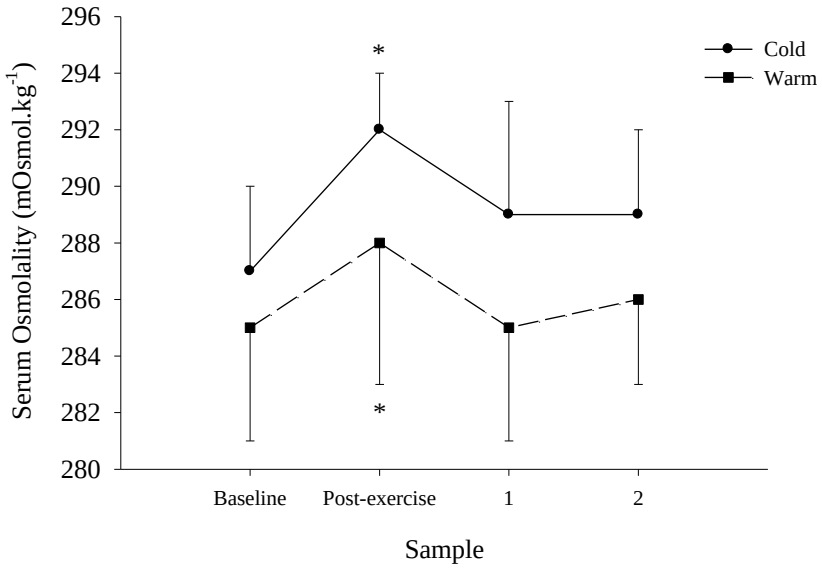

Figure 5.5. Serum osmolality over the duration of each trial ($mOsmol.kg^{-1}$). * different to baseline ($p<0.05$). Mean ± SD.

Reported feelings of thirst were similar between cold and warm trials at baseline, post-exercise and after one and two hours of recovery ($p>0.05$) (Figure 5.6a). No difference between sample points was observed in the cold trial, however in the warm trial reported sensations of thirst were higher post-exercise compared to one ($p=0.012$) and two hours of recovery ($p=0.006$). Reported sensations of mouth dryness were not different between trials and sample points (Figure 5.6b).

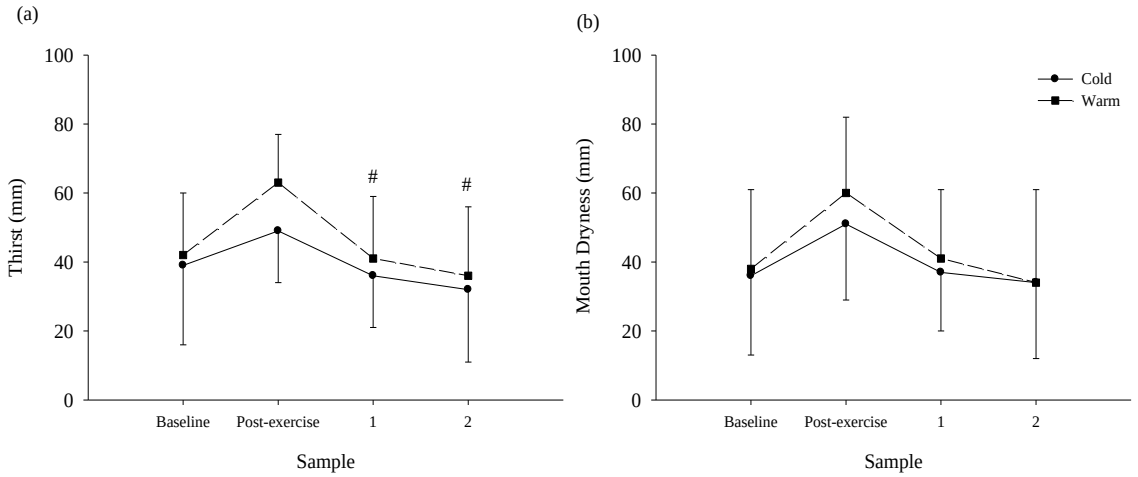

Figure 5.6. Subjective feelings of thirst (a) and mouth dryness (b) over the duration of each trial. 0mm = not at all thirsty / mouth not at all dry, 100mm = very thirsty / mouth very dry. # different to post-exercise ($p<0.05$). Mean ± SD.

Urine sodium concentrations were 51 ± 41 v 65 ± 36 mmol.l^{-1} at baseline, 64 ± 26 v 59 ± 13 mmol.l^{-1} post exercise, 60 ± 30 v 69 ± 33 mmol.l^{-1} after one hour of recovery and 62 ± 45 v 56 ± 38 mmol.l^{-1} after two hours of recovery in the cold and warm trials respectively ($p>0.05$). Urine potassium concentrations were 51 ± 35 v 61 ± 29 mmol.l^{-1} at baseline, 93 ± 38 v 91 ± 28 mmol.l^{-1} post exercise, 79 ± 50 v 103 ± 49 mmol.l^{-1} after one hour of recovery and 70 ± 45 v 75 ± 54 mmol.l^{-1} after two hours of recovery in the cold and warm trials respectively ($p>0.05$). The amount of total sodium excreted was similar at post-exercise (13 ± 14 v 8 ± 2 mmol), and one (58 ± 45 v 30 ± 11 mmol) and two hours (129 ± 86 v 87 ± 39 mmol) between cold and warm trials ($p>0.05$) whilst the total amount of potassium excreted was similar post-exercise (16 ± 9 v 11 ± 4 mmol) but greater in the cold trial after one (70 ± 25 v 44 ± 14 mmol) and two hours of the recovery period (156 ± 44 v 122 ± 47 mmol) ($p<0.05$).

There was no difference in serum sodium concentrations between trials or over the duration of the study ($p<0.05$). Serum sodium concentrations were 142 ± 1 v 142 ± 1 mmol.l^{-1} at baseline, 142 ± 1 v 141 ± 2 mmol.l^{-1} post exercise, 142 ± 1 v 142 ± 1 mmol.l^{-1} after one hour of recovery and 142 ± 1 v 141 ± 1 mmol.l^{-1} after two hours of recovery in the cold and warm trials respectively ($p>0.05$). Serum potassium concentrations were higher post-exercise (5.0 ± 0.4 and 5.0 ± 0.3 mmol.l^{-1}; cold and warm trial respectively) and after one hour of recovery (5.1 ± 0.4 and 4.9 ± 0.2 mmol.l^{-1}; cold and warm trial respectively) compared to baseline (4.6 ± 0.3 and 4.5 ± 0.3 mmol.l^{-1}; cold and warm trial respectively) ($p<0.05$). No differences were observed between trials at each sample point.

5.4.4 Blood analysis

Haemoglobin concentration, haematocrit and blood glucose concentrations are presented in Figure 5.7. Haemoglobin concentrations increased in both trials following exercise ($p<0.05$) and remained elevated in the cold trial ($p<0.05$). In the warm trial values returned to baseline after one hour of recovery but were elevated above baseline values following two hours of recovery ($p=0.018$). Haematocrit values were similar between trials ($p>0.05$) but within trials were elevated at all sample points compared to baseline in the cold trial and post-exercise and after one hour of recovery in the warm trial ($p<0.05$). Following one and two hours of recovery in the cold trial, although values did not return to baseline, they were lower than post-exercise samples ($p<0.05$).

Plasma volume change from baseline was similar at post-exercise (-11.3 ± 2.0 v -9.9 ± 5.5% for the cold and warm trial respectively; $p>0.05$) and after one (-5.9 ± 3.2 v -2.0 ± 1.6% for the cold and warm trial respectively; $p>0.05$) and two hours of the recovery period (-6.6 ± 3.8 v -5.5 ± 2.7% for the cold and warm trial respectively; $p>0.05$). In the cold trial plasma volume change from baseline was greater at post-exercise compared to the change after one and two hours of recovery ($p<0.05$). A significant change from baseline occurred at post-exercise ($p<0.05$); after one and two hours of the recovery period plasma volume had returned to baseline values ($p>0.05$). In the warm trial plasma volume changed from baseline post-exercise ($p<0.05$) but had returned to baseline values after one and two hours of the recovery period ($p>0.05$). The plasma

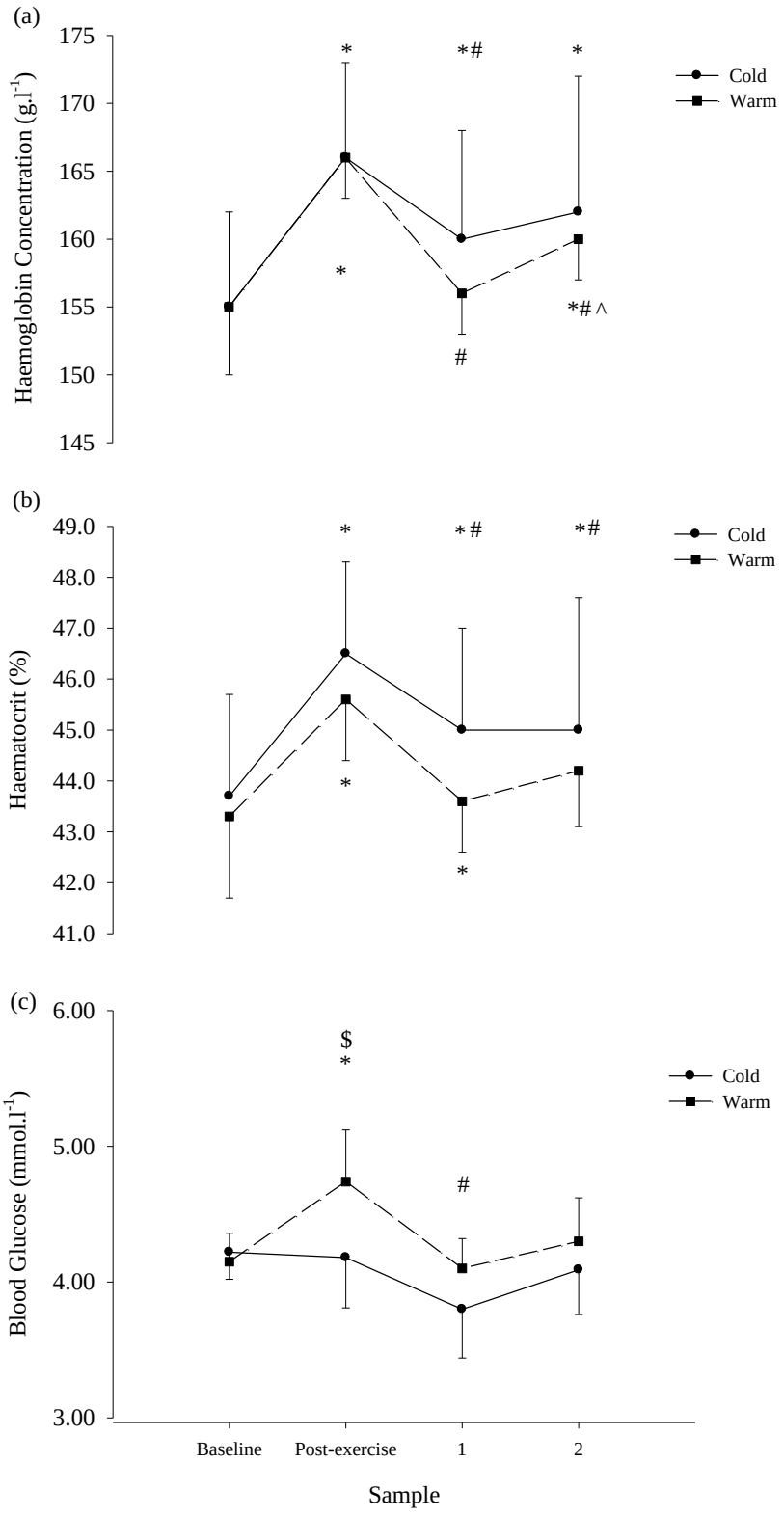

Figure 5.7. Haemoglobin concentration (a), haematocrit (b) and blood glucose concentration (c) over the duration of each trial. * different to baseline ($p<0.05$). # different to post-exercise ($p<0.05$). ^ different to one hour of recovery ($p<0.05$). $ different between trials ($p<0.05$). Mean ± SD.

volume change from baseline after one hour of recovery was less than the change from baseline to post-exercise and from baseline to two hours of recovery ($p<0.05$).

Blood volume decrease from baseline was greater in the cold trial compared to the warm trial after one hour of the recovery period (-3.3 ± 2.2 v -0.6 ± 1.0%) ($p<0.05$) but was similar at post-exercise (-6.5 ± 1.4 v -6.3 ± 3.3%) and after two hours (-3.9 ± 2.6 v -3.1 ± 1.7%) of the recovery period ($p>0.05$). In the cold trial decreases in blood volume occurred at post-exercise and after one and two hours of the recovery period, whilst decreases only occurred at post-exercise and after two hours in the warm trial ($p<0.05$). Red blood cell volume change from baseline was similar between trials at post-exercise (-0.3 ± 1.3 v -1.3 ± 1.1%) and after one (0.1 ± 1.6 v 1.2 ± 1.2%) and two hours of the recovery (-0.4 ± 2.1 v 0.0 ± 0.5%) ($p>0.05$). In the warm trial there was a greater decrease from baseline compared to the cold trial ($p<0.05$). In the cold trial, blood glucose concentrations did not change from baseline ($p>0.05$) (Figure 5.7c). In the warm trial, post-exercise concentrations were higher than baseline ($p<0.0001$) and compared to post-exercise concentrations in the cold trial ($p=0.016$). During recovery, blood glucose concentrations returned to baseline ($p>0.05$). Vasopressin analysis was conducted on two enzoimmunoassay plates. Results were not considered physiologically possible due to a lack of binding on the plate and therefore have not been included.

5.4.5 Correlations

Total water intake was positively related to cumulative urine output in both the cold ($r=0.851$, $p=0.002$) and the warm trials ($r=0.949$, $p<0.0001$), however water intake during each hour of the trial was not related to corresponding urine output volume in both trials (cold, $r=0.218$, $p=0.246$; warm, $r=0.130$, $p=0.492$). No relationship was observed between serum osmolality, subjective feelings of thirst and mouth dryness and the subsequent water intake in the following monitored time period. Serum osmolality was positively related to feelings of thirst ($r=0.429$, $p=0.011$) and mouth dryness ($r=0.470$, $p=0.005$) in the cold trial but there was no relationship with feelings of thirst ($r=0.267$, $p=0.127$) and mouth dryness ($r=0.145$, $p=0.412$) in the warm trial.

5.4.6 Core and skin temperature

Core temperatures were similar between trials ($p>0.05$). In both trials, during exercise, core temperature rose ($p<0.05$) (Figure 5.8a) before returning to baseline values during the recovery period. Mean weighted skin temperature was similar throughout the warm trial ($p>0.05$) (Figure 5.8b). During the cold trial, skin temperature decreased during the exercise period ($p<0.05$) but returned to baseline values on exiting the environmental chamber ($p>0.05$).

5.4.7 Rating of perceived exertion, heart rate and thermal sensation

During exercise, RPE values were similar between trials, however after 30 minutes RPE values were lower in the cold trial (14 ± 1) compared to the warm trial (15 ± 1)

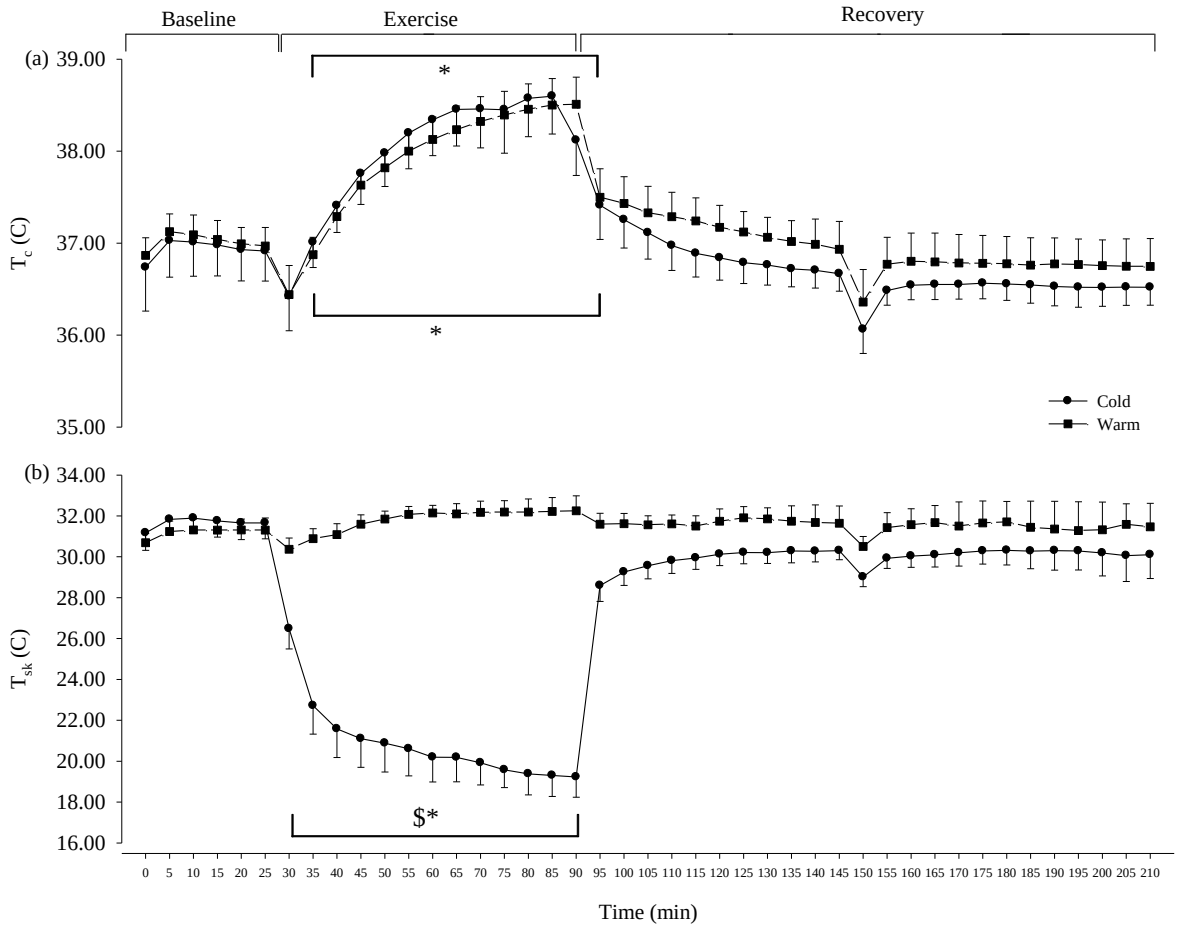

Figure 5.8. Core temperature (a) and mean weighted skin temperatures (b) over the duration of each trial. Mean ± SD. * Different to baseline, $ different between trials ($p<0.05$). At 30 and 150 min the decreases in core and skin temperature were caused when the Biopac connection was interrupted to allow for body mass measurement.

($p<0.0001$). Heart rate values were lower after 20 (65 ± 11v 72 ± 12 beats.min^{-1}; $p<0.0001$) and 40 minutes (61 ± 10 v 72 ± 11 beats.min^{-1}; $p=0.006$) of the recovery period in the cold trial. No difference was observed during other time points ($p>0.05$). During exercise, mean heart rate values were 144 ± 11 beats.min^{-1} and 154 ± 13 beats.min^{-1} in the cold and warm trials respectively. During exercise thermal sensation was lower in the cold after 10 (-4 ± 2 v 3 ± 1; $p<0.0001$), 20 (-4 ± 2 v 4 ± 1; $p<0.0001$), 30 (-4 ± 2 v 4 ± 2; $p<0.0001$), 40 (-4 ± 2 v 5 ± 1; $p<0.0001$), 50 (-4 ± 2 v 5 ± 2; $p<0.0001$) and 60 minutes (-4 ± 2 v 5 ± 1; $p<0.0001$).

5.5 Discussion

The results of this study suggested that in both warm and cold conditions, voluntary water intake was sufficient to prevent potentially detrimental levels of dehydration occurring. When water intake was factored out, 4 subjects in the cold and 6 in the warm condition would have experienced body mass losses greater than 2%, but due to voluntary water intake were able to prevent this.

Ad libitum water intake appeared to prevent body mass losses greater than 2% occurring during and following exercise in cold and warm conditions. It has been shown that during exercise *ad libitum* water intake, when compared to prescribed volumes of water replacement, can prevent body mass losses of greater than 2% (Dugas *et al.*, 2009). *Ad libitum* water intake is believed to be largely driven by sensations of thirst, and this has thought to have been sufficient to replace water losses (Greenleaf, 1992). However, *ad libitum* water intake can be affected by inappropriate sensations and/or inappropriate interpretations of thirst (Maughan & Shirreffs, 2010). If *ad libitum* water intake results in too little water consumed then dehydration levels may be greater than a 2% body mass loss (Cheuvront & Haymes, 2001) but if too much is consumed, so that there is a gain in body mass, then there is often an accompanying increase in urine output and increased water losses. In the current study all subjects prevented a 2% body mass loss from occurring, and with the exception of one subject in the cold trial and two in the warm trial, did not consume so much water for weight gain to occur during the exercise period (one subject in the cold and two in the warm trial gained weight following the exercise period and so consumed more water than they lost). By the completion of the trials all subjects were in negative water balance but not at a level that was likely to affect endurance performance (>2% body mass loss) (Sawka *et al.*, 2007).

Despite the difference in water intake between the cold and warm trials, body mass loss was similar in both trials, suggesting that water intake was adjusted to suit the physiological responses to the environment and therefore was appropriate for the situation. Subjects were able to consume enough water to offset a sufficient volume of the water losses through sweating, respiration and urine output. In addition similar values for serum osmolality, urine and serum sodium and potassium concentrations and

plasma volume changes between trials confirmed that water intake within each trial was sufficient to prevent large levels of dehydration.

Individual water intake patterns varied within and between trials. In the cold trial two subjects consumed no water during the exercise period, whilst one subject consumed 0.959 l, equating to a 177% replacement of the water lost. In the warm trial, individual water intake patterns mirrored the cold trial with those consuming little in the cold trial consuming more in the warm trial. Maughan *et al.* (2005) found large variation in individual water intake patterns during a 90 minute football training session in the cold (5.1 ± 0.7°C, 81 ± 6% RH) (mean intake 0.423 ± 0.215 l, range (0.044 – 0.951 l), whilst Maughan & Shirreffs (2008) have recommended that athletes create individualised hydration strategies. In the present study those that consumed smaller amounts repeated the trend in the second trial. In a practical setting during exercise in the cold or in the warm it is important to cater to individual needs and identify those that may be consuming too much water. This could potentially lead to unnecessary weight gain which can often be conflicting for maximal sporting performance and to increased urine output, which can increase water losses and may provide inconvenience through increased frequency of urination. In addition it is also important to identify those that are not completely responding to thirst signals or have incorrect thirst signals and are not consuming enough water to prevent dehydration from going beyond a 2% body mass loss. However, identification of these individuals is difficult as it was not known whether subjects were drinking in response to sensations of thirst or not and instead due to a habitual response. Asking them this question may have influenced water consumption.

A greater cumulative urine output was observed in the cold trial ($p=0.036$). O'Brien *et al.* (1998) observed an increase in urine flow rate following exposure to 7°C (0.76 ± 0.17 to 2.70 ± 0.54 $ml.min^{-1}$). This only occurred when the participant was in a euhydrated condition suggesting than in states of hypohydration urine output was reduced to prevent water loss. Release of vasopressin activates V2R receptors in the kidney, increasing reabsorption of water in the kidneys by increasing permeability to water of the collecting ducts (Bankir, 2001). In the cold, vasoconstriction of the peripheral blood vessels and redistribution of blood volume to the central areas of the body causes an increase in central blood pressure. The increase in pressure is detected

as increased extracellular water and therefore is removed resulting in increased urine production (Stricker & Verbalis, 1988). The lack of difference in individual urine outputs between the trials may be attributed to the time spent in the different environmental conditions. Subjects did not rest in the cold environment and so once the exercise protocol had finished, the effect of the environment on causing cold-induced diuresis was diminished. Despite subjects not resting in the cold environment following the exercise period, it appeared that the effect of cold-induced diuresis as indicated by the greater cumulative urine output at the end of the cold trial was having a continued presence.

Serum osmolality values were higher post-exercise in both trials compared to baseline. In addition, the serum osmolality values in the cold trial post-exercise were greater than the threshold for thirst outlined by Phillips *et al.* (1985) of 290 mOsmol.kg^{-1}, yet water intake was lower and reported sensations of thirst were similar, compared to values in the warm trial. Above this threshold value, the sensation of thirst occurs, ultimately resulting in a desire to drink. In the cold it has been suggested that there is a blunted thirst response which may affect water intake volumes (Kenefick *et al.*, 2008). Kenefick and colleagues examined plasma osmolality and thirst responses to 30 min exposure to 24°C and 50% RH then 45 minutes of exposure to 4°C and 50% RH following either dehydration to 4% and consumption of sodium chloride or a placebo prior to exposure or participants remained euhydrated and consumed a placebo drink. They found that the sensation of thirst was attenuated in the cold to a threshold of approximately 304 mOsmol.kg^{-1} but this attenuation of thirst, resulting from cold-exposure, could be negated by an increase in plasma osmolality, in this instance, through sodium chloride ingestion. However, unlike in the current study they did not examine the subsequent effect of thirst sensations on water intake behaviours. In the current study the reduced water intake in the cold trial, despite similar rises in serum osmolality would suggest that there was a blunting of the thirst response, however due to the small duration of the exercise protocol there was not sufficient time for the blunted thirst response to have a negative impact on hydration status.

Previous studies have examined the response to cold exposure without periods of exercise (Kenefick *et al.*, 2008, O'Brien *et al.*, 1998 and O'Brien *et al.*, 2005) and have not combined this with a recovery period allowing *ad libitum* water rehydration to be

monitored. Although in this study, the recovery period was at a temperature of 22.2 ± 1.2°C and 50.5 ± 7.8% RH; therefore not causing continual exposure to the cold environment, this situation was felt to occur more readily in a sporting situation. Often, following completion of exercise, individuals retreat to warmer environments and only remain exposed to the cold when a warmer environment is not accessible.

The cause of the failure to obtain results from the vasopressin analysis was difficult to determine. The nature of the results produced would suggest that there was low binding to the wells of the analysis plate but the reason behind this was unclear. Previous practice testing of vasopressin combined with a large number of successful results in Chapter 4, indicate that the method used has produced results and that the problem must lie elsewhere. Vasopressin analysis would have allowed a greater insight into water intake behaviour and may have provided greater understanding of the slightly blunted thirst response in the cold trial. Kenefick *et al.* (2004a) found a decrease in vasopressin concentrations following 60 min exposure to 4°C compared to exposure to 27°C and attributed this to volume factors (increased central blood volume in response to redistribution of blood flow from peripheral areas) and not to changes in plasma osmolality.

5.6 Conclusion

Voluntary water intake was less in the cold environment, however in both the warm and cold environment, *ad libitum* water intake was sufficient to ensure an appropriate state of hydration. In the cold there appeared to a blunted thirst response, however the severity of the cold exposure was not enough to exacerbate this problem in relation to hydration status.

CHAPTER 6

The effects of high intensity intermittent exercise compared to continuous exercise on voluntary water ingestion

6.1 Abstract

Voluntary water intake after high intensity intermittent exercise (HIIE) has not been assessed. Increased blood lactate concentrations resulting from HIIE may increase serum osmolality, thus increasing vasopressin release and thirst. The aim was to assess voluntary water intake after HIIE. Ten males (age 22 ± 2 years, mass 75.6 ± 6.9 kg, height 1.78 ± 0.08 m, $\dot{V}O_{2peak}$ 57.3 ± 11.4 ml.kg^{-1}.min^{-1}) took part in two trials (7-14 days apart) after familiarisation. In each trial, subjects sat for 30 min then completed an exercise period involving 2 min of rest followed by 1 min at 100% $\dot{V}O_{2peak}$ repeated until 60 min had passed (HI). In total twenty 1 min bouts of HIIE were performed. In the LO trial 60 min at 33% $\dot{V}O_{2peak}$ was performed. Subjects then sat for 60 min and were allowed a*d libitum* water intake. Body mass was measured at the start and end of the trial. Serum osmolality, blood lactate and sodium concentrations, sensations of thirst and mouth dryness were measured at baseline, post-exercise and after 5, 15, 30 and 60 min of the recovery. Plasma volume changes were calculated relative to baseline. Vasopressin and aldosterone concentrations were measured at baseline, post-exercise, 5 and 30 min. Body mass loss over the whole trial was similar between trials (HI: 0.77 ± 0.50; LO: 0.85 ± 0.55%) ($p=0.124$). Volume of sweat lost during exercise (0.78 ± 0.22 v 0.66 ± 0.26 l) and voluntary water intake during the recovery period (0.416 ± 0.299 v 0.294 ± 0.295 l) ($p<0.05$) were greater in the HI trial. Serum osmolality (297 ± 3 v 288 ± 4 mOsmol.kg^{-1}), blood lactate (8.5 ± 2.7 v 0.7 ± 0.4 mmol.l^{-1}), serum sodium (146 ± 1 v 143 ± 1 mmol.l^{-1}) and vasopressin (9.91 ± 3.36 v 4.43 ± 0.86 pg.ml^{-1}) concentrations were higher post-exercise in the HI trial ($p<0.05$) and thirst (84 ± 7 v 60 ± 21) and mouth dryness (87 ± 7 v 64 ± 23) tended to be higher ($p=0.060$). Plasma volume decrease was greater in the HI trial at post-exercise, 5, 15 and 30min ($p<0.05$). There was no change or difference in aldosterone concentrations ($p>0.05$). Greater voluntary water intake after HIIE was mainly caused by increased water loss but was also, in part, attributed to increased blood lactate concentrations causing an increase in serum osmolality and thus vasopressin release.

6.2 Introduction

An increase in serum osmolality causing an increased release of vasopressin has been proposed as one of the mechanisms resulting in the sensation of thirst and water replacement (Stricker & Verbalis, 1988). Following the onset of exercise loss of water from the vascular space results in a rise in serum osmolality (Convertino *et al.*, 1981). During and following continuous exercise, the resultant effect of increased osmolality and AVP release on voluntary water intake has been extensively studied (Cheuvront & Haymes, 2001; Dugas *et al.*, 2009; Wong *et al.*, 1998), yet the effect on resultant water intake following a bout of high intensity intermittent exercise (HIIE) is less well known. During and following HIIE, there is often an increase in blood lactate concentration, either through increased production, reduced clearance or a combination of both, which has been linked to the prevention of serum sodium release from the vascular space to the intracellular space, thus causing an increase in serum osmolality (Nose *et al.*, 1991). Nose *et al.*, (1991) suggested that the negatively charged lactate ions were preventing efflux of the positively charged sodium ions from the vascular space to the intracellular space. In addition bouts of high intensity exercise have resulted in an increase in AVP release (Hew-Butler *et al.*, 2008). They found that on completion of a $\dot{V}O_{2peak}$ test on the treadmill, AVP concentrations had increased by 10.9 ± 5.0 $pg.ml^{-1}$ compared to an increase of 0.7 ± 0.3 $pg.ml^{-1}$ following a steady state run at 60% of $\dot{V}O_{2peak}$. However, work performed was not matched and so is difficult to make a direct comparison and in addition the effect this rise in vasopressin concentration had on water intake was not assessed. Despite the known effect of HIIE on the rise in serum osmolality and an increase in vasopressin release, the effect on subsequent voluntary water intake is unknown.

A rise in osmolality above the AVP release threshold of approximately 285 $mOsmol.kg^{-1}$ (Thompson *et al.*, 1986) will lead to maximal anti-diuresis occurring, resulting in an osmotically driven thirst signal, thus facilitating water intake. Following a bout of HIIE, the increased serum osmolality values above the values experienced following continuous exercise of relative intensity, may result in greater osmotic signals, ultimately leading to increased water intake. Excessive water intake can result in exercise associated hyponatraemia, which may lead to death in severe cases (Beltrami *et al.*, 2008; Hew-Butler, 2010; Kipps *et al.*, 2011). Therefore, from a health

perspective understanding the voluntary water intake response to elevated levels of serum osmolality is of importance.

In addition to water losses through sweat, HIIE is likely to result in an increase in ventilation and therefore respiratory water losses. Although different respiratory losses will not affect interpretation of changes in hydration status the accurate calculation of sweat loss may be affected (Maughan *et al.*, 2007b). However, the shortness in duration of the exercise period and the relative humidity expected to be experienced (~50%) are unlikely to result in a significant contribution to total water losses through respiration (Maughan *et al.*, 2007b).

It was hypothesised that the increase in lactate concentration, resulting from the high intensity intermittent exercise, would increase serum sodium concentration and thus, serum osmolality, in turn causing an increase in vasopressin release and subsequent voluntary water intake. It was also hypothesised that this increase in voluntary water intake would be driven by increased sensations of thirst.

6.3 Methods

6.3.1 Subjects

Ten healthy male subjects (age 22 ± 2 years, mass 75.6 ± 6.9 kg, height 1.78 ± 0.08 m, $\dot{V}O_{2peak}$ 57.3 ± 11.4 ml.kg^{-1}.min^{-1}) were recruited to take part in two trials, undertaken in a randomised order. All subjects had the experimental protocol explained to them verbally and in writing. Subjects provided written informed consent and the experiment was approved by the Loughborough University Ethical Advisory Committee (R10-P132).

6.3.2 Experimental protocol

Subjects were asked to visit the laboratory on four separate occasions for a $\dot{V}O_{2peak}$ test, a familiarisation trial and two experimental trials; high intensity intermittent (HI) and continuous (LO) exercise. During the first visit, $\dot{V}O_{2peak}$ was measured (details in Chapter 2). Subjects visited the lab a further three times for the familiarisation trial and two experimental trials. The familiarisation trial was identical to the HI trial. Prior to each experimental trial subjects were asked to perform the pre-trial standardisation outlined in Chapter 2.

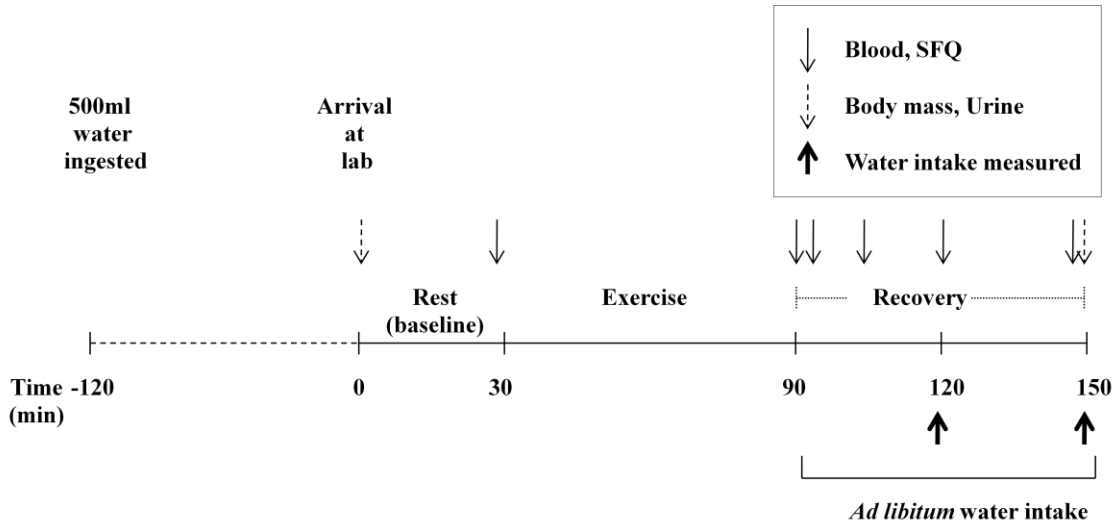

Figure 6.1. Schematic diagram indicating the testing protocol. Arrows represent sampling points. SFQ denotes subjective feelings questionnaire.

The experimental trials were separated by a period of 7-14 days and began in the morning at the same time for each subject. Experimental trials were identical apart from the exercise performed. A schematic outline of the experimental trial is presented in Figure 6.1. Experimental trial order was created by incomplete Latin square design and subjects did not know which trial they were participating in when arriving at the laboratory for the first trial.

In each trial, on arrival, subjects voided and the whole volume was measured and a 5 ml sample retained for later analysis and had nude body mass measured. Subjects were asked to insert a rectal thermistor 10 cm past the anal sphincter to measure core temperature and skin thermistors and a heart rate monitor were positioned (see Chapter 2 for details). Subjects sat for 30 minutes at 19.7 ± 1.1°C and 30.7 ± 10.5% relative humidity (RH). Baseline heart rate values every 10 minutes were recorded and a 100 mm visual analogue subjective feelings questionnaire comprising of thirst and dry mouth scales was administered at the completion of the 30 minutes seated rest (0 mm = not all thirsty/mouth not at all dry, 100 mm = very thirsty/mouth very dry) (Appendix E). During the rest period a 21g cannula (Surflo, Terumo, Leuven, Belgium) was inserted into a superficial vein on the forearm to allow venous blood sampling. The line was flushed with 2-3 ml of heparinised saline. A baseline blood sample (7.5 ml) was collected at the end of the rest period. Subjects then cycled for a period of 60 minutes in 24.9 ± 0.7°C and 51.1 ± 2.1% RH. In the HI trial they rested for 2 minutes and then performed 1 minute of cycling at a power output equal to the maximum power achieved when achieving $\dot{V}O_{2peak}$. This was repeated 20 times during the 60 minute period. In the LO trial work performed was matched with subjects cycling continuously at 33% of their peak power output for 60 minutes. Every 10 minutes heart rate was recorded and subjects were asked to rate their perceived exertion and thermal sensation. Immediately following completion of exercise a blood sample (7.5 ml) was collected and thirst and dry mouth subjective feelings questionnaires were completed. Subjects were then seated for 60 minutes in 21.2 ± 1.8°C and 29.5 ± 10.3% RH with tap water (11 ± 3°C) intake measured during each 30 minute period. The amount of water consumed was measured but the subject was not made aware of the volume or that the volume was being measured. They were provided with no external cues to drink and informed at the start that they could drink as they wanted and that the bottle would be refilled if

necessary. Heart rate and thermal sensation were measured every 10 minutes. At 5, 15, 30 and 60 minutes a blood sample (7.5 ml) was collected and thirst and dry mouth subjective feelings questionnaires were completed. Subjects voided, the volume was measured and a 5 ml sample was retained for later analysis and they then had nude body mass measured. After completion of the body mass measurement, subjects were allowed to leave the laboratory. Ambient temperature and relative humidity was measured at 10 minute intervals (RH85 Digital Thermo-Hygrometer; Omega, Manchester, UK).

6.3.3 Sample analysis

Blood was analysed for haemoglobin concentration, haematocrit, blood glucose concentration, plasma volume change, blood volume change, red blood cell volume change, serum sodium and potassium concentration, serum osmolality and vasopressin and aldosterone concentration (see Chapter 2 for details).

The total volume of each urine sample was measured and a 5 ml sample was retained and analysed for osmolality and sodium and potassium concentration (see Chapter 2 for details).

6.3.4 Statistical analysis

Data were checked for normality of distribution using Shapiro-Wilks tests. All samples were normally distributed and subsequently either paired samples t-tests or repeated measures ANOVA was performed and if a significant ANOVA result was found, a paired samples t-tests with Bonferroni correction were performed to identify where the statistical differences occurred and to find differences in significant and non-significant interaction effects. Linear regression values and Pearson's product moment correlation coefficients were calculated when appropriate. Correlation analysis performed between variables that were deemed to be related in terms of water balance and the mechanism identified by Nose *et al.* (1991) (Serum osmolality/serum sodium/blood lactate). Statistical significance was accepted when $p<0.05$. When post-hoc tests were conducted, p values presented were multiplied to correct for repeated samples. Data is expressed as mean ± SD.

6.4 Results

Blood samples were collected from eight subjects due to cannulation problems in two subjects. All subjects who provided blood samples provided all six samples in each trial except one subject whose cannula closed down after exercise and therefore venepunctures from an antecubuital vein were taken post-exercise and after 15 minutes of the recovery period.

6.4.1 Baseline values

There was no difference in baseline body mass between the HI (75.57 ± 7.28 kg) and LO trial (75.71 ± 6.98 kg) ($p=0.496$). Similar baseline values for urine osmolality (510 ± 248 v 507 ± 270 mOsmol.kg^{-1} for HI and LO trials respectively), serum osmolality (285 ± 4 v 284 ± 3 mOsmol.kg^{-1} for HI and LO trials respectively) and subjective feelings of thirst (53 ± 21 v 40 ± 15 for HI and LO trials respectively) and mouth dryness (52 ± 22 v 42 ± 16 for HI and LO trials respectively) were found, suggesting subjects arrived in a similar state of euhydration (Sawka *et al.*, 2007) ($p>0.05$).

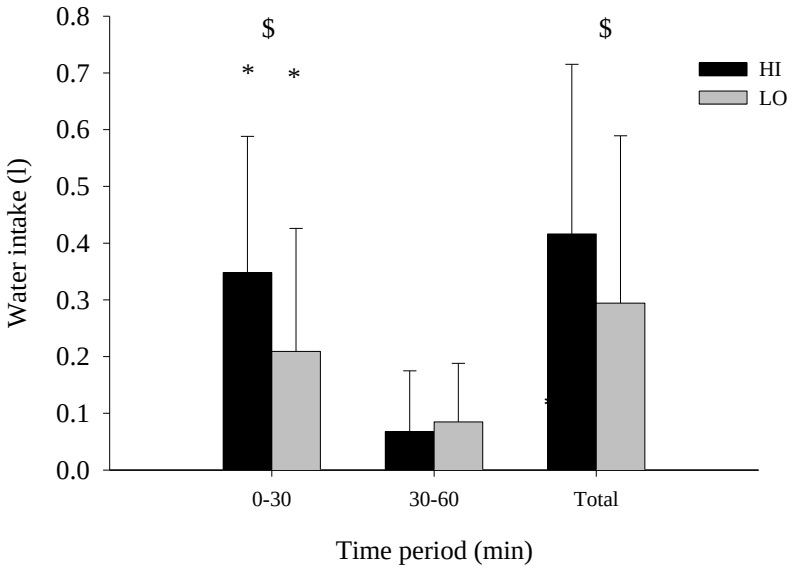

Figure 6.2. Voluntary water intake during each trial. $ denotes difference between trials, * denotes different from 30-60min ($p<0.05$).

6.4.2 Water balance

Body mass loss was similar between trials (0.77 ± 0.50 v 0.85 ± 0.55% for HI and LO trials respectively) ($p=0.124$). Sweat loss was greater in the HI trial (0.78 ± 0.22 l)

compared to the LO trial (0.66 ± 0.26 l) (p=0.009). In the HI trial subjects consumed more water during the recovery period (p<0.0001) (Figure 6.2) but this difference was solely due to a higher water intake during the first 30 minutes of recovery (0.348 ± 0.240 v 0.209 ± 0.217 l for the HI and LO trial respectively; p=0.006) whilst during the final 30 minutes of the recovery period, water intake was similar (0.068 ± 0.107 v 0.085 ± 0.103 l for the HI and LO trial respectively; p=0.094). Expressed as a percentage, the amount of water lost that was replaced was higher in the HI trial compared to the LO trial (44 ± 29 v 35 ± 34%; p=0.012). In the HI trial, one subject drank more than the water lost (104%), with the next highest replacement value was 77%. In the LO trial, two subjects replaced 90-100% with the remaining subjects replacing less than 51% of the water lost during exercise. Negating water intake, body mass losses from baseline would have been similar: 1.34 ± 0.36% in the HI trial and 1.26 ± 0.39% in the LO trial, with only one subject in both trials losing enough water to elicit a greater than 2% body mass loss. There was no difference in urine output at the end of the trial (0.23 ± 0.12 v 0.28 ± 0.12 l for HI and LO trials respectively; p=0.203).

Serum osmolality values were higher in the HI trial post-exercise (p=0.06) and after 5 (p=0.048) and 30 minutes (p<0.001) of the recovery period (Figure 6.3). Serum osmolality values were similar across all sample points in the LO trial (p>0.05) but were elevated above baseline and the recovery period samples post-exercise in the HI

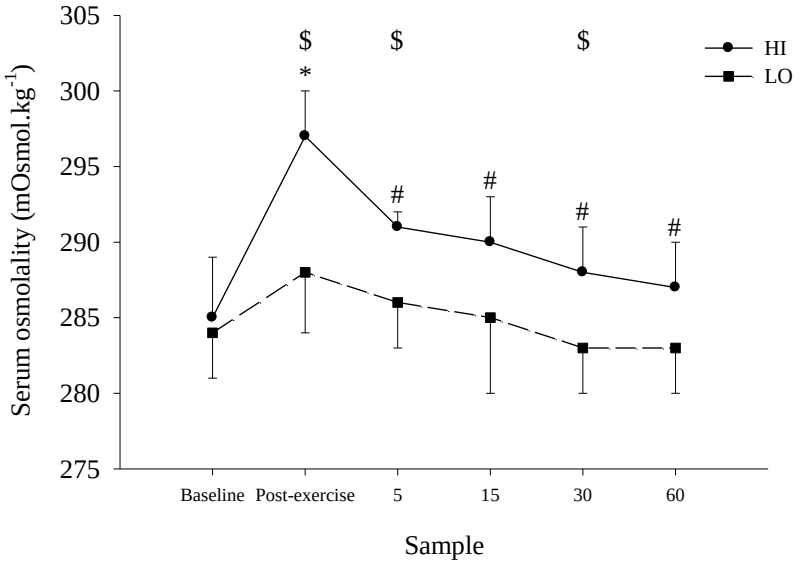

Figure 6.3. Serum osmolality values over the duration of each trial. $ different between trials, * different from baseline, # different to post-exercise (p<0.05).

trial ($p \leq 0.015$). In the HI trial values had returned to baseline following 5 minutes of recovery ($p > 0.05$).

Serum sodium concentrations post-exercise were higher in the HI trial compared to the LO trial ($p = 0.018$) (Figure 6.4). Concentrations at other sample points were not different ($p > 0.05$). In the HI trial, post-exercise concentrations were greater post-exercise compared to baseline and during the recovery period ($p \leq 0.015$) and had returned to baseline after 5 minutes of the recovery period ($p > 0.05$). In the LO trial serum sodium concentrations did not increase above baseline ($p > 0.05$). Serum potassium concentrations were similar between trials and sample points (baseline: 4.4 ± 0.3 v 4.4 ± 0.3 mmol.l^{-1}; post-exercise: 5.1 ± 0.3 v 4.9 ± 0.4 mmol.l^{-1}; 5min: 4.4 ± 0.3 v 4.5 ± 0.3 mmol.l^{-1}; 15min: 4.6 ± 0.3 v 4.5 ± 0.3 mmol.l^{-1}; 30min: 4.5 ± 0.3 v 4.5 ± 0.3 1mmol.l^{-1} and 60min: 4.6 ± 0.3 v 4.4 ± 0.2 mmol.l^{-1}) ($p > 0.05$).

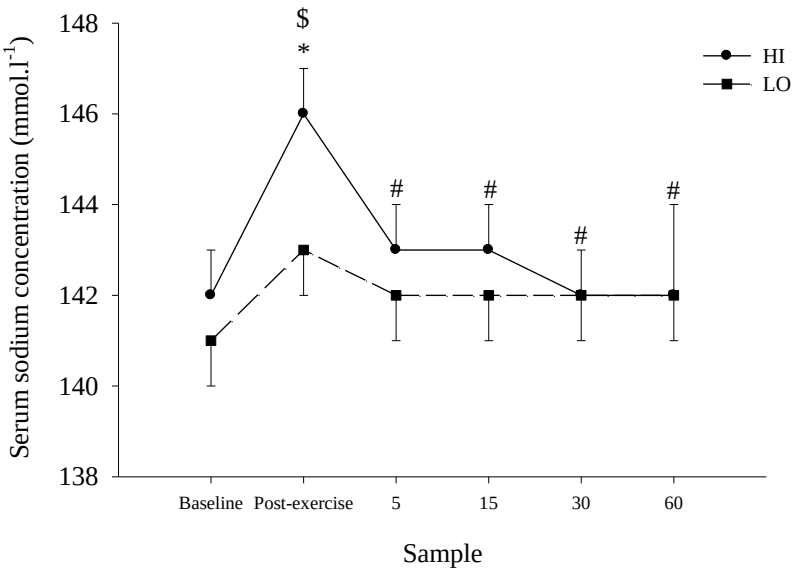

Figure 6.4. Serum sodium concentration over the duration of each trial. $ different over the duration of each trial, * different from baseline, # different to post-exercise ($p < 0.05$).

Urine sodium concentrations at the end of the trials were similar between the HI (95 ± 44 mmol.l^{-1}) and LO (83 ± 34 mmol.l^{-1}) trials ($p = 0.155$). Urine potassium concentrations at the end of the trials were similar between the HI (101 ± 32 mmol.l^{-1}) and LO (99 ± 41 mmol.l^{-1}) trials ($p = 0.673$). Baseline concentrations were similar between trials for sodium (66 ± 40 v 57 ± 37 mmol.l^{-1} for HI and LO trial respectively)

and potassium concentrations (68 ± 35 v 60 ± 41 mmol.l^{-1} for HI and LO trial respectively) ($p>0.05$).

6.4.3 Blood analysis

Haemoglobin concentrations were higher post-exercise in the HI trial compared to the LO trial ($p=0.012$) (Figure 6.5a). No difference was found at the other sample points ($p>0.05$). In the HI trial post-exercise concentrations were greater than at all other sample points ($p<0.0001$) and remained elevated after 5 minutes of recovery compared to baseline and the remaining recovery samples ($p\leq0.015$). Samples taken after 5 minutes of recovery had returned to baseline ($p>0.05$). In the LO trial post-exercise haemoglobin concentrations were elevated compared to all other sample points ($p\leq0.015$) but had returned to baseline following the commencement of the recovery period ($p>0.05$).

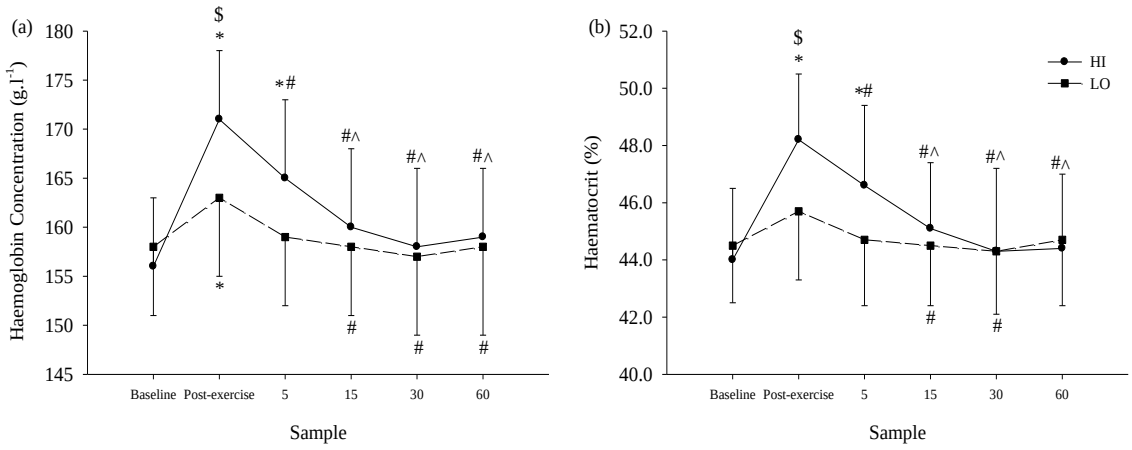

Figure 6.5. Haemoglobin concentration (a) and haematocrit values (b) over the duration of each trial. $ different between trials, * different to baseline, # different to post-exercise, ^ different to 5min ($p<0.05$).

Haematocrit was greater in the HI trial post-exercise compared to the LO trial ($p=0.006$) (Figure 6.5b). Baseline and recovery samples were similar between trials ($p>0.05$). Haematocrit values post-exercise in the HI trial were elevated above baseline and recovery samples ($p\leq0.015$). After 5 minutes of the recovery period, haematocrit values were still elevated above baseline and the remaining recovery samples ($p\leq0.015$) but had decreased compared to post-exercise ($p=0.015$). In the LO trial post-exercise values were greater compared to samples taken after 15 and 30 minutes of recovery ($p>0.05$).

At baseline, blood lactate concentrations were similar between trials (p=0.914) but increased during exercise and remained elevated throughout the recovery period ($p \leq 0.006$) (Figure 6.6). In the HI trial, blood lactate concentrations peaked post-exercise and remained elevated above baseline values until 30 minutes of the recovery period ($p \leq 0.015$). Post-exercise concentrations and after 5 minutes of the recovery period were greater than after 15, 30 and 60 minutes of the recovery period ($p \leq 0.045$), whilst concentrations recorded after 30 and 60 minutes of the recovery period were less than after 15 minutes ($p \leq 0.030$).

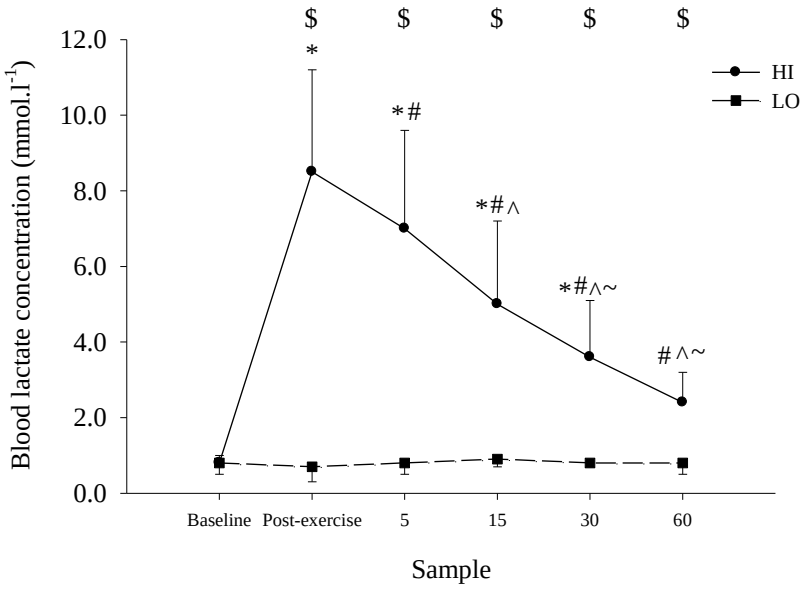

Figure 6.6. Blood lactate concentrations over the duration of each trial. $ different between trials, * different to baseline, # different to post-exercise, ^ different to 5min, ~ different to 15min ($p<0.05$).

Blood glucose concentrations were similar between trials and sample points (baseline: 4.09 ± 0.22 v 4.17 ± 0.18 mmol.l^{-1}; post-exercise: 4.21 ± 0.38 v 4.24 ± 0.34 mmol.l^{-1}; 5 min: 4.42 ± 0.41 v 4.34 ± 0.21 mmol.l^{-1}; 15 min: 4.34 ± 0.39 v 4.21 ± 0.30 mmol.l^{-1}; 30 min: 4.20 ± 0.38 v 4.32 ± 0.21 mmol.l^{-1} and 60 min: 4.25 ± 0.29 v 4.24 ± 0.30 mmol.l^{-1}) ($p>0.05$).

Plasma vasopressin and aldosterone concentrations are presented in Figure 6.7a and Figure 6.7b respectively. Plasma vasopressin concentrations were higher in the HI trial

at post-exercise and after 5 and 30 minutes of the recovery period ($p<0.05$). In the HI trial, vasopressin concentration increased from baseline concentrations post-exercise ($p=0.048$), had a tendency to remain elevated above baseline after 5 minutes of recovery ($p=0.054$) and were elevated above baseline values after 30 minutes of the recovery period ($p<0.05$). In the LO trial concentrations did not change from baseline ($p>0.05$). In both the HI and LO trials, aldosterone concentration did not change from baseline ($p>0.05$) but after 30 minute of the recovery period aldosterone concentrations were greater in the HI trial compared to the LO trial ($p=0.048$).

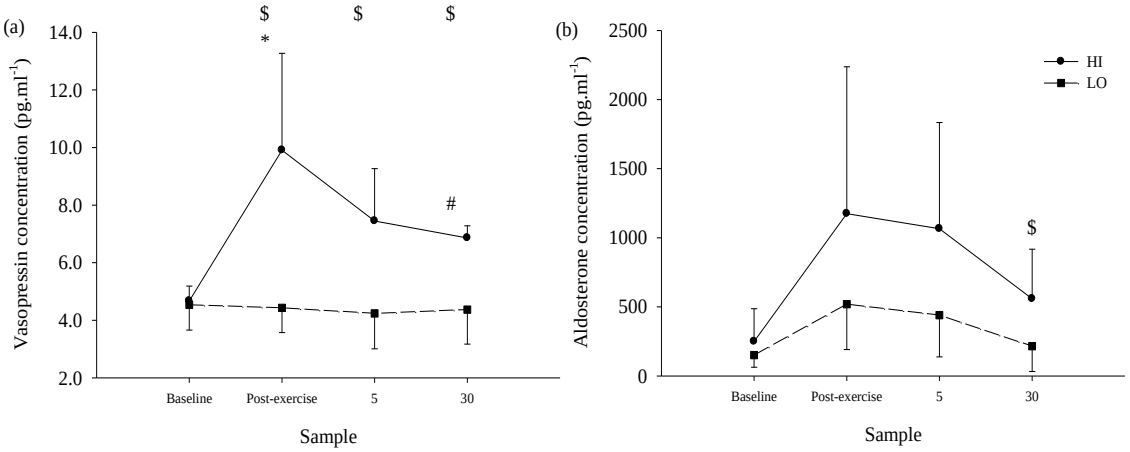

Figure 6.7. Vasopressin (a) and aldosterone (b) concentration over the duration of each trial. $ different between trials, * different to baseline, # different to post exercise ($p<0.05$).

Plasma volume change from baseline was greater in the HI trial compared to the LO trial at post-exercise and after 5, 15 and 30 minutes of the recovery period ($p<0.05$) (Figure 6.8). In the HI trial plasma volume was different compared to baseline at post-exercise and after 5 and 15 minutes of the recovery period ($p<0.0001$) before plasma volume returned to baseline values. The change from baseline to post-exercise was greater than the change from baseline to 5, 15, 30 and 60 minutes, whilst the change from baseline to 5 minutes of the recovery period was greater than the change from baseline to 15, 30 and 60 minutes ($p<0.0001$). In the LO trial plasma volume changes from baseline at each sample point were similar ($p>0.05$). The change from baseline to post-exercise was greater than the changes from baseline to 15, 30 and 60 minutes of the recovery period ($p<0.05$).

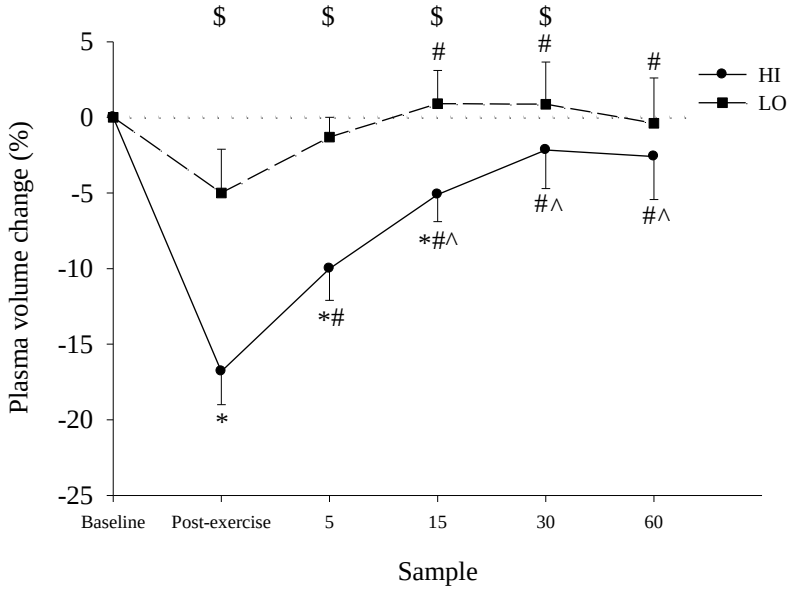

Figure 6.8. Plasma volume change from baseline values over the duration of each trial. $ different between trials, * different to baseline values, # different to the post exercise change, ^ different to the baseline to 5 min change ($p<0.05$).

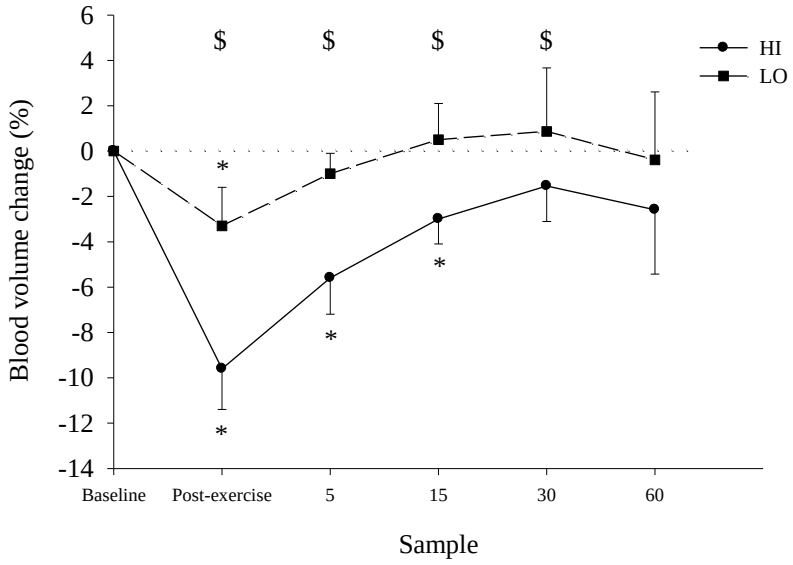

Figure 6.9. Blood volume change from baseline values over the duration of each trial. $ different between trials, * different to baseline values ($p<0.05$).

Blood volume changes from baseline were greater in the HI trial compared to the LO trial at post-exercise and after 5, 15 and 30 minutes of the recovery period ($p<0.05$) (Figure 6.9). In the HI trial, blood volume decreased from baseline at post-exercise and after 5 and 15 minutes of the recovery period ($p<0.0001$) before returning to baseline values. In the LO trial blood volume had decreased from baseline at post-exercise

(p<0.05) but had returned to baseline values by 5 minutes of the recovery period (p>0.05). Red blood cell volumes were similar when comparing HI and LO trials at post-exercise (-0.4 ± 1.0 v -1.2 ± 2.0%), 5 (0.0 ± 1.6 v -0.6 ± 1.9%), 15 (-0.4 ± 0.8 v -0.1 ± 1.7%), 30 (-0.7 ± 1.5 v -0.4 ± 1.9%) and 60 min (-1.0 ± 1.0 v 0.0 ± 1.6%) of the recovery period (p>0.05). There was no difference from baseline values within trials (p>0.05).

6.4.4 Subjective feeling questionnaires

Reported sensations of thirst peaked in both trials post-exercise (Figure 6.10a) and tended to be higher in the HI trial compared to the LO trial at the post-exercise sample (p=0.060). There was no difference between trials at the other sample points (p>0.05). In the HI trial, post-exercise reported sensations of thirst were greater than baseline and during the recovery period (p<0.0001). After 5 minutes of the recovery period, reported sensations of thirst remained greater compared to 15, 30 and 60 minutes of the recovery period (p<0.0001). After 60 minutes of recovery, reported sensations of thirst had decreased below baseline values (p=0.015). Reported sensations of mouth dryness peaked in both trials post-exercise (Figure 6.10b) and tended to be higher in the HI trial compared to the LO trial at post-exercise (p=0.060). There was no difference between trials at the other sample points (p>0.05). In the HI trial post-exercise reported sensations of mouth dryness were greater than baseline and during the recovery period (p<0.0001). After 5 minutes of the recovery period, reported sensations of mouth dryness remained greater compared to 15, 30 and 60 minutes of the recovery period

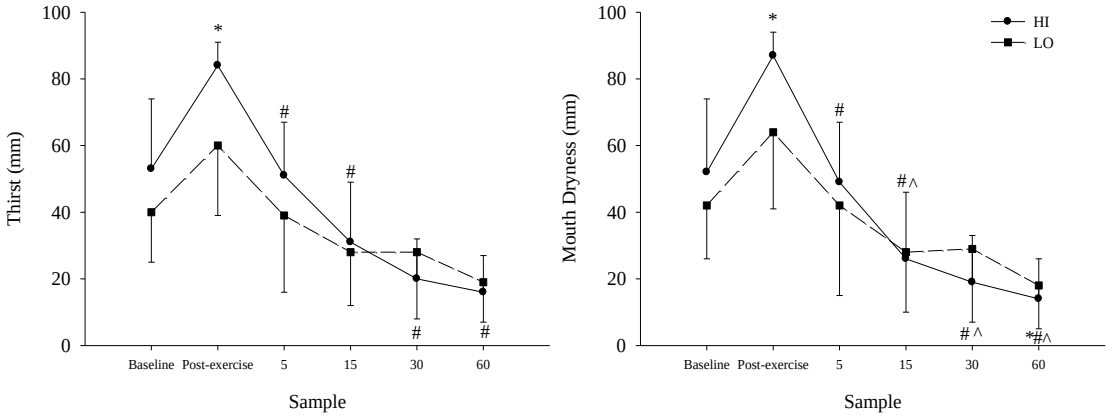

Figure 6.10. Sensations of thirst (a) and mouth dryness (b) over the duration of each trial. * different to baseline in HI trial, # different to post exercise in HI trial, ^ different to 5min in HI trial (p<0.05).

($p<0.0001$). After 60 minutes of recovery reported sensations of mouth dryness had decreased below baseline values ($p=0.015$).

6.4.5 Correlations

In the HI trial serum sodium concentrations were positively correlated to blood lactate concentrations and serum osmolality (Table 6.1). Serum osmolality was positively correlated to blood lactate concentrations, vasopressin and aldosterone concentrations and sensations of thirst and mouth dryness. Vasopressin concentrations were positively correlated to blood lactate concentrations but there was no correlation with aldosterone concentrations. In the LO trial correlations were not found between serum osmolality, serum sodium concentrations, blood lactate concentrations and vasopressin and aldosterone concentrations.

Table 6.1. Correlation coefficients between measured variables in each trial. * denotes significant ($p<0.05$).

		HI		LO	
Variables		**r**	**p**	**r**	**p**
Serum osmolality	Serum sodium concentration	0.470	0.001*	0.223	0.142
	Blood lactate concentration	0.655	<0.0001*	0.170	0.265
	Vasopressin concentration	0.661	<0.0001*	-0.195	0.320
	Aldosterone concentration	0.545	0.003*	0.373	0.050
	Thirst	0.419	0.004*	0.293	0.051
	Mouth dryness	0.411	0.005*	0.211	0.165
Serum sodium concentration	Blood lactate concentration	0.608	<0.0001*	0.201	0.184
	Vasopressin concentration	0.663	<0.0001*	0.131	0.506
	Aldosterone concentration	0.415	0.028*	0.207	0.506
	Thirst	0.521	<0.0001*	0.264	0.079
	Mouth dryness	0.552	<0.0001*	0.243	0.108
Blood lactate concentration	Vasopressin concentration	0.657	<0.0001*	-0.307	0.112
	Aldosterone concentration	0.476	0.010*	-0.096	0.628
	Thirst	0.518	<0.0001*	0.270	0.073
	Mouth dryness	0.468	0.001*	0.265	0.078
Vasopressin concentration	Aldosterone concentration	0.317	0.100	-0.119	0.547
	Thirst	0.517	0.005*	-0.242	0.214
	Mouth dryness	0.517	0.004*	-0.270	0.164
Aldosterone concentration	Thirst	0.226	0.248	0.309	0.110
	Mouth dryness	0.159	0.419	0.262	0.177
Thirst	Mouth dryness	0.976	<0.0001*	0.972	<0.0001*

6.4.6 Core and skin temperature

Core temperature was greater at 30, 40, 50 and 60 minutes of the exercise period and remained elevated after the first 10 minutes of the recovery period in the HI trial compared to the LO trial ($p<0.05$) (Figure 6.11a). In the HI trial, core temperature was elevated above final baseline values after 20 minutes of the exercise period and remained elevated above baseline until 10 minutes of the recovery period ($p<0.05$). During the final 30 minutes of the exercise period, core temperature was higher than the final 50 minutes of the recovery period ($p<0.05$). Core temperature continually increased at each 10 minute time point from 10 minutes of the exercise period until a plateau was reached after 50 minutes of exercise, before there was a continual decrease throughout the recovery period ($p<0.05$). In the LO trial during the exercise period, core temperature increased from 10 to 40 minutes before a plateau was reached ($p<0.05$). Core temperature was higher than final baseline values during the final 30 minutes of the exercise period, whilst core temperature values recorded after 50 and 60

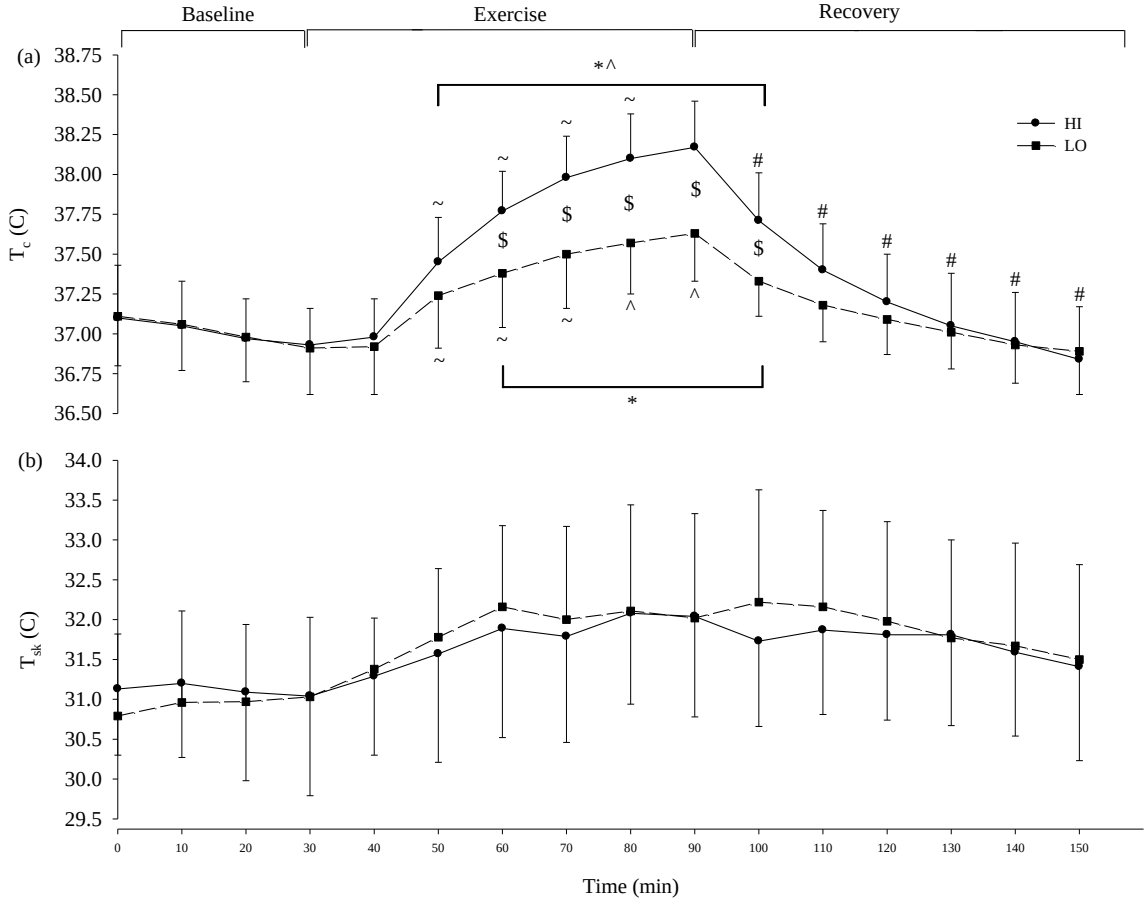

Figure 6.11. Core (a) and skin (b) temperature over the duration of each trial. $ different between trials, * different to baseline, ~ increase from previous time point, # decrease from previous time point, ^ different to 110-150 min ($p<0.05$).

minutes of the exercise period were higher than the final 40 minutes of the recovery period ($p<0.05$). Skin temperatures were similar between trials ($p>0.05$) (Figure 6.11b).

6.4.7 Heart rate, rating of perceived exertion and thermal sensation

During the exercise period of the trials, heart rate was significantly higher during the HI trial (158 ± 12 v 110 ± 10 beats.min^{-1}, $p<0.0001$), with differences occurring at 10, 20, 30, 40, 50 and 60 minutes of exercise. Heart rate remained higher in the HI trial after 10 (85 ± 8 v 62 ± 8 beats.min^{-1}, $p<0.0001$), 20 (77 ± 8 v 61 ± 7 beats.min^{-1}, $p<0.0001$), 30 (73 ± 4 v 60 ± 6 beats.min^{-1}, $p<0.0001$) and 40 (70 ± 6 v 60 ± 6 beats.min^{-1}, $p<0.0001$) minutes of the recovery period. Thermal sensation was higher after 20 (5 ± 1 v 3 ± 1, $p<0.0001$), 30 (6 ± 1 v 4 ± 1, $p<0.0001$), 40 (6 ± 1 v 4 ± 1, $p<0.0001$), 50 (6 ± 1 v 4 ± 1, $p<0.0001$) and 60 minutes (6 ± 2 v 4 ± 1, $p<0.0001$) of the exercise period in the HI trial. There was no difference in thermal sensations during the baseline and recovery periods ($p>0.05$). Ratings of perceived exertion were higher in the HI trial after 10 (14 ± 2 v 10 ± 2, $p=0.006$), 20 (15 ± 1 v 11 ± 2, $p<0.0001$), 30 (16 ± 2 v 11 ± 2, $p<0.0001$), 40 (16 ± 2 v 11 ± 3, $p<0.0001$), 50 (17 ± 2 v 11 ± 2, $p<0.0001$) and 60 minutes (17 ± 2 v 11 ± 2, $p<0.0001$) of the exercise period.

6.5 Discussion

In the present study HIIE resulted in increased voluntary water intake during a 60 minute recovery period compared to a bout of continuous exercise matched for work performed. In addition there was also increased serum osmolality, blood lactate, serum sodium and plasma vasopressin concentrations following the period of HIIE.

Voluntary water intake was greater during the recovery period after HIIE. The increase was apparent during the first 30 minutes following exercise. Despite the greater water intake, body mass loss was similar between trials primarily due to the increased sweat loss experienced in the HI trial. It appeared that the increased water intake could be mainly related to the increased water losses. The body mass loss experienced in each trial did not reach the much outlined level of 2% (Sawka *et al.*, 2007), and would not have, with the exception of one subject, even without water intake, yet all subjects consumed water. This raises the question of the necessity of water intake in the current study. In the ACSM position statement on water replacement, Sawka *et al.* (2007) suggested consuming 150% of the volume of water lost when rapid water replacement is required (less than 12 hours) following excessive dehydration. When excessive dehydration is not apparent, then euhydration can be restored through normal meals and snacks with sufficient volumes of plain water. As dehydration in the current study was not excessive, water intake could be considered largely unnecessary. When combined with the normal meals, snacks and water likely to be consumed during the remainder of the day following the trial that should restore euhydration, this could lead to overdrinking, which in turn could lead to increased urine output (Shirreffs & Maughan, 1998; Wong *et al.*, 1998). This may not only, act as an inconvenience through increased frequency of urination, but may result in increased water losses. In the present study, there was no difference in urine output at the end of the trial, however, over a longer period of time, with increased opportunity to drink, this may have become different. Wong *et al.* (1998) found that following a four hour recovery period with *ad libitum* CHO-E replacement, only 43% of water had been retained due to urine losses despite replacing almost 100% of water lost from a 90 min run at 70%$\dot{V}O_{2max}$. In extreme cases, excessive water consumption may result in hyponatraemia (Almond *et al.*, 2005), however in the current study, water intake volumes, in conjunction with

serum sodium concentrations, did not appear to suggest that subjects were at risk from hyponatraemia.

Examining the individual response to water intake there was a wide range in the amount of water replaced with ranges of 12-104% in the HI trial and 0-94% in the LO trial, indicating that water intake replacement was highly variable. The replacement of water helped alleviate thirst sensations in the HI trial indicated by the reduction in reported sensations of thirst following the onset of the water intake period. This adds strength to the notion that when *ad libitum* water is allowed individuals consume sufficient amounts to alleviate sensations of thirst despite not replacing all of the water lost during exercise.

The osmolality threshold for sensations of thirst to be experienced has been suggested as a value greater than 290 mOsmol.kg^{-1} (Phillips *et al.*, 1985) and may provide explanation as to why subjects consumed water despite body mass losses not greater than 2%. In the HI trial, osmolality values decreased below this threshold value between 15 and 30 minutes of the recovery period, whilst in the LO trial, osmolality values did not increase above 290 mOsmol.kg^{-1} throughout the trial. In the HI trial sensations of thirst had decreased from peak post-exercise samples after 5 minutes of the recovery period returning to sensation levels experienced during the baseline period, this despite osmolality values still being elevated above threshold values after 5 and 15 minutes of the recovery period. It appears that the premise of a threshold for thirst works only to initiate drinking and once this has occurred then the effect of the threshold for thirst is diminished. As a result, water intake during the final 30 minutes of the HI trial was similar to the LO trial. It has been shown that satiation of water intake occurs quickly following an initial bout of drinking (Rolls *et al.*, 1980). A greater breakdown of the drinking period would have allowed greater dissection of water intake behaviour in response to exercise, particularly during the first 5 minutes of the recovery period.

It was hypothesised that water intake in the HI trial would be increased and due to increased serum osmolality values, intake would be above what may have been typically expected for a similar exercise period without HIIE. There was concern that the result would be that of increased water intake above what was required due to

inflated osmotic signals driving increased sensations of thirst. As mentioned, the amount of water replaced was generally less than 100% in the HI trial, however only one subject would have exhibited body mass losses greater than 2% without water intake, and therefore it would appear that any water intake was unnecessary. With elevated serum osmolality levels there may still be cause for concern that the resultant water intake will be increased further. Over a protracted period of time, particularly if water intake was permitted during exercise where it would be expected to see osmolality levels rise following intermittent satiety of sensations of thirst, the increased signals causing a desire to drink may lead to increased water intake and subsequent increased urine output. It appears that the human body is well versed in regulating water homeostasis following HIIE but in extreme cases it may be beneficial to monitor at an individual level.

The increase in serum osmolality following HIIE has been attributed to the effect that increased blood lactate concentrations have on serum sodium concentrations (Nose *et al.*, 1991). It has been hypothesised that the efflux of sodium ions from the vascular space to the intracellular space is reduced by the negatively charged lactate ions produced following HIIE, thus causing an increase in serum sodium concentration and subsequently, serum osmolality values. In the current study the difference in serum osmolality at post-exercise between trials was approximately 10 $mOsmol.kg^{-1}$, whilst the difference in serum sodium concentration was approximately 3 $mmol.l^{-1}$. Using the formula assessed by Worthley *et al.* (1987) (Serum osmolality = $2[Na^{+}]$ + [BUN] + [Glucose] + [lactate] where BUN is blood urea nitrogen), the change in serum osmolality was not completely accounted for by the change in serum sodium concentration. Therefore it appeared that the change in blood lactate concentration was a direct contributing factor to the increase in serum osmolality (contribution of 4 $mOsmol.kg^{-1}$) as blood glucose concentration did not change and BUN would likely have little contribution. At later samples, during the recovery period the effect of blood lactate concentration on serum osmolality values became more apparent. Serum sodium concentrations between trials during the recovery period were similar despite serum osmolality values being greater in the HI trial at 5 and 15 minutes of the recovery period. Blood lactate concentrations remained different between trials throughout the recovery period. The r^2 values of 0.43 and 0.22 between serum osmolality and blood lactate concentrations and between serum osmolality values and serum sodium

concentrations respectively provided further information regarding the amount of variation in serum osmolality that could be explained by each variable. The effect of blood lactate concentrations on serum osmolality values would have contributed to the osmotically driven release of vasopressin and potentially helped contribute to the increased water intake in the HI trial (Phillips *et al.*, 1985).

As mentioned previously the onset of thirst sensations to stimulate water intake occurs when serum osmolality values are greater than approximately 290mOsmol.kg^{-1} (Phillips *et al.*, 1985) but prior to this vasopressin is released from the posterior pituitary gland to increase water reabsorbtion in the kidneys (Bankir, 2001). The threshold of vasopressin release has been outlined at approximately 285 mOsmol.kg^{-1} and increases until maximum anti-diuresis has been reached (Thompson *et al.*, 1986). In the current study there was a large increase in plasma vasopressin concentration following the high intensity exercise when serum osmolality values were above the reported threshold value. Vasopressin concentration remained elevated, or had a tendency to remain elevated, above baseline values at all sample points measured in the HI trial and this was consistent with serum osmolality levels remaining above the threshold value outlined. Vasopressin concentration has been widely shown to decrease quickly (2.5 – 15 minutes) following initiation of drinking (Burrell *et al.*, 1991; Figaro & Mack, 1997; Geelen *et al.*, 1984; Seckl *et al.*, 1986), however within these studies serum osmolality has decreased at a similar rate (Burrell *et al.*, 1991) and at a slightly delayed rate (30-60 minutes) (Seckl *et al.*, 1986). Whilst vasopressin concentration remained elevated in the present study, the accompanying decreases in thirst sensations after the initial post-exercise peak, and along with the decrease in water intake during the final 30 minute period, would indicate that the increased blood lactate concentration and serum osmolality relationship may have had an effect on maintaining vasopressin concentrations.

In the current study, linear regression showed that for every change in osmolality of 1 mOsmol.kg^{-1} there was a change in plasma vasopressin concentration of 0.4 pg.ml^{-1}. This is consistent with previous research (Thompson *et al.*, 1986; Verbalis, 2007); however results were calculated based on increasing vasopressin concentrations with concomitant increases in serum osmolality. Assessing the increased vasopressin concentration compared to baseline values, the two-fold increase was similar to that of

Hew-Butler *et al.* (2008), however, the four-fold increase experienced by those authors was slightly larger and may have been attributed to two possible anomalous results that appear to be present in their data set. As in the present study, Hew-Butler *et al.* (2008) found that the increase in vasopressin concentration following exercise was accompanied by a similarly disproportionately small increase in serum sodium concentrations. This was attributed to either an enhanced osmotic response to high intensity exercise and/or the effect of non-osmotic stimuli.

It was expected that plasma aldosterone concentrations would be increased in the HI trial following exercise, particularly when an increase in vasopressin concentrations and serum osmolality values and a greater decreases in plasma volume was experienced post-exercise. Previous research has shown a threefold increase in aldosterone concentration following high intensity exercise (Hew-Butler *et al.*, 2008), however, it was difficult to determine why the expected change in aldosterone did not occur in the current study, but possibly may be explained by the large variability between subjects. All subjects had an increase in aldosterone concentrations following exercise but in the smallest response an increase from 28 to 91 $pg.ml^{-1}$ was found whilst the largest increase in aldosterone concentration was from 644 to 2738 $pg.ml^{-1}$, both similar to the magnitude of increase seen by Hew-Butler *et al.* (2008).

In an attempt to match work performed during the exercise period, subjects cycled at a set work rate designed to elicit the same average power output over the duration of the exercise period. Despite this, sensations of thirst and mouth dryness were higher at the end of exercise and average RPE, thermal comfort and heart rate values were higher during the exercise period in the HI trial. It was possible that working at a more intense effort for short periods increased mouth dryness through an increase in breath frequency, and therefore stimulating greater water intake to satisfy these sensations. Brunstrom *et al.* (2000) found an increase in water intake when cotton wool balls were placed in the mouth to absorb salivary output following 20 min of self selected exercise. As mentioned previously, a greater breakdown of the water intake periods would allow for improved understanding of the effect of satiety of mouth dryness on drinking behaviour.

During the recovery period it appeared that two main variables were influencing the decrease in serum osmolality from post-exercise peak values. Both the clearance and reduced production of blood lactate concentration and the intake of water will both contribute to decreasing serum osmolality values. As the effect of no water intake was not assessed, determining the contributing effect of each variable is difficult. Future work would need to examine the effect of blood lactate clearance on serum osmolality values when water intake is prevented and also when delayed. This would help assess the impact of blood lactate concentration on variables influencing drinking initiation.

6.6 Conclusion

In conclusion, water intake following a period of HIIE was greater than an exercise period of low intensity continuous exercise performing the same average power output. The increased water intake in the HI trial was mainly attributed to the increased water losses. In addition, the result of an increase in serum osmolality and subsequent vasopressin release caused by an increased blood lactate concentration in combination with an increased serum sodium concentration may have also contributed to the increased water intake. Further work is required to assess the relative effect of water intake and/or decreased blood lactate concentrations on serum osmolality levels.

CHAPTER 7

The effect on serum osmolality following high intensity intermittent exercise when voluntary water intake was allowed, delayed and not allowed

7.1 Abstract

Following a period of high intensity intermittent exercise (HIIE) there is an increase in serum osmolality caused by an increase in blood lactate concentrations, serum sodium concentrations and decreased plasma volumes resulting in a subsequent increase in vasopressin concentrations. The individual effect of either voluntary water intake or reduced blood lactate concentrations on serum osmolality has not been assessed. The aim of the study was to examine serum osmolality after HIIE during a recovery period when *ad libitum* water intake was allowed, delayed and prevented. Twelve males (age 26 ± 4 years, mass 80.1 ± 9.3 kg, height 1.81 ± 0.05 m, $\dot{V}O_{2peak}$ 60.1 ± 8.9 ml.kg^{-1}.min^{-1}) participated in three trials (7 – 14 days apart) following familiarisation. Subjects sat for 30 min then completed an exercise period involving 2 mins of rest followed by 1 min at 100% $\dot{V}O_{2peak}$ repeated until 60 min had passed (a total of twenty 1 min bouts) followed by 60 min of recovery in which *ad libitum* water intake was either allowed for 60 min (W), after 30 min for 30 min (W30) or not allowed (NW). Body mass was measured at the start and end of the trial. Serum osmolality, blood lactate and serum sodium concentrations and sensations of thirst and mouth dryness were measured at baseline, post-exercise and after 5, 15, 30, 35 and 60 min of the recovery period. Plasma volume changes were calculated relative to baseline. Vasopressin and aldosterone concentrations were measured at baseline, post-exercise and after 30 and 60 min of the recovery period. Body mass loss over the duration of the trial was different between all trials (0.25 ± 0.45 v 0.49 ± 0.37 v 1.29 ± 0.37%) ($p<0.05$). Total voluntary water intake was greater in the W trial compared to W30 (0.846 ± 0.417 v 0.630 ± 0.277 l) ($p<0.05$) but water intake during the first 30 min period of allowed drinking was similar (0.618 ± 0.297 v 0.630 ± 0.277 l) ($p>0.05$). Serum osmolality (299 ± 6 v 298 ± 5 v 298 ± 3 mOsmol.kg^{-1}), blood lactate (7.1 ± 1.1 v 7.2 ± 1.1 v 7.1 ± 1.2 mmol.l^{-1}) serum sodium (142 ± 2 v 145 ± 2 v 145 ± 2 mmol.l^{-1}) and vasopressin (8.27 ± 2.61 v 7.17 ± 2.25 v 7.13 ± 2.16 pg.ml^{-1}) and aldosterone (1229 (106-4001) v 652 (91-4503) v 1326 (113-4967) pg.ml^{-1}) concentrations peaked post-exercise in each trial (W v W30 v NW) ($p<0.05$) but were not different between trials across all sample points ($p>0.05$). Sensations of thirst and mouth dryness decreased in the W and W30 trials following water ingestion ($p<0.05$) but remained at peak post-exercise values throughout the recovery period in the NW trial. Plasma volume had been restored to baseline by 15 min after exercise in each trial. Serum osmolality decreased at the same rate during the

recovery period suggesting a reduction in blood lactate and serum sodium concentrations, restoration of plasma volume, reduction in hydrostatic pressure in the capillary beds and water intake will all have contributed.

7.2 Introduction

Following a period of high intensity exercise water has been shown to shift from the vascular to the interstitial and intracellular spaces (Convertino *et al.*, 1981; Nose *et al.*, 1991; Sjøgaard *et al.*, 1985). In addition, high intensity intermittent exercise (HIIE) often results in an increase in blood lactate concentration either through reduced clearance, increased production or a combination of both (Pilegaard *et al.*, 1999). The increase in blood lactate concentration has been positively related to an increase in serum osmolality levels and subsequent vasopressin release (Hew-Butler *et al.*, 2008; Nose *et al.*, 1991; Sjøgaard *et al.*, 1985). It has been hypothesised that the negatively charged lactate ions reduce sodium release from the vascular space thus increasing serum sodium concentrations and subsequent osmolality levels (Nose *et al.*, 1991).

An increase in serum osmolality levels has been shown to cause an increase in the release of the water regulatory hormone arginine vasopressin (Verbalis, 2007). Vasopressin release tends to increase when osmolality levels are greater than 285 $mOsmol.kg^{-1}$ (Thompson *et al.*, 1986). In addition, above an osmolality value of approximately $290mOsmol.kg^{-1}$, a threshold for thirst sensation has been suggested, causing a desire to drink (Phillips *et al.*, 1985). The threshold for thirst has been suggested when osmolality values are influenced through changes in cell tonicity, however, the impact on thirst and drinking behaviours have not been assessed when osmolality levels are influenced by other parameters such as changes in blood lactate concentrations.

Data from Chapter 6 of this thesis suggested that following high intensity intermittent exercise there was an increase in *ad libitum* water intake compared to continuous exercise. However, as *ad libitum* water intake was permitted immediately after exercise it was difficult to ascertain the relative influence that both drinking and the combination of reduced blood lactate and serum sodium concentrations and plasma volume restoration had on subsequent changes in serum osmolality and drinking behaviours. Therefore, the aim of this study was to restrict the time *ad libitum* water was allowed during a recovery period following HIIE to assess voluntary water intake and associated blood parameters. It was hypothesised that water intake would be greater immediately after exercise compared to when there was a delay in allowing *ad libitum* water intake,

and that *ad libitum* water intake during the whole recovery period would cause a greater decrease in serum osmolality compared to when *ad libitum* water intake was delayed or not allowed.

7.3 Methods

7.3.1 Subjects

Twelve healthy male subjects (age 26 ± 4 years, mass 80.1 ± 9.3 kg, height 1.81 ± 0.05 m, $\dot{V}O_{2peak}$ 60.1 ± 8.9 ml.kg^{-1}.min^{-1}) were recruited to take part in three experimental trials, undertaken in a randomised order. All subjects had the experimental protocol explained to them verbally and in writing. Subjects provided written informed consent and the experiment was approved by the Loughborough University Ethical Advisory Committee (R11-P45).

7.3.2 Experimental protocol

Subjects were asked to visit the laboratory on five separate occasions for a $\dot{V}O_{2peak}$ test, a familiarisation trial and three experimental trials differing by the time period during which *ad libitum* water intake was allowed following exercise; water immediately after exercise until the end of the recovery period (W), water 30 minutes after exercise until the end of the recovery period (W30) and no water after exercise during the recovery period (NW).

During the first visit $\dot{V}O_{2peak}$ was measured (details in Chapter 2). Subjects visited the

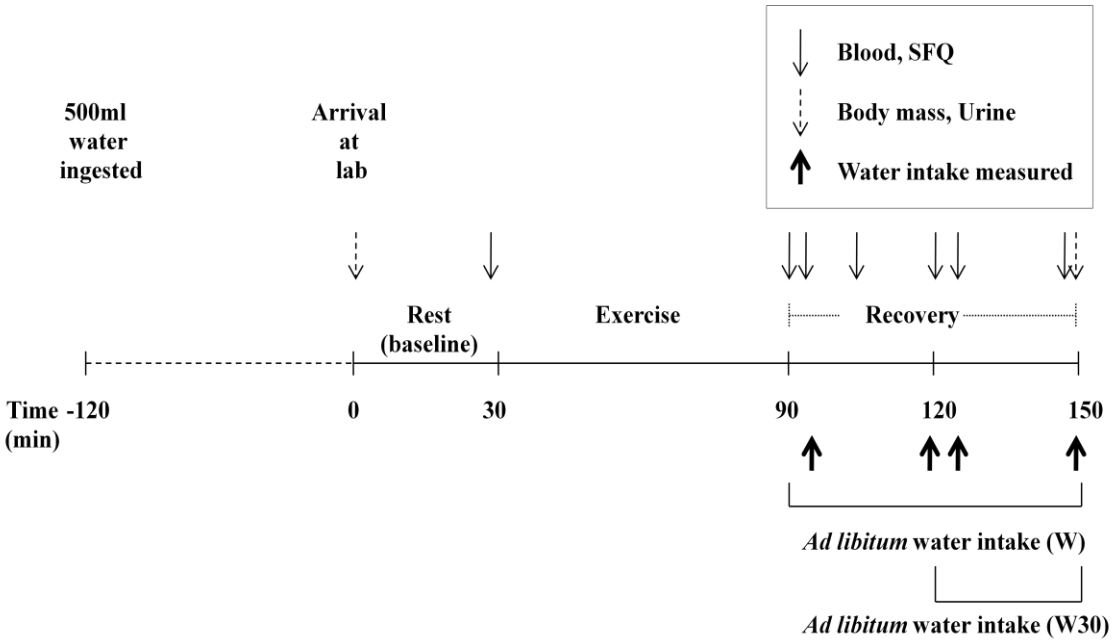

Figure 7.1. Schematic diagram indicating the testing protocol. Arrows represent sampling points. SFQ denotes subjective feelings questionnaire.

lab a further four times for a familiarisation and three experimental trials. The first of these acted as a familiarisation and was identical to the W trial. Subjects were asked to follow the pre-trial standardisation procedure outlined in Chapter 2.

The experimental trials were separated by a period of 7-14 days and began in the morning at the same time for each subject. Experimental trials were identical apart from the *ad libitum* water intake following each exercise period. A schematic outline of the trial is shown in Figure 7.1. Experimental trial order was created by incomplete Latin square design and subjects did not know which trial they were participating in when arriving at the laboratory for the first and second experimental trials.

In each trial, on arrival, subjects voided and the whole volume was measured and a 5 ml sample retained for later analysis and had nude body mass measured. Subjects were asked to insert a rectal thermistor 10 cm past the anal sphincter to measure core temperature and skin thermistors and a heart rate monitor were positioned (see Chapter 2 for details). Subjects sat for 30 minutes at 22.3 ± 0.4°C and 47.5 ± 9.3% relative humidity (RH). Baseline heart rate values every 10 minutes were recorded and a 100 mm visual analogue subjective feelings questionnaire comprising of thirst and dry mouth scales was administered at the completion of the 30 minutes seated rest (0 mm = not all thirsty/mouth not at all dry, 100 mm = very thirsty/mouth very dry) (Appendix E). During the baseline period a 21 g cannula (Surflo, Terumo, Leuven, Belgium) was inserted into a superficial vein on the forearm to allow venous blood sampling. The line was flushed with 2-3 ml of heparinised saline. A baseline blood sample (7.5 ml) was collected at the end of the rest period. Subjects then had an exercise period of 60 minutes in 23.0 ± 0.4°C and 48.4 ± 10.2% RH. In the each trial they rested for 2 minutes and then performed 1 minute of cycling at a power output equal to the maximum power achieved when recording $\dot{V}O_{2peak}$. This was repeated 20 times during the 60 minute period. Heart rate, rating of perceived exertion and thermal sensation were reported every 10 minutes. Immediately following completion of exercise a blood sample (7.5 ml) was collected and thirst and dry mouth subjective feelings questionnaires were completed. Subjects were then seated for 60 minutes in 22.7 ± 0.3°C and 47.5 ± 10.5% RH. In the W trial *ad libitum* water intake (10 ± 3°C) was allowed for the whole duration of the recovery period with measurements of water

intake taken between 0-5 minutes, 5-30 minutes and 35-60 minutes. In the W30 trial, *ad libitum* water intake was delayed until 30 minutes of the recovery period had passed. Water intake was then measured between 30-35 minutes and 35-60 minutes. In the NW trial no water was allowed during the recovery period. The subject was not made aware of the volume or that the volume was being measured. They were provided with no external cues to drink and informed at the start that they could drink as they wanted and that the bottle would be refilled if necessary. HR and thermal sensation were measured every 10 minutes. At 5, 15, 30, 35 and 60 minutes a blood sample (7.5 ml) was collected and thirst and dry mouth subjective feelings questionnaires were completed. At the end of the recovery period following the final blood sample, subjects voided, the volume was measured and a 5 ml sample was retained for later analysis and they then had nude body mass measured. After completion of the body mass measurement, subjects were allowed to leave the laboratory. Ambient temperature and relative humidity was measured at 10 minute intervals (RH85 Digital Thermo-Hygrometer; Omega, Manchester, UK).

7.3.3 Sample analysis

Blood was analysed for haemoglobin concentration, haematocrit, blood glucose concentration, plasma volume change, blood volume change, red blood cell volume change, serum sodium and potassium concentration, serum osmolality and vasopressin and aldosterone concentration (see Chapter 2 for details).

The total volume of each urine sample was measured and a 5 ml sample was retained and analysed for osmolality and sodium and potassium concentration (see Chapter 2 for details).

7.3.4 Statistical analysis

Data were checked for normality of distribution using Shapiro-Wilks tests. All samples were normally distributed and subsequently either paired samples t-tests or repeated measures ANOVA was performed and if a significant ANOVA result was found, a paired samples t-tests with Bonferroni correction were performed to identify where the statistical differences occurred. Non-parametric data were examined using Friedman's

ANOVA and Wilcoxon signed-rank tests. Post-hoc tests were performed when significant and non-significant interaction effects were found. Linear regression values and Pearson's product moment correlation coefficients and Spearmon's ranked correlation coefficients were calculated when appropriate. Correlation analysis performed between variables that were deemed to be related in terms of water balance and the mechanism identified by Nose *et al.* (1991) (Serum osmolality/serum sodium/blood lactate). Statistical significance was accepted when $p<0.05$. When post-hoc tests were conducted, p values presented were multiplied to correct for repeated samples. Parametric data is expressed as mean ± SD and non-parametric data as median (range). Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point.

7.4 Results

7.4.1 Baseline measures

There was no difference in body mass measured at baseline between trials (Table 7.1) ($p>0.05$). Serum osmolality (284 ± 3 v 284 ± 3 v 285 ± 3 mOsmol.kg^{-1} for W, W30 and NW respectively) and urine osmolality (409 ± 221 v 434 ± 256 v 454 ± 238 mOsmol.kg^{-1} for W, W30 and NW respectively) ($p>0.05$) were similar between trials indicating subjects arrived in a similar state of hydration which could be interpreted as euhydrated (Sawka *et al.*, 2007).

7.4.2 Water balance

Water balance variables are presented in Table 7.1. Body mass decreased from the start to the end of the trial in the W30 and NW trial ($p<0.05$), whilst percentage decrease in body mass was greater in the W30 and NW trial compared to the W trial and greater in the NW trial compared to the W30 trial ($p<0.05$). One subject in the NW trial lost greater than 2% of their body mass, whilst in the W and W30 trials, if water intake was removed no subject would have experienced a 2% body mass loss. Sweat loss and post-exercise urine output were similar between trials ($p>0.05$).

Total water intake was greater in the W trial compared to the W30 trial ($p=0.009$) (Figure 7.2). In the W trial, less water was consumed between 30-35 minutes compared to between 0-5, 5-30 and 35-60 minutes ($p<0.05$). Water intake was similar between 0-5 and 5-30 minutes and between 0-5 and 35-60 minutes ($p>0.05$) but more water was consumed between 5-30 minutes compared to 35-60 minutes ($p<0.05$). The first two initial periods of drinking were compared between the W and W30 trial. There was no difference between water consumption during 0-5 minutes in the W trial and 30-35 minutes in the W30 trial and between 5-30 minutes in the W trial and 35-60 minutes in the W30 trial ($p>0.05$). The amount of water lost through sweating that was replaced was greater in the W trial compared to the W30 trial ($p=0.08$). Four subjects replaced greater than 100% of water lost in the W trial.

Table 7.1. Body mass (BM) and water variables for all trials. † denotes pre to post measurement difference, * denotes different to W trial, # denotes different to W and W30 trials ($p<0.05$). BM = body mass.

Trial	Pre BM (kg)	Post BM (kg)	BM change (%)	Water intake (l)	Sweat loss (l)	Urine Output (l)	Water replaced (%)
W	79.9 ± 8.9	79.7 ± 8.9	-0.25 ± 0.54	0.85 ± 0.42	0.83 ± 0.18	0.21 ± 0.10	87 ± 37
W30	79.8 ± 8.9	79.4 ± 8.9†	-0.49 ± 0.37	0.63 ± 0.28*	0.82 ± 0.23	0.21 ± 0.08	60 ± 26*
NW	80.2 ± 9.0	79.2 ± 9.0†*	-1.29 ± 0.37#	-	0.84 ± 0.24	0.21 ± 0.10	-

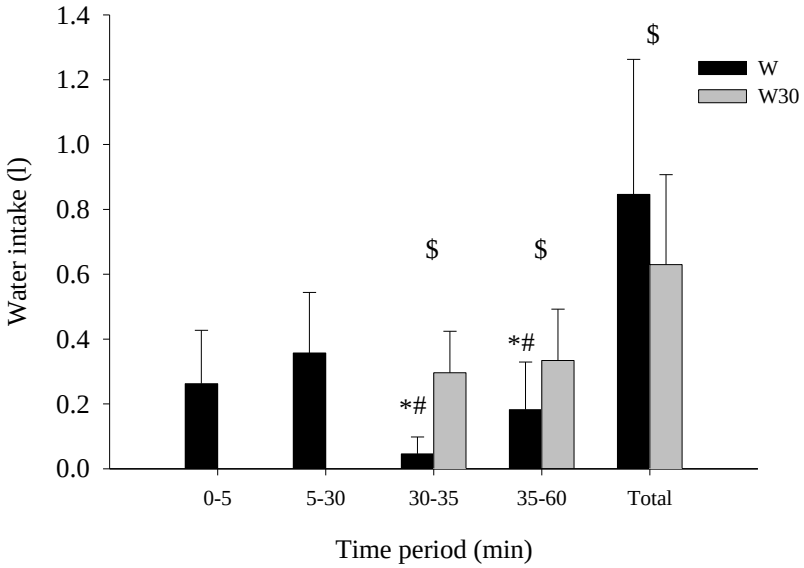

Figure 7.2. Water intake (l) during each trial. Comparison between W and W30 trials. $ denotes difference between trials, * denotes difference to 0-5 min (W) and # denotes different to 5-30 min (W) ($p<0.05$).

No difference in serum osmolality was found between trials ($p>0.05$) (Figure 7.3). In the W trial osmolality was elevated above baseline post-exercise and after 5 and 15 minutes of the recovery period ($p<0.05$) before returning to baseline values. Peak serum osmolality occurred at post-exercise and was greater than all other sample points ($p<0.0001$). After 5 minutes, osmolality was higher than at 15, 30, 35 and 60 minutes of the recovery period ($p<0.05$). Values at 60 minutes were lower than at 15 minutes ($p<0.05$). Similar results were found in the W30 trial except that osmolality at 15 minutes was different to baseline, 5 and 60 minutes ($p>0.05$). In the NW trial osmolality values were elevated above baseline at post-exercise and after 5 and 15 minutes of the recovery period ($p<0.05$) but had returned to baseline by 30 min. Post-

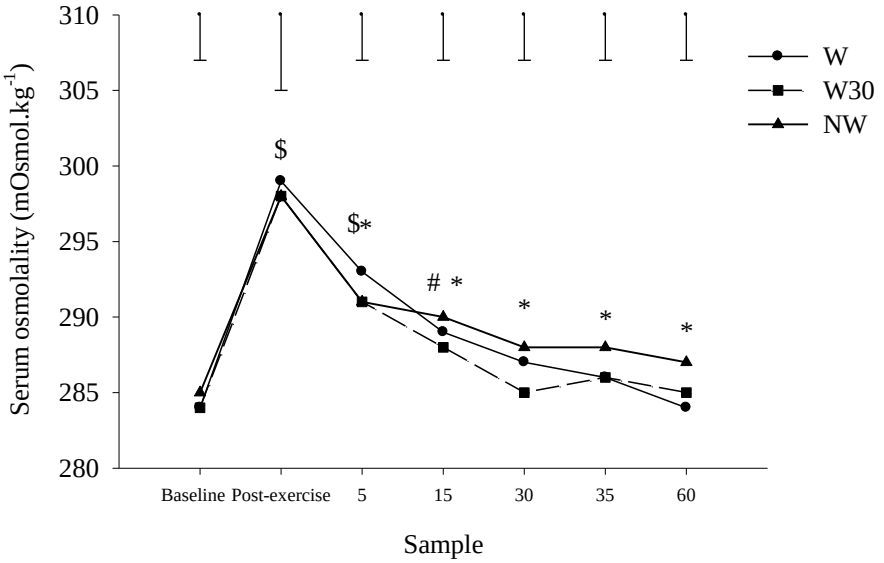

Figure 7.3. Serum osmolality over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. $ denotes different to baseline values in all trials, # denotes different to baseline values in W and NW trial, * denotes different to post-exercise values in all trials ($p<0.05$).

exercise osmolality was greater than all other samples in the recovery period ($p<0.0001$). Values at 60 minutes were lower than at 5 and 15 minutes ($p<0.05$).

Serum sodium concentrations were similar between trials ($p>0.05$) (Figure 7.4). In the W trial, post-exercise concentrations were elevated above baseline and recovery

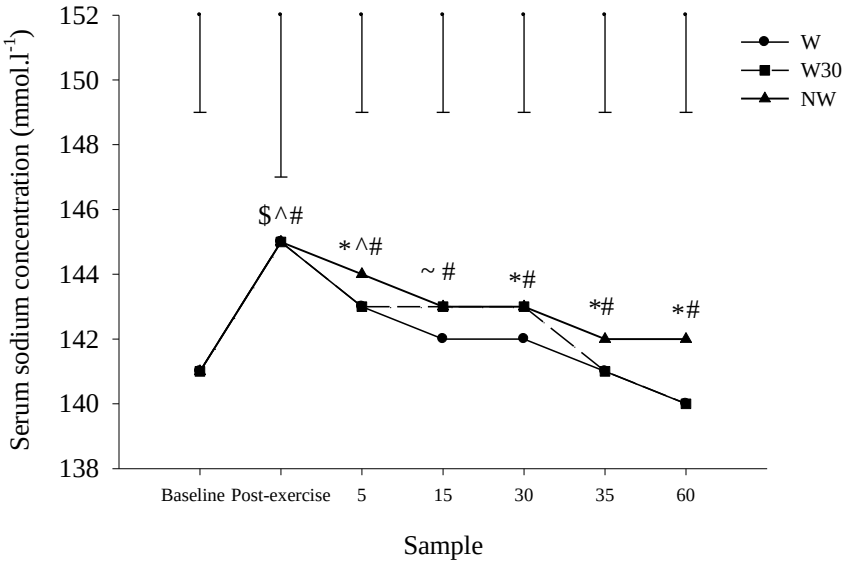

Figure 7.4. Serum sodium concentrations over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. $ denotes different to baseline values in W, ^ denotes different to baseline values in W30, # denotes different to baseline values in NW trial, * denotes different to post-exercise value in all trials, ~ denotes different to post-exercise value in W and NW trial ($p<0.05$).

samples ($p<0.05$). Following the start of the recovery period serum sodium concentrations returned to baseline. In the W30 trial post-exercise concentrations were greater than baseline, 5, 30, 35 and 60 minutes ($p<0.05$). By 15 minutes serum sodium concentrations had returned to baseline ($p>0.05$). Concentrations at 60 minutes of the recovery period were lower than after 5, 15 and 30 minutes ($p<0.05$). In the NW trial serum sodium concentrations increased from baseline levels after exercise and remained elevated during the recovery period ($p<0.05$). Post-exercise concentrations were higher than all recovery samples ($p<0.05$) and after 5 minutes concentrations were greater compared to 35 and 60 minutes ($p<0.05$).

Serum potassium concentrations were similar between trials and sample points (baseline: 4.3 ± 0.3 v 4.2 ± 0.3 v 4.3 ± 0.3 $mmol.l^{-1}$; post-exercise: 5.1 ± 0.4 v 4.9 ± 0.4 v 5.0 ± 0.3 $mmol.l^{-1}$; 5 min: 4.1 ± 0.2 v 4.2 ± 0.2 v 4.3 ± 0.5 $mmol.l^{-1}$; 15 min: 4.3 ± 0.3 v 4.3 ± 0.3 v 4.3 ± 0.3 $mmol.l^{-1}$; 30 min: 4.3 ± 0.2 v 4.3 ± 0.2 v 4.4 ± 0.5 $mmol.l^{-1}$; 35 min: 4.4 ± 0.2 v 4.2 ± 0.2 v 4.4 ± 0.3 $mmol.l^{-1}$ and 60 min: 4.3 ± 0.1 v 4.3 ± 0.2 v 4.4 ± 0.3 $mmol.l^{-1}$ for W, W30 and NW trials respectively) ($p>0.05$). In all three trials, serum potassium concentration was significantly elevated above baseline and recovery period samples at post-exercise ($p<0.05$) before returning to baseline levels during the recovery period.

7.4.3 Blood analysis

Haemoglobin concentrations were similar between trials ($p>0.05$) (Figure 7.5a). In the W trial haemoglobin concentration was greatest at post-exercise compared to baseline and all other recovery sample points after 5 minute of the recovery period ($p<0.0001$). After 15 minutes of the recovery period haemoglobin concentration was greater than at 30 and 60 minute ($p<0.05$). Haemoglobin concentration had returned to baseline concentrations after 15 min of the recovery period ($p>0.05$). Similar results were found in the W30 and NW trials.

Similar haematocrit values were found between trials ($p>0.05$) (Figure 7.5b). In the W trial, haematocrit was elevated at post-exercise above baseline and all recovery samples ($p<0.0001$). Values at 5 minutes were greater than at 15, 30, 35 and 60 minutes of the recovery period and after 15 minutes, haematocrit was higher compared to 30 and 35

minutes ($p<0.05$). Haematocrit had returned to baseline after 15 minutes of the recovery period ($p>0.05$). A similar pattern was observed in both the W30 and NW trials. In the NW trial, haematocrit returned to baseline after 15 minutes but then decreased further at 35 and 60 minutes ($p<0.05$).

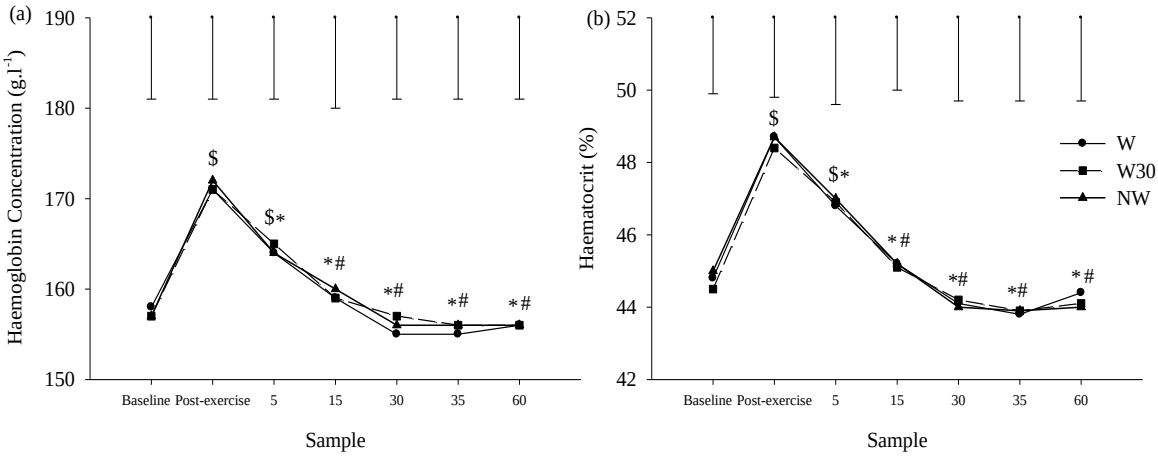

Figure 7.5. Haemoglobin concentrations (a) and haematocrit values (b) over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. $ denotes different to baseline values in all trials, * denotes different to post-exercise values in all trials, # denotes different to 5 min values in all trials ($p<0.05$).

Plasma volume changes from baseline were similar between trials at all sample points ($p>0.05$) (Figure 7.6). In all trials there was a decrease in plasma volume from baseline values at post-exercise and after 5 minutes of the recovery period ($p<0.05$) before plasma volume returned to baseline ($p>0.05$). The plasma volume change post-exercise compared to baseline was greater than all other sample points in all trials ($p<0.0001$), whilst the change from baseline to 5 minutes was greater than the remaining samples in the recovery period in all trials ($p<0.0001$). In all trials the change from baseline to 15 minutes of the recovery period was greater than the changes to 30, 35 and 60 minutes of the recovery period and the change from baseline to 30 minutes was greater than the changes from baseline to 35 and 60 minutes respectively ($p<0.05$). Blood volume changes from baseline values were similar between trials at all sample points except at 30 min when there was a decrease in blood volume in the W30 trial compared to an increase in the NW trial (Figure 7.7). Decreases from baseline values were observed in all trials at post-exercise and after 5 min of the recovery period ($p<0.0001$) before blood

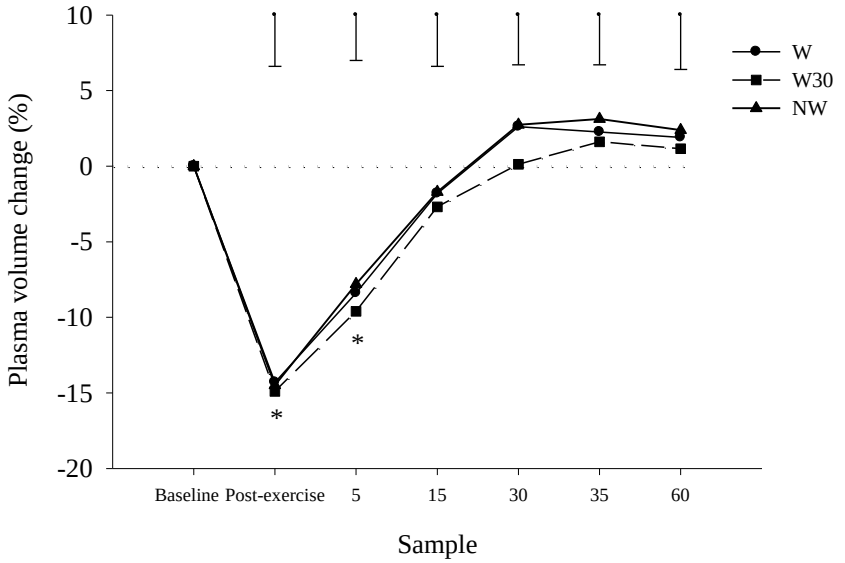

Figure 7.6. Plasma volume changes compared to baseline values over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. * denotes different to baseline values in all trials ($p<0.05$).

volumes returned to baseline values. Red blood cell volume changes compared to baseline were not different between trials (post-exercise: 0.2 ± 1.9 v -0.5 ± 1.0 v -0.8 ± 2.1%; 5 min: 0.2 ± 1.7 v -0.2 ± 1.0 v -0.3 ± 1.4%; 15 min: -0.1 ± 1.3 v -0.5 ± 1.1 v -0.9 ± 1.4%; 30 min: -0.2 ± 1.8 v -1.0 ± 1.4 v -1.1 ± 1.5%; 35 min: -0.5 ± 1.7 v -1.0 ± 1.1 v -1.4 ± 1.2% for W, W30 and NW trials respectively) ($p>0.05$) except at 60 min when an increase was observed in the W trial (0.4 ± 1.4%) compared to a decrease in the NW trial (-1.5 ± 1.2%) ($p<0.05$). In each trial there was no difference in red blood cell volume change from baseline ($p>0.05$) except in the NW trial when a decrease from baseline occurred at 35 and 60 min ($p<0.05$).

Blood lactate concentrations were similar between trials ($p>0.05$) (Figure 7.8). In the W trial, blood lactate concentration was significantly elevated above baseline at all sample points and a significant peak occurred post-exercise ($p<0.05$). There were significant decreases in blood lactate concentration at all sample points ($p<0.05$) except between post-exercise and 5 minutes and between 30 and 35 minutes ($p>0.05$). In the W30 and NW trials a similar response was found except that there was a significant decrease in blood lactate concentration from post-exercise to 5 minutes ($p<0.05$) and that in the W30 trial there was no difference in blood lactate concentration between 30 and 60 minutes ($p>0.05$).

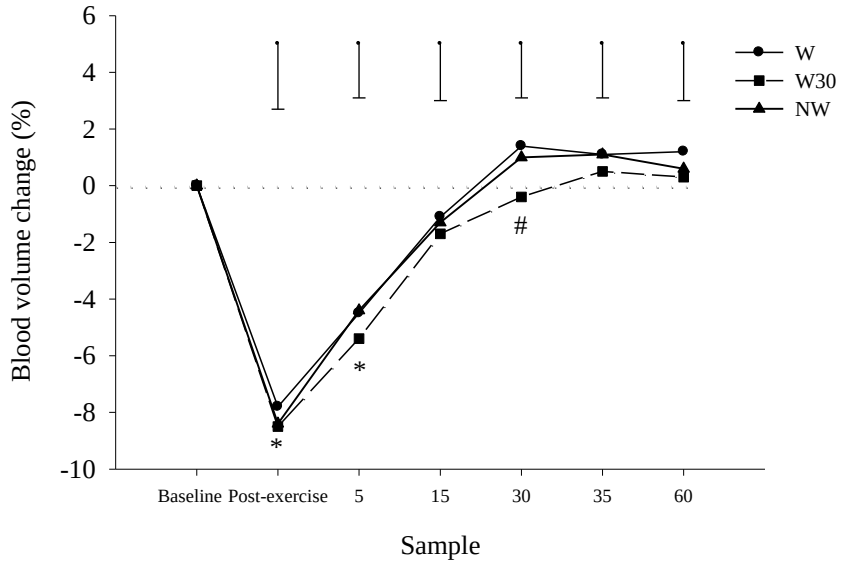

Figure 7.7. Blood volume changes compared to baseline values over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. * denotes different to baseline values in all trials, # denotes NW trial different to W30 ($p<0.05$).

Concentrations of vasopressin were similar between trials ($p>0.05$) (Figure 7.9a). In the W trial, vasopressin concentration was elevated above baseline concentrations at

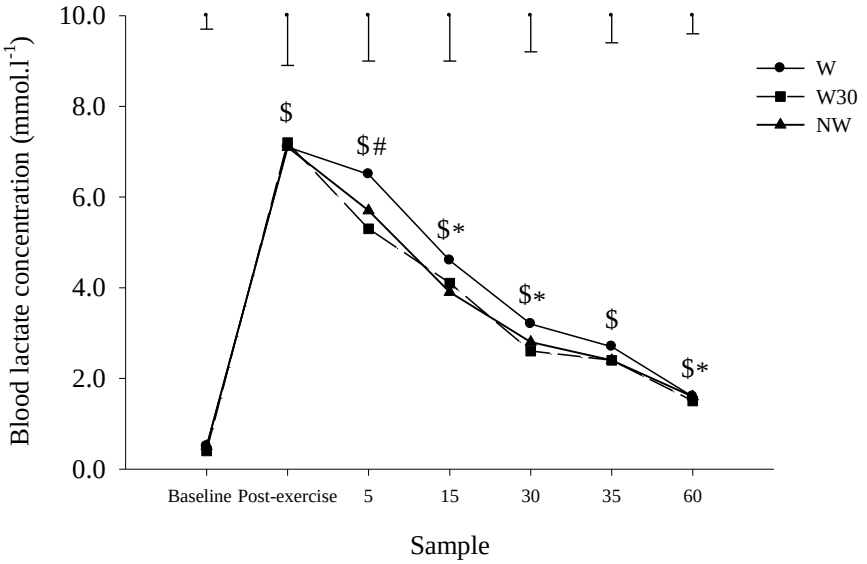

Figure 7.8. Blood lactate concentrations over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. $ denotes different to baseline values in all trials, * denotes decrease from previous sample in all trials and # denotes decrease from previous sample in W30 and NW trials ($p<0.05$).

post-exercise and after 30 and 60 minutes of the recovery period ($p<0.05$). Post-

exercise concentrations were also higher than concentrations found after 60 minutes of the recovery period ($p<0.05$). In the W30 trial baseline concentrations were lower than post-exercise concentrations and after 60 minutes of the recovery period ($p<0.05$). In the NW trial only post-exercise concentrations were higher than baseline ($p<0.05$). All other concentrations were similar ($p>0.05$).

Similar concentrations of aldosterone were found between trials ($p>0.05$) (Figure 7.9b). In the W trial, aldosterone concentration increased after exercise compared to baseline and remained elevated after 30 minutes of the recovery period before returning back to baseline ($p<0.05$). A decrease in aldosterone concentration was observed from post-exercise to the end of the trial ($p<0.05$). In the W30 trial, aldosterone concentration increased after exercise compared to baseline concentrations and remained elevated after 30 minutes of the recovery period before returning back to baseline ($p<0.05$). A decrease in aldosterone concentration was observed from post-exercise to 30 and 60 minutes of the recovery period ($p<0.05$). In the NW trial, aldosterone concentration was higher than baseline concentrations at post-exercise ($p<0.05$) before returning to baseline by 30 minutes of the recovery period ($p>0.05$). Aldosterone concentration was lower at the end of the trial compared to post-exercise concentrations and concentrations after 30 minutes of the recovery period ($p<0.05$).

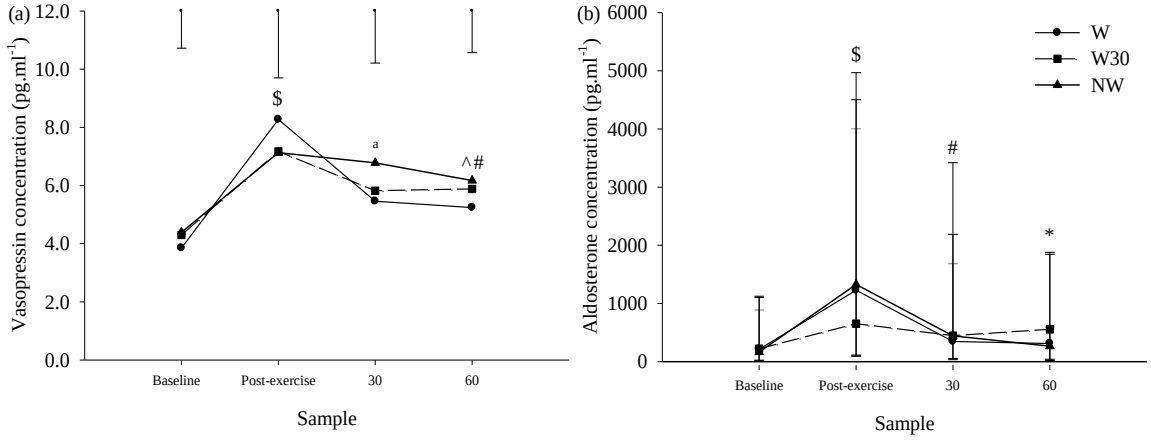

Figure 7.9. Vasopressin (a) (mean ± SD) and aldosterone (b) (median (range)) concentrations over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. $ denotes different to baseline values in all trials, * denotes different to post-exercise in all trials, # denotes different to baseline in W and W30, [a] denotes different to baseline in W and ^ denotes different to post-exercise in W ($p<0.05$).

Blood glucose concentrations were similar between trials and sample points (baseline: 4.21 ± 0.23 v 4.13 ± 0.20 v 4.32 ± 0.20 mmol.l^{-1}; post-exercise: 4.31 ± 0.34 v 4.28 ± 0.32 v 4.39 ± 0.47 mmol.l^{-1}; 5 min: 4.33 ± 0.38 v 4.26 ± 0.27 v 4.51 ± 0.54 mmol.l^{-1}; 15 min: 4.24 ± 0.33 v 4.21 ± 0.27 v 4.41 ± 0.40 mmol.l^{-1}; 30 min: 4.10 ± 0.21 v 4.22 ± 0.19 v 4.33 ± 0.31 mmol.l^{-1}; 35 min: 4.14 ± 0.30 v 4.19 ± 0.17 v 4.30 ± 0.23 mmol.l^{-1} and 60 min: 4.11 ± 0.28 v 4.09 ± 0.27 v 4.31 ± 0.35 mmol.l^{-1} for W, W30 and NW trials respectively) ($p>0.05$).

7.4.4 Urine analysis

Urine osmolality was similar between trials ($p>0.05$). In the W trial osmolality tended to increase from the start to the end of the trial (409 ± 221 v 554 ± 138 mOsmol.kg^{-1}) ($p=0.05$). In the NW trial there was an increase in urine osmolality at the end of the trial (454 ± 238 v 650 ± 168 mOsmol.kg^{-1}) ($p=0.003$), whilst there was no difference in the W30 trial (434 ± 256 v 577 ± 165 mOsmol.kg^{-1}) ($p=0.052$). Urine sodium concentration was similar between trials and between start and end concentrations in the W trial (66 ± 34 v 87 ± 34 mmol.l^{-1}; $p>0.05$) but was greater at the end of the trial compared to the start in both the W30 (61 ± 37 v 84 ± 33 mmol.l^{-1}; $p=0.012$) and NW trials (64 ± 33 v 89 ± 30 mmol.l^{-1}; $p=0.027$). Urine potassium concentrations were greater at the end of the trial compared to the start in the W (57 ± 28 v 87 ± 16 mmol.l^{-1}; $p=0.015$), W30 (47 ± 28 v 78 ± 17 mmol.l^{-1}; $p=0.015$) and NW trials (54 ± 22 v 98 ± 21 mmol.l^{-1}; $p=0.003$). Potassium concentrations at the end of the NW trial were greater compared to the W30 trial ($p<0.05$), but were similar when comparing start and end concentrations between other trials ($p>0.05$).

7.4.5 Subjective feeling questionnaires

Recorded perceived sensations of thirst at baseline were similar between W and NW trials and W30 and NW trials ($p>0.05$) but were lower in the W30 trial compared to the W trial ($p<0.0001$) (Figure 7.10a). Peak sensations of thirst were reported in all trials at post-exercise but were not different between trials. Following the onset of water intake, sensations of thirst were reportedly lower in the W trial compared to the NW trial at 5, 15, 30, 35 and 60 minutes of the recovery period and to the W30 trial 15, 30 and 35 minutes of the recovery period ($p<0.05$). Sensations of thirst were lower in the W30

trial compared to the NW trial once water was allowed at sample points 35 and 60 minutes ($p<0.05$). In the W trial, post-exercise sensations of thirst were greater than at all other sample points, but had returned to baseline values after allowing *ad libitum* water intake. Sensations of thirst were lower than baseline after 30, 35 and 60 minutes

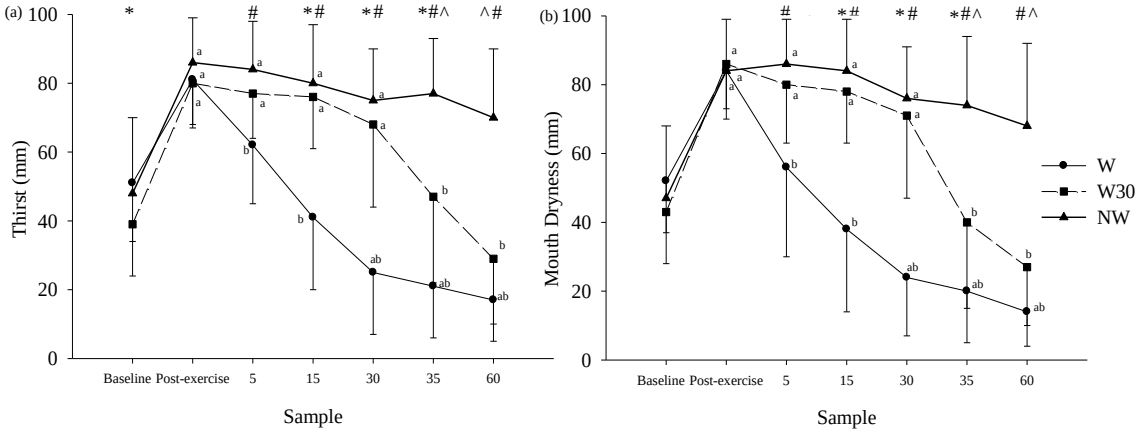

Figure 7.10. Subjective feeling questionnaire responses for (a) thirst and (b) mouth dryness over the duration of each trial. * denotes difference between W and W30 trials, # denotes difference between W and NW trials, ^ denotes difference between W30 and NW trials, [a] denotes difference within the trial compared to baseline and [b] denotes difference within the trial compared to post-exercise ($p<0.05$).

($p<0.05$), with sensations reaching a plateau at 30 minutes. Reported sensations were elevated compared to remaining samples at both 5 and 15 minutes of the recovery period ($p<0.05$). In the W30 trial sensations of thirst were elevated above baseline at post-exercise and after 5, 15 and 30 minutes of the recovery period ($p<0.05$) before returning to baseline levels. Sensations of thirst were lower after 35 and 60 minutes of the recovery period compared to post-exercise, 5, 15 and 30 minutes, whilst there was a further decrease in reported sensations of thirst after 60 minutes of the recovery period compared to 35 minutes ($p<0.05$). Subjective feelings of thirst were elevated above baseline at post-exercise and after 5, 15 and 30 minutes of the recovery period in the NW trial ($p<0.05$). No difference was found between remaining sample points ($p>0.05$).

Baseline and peak values of sensations of mouth dryness reported at post-exercise were similar between all trials ($p>0.05$) (Figure 7.10b). Lower sensations of mouth dryness were reported in the W trial compared to the W30 trial at 15 and 30 minutes and to the NW trial at 5, 15, 30, 35 and 60 minutes of the recovery period ($p<0.05$). Perceived

mouth dryness was lower at 35 and 60 minutes of the recovery period in the W30 trial compared to the NW trial ($p<0.05$). In the W trial, sensations of mouth dryness at post-exercise were greater than baseline and all samples during the recovery period ($p<0.05$). Following the commencement of the recovery period, mouth dryness returned to baseline ($p>0.05$), but then decreased below baseline values at 30, 35 and 60 minutes of the recovery period. Perceived sensations of mouth dryness at 30 minutes were lower than baseline, post-exercise and 5 minute samples but greater than at 60 minutes ($p<0.05$). At 35 and 60 minutes of the recovery period sensations of mouth dryness were lower than baseline, post-exercise, 5 and 15 minutes ($p<0.05$). In the W30 trial sensations of mouth dryness remained elevated above baseline until 35 min ($p<0.05$) and remained at peak post-exercise levels until 35 min when water consumption had been allowed. In the NW trial sensations of mouth dryness were elevated above baseline until 35 min ($p<0.05$). Sensations of mouth dryness remained at peak post-exercise levels throughout the recovery period ($p>0.05$).

7.4.6 Correlations

Serum osmolality was positively correlated to serum sodium concentrations, blood lactate concentrations, and aldosterone concentrations in all trials (Table 7.2). Serum osmolality was positively correlated to vasopressin concentrations in the W and NW trial. Serum sodium concentrations were positively correlated to blood lactate concentrations in all trials and vasopressin and aldosterone concentrations in the W trial. Vasopressin concentrations were positively related to aldosterone concentrations in the W and NW trials. Strong positive correlations between sensations of thirst and mouth dryness occurred in all trials.

7.4.7 Core and skin temperature

Core temperature was similar between trials ($p>0.05$) (Figure 7.11a). In each trial core temperature was elevated above baseline values after 20 minutes of the exercise period had elapsed and remained elevated above baseline values until 10 minutes into the recovery period in the W trial and 40 minutes in the W30 and NW trial ($p<0.05$). During the exercise period, in all three trials, core temperature progressively increased from 10 minutes until 50 minutes, when a plateau was reached ($p<0.05$). For the first

40 minutes of the recovery period, core temperature progressively decreased in all three trials ($p<0.05$). No differences were observed within and between trials for skin temperature measurements ($p>0.05$) (Figure 7.11b).

Table 7.2. Correlation coefficients (r) and significance levels (p) for measured variables in each trial. (conc. denotes concentration). *denotes significant ($p<0.05$). Correlations involving aldosterone are Spearmon's rank correlations, remaining correlations are Pearson's product moment correlations.

		W		W30		NW	
Variables		**r**	**p**	**r**	**p**	**r**	**p**
Serum osmolality	Serum sodium conc.	0.646	<0.0001*	0.555	<0.0001*	0.424	<0.0001*
	Blood lactate conc.	0.824	<0.0001*	0.773	<0.0001*	0.813	<0.0001*
	Vasopressin conc.	0.621	<0.0001*	0.218	0.170	0.443	0.003*
	Aldosterone conc.	0.321	0.034*	0.313	0.039*	0.416	0.005*
	Thirst	0.562	<0.0001*	0.314	0.005*	0.292	0.01*
	Mouth dryness	0.567	<0.0001*	0.406	<0.0001*	0.119	0.301
Serum sodium conc.	Blood lactate conc.	0.607	<0.0001*	0.648	<0.0001*	0.616	<0.0001*
	Vasopressin conc.	0.501	0.001*	0.218	0.171	0.183	0.247
	Aldosterone conc.	0.412	0.005*	0.194	0.206	0.197	0.200
	Thirst	0.535	<0.0001*	0.554	<0.0001*	0.499	<0.0001*
	Mouth dryness	0.518	<0.0001*	0.610	<0.0001*	0.560	<0.0001*
Blood lactate conc.	Vasopressin conc.	0.720	<0.0001*	0.471	0.002*	0.470	0.002*
	Aldosterone conc.	0.431	0.004*	0.379	0.011*	0.446	0.002*
	Thirst	0.494	<0.0001*	0.528	<0.0001*	0.421	<0.0001*
	Mouth dryness	0.466	<0.0001*	0.525	<0.0001*	0.373	0.001*
Vasopressin conc.	Aldosterone conc.	0.380	0.012*	0.174	0.276	0.313	0.044*
	Thirst	0.376	0.013*	0.456	0.003*	0.226	0.150
	Mouth dryness	0.398	0.008*	0.328	0.037*	0.115	0.469
Aldosterone conc.	Thirst	0.268	0.079	0.173	0.261	0.099	0.524
	Mouth dryness	0.271	0.076	0.131	0.396	0.070	0.653
Thirst	Mouth dryness	0.959	<0.0001*	0.921	<0.0001*	0.775	<0.0001*

7.4.8 Heart rate, thermal sensation and rating of perceived exertion

Heart rate was similar between trials at all time points except between the W30 and NW trial after 50 minutes of the recovery period when a higher heart rate was recorded in the NW trial (63 ± 8 v 70 ± 9 beats.min^{-1} W30 and NW trials respectively). Peak heart rates were observed during the exercise period of the trials (163 ± 13 v 163 ± 12 v 162 ± 13 beats.min^{-1} for W, W30 and NW trials respectively) and were elevated from baseline values (61 ± 10 v 57 ± 8 v 63 ± 7 beats.min^{-1} for W, W30 and NW trials respectively) ($p<0.05$). Heart rate had returned to baseline values by 30 minutes of the

recovery period in the W and W30 trials and by 40 minutes in the NW trial ($p>0.05$). In the W trial during the recovery period heart rate was higher after 10 minutes (87 ± 10 beats.min^{-1}) compared to 20 (78 ± 11 beats.min^{-1}), 30 (73 ± 9 beats.min^{-1}), 40 (67 ± 10 beats.min^{-1}), 50 (65 ± 8 beats.min^{-1}) and 60 minutes (65 ± 9 beats.min^{-1}), after 20 minutes compared to 40, 50 and 60 minutes and after 30 minutes compared to 40, 50 and 60 minutes ($p<0.05$). In the W30 trial during the recovery period heart rate was higher after 10 minutes (87 ± 11 beats.min^{-1}) compared to 40 (69 ± 9 beats.min^{-1}), 50 (63 ± 8 beats.min^{-1}) and 60 minutes (66 ± 7 beats.min^{-1}), after 20 minutes (82 ± 11 beats.min^{-1}) compared to 40, 50 and 60 minutes, after 30 minutes (77 ± 11 beats.min^{-1}) compared to 40, 50 and 60 minutes and after 40 minutes compared to 50 minutes ($p<0.05$). In the NW trial during the recovery period heart rate was higher after 10 minutes (86 ± 10 beats.min^{-1}) compared to 30 (77 ± 8 beats.min^{-1}), 40 (73 ± 7 beats.min^{-1}), 50 (70 ± 9 beats.min^{-1}) and 60 minutes (67 ± 7 beats.min^{-1}), after 20 minutes (79 ± 6 beats.min^{-1}) compared to 50 and 60 minutes and after 30 minutes compared to 60 minutes ($p<0.05$).

RPE values were similar between W, W30 and NW trials at 10 (14 ± 2 v 14 ± 2 v 14 v 2), 20 (14 ± 2 v 14 ± 1 v 14 v 1), 30 (15 ± 1 v 15 ± 1 v 15 v 1), 40 (15 ± 2 v 16 ± 1 v 15 v 3), 50 (16 ± 2 v 16 ± 1 v 16 v 1) and 60 minutes (17 ± 2 v 17 ± 2 v 17 v 1) ($p<0.05$). In the W trial RPE was greater at 60 minutes compared to all time points, whilst RPE at 50 minutes was higher than at 20 and 30 minutes ($p<0.05$). In the W30 trial RPE at 60 minutes was higher than 10, 20 and 30 minutes, whilst RPE at 50 minutes was higher than 20 and 30 minutes and reported values at 40 minutes were higher compared to 20 minutes ($p<0.05$). In the NW trial RPE values at 50 and 60 minutes were greater than at 10, 20 and 30 minutes ($p<0.05$).

Thermal sensations were similar between trials ($p>0.05$). In all trials peak thermal sensations were found during the exercise period (7 ± 2 v 6 ± 2 v 7 ± 1 for W, W30 and NW trials respectively) and were higher compared to final baseline recordings (1 ± 1 v 1 ± 1 v 1 ± 1 for W, W30 and NW trials respectively) ($p<0.05$). Thermal sensations had returned to baseline values by the first 10 minutes of the recovery period in the W and NW trials and by 20 minutes of the recovery period in the W30 trial.

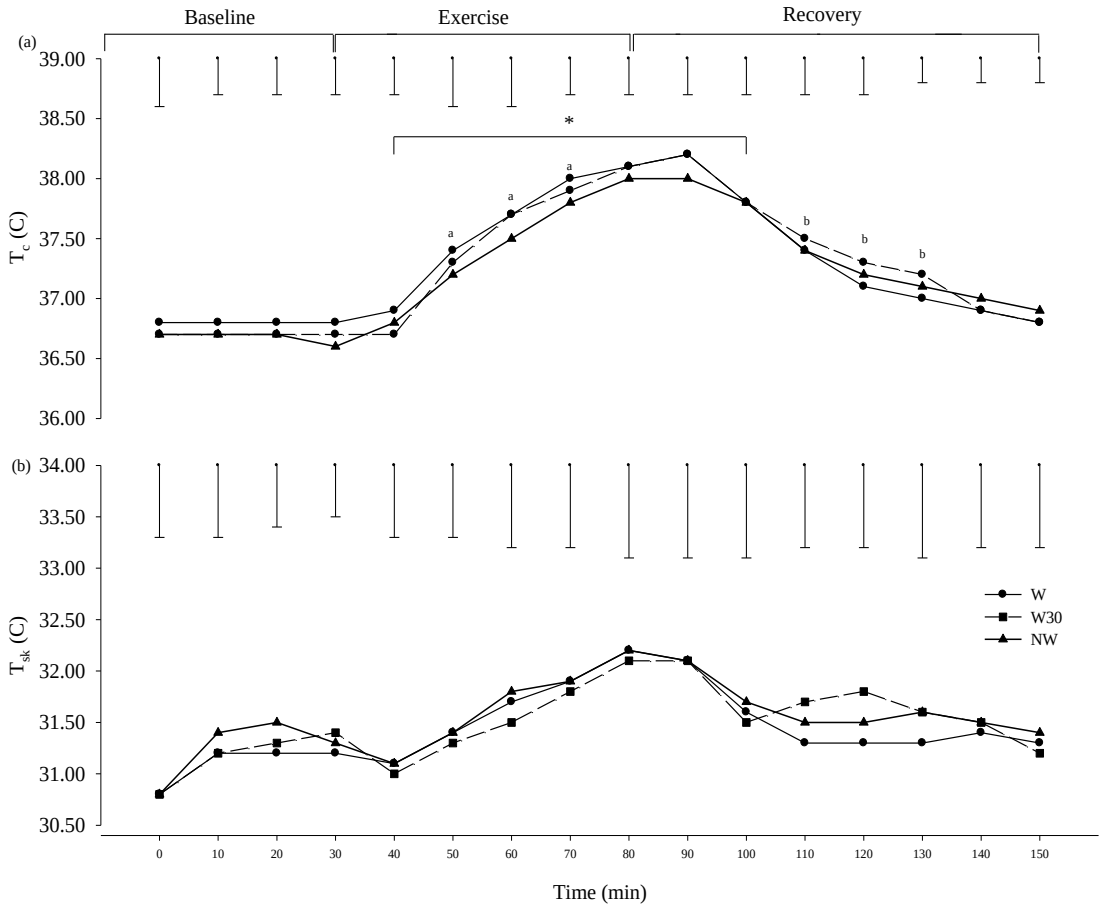

Figure 7.11. Core (a) and skin (b) temperatures over the duration of each trial. Error bars plotted above time points represent the standard deviation of all samples in all trials at that time point. * denotes different to baseline in all trials, [a] denotes an increase from the previous time point in all trials and [b] denotes different to baseline in W30 and NW trials ($p<0.05$).

7.5 Discussion

Following a bout of HIIE, allowing *ad libitum* water intake immediately after exercise, after a delay or not at all resulted in similar decreases in serum osmolality, blood lactate and serum sodium concentrations and sensations of thirst and mouth dryness and restoration of plasma volume during a recovery period. Delaying *ad libitum* water intake resulted in similar quantities of water intake for a 30 minute period compared to when *ad libitum* intake was allowed immediately post-exercise despite lower serum osmolality values. Water intake did not appear to affect serum osmolality values.

As expected, due to the increased time of availability, water intake was greater in the W trial compared to the W30 trial. When the periods in the W trial between 0-5 min and 5-30 min were compared against the 30-35 and 35-60 min periods in the W30 trial, water intake was similar, in spite of a large difference in serum osmolality at the point at which drinking could commence. In the W trial at post-exercise, serum osmolality was 299 ± 6 mOsmol.kg^{-1} compared to values of 285 ± 3 mOsmol.kg^{-1} in the W30 trial after 30 min of the recovery period. Serum osmolality at 30 min in the W30 trial was below values typically associated with sensations of thirst and subsequent voluntary water intake (~290 mOsmol.kg^{-1}) (Phillips *et al.*, 1985). This resulted in similar water intake compared to the W trial between 0-30 min when serum osmolality was above the threshold and also was greater than the W trial between 30-60 min when serum osmolality was similar between trials. This raises the suggestion that once serum osmolality has risen above the threshold to stimulate sensations of thirst and when a desire to drink has been experienced; this desire remains until satiation occurs regardless of the current serum osmolality levels. This implicates that advice regarding drinking to thirst may be inappropriate, particularly if dehydration levels, indicated by body mass losses, do not correlate with large increases in serum osmolality that are thought to contribute to sensations of thirst.

In addition, sensations of thirst in all three trials corresponded to water intake, with elevated sensations of thirst until water intake was permitted. Despite decreased serum osmolality values at 30 min in the W30 trial compared to post-exercise in the W trial, similar sensations of thirst were experienced, which, again, suggests that once thirst has been experienced, it remains elevated until satiated by water intake.

Within both the W and W30 trials there was no difference in the amount of water consumed in the first 5 min period compared to the next 25 minute period despite there being a large difference in time allowed for water intake. In studies examining voluntary water intake following a period of dehydration through exercise or water restriction, water intake predominantly occurs at the onset of the drinking period to reduce sensations of mouth dryness and thirst (Brunstrom *et al.*, 2000; Guest *et al.*, 2006). In the current study there were significant decreases in sensations of mouth dryness and thirst after *ad libitum* water intake was allowed. These differences, combined with similar water intake volumes between both 0-5 and 5-30 min in the W trial and 30-35 and 35-60 min in the W30 trial, would confirm that that the osmoreceptors in the oropharyngeal region play an important role in governing water intake behaviour.

It has been shown that increased blood lactate and serum sodium concentrations, decreased plasma volume and increased hydrostatic pressure in the capillary bed in response to HIIE will all contribute to an increased serum osmolality (Convertino *et al.*, 1981; Freund *et al.*, 1987; Nose *et al.*, 1991). The effect this has on subsequent water intake and whether serum osmolality values still reflect hydration status is unclear. Presuming water loss was similar in all three trials due to similar sweat losses, body mass loss compared to baseline values in the NW trial was 1.29 ± 0.37% with one subject losing greater than 2% body mass loss. It would therefore seem that osmolality values and the subsequent effects of sensations of thirst and ultimately water intake were greater than what would be typically expected from the experienced body mass loss (Popowski *et al.*, 2001). This may have impacted on the amount of water consumed; however, water intake replaced only 87 ± 37% and 60 ± 26% of the water lost in the W and W30 trials respectively. If only one subject lost greater than 2% body mass in the NW trial and no subjects would have lost 2% body mass in the other trials without water replacement, the necessity of water replacement could be questioned. Sawka *et al.* (2007) have suggested that if dehydration was not severe then a return to euhydration could occur through normal meals, snacks and plain water intake. Consumption of excess water may lead to increased urine output, particularly if a large volume of water is replaced, which may result in less water retained (Wong *et al.*, 1998). In the current study four subjects in the W trial did replace over 100% of water,

however, there was no difference in urine output between trials. The results would suggest that water intake was governed by the satiation of thirst sensations.

In all three trials, serum osmolality, blood lactate and serum sodium concentrations, and plasma volume changes were all similar. HIIE appeared to play a pivotal role in creating large increases in the variables when measured post-exercise. The peak values were similar to those observed in Chapter 6, when a similar protocol was followed and in research conducted by Convertino *et al.* (1981) and Freund *et al.* (1987). During the recovery period, although between trials there was no difference, statistical differences in serum sodium concentrations occurred or tended to occur within trials at the point water intake was allowed. In the W trial, to cause a decrease in the serum sodium concentration of 2 $mmol.l^{-1}$, found between post-exercise and 5 min of the recovery period, through water ingestion, approximately 0.30 l of water would need to be ingested, however the actual volume was only 0.26 l and does not take the effect of gastric emptying into account (Coyle *et al.*, 1978). Gastric emptying would slow the availability of water into the vascular space and therefore any decrease in serum sodium would not likely be immediately caused by the recently ingested bolus of water. Serum sodium concentrations would, in part, have decreased due to restoration of water into the vascular space but the reason why the decrease occurred at the same time as the commencement of water intake was unclear, particularly as similar responses were not seen with serum osmolality values. This suggested that water intake in this study did not affect the rate of recovery of serum sodium concentrations and therefore serum osmolality, back to baseline. It would appear probable that the accompanying decrease in blood lactate concentrations would have also contributed to the decrease in serum sodium concentrations (Nose *et al.*, 1991); however this would not explain the decrease in serum sodium concentrations at the exact time that water was first ingested.

Serum osmolality returned to baseline at a similar rate in all three trials despite varied water intake time allowances and accompanying blood lactate concentrations, which remained elevated above baseline values throughout the recovery period. Following HIIE increased blood lactate and serum sodium concentrations and haemoconcentration have been positively related to rises in serum osmolality (Convertino *et al.*, 1981; Freund *et al.*, 1987; Nose *et al.*, 1991). In particular, blood lactate concentrations have been shown to prevent serum sodium release into the vascular space, resulting in an

increase in osmolality levels (Nose *et al.*, 1991). In the previous chapter, blood lactate concentration was shown as a key contributor to an increase in serum osmolality. Similarly in the current study, again using the formula assessed by Worthley *et al.* (1987) (Serum osmolality = $2[Na^+]$ + [BUN] + [Glucose] + [lactate]), the change in osmolality from baseline to post-exercise of 13-15 $mmol.l^{-1}$ would have been caused by the increase in serum sodium concentration (contribution of 2 x 4 $mOsmol.kg^{-1}$) and also blood lactate concentration (contribution of 5-7 $mOsmol.kg^{-1}$). Following the peak in serum osmolality post-exercise caused primarily by increased blood lactate concentrations, the reason for the decrease during the recovery period was unclear but may be related to several influencing factors or a combination. Although blood lactate concentrations remained elevated above baseline values throughout the recovery period, there was a steady decrease from peak post-exercise concentrations, and therefore there was less contribution to serum osmolality as the recovery period progressed.

The most likely explanation for the similar reductions in osmolality during the recovery period, despite *ad libitum* water intake variations, was probably due to a combination of factors. The completion of the exercise period, reduction in blood lactate and serum sodium concentrations, restoration of plasma volume, reduction in hydrostatic pressure in the capillary beds and a slight effect from water intake will all have contributed to some extent. Correlation values revealed reasonably strong correlations between serum osmolality and the variables (serum sodium concentration and blood lactate concentrations) with significant r values ranging from 0.424 to 0.824 ($p<0.05$). Values are consistent with previous studies (Convertino *et al.*, 1981; Freund *et al.*, 1987).

In conjunction with an increase in serum osmolality post-exercise, there was an increase in plasma vasopressin concentrations in all three trials. Similar responses to HIIE have been reported by Covertino *et al.* (1981), Freund *et al.* (1987), Hew-Butler *et al.* (2008) and Nose *et al.* (1991). Despite the differences in water intake and the corresponding thirst sensations between trials, vasopressin concentration was similar between trials and followed the trend of decreasing serum osmolality values during the recovery period. The release of vasopressin has been shown to occur above an osmolality threshold of approximately 285 $mOsmol.kg^{-1}$ (Thompson *et al.*, 1986) but following HIIE the threshold has been shown to shift right with greater osmolality required to elicit a similar vasopressin response mainly due to increased blood pressure (Nose *et*

al., 1991). In the current study serum osmolality was considerably above the threshold and therefore it was difficult to assess the impact of HIIE on vasopressin release and also the subsequent effect of water retention and water intake.

Similar responses were also found for aldosterone concentrations, which is consistent with previous work (Covertino *et al.*, 1981; Freund *et al.*, 1987). However in the W and W30 trials, concentrations were still elevated above baseline concentrations after 30 minutes of the recovery period whilst in the NW trial they had returned to baseline. The reason for this is unclear as variables known to affect aldosterone release (increased serum osmolality, increased serum sodium concentration and decreased plasma volume) were similar between trials. The difference may be attributed to a prevention of a statistical difference due to large variability in the hormonal response.

In the W trial, core temperature returned to baseline following *ad libitum* water intake whilst in the W30 and NW trials it remained elevated above baseline during the recovery period. The water ingested was cool (10 ± 3°C) and therefore a decrease would have been expected (Gisolfi & Copping, 1974). The reason why a similar pattern did not occur after 30 min in the W30 trial compared to the NW trial may be a result of an already significant decrease in core temperature following the peak temperatures measured post-exercise and the already close proximity to baseline values. A decrease in core temperature has been shown to increase (Takamata *et al.*, 1995) and also have no effect (Montain *et al.*, 1997) on vasopressin release and therefore, possibly voluntary water intake; however despite the decreases in core temperature throughout the recovery period, vasopressin concentrations were similar, whilst water intake between 0-30 min in the W trial and 30-60 min in the W30 trial were also similar.

7.6 Conclusion

In conclusion serum osmolality was significantly raised following a bout of HIIE and decreased progressively during the recovery period due a combination of factors. Delaying water intake resulted in a similar voluntary water intake despite reduced serum osmolality values suggesting that once the desire to drink arises, sensations will remain until satiated. The elevated serum osmolality values post-exercise did not result in hyperhydration as voluntary water intake was adjusted to maintain similar body mass losses.

CHAPTER 8

General discussion and conclusions

8.1 General Discussion

8.1.1 Summary

Examining voluntary water intake at work and during and following exercise has provided an indication of the variability that can occur both between individuals and in different situations relating to everyday life and exercise.

The purpose of this thesis was to assess the physiological and behavioural mechanisms that can influence voluntary water intake and hydration status and to analyse capillary blood sampling to allow an alternative assessment of hydration status using blood parameters in a field environment.

Following assessment of water intake and hydration status using urinary indices in the general population at places of work, the planned progression was to analyse the use of capillary blood sampling as an alternative to the more restrictive and invasive method of venous blood sampling. Positive results would have allowed greater indication of hydration status through assessment of plasma/serum osmolality, serum sodium concentrations and vasopressin concentrations, in a more natural environment compared to the laboratory in both the general population and in exercise settings. Results from Chapter 4 were inconsistent and did not instil enough confidence in reliability of the results to suggest the method could be consistently used. This was mainly attributed to the attainment of the sample and although it was attempted to be minimised, 'milking' of the finger resulting from the difficulty in obtaining a free-flowing sample, may have contributed to the inconsistent results. In the final three chapters the focus of the thesis was to increase understanding of the physiological and behavioural mechanisms that govern voluntary water intake during and following a period of exercise in different environmental conditions and in response to different exercise intensities.

8.1.2 Voluntary water intake and sensations of thirst

Voluntary water intake appears to be largely stimulated by sensations of thirst (Greenleaf, 1992). In Chapter 3, when water intake was recorded during a shift and hydration status was assessed through urine osmolality and USG at the start and end of a shift, using self report questionnaires, 117 out of 156 subjects experienced a sensation

of thirst with 85% of those consuming a beverage (median volume 0.2 l) to alleviate the sensations. The laboratory studies (Chapters 5, 6 and 7) with measured water intake showed that the largest intakes of water usually followed a high reported sensation of thirst that was above a previously reported euhydrated baseline value. Describing the control of thirst requires a complex model and it has been shown that sensations are initially derived from cerebral osmoreceptors and the release of vasopressin (Stricker & Sved, 2000). Sensations of thirst are also controlled and influenced by decreases in blood volume (McKinley & Johnson, 2004). Stricker and Verbalis (1988) describe thirst as the motivation to seek and consume water driven by perceived sensations. It is this drive that leads to the assessment of voluntary water intake and the accompanying physiological responses. The resulting effect is of consumption of water to satiate sensations of thirst.

The contribution that sensations of thirst provide to total *ad libitum* water intake is difficult to quantify. It is largely regarded in the literature that thirst is a key contributor to water intake (Greenleaf, 1992) and this was shown in Chapters 3, 5, 6 and 7, however following satiation of thirst, water intake often continued, albeit at a reduced rate and volume. In Chapter 7, following the onset of *ad libitum* water intake, sensations of thirst decreased from peak post-exercise values and remained at this level throughout the recovery period, whilst voluntary water intake continued. In Chapter 3 a minority of subjects (n=39) did not experience sensations of thirst yet still consumed water in some capacity. Drinking has been suggested to be governed by habit as well as through sensations of thirst and in large scale assessments of dietary intake, the majority of beverage consumption occurs in accompaniment with meal times (McKiernan *et al.*, 2008), but in order to fully understand why this was apparent a concurrent examination of sensations of thirst would be required. McKiernan *et al.* (2008) did find that sensations of thirst oscillated throughout the day with peak values at midday and evening meal times, however this increased sensation may be more of a retrospective sensation, associating meal times with food and beverage and realising that both are desired.

Using sensations of thirst as indicator of when to drink during exercise has been widely debated in the literature (Maughan & Shirreffs, 2010; Noakes, 2003; 2007; 2010; Pitts *et al.*, 1944). When examining the amount sensations of thirst contribute, the necessity

of water intake must also be taken into consideration as should the effect of confusion with other sensations such as mouth dryness influencing thirst. The main arguments against drinking to thirst are that water intake can be delayed when there are high rates of water loss and thirst is not experienced until a substantial volume of water has been lost, resulting in a water balance deficit, which is difficult to restore, particularly when water losses continue. When employing a drinking to thirst strategy, water may not be always available when thirst is experienced and vice versa, which can be particularly apparent in races with drinks stations. In addition, inappropriate signals or inappropriate interpretations of these signals can lead to incorrect drinking strategies with either too much consumed and an increase in urine output volume and frequency (Kovacs *et al.*, 2002) or too little and dehydration can increase (Greenleaf, 1992). During exercise, the ACSM position stance suggested consuming enough water to prevent greater than a 2% body mass loss from occurring, without consuming so much that weight is gained, whilst following exercise, unless rapid rehydration was required and/or dehydration was severe (>2% body mass loss), normal dietary intake of food and water should be sufficient to ensure a return to a state of euhydration (Sawka *et al.*, 2007). In Chapters 5, 6 and 7 rehydration occurred through *ad libitum* water intake in a two hour (Chapter 5) and one hour (Chapters 6 and 7) recovery period. In all three chapters body mass loss did generally not reach levels above 2% except in isolated cases. In accordance with the ACSM recommendations, water intake could have been considered unnecessary. This would have been of greater concern if an increase in urine output had resulted, however in Chapters 6 and 7, there was no difference in urine volume between trials, whilst in Chapter 5, there was greater cumulative urine output in the cold trial after two hours of the recovery period but this did not appear to have a significant effect on hydration status.

8.1.3 Water replacement

Although water intake in Chapters 5, 6 and 7 may be classed as unnecessary and increases the prevalence of overdrinking, a gain in body mass was not commonly recorded and occurred in one subject in Chapter 6 and four subjects in Chapter 7. Water replacement tended to be less than 100% suggesting that voluntary water intake is terminated before complete rehydration after the initial commencement driven by sensations of thirst. Termed voluntary dehydration (Adolph *et al.*, 1954), the failure to

completely replace water losses has been frequently found (Godek *et al.*, 2005; Mack *et al.*, 1994; Passe *et al.*, 2007; Peacock *et al.*, 2011). If voluntary water intake is initiated by sensations of thirst, the reason for termination is unclear particularly as sensations of thirst in Chapters 6 and 7 had returned to baseline yet water intake continued. The desire to terminate drinking during a recovery period appears to assist in the prevention of hyperhydration.

8.1.4 Physiological responses

Following the onset of *ad libitum* water intake, physiological responses were similar between Chapters 4, 5, 6 and 7 and similar to previous work (Convertino *et al.*, 1981, Kenefick *et al.*, 2004a; Kenefick *et al.*, 2008; Nose *et al.*, 1991; Sjøgaard *et al.*, 1985). When voluntary water intake was only allowed during a recovery period there was a noticeable peak in variables (serum osmolality, plasma volume changes, haemoglobin concentration, haematocrit and serum sodium concentrations) post-exercise followed by a progressive decrease throughout the duration of the recovery periods. In Chapter 5 when water intake was allowed during exercise and the recovery period, serum osmolality increased above baseline post-exercise but there was no difference after one and two hours of the recovery, however haemoglobin concentration and haematocrit did decrease after one hour of the recovery period compared to post-exercise peak values. The effect of water intake on the decrease in variables was difficult to determine as the time for gastro-intestinal absorption must also be considered. When absorbed, water will restore decreases in plasma volume and will cause a decrease in serum osmolality and serum sodium concentrations through a restoration of extracellular water (Figaro & Mack, 1997; Geelen *et al.*, 1984; Rolls *et al.*, 1980). In Chapter 7 when no water was allowed, similar decreases in the measured variables were found compared to when water was allowed. It may be that the completion of the exercise bout allowed restoration of many of the variables back to or towards baseline values, due to the large reduction in physical stress (Sjøgaard *et al.*, 1985).

8.1.5 General population

When assessing the volume of voluntary water intake in the general population consideration of recommendations, requirements or suggested adequate intakes is

important. Comprehending these suggestions may allow for greater understanding of the necessity of water intake and replacement. In the general population adequate intake values for water through food and beverages are typically used (EFSA, 2010; Food Standards Agency, 2010; Institute of Medicine, 2004). The amount of water reportedly consumed in Chapter 3 follows the trend of the adequate intake values and studies involving water intake in the general population (Manz *et al.*, 2011), however it is difficult to make a direct comparison as water intake, in Chapter 3, was only reported for the period spent at the place of work, did not contain water obtained from food, and would likely not include two main meals. The results in this thesis provided an indication of the variability in reported water intake in different working groups and suggest that further research may be useful to enable the examination of the effect water consumption and the resulting hydration status may have on work productivity and other factors relating to cognitive performance.

8.1.6 Voluntary water intake influences

Palatability of a beverage (temperature and flavour) has been shown to influence *ad libitum* consumption (Szlyk *et al.*, 1989). In Chapters 5, 6 and 7 plain water at a cool temperature (mean temperature approximately 10-11°C) was provided for the subjects. The coolness of the water would likely have caused an increase in voluntary water intake compared to the provision of warmer water, particularly immediately after exercise when core temperature peaked. In contrast, thermal sensation decreased throughout the recovery period in many subjects, possibly rendering the cool water undesirable following the accompanying decrease in core temperature. In Chapter 3 free choice of beverages were allowed, as subjects followed a normal day in their place of work. The ability to freely choose their own beverage may have had an influence on the amount consumed as subjects would have been more likely to choose and consume beverages that they desired.

8.1.7 Measuring and self reporting water intake

When using dietary recall to assess voluntary water intake as used in Chapter 3, the possibility of recall bias may have influenced reported values (Beaton *et al.*, 1979). Ideally, weighed intake of all food and beverages consumed throughout the day would

have allowed a greater understanding of water intake, however, in addition, to examine water balance, urine output would also have been required. The problem associated with weighed food and water intake and measuring water loss is the impact on the normality of the day and may prove an inconvenience. Measuring water intake and water losses in Chapters 4, 5, 6 and 7 provided a more accurate assessment but there is still potential for bias in the volume of water consumed. The Hawthorne Effect describes how behaviour of subjects alters when they know they are being observed (Roethlisberger & Dickson, 1939). The measurement of water intake was not made aware to the subjects until the completion of the final trial and water bottles were not weighed in the presence of the subjects, but some subjects may still have been aware that their water intake responses were being monitored and thus may have altered their water intake to produce perceived desirable results.

8.1.8 Capillary blood sampling

Capillary blood sampling has been shown to provide strong agreement with venous sampling when measuring human growth hormone concentrations (Godfrey *et al.*, 2004), haemoglobin concentrations (Morris *et al.*, 1999) and blood glucose concentrations (Martin *et al.*, 2005) and therefore it would seem reasonable to expect similar results with measures of plasma osmolality and vasopressin concentrations. An attempt to reason with results to the contrary has been provided in Chapter 4. The required aim of capillary sampling was to allow replication of similar trials to those presented in Chapters 5, 6 and 7 in a more natural exercise environment without any constraints the laboratory may impart. The effect the laboratory may have had on water intake behaviours has been previously discussed and therefore understanding the physiology accrued from laboratory data may not always be indicative of a field setting. Many studies pertaining to voluntary water intake have occurred outside of the laboratory and have been limited in measures of physiological variables (Bergeron *et al.*, 2006; Cox *et al.*, 2002; Maughan *et al.*, 2004; Osterberg *et al.*, 2009). These studies focussed on urinary markers to assess hydration status, when additional analysis of blood parameters may have allowed for greater understanding, particularly as they are often viewed as the preferred method of hydration status assessment (Cheuvront *et al.*, 2010; Popowski *et al.*, 2001). In Chapters 5, 6 and 7, measures of osmolality, plasma volume changes, and hormonal concentrations were able to provide a greater overview

of voluntary water intake influences, however despite familiarisation trials to minimise the unfamiliar and unnatural environment the laboratory provided, the laboratory environment may have created a misrepresentative impression of voluntary water intake and the influencing factors.

8.2 Conclusions

Several conclusions can be made from this thesis.

- Water intake was generally initiated by sensations of thirst and reduced once satiated. This appeared to occur even when serum osmolality had decreased below, or was lower that the threshold for thirst (<290 mOsmol.kg^{-1}), suggesting that thirst and desire to drink are strongly influenced by behaviour.
- *Ad libitum* water intake was lower during exercise in the cold compared to a warmer environment and there was a slight blunted thirst response in the cold.
- HIIE increased serum osmolality, blood lactate concentrations, serum sodium concentrations, plasma volume changes, sensations of thirst and vasopressin and aldosterone concentrations and resulted in increased water intake during a one hour recovery period compared to continuous exercise.
- Delaying *ad libitum* water intake following HIIE resulted in similar volumes of water intake despite lower serum osmolality and sodium concentrations compared to when *ad libitum* water intake was allowed immediately after exercise.
- Voluntary water intake differs between individuals, between work environments, during and following exercise in different environments and following different exercise intensities. Many explanations as the why these differences occur have been explained within the thesis.
- Using capillary blood sampling compared to venous blood sampling to track changes in hydration status and measure vasopressin concentration is inconsistent due to the increased frequency of 'milking' and forcing the sample.

8.3 Future research

Understanding voluntary water intake is still not completely clear. In the general population, in different places of work, there was large variation in water intake but the impact that hydration status had on water intake and the resultant effect on hydration status require further research. Ideally the development of capillary blood sampling would also aid this research, particularly with analysis of plasma/serum osmolality and vasopressin concentrations. An improved reliability of capillary sampling would also permit analysis of the voluntary water intake response to exercise intensity and environments in a natural field setting, thus reducing any influences of the laboratory. Is addition, development of assessing hydration status through saliva may also provide additional information is similar environments.

The role that HIIE has on voluntary water intake requires further research. Expanding on the results from Chapters 6 and 7, removing the influence of body mass loss may identify key mechanisms governing water intake and possibility of overdrinking, which may result in increased urine output and in extreme cases, hyponatraemia. It was difficult to separate the physiological responses from HIIE and the physiological and psychological effects of water loss on voluntary water intake. In the majority of trials, body mass loss was not at a level to require a specific rehydration strategy (Sawka *et al.*, 2007), but was at a level where the subject felt it important to replace water losses. In contrast to the previous suggestion, increasing the exercise period to elicit larger body mass losses (>2%) in all subjects should be explored further along with allowing water intake during the exercise period to recreate a situation that would typically arise in a sporting context.

CHAPTER 9

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APPENDICES

Appendix A

Borg (1970) – Rating of perceived exertion

6

7 Very, very light

8

9 Very light

10

11 Fairly light

12

13 Somewhat hard

14

15 Hard

16

17 Very hard

18

19 Very, very hard

20

Appendix B

Thermal sensation scale (adapted from Hardy, 1970)

10	**Heat impossible to bear**
9	
8	**Very Hot**
7	
6	**Hot**
5	
4	**Warm**
3	
2	**Slightly Warm**
1	
0	**Neutral**
-1	
-2	**Slightly Cool**
-3	
-4	**Cool**
-5	
-6	**Cold**
-7	
-8	**Very Cold**
-9	
-10	**Cold impossible to bear**

Appendix C

Subjective feelings questionnaires used in Chapter 3

Subject:_____ **Trial:_____**
Place: _____ **Date:_____**
Time:_____

Subjective Feelings Questionnaire

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

How thirsty do you feel now?

_____

Not at all thirsty Very thirsty

How dry does your mouth feel now?

_____

Not at all dry Very dry

How tired do you feel now?

_____

Not at all tired Very tired

How hungry do you feel now?

_____

Not at all hungry Very hungry

How well can you concentrate just now?

_____

Not at all well Very well

How much energy do you feel you have now?

_____

No energy Full of energy

Appendix D

Subjective feelings questionnaire used in Chapter 4

Subject:_____ **Trial:_____**
Time:_____

Subjective Feelings Questionnaire

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

How thirsty do you feel now?

_____

Not at all thirsty — Very thirsty

How full does your stomach feel now?

_____

Very full — Not at all full

How pleasant does mouth taste now?

_____

Not at all pleasant — Very pleasant

How bloated do you feel now?

_____

Very bloated — Not at all bloated

How hungry do you feel now?

_____

Not at all hungry — Very hungry

How tired do you feel now?

_____

Very tired — Not at all tired

How dry does your mouth feel now?

Not at all dry — Very dry

How scratchy does your throat feel now?

Very scratchy — Not at all scratchy

How alert do you feel now?

Not at all alert — Very alert

How does your head feel now?

Very sore — Not at all sore

How well can you concentrate just now?

Not at all well — Very well

How much energy do you feel you have now?

Full of energy — No energy

Appendix E

Subjective feelings questionnaire used in Chapter 5, 6 and 7

Subject:_____ **Trial:_____**
Date:_____ **Time:_____**

Subjective Feelings Questionnaire

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

How thirsty do you feel now?

_____

Not at all thirsty Very thirsty

How dry does your mouth feel now?

_____

Not at all dry Very dry

Appendix F

Pre and post shift questionnaires used in Chapter 3

Subject:_____ **Trial:**_____
Place: _____ **Date:**_____
Time:_____

Assessing hydration status in typical lifestyle environments
Pre shift questionnaire

1) Do you have access to drinks during your typical shift?

YES **NO**

If **YES**, what form is this in? (e.g. bottle of water, water cooler / fountain, tea / coffee machine etc.)
……
2) Does anything influence your drinking during your shift? (e.g. access to drinks, timing of breaks, toilet facilities mouth dryness etc.)

YES **NO**

If **YES**, please list
……...

3) How much fluid do you typically consume during your shift?
……
……...

4) Do you often experience a feeling of thirst during your shift?

YES **NO**

5) Does your concentration become affected if you do not drink?

YES **NO**

Subject:_____ **Trial:**_____
Place: _____ **Date:**_____
Time:_____

Assessing hydration status in typical lifestyle environments
Post shift questionnaire

1) Did you have access to drinks during your shift?
YES **NO**

2) What did you drink during your shift?
……

3) Did you feel thirsty at any point?
YES **NO**

4) If you felt thirsty did you have a drink?
YES **NO**

If **YES**, how much and what did you drink?
……
If **NO**, why was this?
……

5) How would you rate your concentration

at the start of your shift

_____

Not at all good Very good

in the middle of your shift

_____

Not at all good Very good

at the end of your shift

_____

Not at all good Very good

6) Did you feel you kept yourself hydrated throughout the shift? **YES** **NO**

7) Was today typical of a normal shift?
YES **NO**
If **NO**, why was this?.....

Appendix G

Data for figures presented in Chapter 3

Table 3.2ab. USG and urine osmolality (mOsmol.kg^{-1}at the start and end of the shift (mean ± SD). * greater than research group, # greater than masters, ^ greater than teachers, ~ greater than firefighters, + greater than office and @ greater than catering ($p<0.05$). x difference between start and end values ($p<0.05$).

	USG		Urine Osmolality (mOsmol.kg^{-1})	
Group	Start	End	Start	End
Research	1.022 ± 0.006 $^{+\wedge@}$	1.015 ± 0.007 ^{x}	749 ± 189 $^{\#\wedge+@}$	538 ± 233 ^{x}
Masters	1.018 ± 0.008	1.021 ± 0.006 $^{*\wedge+}$	600 ± 250	699 ± 199 $^{*\wedge+}$
Teachers	1.017 ± 0.008	1.015 ± 0.008	563 ± 237	517 ± 272
Security	1.021 ± 0.007	1.019 ± 0.005 $^{+}$	714 ± 226 $^{\wedge}$	651 ± 215
Firefighters	1.023 ± 0.006 $^{\wedge+@}$	1.016 ± 0.007 ^{x}	754 ± 198 $^{\#\wedge+}$	573 ± 230 ^{x}
Office	1.017 ± 0.007	1.013 ± 0.007	575 ± 246	441 ± 234
Catering	1.017 ± 0.007	1.016 ± 0.007	607 ± 248	591 ± 260

Table 3.2cd. Urine sodium and potassium concentrations (mmol.l^{-1}) at the start and end of the shift (mean ± SD). * greater than research group, # greater than masters, ^ greater than teachers, ~ greater than firefighters, + greater than office and @ greater than catering ($p<0.05$). x difference between start and end values ($p<0.05$).

	Urine Sodium Concentration (mmol.l^{-1})		Urine Potassium Concentration (mmol.l^{-1})	
Group	Start	End	Start	End
Research	104 ± 33 $^{\wedge}$	101 ± 44 $^{\wedge}$	98 ± 28	78 ± 32 ^{x}
Masters	84 ± 39	84 ± 39	82 ± 33	99 ± 37
Teachers	83 ± 30	78 ± 40	87 ± 34	81 ± 37
Security	117 ± 45 $^{\#\wedge}$	134 ± 41 $^{*\#\wedge\sim+}$	97 ± 29	81 ± 24
Firefighters	112 ± 45 $^{\#\wedge}$	99 ± 43	98 ± 28	86 ± 36
Office	99 ± 46	77 ± 36	93 ± 41	78 ± 35
Catering	108 ± 34 $^{\wedge}$	118 ± 48 $^{\#\wedge\sim}$	98 ± 28	102 ± 26

Table 3.4. Reported water intakes for each group in all subjects, males and females during the shift (median (range)). * greater than research group, # greater than masters, ^ greater than teachers, $ greater than security, + greater than office and @ greater than catering ($p<0.05$).

	Reported water intake (l)		
Group	All	Males	Females
Research	1.1 (0.3-3.3) [#^]	1.2 (0.4-3.3) [#]	0.9 (0.3-1.9) [#]
Masters	0.5 (0.0-1.4	0.7 (0.0-1.1)	0.5 (0.0-1.4)
Teachers	0.6 (0.0-2.5)	0.8 (0.4- 2.5)	0.6 (0.0-1.2)
Security	0.9 (0.3-2.0) [#^]	0.9 (0.3-2.0)	1.4 (0.8-2.0) [#^]
Firefighters	2.1 (0.5-3.0) [*#^$+@]	2.1 (0.5-3.0) [#*^$]	-
Office	1.1 (0.5-3.0) [#^]	1.3 (0.5-3.0) [#]	0.8 (0.5-1.5) [#]
Catering	0.9 (0.4-2.0) [#^]	1.8 (0.4-2.0)	0.8 (0.4-1.4) [#^]

Appendix H

Data for figures presented in Chapter 4

Table 4.1. Body mass change from baseline over the duration of the trials (%) (mean ± SD). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning and ~ denotes different from recovery ($p<0.05$).

	Body mass change from baseline (%)	
Sample	EU	HY
Baseline	0.00 ± 0.00	0.00 ± 0.00
Morning	-0.17 ± 0.61	-0.76 ± 0.19 $^{\#*}$
Post-exercise	-0.92 ± 0.60 $^{\#\wedge\sim}$	-1.96 ± 0.15 $^{\#\wedge\sim *}$
Recovery	- 0.17 ± 0.62	-0.77 ± 0.22 $^{\#*}$

Table 4.2. Plasma, serum and urine osmolality over the duration of each trial. Plasma osmolality is compared between sampling technique. Serum and urine osmolality are compared between trials (mean ± SD). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning and ~ denotes different from recovery ($p<0.05$).

	Plasma osmolality ($mOsmol.kg^{-1}$)		Serum osmolality ($mOsmol.kg^{-1}$)		Urine osmolality ($mOsmol.kg^{-1}$)	
Sample	Venous	Capillary	EU	HY	EU	HY
Baseline	292 ± 6	296 ± 8	284 ± 4	284 ± 4	535 ± 225	479 ± 269
Morning	294 ± 5	298 ± 5 *	282 ± 3 $^{\#}$	287 ± 4 $^{\#*}$	410 ± 246	884 ± 123 *
Post-exercise	296 ± 6 $^{\#\wedge\sim}$	300 ± 6 $^{\#\wedge\sim *}$	283 ± 3	290 ± 5 $^{\#\wedge\sim *}$	687 ± 154 $^{\wedge\sim}$	886 ± 107 $^{\#}$
Recovery	294 ± 6	297 ± 6 *	282 ± 3 $^{\#}$	288 ± 3 $^{\#*}$	318 ± 233	826 ± 145 *

Table 4.3. Vasopressin concentration over the duration of the trials. Compared between sampling technique and trial (median and range). * denotes different between technique or trial, # denotes different from baseline and ^ denotes different from morning ($p<0.05$).

	Vasopressin concentration ($pg.ml^{-1}$)		Vasopressin concentration ($pg.ml^{-1}$)	
Sample	Venous	Capillary	EU	HY
Baseline	0.38 (0.00-3.12)	1.23 (0.20-3.09)	0.66 (0.00-2.21)	0.28 (0.00-3.95)
Morning	0.59 (0.00-7.05)	1.82 (0.05-4.85) $^{\#*}$	0.76 (0.00-2.37)	2.22 (0.00-7.05) $^{\#\wedge *}$
Post-exercise	1.25 (0.00-5.85)	2.61 (0.00-9.57) $^{\#}$	1.21 (0.00-2.31)	3.26 (0.00-7.29) $^{\#*}$
Recovery	1.53 (0.00-3.04	2.16 (0.00-3.84)	0.65 (0.00-2.66)	2.27 (0.00-4.89) *

Table 4.4. Comparison between sampling techniques for haemoglobin concentration ($g.l^{-1}$), haematocrit (%) and blood glucose concentration ($mmol.l^{-1}$) (mean ± SD). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning, $ denotes different from post-exercise and ~ denotes different from recovery ($p<0.05$).

	Haemoglobin concentration ($g.l^{-1}$)		Haematocrit (%)		Blood glucose concentration ($mmol.l^{-1}$)	
Sample	Venous	Capillary	Venous	Capillary	Venous	Capillary
Baseline	161 ± 6*	157 ± 8	45.7 ± 1.9	45.2 ± 2.0*	3.92 ± 0.26	4.09 ± 0.28
Morning	162 ± 6$^{\sim *}$	160 ± 8$^{\sim}$	45.6 ± 1.9$^{\#}$	46.1 ± 2.0$^{\sim *}$	4.19 ± 0.23	4.21 ± 0.26
Post-exercise	164 ± 6$^{\#\wedge\sim}$	163 ± 6$^{\#\wedge\sim}$	45.7 ± 1.7$^{\#\sim}$	46.1 ± 1.9$^{\wedge\sim *}$	4.15 ± 0.29	4.17 ± 0.36
Recovery	160 ± 6$^{\#}$	160 ± 7	45.1 ± 1.9$^{\#\sim}$	45.7 ± 1.9$^{\#*}$	5.27 ± 0.60	5.29 ± 0.63

Table 4.5. Comparison of serum potassium, sodium and chloride concentrations between trials (mean ± SD). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning and ~ denotes different from recovery ($p<0.05$).

	Serum potassium concentration ($mmol.l^{-1}$)		Serum sodium concentration ($mmol.l^{-1}$)		Serum chloride concentration ($mmol.l^{-1}$)	
Sample	EU	HY	EU	HY	EU	HY
Baseline	4.3 ± 0.3	4.2 ± 0.2	142 ± 1	142 ± 1	103 ± 2	103 ± 2
Morning	4.2 ± 0.4	4.3 ± 0.3	142 ± 1	143 ± 1$^{\#*}$	103 ± 4	106 ± 2$^{\#\sim *}$
Post-exercise	4.5 ± 0.6	4.5 ± 0.2 $^{\#\sim}$	142 ± 2	144 ± 1$^{\#\wedge\sim *}$	105 ± 2$^{\#\sim}$	107 ± 4$^{\#}$
Recovery	4.3 ± 0.3	4.3 ± 0.3	142 ± 1	143 ± 1$^{\#*}$	104 ± 2	105 ± 2$^{\#}$

Table 4.6. Subjective feelings responses for thirst and mouth dryness over the duration of the trials (median and range). * denotes different between trials, # denotes different from baseline, ^ denotes different from morning and ~ denotes different from recovery ($p<0.05$).

	Thirst (mm)		Mouth Dryness (mm)	
Sample	EU	HY	EU	HY
Baseline	28 (13-40)	57 (0-65)	42 (0-54)	35 (3-75)
Morning	32 (13-63)$^{\#\sim}$	76 (26-94)$^{\#\sim}$	25 (3-64)$^{\sim}$	67 (19-86)$^{\#\sim *}$
Post-exercise	73 (38-88)$^{\#\wedge\sim}$	87 (60-100)$^{\#\wedge\sim *}$	49 (10-88)$^{\sim}$	90 (49-100)$^{\#\wedge\sim *}$
Recovery	9 (4-37)	19 (12-79)^{8}	22 (0-34)$^{\#}$	25 (5-70)*

Appendix I

Data for figures presented in Chapter 5

Table 5.2. Body mass change from baseline on each trial (%). * different to baseline ($p<0.05$). 'a' different to two hours of recovery ($p<0.05$). Mean ±SD.

	Body mass change from baseline (%)	
Sample	Cold	Warm
Baseline	0.00 ± 0.00	0.00 ± 0.00
Post-exercise	-0.50 ± 0.50*a	-0.63 ± 0.56*a
1	-0.82 ± 0.38*a	-0.63 ± 0.51*a
2	-1.15 ± 0.26*	-1.03 ± 0.26*

Table 5.3. Cumulative urine output (l) over the duration of each trial. $ different between trials ($p<0.05$).

	Cumulative urine output (l)	
Sample	Cold	Warm
Baseline	0.00 ± 0.00	0.00 ± 0.00
Post-exercise	0.22 ± 0.19	0.13 ± 0.04
1	0.52 ± 0.34	0.24 ± 0.07
2	0.81 ± 0.46$	0.54 ± 0.31

Table 5.4. Water intake (l) on each trial. * different to exercise period ($p<0.05$). # different to 0-30 min ($p<0.05$). $ different between trials ($p<0.05$). Mean ± SD.

	Water intake (l)	
Time	Cold	Warm
Exercise	0.269 ± 0.337	0.522 ± 0.335$
0-30	0.046 ± 0.057	0.111 ± 0.087*
30-60	0.078 ± 0.095	0.090 ± 0.106*
60-90	0.086 ± 0.112	0.061 ± 0.062*#
90-120	0.025 ± 0.033	0.025 ± 0.025*#

Table 5.5. Serum osmolality over the duration of each trial ($mOsmol.kg^{-1}$). * different to baseline ($p<0.05$). Mean ± SD.

	Serum osmolality ($mOsmol.kg^{-1}$)	
Sample	Cold	Warm
Baseline	287 ± 3	285 ± 4
Post-exercise	292 ± 2*	288 ± 5*
1	289 ± 4	285 ± 4
2	289 ± 3	286 ± 3

Table 5.6. Subjective feelings of thirst and mouth dryness over the duration of each trial. 0mm = not at all thirsty / mouth not at all dry, 100mm = very thirsty / mouth very dry. # different to post-exercise ($p<0.05$). Mean ± SD.

	Thirst (mm)		Mouth Dryness (mm)	
Sample	Cold	Warm	Cold	Warm
Baseline	39 ± 23	42 ± 18	36 ± 23	38 ± 23
Post-exercise	49 ± 15	63 ± 14	51 ± 22	60 ± 22
1	36 ± 15	41 ± 18$^{\#}$	37 ± 17	41 ± 20
2	32 ± 21	36 ± 20$^{\#}$	34 ± 22	34 ± 27

Table 5.7. Haemoglobin concentration, haematocrit and blood glucose concentration over the duration of each trial. * different to baseline ($p<0.05$). # different to post-exercise ($p<0.05$). ^ different to one hour of recovery ($p<0.05$). $ different between trials ($p<0.05$). Mean ± SD.

	Haemoglobin concentration ($g.l^{-1}$)		Haematocrit (%)		Blood glucose concentration ($mmol.l^{-1}$)	
Sample	Cold	Warm	Cold	Warm	Cold	Warm
Baseline	155 ± 7	155 ± 5	43.7 ± 2.0	43.3 ± 1.6	4.22 ± 0.20	4.15 ± 0.21
Post-exercise	166 ± 7*	166 ± 3*	46.5 ± 1.8*	45.6 ± 1.2*	4.18 ± 0.37	4.74 ± 0.38$^{*\$}$
1	160 ± 8$^{*\#}$	156 ± 3$^{\#}$	45.0 ± 2.0$^{*\#}$	43.6 ± 1.0*	3.80 ± 0.36	4.10 ± 0.22$^{\#}$
2	162 ± 10*	160 ± 3$^{*\#\wedge}$	45.0 ± 2.6$^{*\#}$	44.2 ± 1.1	4.09 ± 0.33	4.30 ± 0.32

Table 5.8. Core temperature and mean weighted skin temperatures over the duration of each trial. Mean ± SD. * Different to baseline, $ different between trials ($p<0.05$). At 30 and 150 min the decreases in core and skin temperature were caused when the Biopac connection was interrupted to allow for body mass measurement.

	Core temperature (°C)		Skin temperature (°C)	
Time (min)	Cold	Warm	Cold	Warm
0	36.74 ± 0.48	36.87 ± 0.19	31.17 ± 0.85	30.69 ± 0.45
5	37.03 ± 0.40	37.12 ± 0.19	31.84 ± 0.76	31.24 ± 0.50
10	37.01 ± 0.37	37.09 ± 0.22	31.90 ± 0.74	31.31 ± 0.54
15	36.98 ± 0.34	37.04 ± 0.21	31.75 ± 0.79	31.31 ± 0.50
20	36.93 ± 0.34	36.99 ± 0.18	31.65 ± 0.81	31.32 ± 0.53
25	36.91 ± 0.33	36.97 ± 0.20	31.65 ± 0.78	31.30 ± 0.60
30	36.42 ± 0.38	36.44 ± 0.32	26.48 ± 0.99*$	30.37 ± 0.55
35	37.01 ± 0.27*	36.87 ± 0.19*	22.72 ± 1.40*$	30.88 ± 0.49
40	37.41 ± 0.29*	37.29 ± 0.16*	21.58 ± 1.40*$	31.09 ± 0.53
45	37.76 ± 0.33*	37.63 ± 0.15*	21.10 ± 1.40*$	31.60 ± 0.46
50	37.98 ± 0.36*	37.82 ± 0.17*	20.88 ± 1.41*$	31.85 ± 0.39
55	38.20 ± 0.39*	38.00 ± 0.21*	20.61 ± 1.32*$	32.08 ± 0.39
60	38.34 ± 0.39*	38.13 ± 0.25*	20.19 ± 1.21*$	32.14 ± 0.38
65	38.45 ± 0.40*	38.23 ± 0.26*	20.19 ± 1.20*$	32.10 ± 0.50
70	38.46 ± 0.42*	38.32 ± 0.27*	19.92 ± 1.08*$	32.17 ± 0.55
75	38.45 ± 0.47*	38.39 ± 0.26*	19.58 ± 0.87*$	32.19 ± 0.56
80	38.57 ± 0.42*	38.46 ± 0.28*	19.38 ± 1.03*$	32.19 ± 0.64
85	38.60 ± 0.41*	38.50 ± 0.29*	19.31 ± 1.03*$	32.22 ± 0.68
90	38.12 ± 0.38*	38.51 ± 0.29*	19.23 ± 1.00*$	32.25 ± 0.73
95	37.41 ± 0.37*	37.50 ± 0.31*	28.59 ± 0.78	31.60 ± 0.53
100	37.26 ± 0.31	37.43 ± 0.29	29.26 ± 0.65	31.62 ± 0.50
105	37.11 ± 0.29	37.33 ± 0.29	29.56 ± 0.63	31.56 ± 0.44
110	36.97 ± 0.27	37.29 ± 0.27	29.81 ± 0.63	31.61 ± 0.43
115	36.89 ± 0.26	37.24 ± 0.25	29.94 ± 0.55	31.51 ± 0.50
120	36.84 ± 0.24	37.17 ± 0.24	30.12 ± 0.55	31.74 ± 0.61
125	36.79 ± 0.23	37.12 ± 0.22	30.21 ± 0.55	31.91 ± 0.54
130	36.76 ± 0.22	37.06 ± 0.22	30.20 ± 0.52	31.85 ± 0.54
135	36.72 ± 0.20	37.02 ± 0.23	30.28 ± 0.58	31.74 ± 0.75
140	36.70 ± 0.19	36.99 ± 0.27	30.27 ± 0.52	31.68 ± 0.86
145	36.67 ± 0.19	36.93 ± 0.30	30.30 ± 0.44	31.64 ± 0.85
150	36.84 ± 0.24	37.17 ± 0.24	30.12 ± 0.55	30.50 ± 0.49
155	36.49 ± 0.16	36.77 ± 0.29	29.93 ± 0.49	31.43 ± 0.73
160	36.54 ± 0.16	36.80 ± 0.31	30.04 ± 0.55	31.57 ± 0.78
165	36.55 ± 0.16	36.80 ± 0.31	30.09 ± 0.60	31.67 ± 0.84
170	36.55 ± 0.16	36.78 ± 0.31	30.20 ± 0.65	31.51 ± 1.18
175	36.56 ± 0.17	36.78 ± 0.30	30.28 ± 0.64	31.66 ± 1.07
180	36.56 ± 0.18	36.78 ± 0.30	30.32 ± 0.72	31.71 ± 1.00
185	36.55 ± 0.20	36.76 ± 0.30	30.28 ± 0.86	31.44 ± 1.28
190	36.53 ± 0.21	36.77 ± 0.28	30.30 ± 0.95	31.36 ± 1.36
195	36.52 ± 0.22	36.77 ± 0.28	30.29 ± 0.93	31.29 ± 1.40
200	36.52 ± 0.21	36.75 ± 0.28	30.19 ± 1.12	31.32 ± 1.36
205	36.52 ± 0.20	36.75 ± 0.30	30.05 ± 1.26	31.59 ± 1.01
210	36.52 ± 0.19	36.75 ± 0.30	30.10 ± 1.17	31.47 ± 1.15

Appendix J

Data for figures presented in Chapter 6

Table 6.2. Voluntary water intake on each trial. $ denotes difference between trials, * denotes different from 30-60min ($p<0.05$).

	Water intake (l)	
Time	HI	LO
0-30	$0.348 \pm 0.240^{*}$	$0.209 \pm 0.217^{*\$}$
30-60	0.068 ± 0.107	0.085 ± 0.103
Total	0.416 ± 0.299	$0.294 \pm 0.295^{\$}$

Table 6.3. Serum osmolality values over the duration of each trial. $ different between trials, * different from baseline, # different to post-exercise ($p<0.05$).

	Serum osmolality ($mOsmol.kg^{-1}$)	
Sample	HI	LO
Baseline	285 ± 4	284 ± 3
Post-exercise	$298 \pm 3^{*\$}$	288 ± 4
5	$291 \pm 1^{\#\$}$	286 ± 3
15	$290 \pm 3^{\#}$	285 ± 5
30	$288 \pm 3^{\#\$}$	283 ± 3
60	$287 \pm 3^{\#}$	283 ± 3

Table 6.4. Serum sodium concentration over the duration of each trial. $ different over the duration of each trial, * different from baseline, # different to post-exercise ($p<0.05$).

	Serum sodium concentration ($mmol.l^{-1}$)	
Sample	HI	LO
Baseline	142 ± 1	141 ± 1
Post-exercise	$146 \pm 1^{*\$}$	143 ± 1
5	$143 \pm 1^{\#}$	142 ± 1
15	$143 \pm 1^{\#}$	142 ± 1
30	$142 \pm 1^{\#}$	142 ± 1
60	$142 \pm 2^{\#}$	142 ± 1

Table 6.5. Haemoglobin concentration and haematocrit over the duration of each trial. $ different between trials, * different to baseline, # different to post-exercise, ^ different to 5min ($p<0.05$).

	Haemoglobin concentration ($g.l^{-1}$)		Haematocrit (%)	
Sample	HI	LO	HI	LO
Baseline	156 ± 7	158 ± 7	44.0 ± 2.5	44.5 ± 2.0
Post-exercise	171 ± 7$^{*\$}$	163 ± 8*	48.2 ± 2.3$^{*\$}$	45.7 ± 2.4
5	165 ± 8$^{*\#}$	159 ± 7	46.6 ± 2.8$^{*\#}$	44.7 ± 2.3
15	160 ± 8$^{\#\wedge}$	158 ± 7$^{\#}$	45.1 ± 2.3$^{\#\wedge}$	44.5 ± 2.1$^{\#}$
30	158 ± 8$^{\#\wedge}$	157 ± 8$^{\#}$	44.3 ± 2.9$^{\#\wedge}$	44.3 ± 2.2$^{\#}$
60	159 ± 7$^{\#\wedge}$	158 ± 9$^{\#}$	44.4 ± 2.6$^{\#\wedge}$	44.7 ± 2.3

Table 6.6. Blood lactate concentrations over the duration of each trial. $ different between trials, * different to baseline, # different to post-exercise, ^ different to 5min, ~ different to 15min ($p<0.05$).

	Blood lactate concentration ($mmol.l^{-1}$)	
Sample	HI	LO
Baseline	0.8 ± 0.2	0.8 ± 0.3
Post-exercise	8.5 ± 2.7$^{*\$}$	0.7 ± 0.4
5	7.0 ± 2.6$^{*\#\$}$	0.8 ± 0.3
15	5.0 ± 2.2$^{*\#\wedge\$}$	0.9 ± 0.2
30	3.6 ± 1.5$^{*\#\wedge\sim\$}$	0.8 ± 0.1
60	2.4 ± 0.8$^{\#\wedge\sim\$}$	0.8 ± 0.3

Table 6.7. Vasopressin and aldosterone concentration over the duration of each trial. $ different between trials, * different to baseline, # different to post exercise ($p<0.05$).

	Vasopressin concentration ($pg.ml^{-1}$)		Aldosterone concentration ($pg.ml^{-1}$)	
Sample	HI	LO	HI	LO
Baseline	4.7 ± 0.5	4.5 ± 0.9	249 ± 237	150 ± 88
Post-exercise	9.9 ± 3.4$^{*\$}$	4.4 ± 0.9	1175 ± 1063	519 ± 327
5	7.5 ± 1.8$^{\$}$	4.2 ± 1.2	1066 ± 768	440 ± 302
30	6.9 ± 0.4$^{\#\$}$	4.4 ± 1.2	558 ± 358	215 ± 183$^{\$}$

Table 6.8. Plasma volume change from baseline values over the duration of each trial. $ different between trials, * different to baseline values, # different to the post exercise change, ^ different to the baseline to 5 min change ($p<0.05$).

	Plasma volume change from baseline (%)	
Sample	HI	LO
Baseline	0.0 ± 0.0	0.0 ± 0.0
Post-exercise	-16.8 ± 2.2$^{*\$}$	-5.0 ± 2.9
5	-10.0 ± 2.1$^{*\#\$}$	-1.3 ± 1.3
15	-5.1 ± 1.8$^{*\#\wedge\$}$	0.9 ± 2.2$^{\#}$
30	-2.2 ± 2.6$^{\#\wedge\$}$	0.9 ± 2.8$^{\#}$
60	-2.6 ± 2.8$^{\#\wedge}$	-0.4 ± 3.0$^{\#}$

Table 6.9. Blood volume change from baseline values over the duration of each trial. $ different between trials, * different to baseline values ($p<0.05$).

	Blood volume change from baseline (%)	
Sample	HI	LO
Baseline	0.0 ± 0.0	0.0 ± 0.0
Post-exercise	-9.6 ± 1.8$^{*\$}$	-3.3 ± 1.7*
5	-5.6 ± 1.6$^{*\$}$	-1.0 ± 0.9
15	-3.0 ± 1.1$^{*\$}$	0.5 ± 1.6
30	-1.5 ± 1.6$^{\$}$	0.9 ± 2.8
60	-2.6 ± 2.8	-0.4 ± 3.0

Table 6.10. Sensations of thirst and mouth dryness over the duration of each trial. * different to baseline, # different to post exercise, ^ different to 5min ($p<0.05$).

	Thirst (mm)		Mouth Dryness (mm)	
Sample	HI	LO	HI	LO
Baseline	53 ± 21	40 ± 15	52 ± 22	42 ± 16
Post-exercise	84 ± 7*	60 ± 21	87 ± 7*	64 ± 23
5	51 ± 16$^{\#}$	39 ± 23	49 ± 18$^{\#}$	42 ± 27
15	31 ± 18$^{\#}$	28 ± 16	26 ± 20$^{\#\wedge}$	28 ± 18
30	20 ± 12	28 ± 20	19 ± 14$^{\#\wedge}$	29 ± 22
60	16 ± 11	19 ± 12	14 ± 12$^{\#\wedge*}$	18 ± 13

Table 6.11. Core and skin temperature over the duration of each trial. $ different between trials, * different to baseline, ~ increase from previous time point, # decrease from previous time point, ^ different to 110-150 min ($p<0.05$).

	Core temperature (°C)		Skin temperature (°C)	
Time (min)	HI	LO	HI	LO
0	37.10 ± 0.33	37.11 ± 0.31	31.13 ± 0.83	30.79 ± 1.03
10	37.05 ± 0.28	37.06 ± 0.29	31.20 ± 0.93	30.96 ± 1.15
20	36.97 ± 0.25	36.98 ± 0.28	31.09 ± 1.11	30.97 ± 0.97
30	36.93 ± 0.23	36.91 ± 0.29	31.04 ± 1.25	31.03 ± 1.00
40	36.98 ± 0.24	36.92 ± 0.30	31.29 ± 0.99	31.38 ± 0.64
50	37.45 ± 0.28$^{\sim *\wedge}$	37.24 ± 0.33$^{\sim}$	31.57 ± 1.36	31.78 ± 0.86
60	37.77 ± 0.25$^{\sim *\wedge \$}$	37.38 ± 0.34$^{\sim *}$	31.89 ± 1.37	32.16 ± 1.02
70	37.98 ± 0,26$^{\sim *\wedge \$}$	37.50 ± 0.34$^{\sim *}$	31.79 ± 1.33	32.00 ± 1..17
80	38.10 ± 0.28$^{\sim *\wedge \$}$	37.57 ± 0.32$^{\wedge *}$	32.08 ± 1.14	32.11 ± 1.33
90	38.17 ± 0.29$^{*\wedge \$}$	37.63 ± 0.30$^{\wedge *}$	32.04 ± 1.26	32.02 ± 1.31
100	37.71 ± 0.30$^{\# *\wedge \$}$	37.33 ± 0.22*	31.73 ± 1.07	32.22 ± 1.41
110	37.40 ± 0.29$^{\#}$	37.18 ± 0.23	31.87 ± 1.06	32.16 ± 1.21
120	37.20 ± 0.30$^{\#}$	37.09 ± 0.22	31.81 ± 1.07	31.98 ± 1.25
130	37.05 ± 0.33$^{\#}$	37.01 ± 0.23	31.81 ± 1.14	31.77 ± 1.23
140	36.95 ± 0.31$^{\#}$	36.93 ± 0.24	31.59 ± 1.05	31.67 ± 1.29
150	36.84 ± 0.33$^{\#}$	36.89 ± 0.27	31.41 ± 1.18	31.50 ± 1.19

Appendix K

Data for figures presented in Chapter 7

Table 7.2. Water intake (l) on each trial. Comparison between W and W30 trials. $ denotes difference between trials, * denotes difference to 0-5 min (W) and # denotes different to 5-30 min (W) ($p<0.05$).

	Water intake (l)	
Time (min)	W	W30
0-5	0.262 ± 0.165	-
5-30.	0.357 ± 0.187	-
30-35	$0.046 \pm 0.147^{*\#\$}$	0.296 ± 0.128
35-60	$0.182 \pm 0.147^{*\#\$}$	0.334 ± 0.158
Total	$0.846 \pm 0.417^{\$}$	0.630 ± 0.277

Table 7.3. Serum osmolality over the duration of each trial. $ denotes different to baseline values in all trials, # denotes different to baseline values in W and NW trial, * denotes different to post-exercise values in all trials ($p<0.05$).

	Serum osmolality ($mOsmol.kg^{-1}$)		
Sample	W	W30	NW
Baseline	284 ± 3	284 ± 3	285 ± 3
Post-exercise	$299 \pm 6^{\$}$	$298 \pm 5^{\$}$	$298 \pm 3^{\$}$
5	$293 \pm 3^{\$*}$	$291 \pm 3^{\$*}$	$291 \pm 3^{\$*}$
15	$289 \pm 3^{*}$	$288 \pm 4^{*\#}$	$290 \pm 3^{*\#}$
30	$287 \pm 4^{*}$	$285 \pm 3^{*}$	$288 \pm 2^{*}$
35	$286 \pm 2^{*}$	$286 \pm 3^{*}$	$288 \pm 3^{*}$
60	$284 \pm 2^{*}$	$285 \pm 2^{*}$	$287 \pm 3^{*}$

Table 7.4. Serum sodium concentrations over the duration of each trial. $ denotes different to baseline values in W, ^ denotes different to baseline values in W30, # denotes different to baseline values in NW trial, * denotes different to post-exercise value in all trials, ~ denotes different to post-exercise value in W and NW trial ($p<0.05$).

	Serum sodium concentration ($mmol.l^{-1}$)		
Sample	W	W30	NW
Baseline	141 ± 1	141 ± 1	141 ± 1
Post-exercise	145 ± 2$^{\$}$	145 ± 2$^{\wedge}$	145 ± 2$^{\#}$
5	143 ± 2*	143 ± 1$^{*\wedge}$	144 ± 1$^{*\#}$
15	142 ± 2$^{\sim}$	143 ± 2	143 ± 1$^{\sim\#}$
30	142 ± 2*	143 ± 2*	143 ± 1$^{*\#}$
35	141 ± 2*	141 ± 1*	142 ± 1$^{*\#}$
60	140 ± 1*	140 ± 1*	142 ± 1$^{*\#}$

Table 7.5. Haemoglobin concentrations and haematocrit values over the duration of each trial. $ denotes different to baseline values, * denotes different to post-exercise values, # denotes different to 5 min values ($p<0.05$).

	Haemoglobin concentration ($g.l^{-1}$)			Haematocrit (%)		
Sample	W	W30	NW	W	W30	NW
Baseline	158 ± 9	157 ± 8	157 ± 9	44.8 ± 2.1	44.5 ± 2.0	45.0 ± 2.3
Post-exercise	171 ± 9$^{\$}$	171 ± 9$^{\$}$	172 ± 9$^{\$}$	48.7 ± 2.4$^{\$}$	48.4 ± 2.2$^{\$}$	48.7 ± 2.2$^{\$}$
5	164 ± 9$^{\$*}$	165 ± 9$^{\$*}$	164 ± 9$^{\$*}$	46.8 ± 2.4$^{\$*}$	46.9 ± 2.3$^{\$*}$	47.0 ± 2.5$^{\$*}$
15	159 ± 10$^{*\#}$	159 ± 9$^{*\#}$	160 ± 11$^{*\#}$	45.2 ± 2.4$^{*\#}$	45.1 ± 2.5$^{*\#}$	45.2 ± 2.5$^{*\#}$
30	155 ± 10$^{*\#}$	157 ± 9$^{*\#}$	156 ± 9$^{*\#}$	44.1 ± 2.6$^{*\#}$	44.2 ± 2.1$^{*\#}$	44.0 ± 2.6$^{*\#}$
35	155 ± 9$^{*\#}$	156 ± 9$^{*\#}$	156 ± 9$^{*\#}$	43.8 ± 2.3$^{*\#}$	43.9 ± 2.3$^{*\#}$	43.9 ± 2.3$^{*\#}$
60	156 ± 9$^{*\#}$	156 ± 10$^{*\#}$	156 ± 10$^{*\#}$	44.4 ± 2.2$^{*\#}$	44.1 ± 2.4$^{*\#}$	44.0 ± 2.4$^{*\#}$

Table 7.6. Plasma volume changes compared to baseline values over the duration of each trial. * denotes different to baseline values in all trials ($p<0.05$).

	Plasma volume change from baseline (%)		
Sample	W	W30	NW
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Post-exercise	-14.3 ± 2.8*	-14.9 ± 4.0*	-14.5 ± 3.7*
5	-8.4 ± 2.1*	-9.6 ± 4.1*	-7.8 ± 2.9*
15	-1.8 ± 2.3	-2.7 ± 3.8	-1.7 ± 4.1
30	2.6 ± 3.3	0.1 ± 2.4	2.7 ± 3.5
35	2.3 ± 3.0	1.6 ± 3.6	3.1 ± 3.3
60	1.9 ± 3.2	1.2 ± 4.4	2.4 ± 3.2

Table 7.7. Blood volume changes compared to baseline values over the duration of each trial. * denotes different to baseline values in all trials, # denotes NW trial different to W30 ($p<0.05$).

	Blood volume change from baseline (%)		
Sample	W	W30	NW
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Post-exercise	-7.8 ± 1.9*	-8.5 ± 2.3*	-8.4 ± 2.8*
5	-4.5 ± 1.6*	-5.4 ± 2.5*	-4.4 ± 1.6*
15	-1.1 ± 1.3	-1.7 ± 2.3	-1.3 ± 2.5
30	1.4 ± 1.9	-0.4 ± 1.4	1.0 ± 2.0$^{\#}$
35	1.1 ± 1.8	0.5 ± 2.1	1.1 ± 2.0
60	1.2 ± 1.6	0.3 ± 2.5	0.6 ± 2.0

Table 7.8. Blood lactate concentrations over the duration of each trial. $ denotes different to baseline values in all trials, * denotes decrease from previous sample in all trials and # denotes decrease from previous sample in W30 and NW trials ($p<0.05$).

	Blood lactate concentration ($mmol.l^{-1}$)		
Sample	W	W30	NW
Baseline	0.5 ± 0.3	0.4 ± 0.3	0.5 ± 0.3
Post-exercise	7.1 ± 1.1$^{\$}$	7.2 ± 1.1$^{\$}$	7.1 ± 1.2$^{\$}$
5	6.5 ± 0.4$^{\$}$	5.3 ± 1.3$^{\$\#}$	5.7 ± 0.9$^{\$\#}$
15	4.6 ± 0.9$^{\$*}$	4.1 ± 1.1$^{\$*}$	3.9 ± 1.0$^{\$*}$
30	3.2 ± 0.7$^{\$*}$	2.6 ± 0.9$^{\$*}$	2.8 ± 0.6$^{\$*}$
35	2.7 ± 0.7$^{\$}$	2.4 ± 0.6$^{\$}$	2.4 ± 0.6$^{\$}$
60	1.6 ± 0.4$^{\$*}$	1.5 ± 0.4$^{\$*}$	1.6 ± 0.4$^{\$*}$

Table 7.9. Vasopressin (mean ± SD) and aldosterone (median (range)) concentrations over the duration of each trial. $ denotes different to baseline values in all trials, * denotes different to post-exercise in all trials, # denotes different to baseline in W and W30, [a] denotes different to baseline in W and ^ denotes different to post-exercise in W ($p<0.05$).

	Vasopressin concentration ($pg.ml^{-1}$)			Aldosterone concentration ($pg.ml^{-1}$)		
Sample	W	W30	NW	W	W30	NW
Baseline	3.85 ± 0.96	4.30 ± 1.60	4.39 ± 1.27	213 (27-889)	221 (8-1123)	164 (22-1100)
Post-exercise	8.27 ± 2.61$^{\$}$	7.17 ± 2.25$^{\$}$	7.13 ± 2.16$^{\$}$	1229 (106-4001)$^{\$}$	652 (91-4503)$^{\$}$	1326 (113-4967)$^{\$}$
30	5.46 ± 1.19^{a}	5.82 ± 1.55	6.78 ± 2.34	347 (57-1684)$^{\#}$	447 (35-2189)$^{\#}$	442 (51-3421)
60	5.24 ± 0.89$^{\wedge\#}$	5.88 ± 1.27$^{\#}$	6.17 ± 1.90	312 (48-595)*	556 (18-1845)*	265 (32-1879)*

Table 7.10. Subjective feeling questionnaire responses for thirst and mouth dryness over the duration of each trial. * denotes difference between W and W30 trials, # denotes difference between W and NW trials, ^ denotes difference between W30 and NW trials, [a] denotes difference within the trial compared to baseline and [b] denotes difference within the trial compared to post-exercise ($p<0.05$).

	Thirst (mm)			Mouth Dryness (mm)		
Sample	W	W30	NW	W	W30	NW
Baseline	51 ± 17	39 ± 15*	48 ± 22	52 ± 15	43 ± 15	47 ± 21
Post-exercise	81 ± 14^{a}	80 ± 12^{a}	86 ± 13^{a}	84 ± 14^{a}	86 ± 13^{a}	84 ± 15^{a}
5	62 ± 17^{b}	77 ± 13^{a}	84 ± 14$^{a\#}$	56 ± 26^{b}	80 ± 17^{a}	86 ± 13$^{a\#}$
15	41 ± 21^{b}	76 ± 15^{a*}	80 ± 17$^{a\#}$	38 ± 24^{b}	78 ± 15^{a*}	84 ± 15$^{a\#}$
30	25 ± 18^{ab}	68 ± 24^{a*}	75 ± 15$^{a\#}$	24 ± 17^{ab}	71 ± 24^{a*}	76 ± 15$^{a\#}$
35	21 ± 15^{ab}	47 ± 25^{b*}	77 ± 16$^{\#\wedge}$	20 ± 15^{ab}	40 ± 25^{b*}	74 ± 20$^{\#\wedge}$
60	17 ± 12^{ab}	29 ± 19^{b}	70 ± 20$^{\#\wedge}$	14 ± 10^{ab}	27 ± 17^{b}	68 ± 24$^{\#\wedge}$

Table 7.11. Core and skin temperatures over the duration of each trial. * denotes different to baseline in all trials, [a] denotes an increase from the previous time point in all trials and [b] denotes different to baseline in W30 and NW trials ($p<0.05$).

	Core temperature (°C)			Skin temperature (°C)		
Time (min)	W	W30	NW	W	W30	NW
0	36.78 ± 0.32	36.74 ± 0.35	36.68 ± 0.41	30.80 ± 0.64	30.79 ± 0.82	30.80 ± 0.65
10	36.84 ± 0.31	36.74 ± 0.31	36.71 ± 0.35	31.21 ± 0.62	31.22 ± 0.85	31.41 ± 0.71
20	36.80 ± 0.32	36.75 ± 0.27	36.66 ± 0.34	31.21 ± 0.60	31.31 ± 0.76	31.46 ± 0.56
30	36.75 ± 0.31	36.75 ± 0.29	36.64 ± 0.31	31.24 ± 0.50	31.42 ± 0.68	31.35 ± 0.44
40	36.87 ± 0.39*	36.75 ± 0.29*	36.78 ± 0.35*	31.14 ± 0.59	31.03 ± 0.76	31.13 ± 0.77
50	37.36 ± 0.39^{*a}	37.31 ± 0.38^{*a}	37.19 ± 0.38^{*a}	31.44 ± 0.65	31.29 ± 0.73	31.44 ± 0.86
60	37.72 ± 0.29^{*a}	37.67 ± 0.41^{*a}	37.54 ± 0.36^{*a}	31.73 ± 0.66	31.50 ± 0.64	31.82 ± 1.02
70	37.97 ± 0.24^{*a}	37.90 ± 0.39^{*a}	37.81 ± 0.32^{*a}	31.90 ± 0.53	31.80 ± 0.95	31.93 ± 0.95
80	38.15 ± 0.19*	38.07 ± 0.38*	37.96 ± 0.31*	32.22 ± 0.73	32.08 ± 1.02	32.20 ± 0.96
90	38.24 ± 0.17*	38.16 ± 0.36*	38.04 ± 0.33*	32.12 ± 0.73	32.13 ± 1.01	32.07 ± 0.93
100	37.79 ± 0.30*	37.80 ± 0.40*	37.81 ± 0.37*	31.62 ± 0.75	31.55 ± 1.11	31.66 ± 0.79
110	37.41 ± 0.23	37.53 ± 0.33	37.44 ± 0.40^{b}	31.33 ± 0.79	31.67 ± 1.01	31.53 ± 0.69
120	37.14 ± 0.23	37.34 ± 0.24	37.22 ± 0.37^{b}	31.32 ± 0.82	31.76 ± 0.93	31.54 ± 0.62
130	36.98 ± 0.22	37.16 ± 0.19	37.11 ± 0.28^{b}	31.29 ± 1.02	31.61 ± 0.92	31.63 ± 0.72
140	36.89 ± 0.21	36.94 ± 0.21	36.98 ± 0.23	31.36 ± 0.92	31.49 ± 0.90	31.50 ± 0.54
150	36.81 ± 0.22	36.81 ± 0.24	36.90 ± 0.22	31.29 ± 0.88	31.22 ± 0.98	31.45 ± 0.63