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Hydration, thirst and fluid balance in resting and exercising individuals

by

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A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

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CERTIFICATE OF ORIGINALITY

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Declaration

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

Jusoh, N., & Shirreffs, S. M. (2009). Thirst as a marker of hydration status? *British Journal of Sports Medicine*, 43(E2), 7. doi:10.1136/bjsm.2009.066886x

Abstract

Adequate fluid consumption is central to human survival. Previous literature suggests that there some misconceptions regarding hydration and fluid balance in some populations. Available data also show that the role of thirst sensations in maintaining fluid balance in different settings is also equivocal. Therefore, this thesis aimed to examine the perception of hydration, thirst and fluid intake in free-living populations, to examine the feasibility of thirst as a marker of hydration status and to investigate the effect of thirst related sensations on fluid balance in resting and exercising individuals under different ambient temperatures. The findings in this thesis (Chapter 3) show that individuals who work within the fitness industry demonstrated substantial knowledge about drinking practices, hydration status and health consequences of water consumption, but lack understanding on the type of beverages that adequately hydrate the body. Further, thirst perception and mood states did not affect ($P>0.05$) the fluid intake in free living individuals (Chapter 4) and resting individuals under cool and warm exposure (Chapter 6), but some other factors such as subjective feelings of mouth dryness and the extent of hydration status might influence the fluid intake behaviour in these populations. In addition, following ingestion of flavoured carbohydrate drinks, thirst sensations was rated lower over time ($P<0.05$) during exercise in the cool, but was higher over time in the warm temperature (Chapter 7). Moreover, subjective feelings related to dehydration such as mouth dryness, thirst perception, desire to drink (water pleasantness) and hunger rating could be used as index of hydration status to signify at least a 1% body mass loss due to food and fluid restriction in resting individuals (Chapter 5). In conclusion, the findings in this thesis provide some new insight with respect to hydration, thirst and fluid balance in different populations under different settings. Nevertheless, some inconclusive findings regarding the role of thirst related sensations in fluid balance require further investigations.

Keywords: **Hydration, Thirst, Fluid intake, Fluid balance, Subjective feelings, Ambient temperatures**

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List of Abbreviations

ANOVA	analysis of variance
beats min^{-1}	beats per minute
BMI	body mass index
CHO	carbohydrate
CI	confidence interval
Cl^{-}	chloride
CV	coefficient of variation
d	day
g	Hedge's *g* effect size
g	grams
h	hour
K^{+}	potassium
K_2EDTA	potassium ethylenediamine tetra acetic acid
kg	kilogram
km	kilometre
L	litre
m	metre
M	mole
mg	milligram
MJ	megajoule
ml	millilitre
mmol	millimole
mOsm	milliosmole
min	minute
Na^{+}	sodium
NaCl	sodium chloride
ppm	part per million
POMS	profile of mood states
r	effect size
rh	relative humidity
s	second

SD	standard deviation
T	temperature
TSS	thermal sensation scale
TBW	total body water
VAS	visual analog scale
VO_{2max}	maximum rate of oxygen uptake
y	years
WT	water turnover
°C	degrees Celsius
^{2}H	deuterium
$^{2}H_{2}0$	deuterium oxide
%	percent
η^2	eta squared

Chapter 1

General Introduction

Historical background of hydration and fluid balance research

The study of hydration and fluid balance is rooted from the field of exercise physiology that is believed to begin over centuries ago. Among the earliest and greatest individuals who shaped this field were the Greek physicians such as Hippocrates (460 to 377 B. C) and Claudius Galenus or Galen (131 to 201 A. D). Hippocrates also known as 'the father of preventive medicine' contributed 87 treatises on medicine, which include several on health and hygiene (Berryman, 1989). Following Hippocrates, Galen emerged as the prominent and influential physician in the history of science and medicine. He strongly believed that medicine is a highly interdisciplinary field that was best practiced by utilizing theory, observation and experimentation to obtain the best results. As a laboratory oriented physiologist, Galen contributed to the enhancement of health and scientific hygiene and wrote numerous essays related to human anatomy, physiology, nutrition and the benefits of exercise and consequences of sedentary life (Green, 1951).

Among the earliest impact of laboratory research in hydration and fluid balance originated from the popularity of marathon running in early 1900s in which athletes were told to abstain from drinking water before and during running because it might impede performance. This popular coaching doctrine of avoiding water consumption continued to be the established practice among athletes in 1940s and 1950s because it was considered harmful to performance, a sign of weakness and 'less manly' (Little, Strayhorn, & Miller, 1949).

On the contrary, numerous research simulating desert and jungle warfare conditions among military personnel during similar period of 1930s to 1950s showed that fluid consumption during physical activity was beneficial by maintaining body fluid balance (Talbort, Edwards, Dill, & Drastich, 1933; Adolph & Dill, 1938; Adolph, 1947). However, these studies failed to change the popular coaching doctrine at the time. Only in late 1960s, the findings from 2 key studies that stimulated the modern interest in hydration and fluid balance before, during and after physical activity. Pugh, Corbett, and Johnson (1967) investigated rectal temperature, weight losses and sweat rates in 77 marathon runners during 42 km marathon race and concluded that successful runners possessed a high tolerance to fluid loss. Wyndham and Strydom (1969) also investigated the effect of inadequate water intake on physiological responses during long distance running in athletes and found that as the athletes became dehydrated by 3% or more of body mass, the post-race rectal temperature increased linearly. Since then, the research into hydration and fluid balance continues to grow and the view about the importance of the role of fluid intake in physical activity and general health has changed significantly.

Knowledge and attitude of hydration

Simple observation of the general population suggests that drinking practices have changed significantly over the past 10 to 20 years. Nowadays, it is common to see people carry a water bottle with them during the day either in sedentary individuals or in people who perform regular physical activity. In fact, there is a substantial amount of information regarding hydration available both in printed or online format, suggesting that people should drink sufficiently either for the benefits of health or exercise performance or both.

However, not all information regarding hydration strategies are backed up with scientific evidence, and therefore could be misleading. Valtin (2002) in his review about the origin of the "drinking at least eight glass of water a day" recommendations for healthy individuals performing at least mild exercise concludes that such a recommendation has no support from scientific evidence. Another common belief, which has been taken literally by the public, is that consuming caffeinated beverages is dehydrating and may compromise hydration status that was based on earlier evidence (Robertson et al., 1978; Passmore, Kondowe, & Johnston, 1987; Neuhauser-Berthold, Verwied & Luhrmann, 1997). Furthermore, despite the recommendation to consume water from variety of sources to prevent dehydration (Institute of Medicine [IOM], 1989), many people tend to believe that the source has to come from plain water only (Kulpa, 2002; Nutri Science Corp., 2005). However, these views have been challenged by scientific evidence that caffeine-containing beverages do not affect fluid balance if consumed in amounts normally found in standard servings of coffee, tea and soft drinks (Maughan & Griffin, 2003) and that the caffeinated beverages hydrate the body as good as plain water (IOM, 2005). In addition, recent investigations by Grandjean, Reimers, Bannick and Haven (2000) and Grandjean, Reimers, Haven and Curtis (2003) provide additional support of the positive effect of caffeinated drinks on hydration status. In the earlier study, Grandjean and colleagues (2000) conducted a counterbalanced, crossover study in 18 healthy males on four occasions where the participants consumed the following drinks; water only, water plus caffeinated caloric cola, water plus caffeinated non-caloric colas and combinations of water, caffeinated caloric cola, caffeinated non-caloric cola and coffee. No significant differences ($P>0.05$) were found between trials on the markers of hydration status such as body weight changes, blood indices of haemoglobin, haematocrit and serum osmolality and urine indices of 24 h urine volume, creatinine, osmolality and specific gravity. Another study by Grandjean et al. (2003) on the effect of two regimes, one with plain water and another without plain water on the hydration status showed the similar results. In that study, twenty-seven healthy sedentary males undertook two trials with two diet regimes, one included plain water and caffeinated drinks as part of the beverages and another trial provided caffeinated drinks without plain water, while diet, physical activity and environment were controlled. They found no significant differences ($P>0.05$) between trials for hydration status markers such as body weight changes, urine osmolality and urine specific gravity, and hence concluded that caffeinated beverages also support hydration.

To deal with this kind of misinformation, sound knowledge seems important so that the correct hydration practice could be instilled. To date, much information about hydration knowledge is from studies on athletes (Felder, Burke, Lowdon, Cameron-Smith, & Collier, 1998; Zawila, Steib, & Hoogenboom, 2003; Rosenbloom, Jonnalagada, & Skinner, 2002; Nichols, Jonnalagada, &

Rosenbloom, 2005; Soo & Naughton, 2007), but such information is lacking in free-living individuals and recreationally active people. In general, the findings in these studies conclude that these populations generally exhibited poor knowledge and attitude towards hydration and needed to be educated for proper fluid replacement and hydration. To demonstrate, Nichols and colleagues (2005) investigated the knowledge, attitudes and behaviours regarding hydration and fluid intake in 139 collegiate athletes and reported that only 5.8% achieved a perfect score of 100% for 17 items of hydration knowledge questions. About one-third (33.1%) of athletes gave a wrong answer for 14 out of 17 questions. In addition, the athletes were knowledgeable about general hydration such as the importance of consuming fluid during and after training and competition, but lacked understanding with regard to an appropriate use of sports drinks. Another study by Felder et al. (1998) on nutritional practices of 10 elite female surfers during training and competition showed that only 50% of the athletes consumed sport drink during competition and 40% drank alcohol before competing on the competition day. The results suggest that the athletes practice the behaviour that could lead to dehydration and consequently might affect their performance.

The athletes become the target population for scientific study probably because the significant role of proper hydration in maintaining sports performance and hydration status can change rapidly. Nevertheless, hydration knowledge and habits among general populations who are sedentary or recreationally active also need attention because proper hydration definitely helps to maintain general health on day-to-day basis (Manz & Wentz, 2005).

Body fluid balance

Under normal physiological conditions, body water content in humans is tightly regulated to maintain water homeostasis. By definition, fluid balance means water input equals water output. Among the sources of water input are drinking beverages, foods and metabolic water, meanwhile water losses through urine, perspiration, respiration and faeces (McArdle, Katch, & Katch, 2009). When water intake equals water loss and water homeostasis is being maintained, individuals are said to be in a state of euhydration, which is represented by a sinusoidal wave that oscillates around an average (Greenleaf, 1992; Oppliger & Bartock, 2002). According to Greenleaf (1992), the volume of total body water is regulated daily within ± 0.22 to ± 0.48% of body weight. When the body water decreased or increased above the euhydrated level, people are hypohydrated and hyperhydrated, respectively. Further, dehydration refers to the process of water loss from the body through sweat, urine, faeces and respiratory which reduces the total body water below the euhydrated level, meanwhile rehydration is the process of gaining body water from ingestions of drinks or foods. Water is stored in different compartments but moves freely within compartments. The largest quantity (approximately 65%) is in the intracellular compartment, whereas the remaining 35% is distributed in the extracellular compartment.

The benefits of maintaining water balance has been long acknowledged for health and survival. It typically makes up the main component of human body which ranges between 50 to 60% of body mass in an average adult. Water is used to transport metabolic end products out of our body, to regulate body temperature and to help in digestion, absorption and transportation of nutrients.

Disturbed water balance may lead to some potential problematic and life threatening events such as dehydration and water intoxication hyponatraemia. Few studies have established that body mass loss of at least 2% can affect cardiovascular and thermoregulatory responses and consequently reduce exercise performance (Rogers, Setliff, & Klopping, 1964; Montain & Coyle, 1992; Gonzalez-Alonso, Mora-Rodriguez, Below, & Coyle, 1995) as well as cognitive function (Maughan, 2003; Wilson & Morley, 2003). Similarly, an excessive water intake over a short period may dilute plasma sodium leading to hyponatreamia and may either be fatal or non-fatal (Noakes & Speedy, 2006).

Furthermore, inadequate hydration status may lead to the development of several health problems. Manz (2007) outlined the positive effects of maintaining adequate hydration status in the prevention of several diseases based on 4 categories of evidence (I-IV). He concluded that adequate fluid intake may be effective in preventing the following diseases; urolithiasis (evidence from at least one randomised controlled trial (Ib)), urinary tract infections (evidence from at least one other type of quasi-experimental study (IIb)), constipation, hypertension and stroke (evidence from descriptive studies such as comparative, correlation and case control studies (III)), and dental disease (evidence from expert committee reports, opinion or clinical experience of respected authorities, or both (IV)).

Body water turnover

Total body water can be assessed by water turnover rate, which is the replacement of body water that is lost over a specific period. Water turnover in normal sedentary adults in a temperate environment is reported to be approximately 2-2.5 L or 5% of the total body water (Shirreffs & Maughan, 2001). There are a number of ways to estimate water turnover, but current consensus is that tracer technique using a stable isotope such as deuterium oxide and tritium oxide provides the best objective measure of total body water (Armstrong, 2007; Schoeller, 1996). These substances are ideal because they are diffusible into all the body fluid compartments within short period, reach a uniform equilibrium at which its concentration can be measured and are not metabolised in the body (Schloerb, Friis-Hansen, Edelman, Solomon, & Moore, 1950).

Factors such as exercise, ambient temperature and age may affect water turnover in humans. Water turnover is reported to be higher with increased level of physical activity. Leiper, Pitsiladis and Maughan (2001) studied the water turnover in cyclists and sedentary men and found that water turnover rate was higher in exercising individuals (3.38 L d^{-1}) than the sedentary group (2.22 L d^{-1}) due to greater non-renal water losses. The findings in this study also reveal that the urinary output was higher ($P<0.05$) in exercising group than the control group, suggesting higher fluid intake in the exercising group. This is likely because exercising individuals normally have increased sweat loss, and if water balance is to be maintained, they need to replenish the loss with fluid intake.

Other studies carried out to compare water turnover rate in physically active subjects versus sedentary subjects also produced the similar findings, which shown higher water turnover rate with increased level of physical activity (Leiper, Carnie, & Maughan, 1996; Shimamoto & Komiya, 2003). Most of the cited studies on the effect of exercise on water turnover were performed in adult men. Therefore, there is limited data on the influence of exercise on water turnover in adult female individuals. Women are known to sweat less than men, and during menstrual period, there is a tendency for water retention in their body (Jurkowski, Jones, Toews, & Sutton, 1981). Thus, effects of these factors on water turnover also warrant further investigations.

Besides exercise, environmental temperature also influences water turnover in humans. A review by Shirreffs and Maughan (2001) compared the water turnover in women exposed to hot and cold temperature. Water turnover in Gambian women (Singh et al., 1989) living in a temperate (23-28°C) climate was compared with women in Cambridge, England, who were exposed to cool (11-19°C) conditions. They reported that Gambian women had higher water turnover rate (5.2 L d^{-1}) than those women in Cambridge (3.2 L d^{-1}), and they concluded the difference was due to warmer weather conditions. This is most probable because people in hot climate have higher sweat rate and may increase fluid intake to compensate for sweat losses, thus higher water turnover rate.

Despite the seasonal effect of water turnover observed in those studies, Leiper, Primrose, Primrose, Phillimore and Maughan (2005) found otherwise. They investigated the water turnover in older people living in community and in nursing homes during summer (11-19°C) and winter (0-7°C). The findings show that the independent group had faster ($P<0.05$) water turnover rate than the dependent group both in summer and winter. However, there was no difference in water turnover rate detected in both groups across the seasons. Likewise, average urine output and calculated non-renal water losses were found similar ($P>0.05$) in both groups regardless of seasonal changes.

Assessment of hydration status

There are numbers of methods that are commonly used to assess hydration status in humans such as objective measures using body weight changes, blood and urine indices and saliva and also subjective measure such as perception of thirst.

A body mass change has been used to quantify total body loss or gain in short period of time based on the assumption that 1 ml of water equals to 1 g (Lentner, 1981). Due to its simplicity, a change in body mass has been commonly used to measure hydration status in exercising individuals particularly in athletes. Changes in pre and post exercise body mass are used to estimate sweat losses that occur during exercise, hence the hydration status. Body mass changes is a reliable marker of hydration status because it provides an accurate estimate of the acute changes in total body water during exercise (Cheuvront, Carter, Montain, & Sawka, 2004; Sawka et al., 2007). A recent investigation by Baker, Lang and Kenney (2009) supports this view. They examined the relationship between changes in body mass and total body water in 8 endurance athletes who completed endurance exercise in the heat. Pre and post exercise body mass were measured and total body water were determined using a dilution technique corrected for D_2O lost in urine, sweat, breath and non-aqueous hydrogen exchange. The results of the study show that the intraclass correlation coefficient between changes in body mass and total body water was 0.76, indicating a strong reliability (Shoukri & Pause, 1999).

However, it is clear that not all weight loss during exercise reflects the changes in total body water because some water bound to glycogen which is released when the glycogen is oxidised as a fuel may provide additional amounts of water to the body water pool and therefore may prevent the body water deficit (Olsson & Saltin, 1970; Speedy et al., 2001). In addition, Maughan, Shirreffs and Leiper (2007) stated that even though body mass loss is the outcome from substrate oxidation, but this oxidation generates water as its by-product and hence contributes to the body water pool and does not reflect the true body mass changes. Furthermore, there is evidence to suggest that body mass changes may not be valid to assess changes in body water over a long period of time because factors such as food and fluid intake or changes in body composition may mask or exacerbate the changes in body weight (O'Brien, Young, & Sawka, 2002).

Blood indices such as plasma and serum osmolality, haematocrit and haemoglobin are among the common markers that have been investigated as indices of hydration status. A valid measurement of haematocrit and haemoglobin requires the standardisation of body posture before the blood collection because a postural change is one of the factor that affects the blood volume and thus haematocrit and haemoglobin (Shirreffs, 2003).

Despite the wide use of plasma or serum osmolality to detect hydration status in different settings, its reliability has been questioned. Armstrong and colleagues (1998) investigated the hydration markers during dehydration, exercise and rehydration in 9 healthy males. The participants were dehydrated equivalent to 4% body mass loss and blood and urine markers were measured at baseline, during dehydration period, after 2 h of exercise, after 4 and 21 h of rehydration, which totalled up to 42 h of observation period. The findings show that plasma osmolality did not track change with dehydration and rehydration as good as urine indices. In contrast, an investigation by Popowski et al. (2001) in 12 male participants who were subjected to 1-5% progressive dehydration concluded that plasma osmolality is sensitive to changes in hydration status during acute dehydration and rehydration. Another study by Oppliger, Magnes, Popowski and Gisolfi (2005) compared the accuracy of urine markers with plasma osmolality in 12 male athletes who were dehydrated till 5% of body mass loss and supported the findings that plasma osmolality responds accurately to progressive dehydration and recovery, while urine specific gravity and urine osmolality are less sensitive and lag behind the plasma osmolality.

Among the common urine markers being used to detect hydration status are urine colour, osmolality and urine specific gravity. Similar to blood indices, the reliability of urine markers in different settings is equivocal. Francesconi et al. (1987) conducted a field study to examine urinary and hematologic indexes of hypohydation at rest in the US army personnel and suggested that urinary indices reflect the hypohydration better than haematological markers. Another study by Armstrong et al. (1994) on the accuracy of urinary indices of hydration status which consisted of two laboratory studies and one field study concluded that urine colour, osmolality and specific gravity are more sensitive to mild hypohydration compared to selected blood indices. However, in situation when a large bolus of hypotonic fluid is ingested rapidly during rehydration period, urine indices may not be valid to assess hydration status. This is because the large amount of water causes the kidney to produce dilute urine and urine markers such as urine specific gravity value may give a misleading result (Armstrong *et al.*, 1998). This is further supported by Kovacs, Senden and Brouns (1999) who examined the accuracy of urine markers as indices of hydration status in 8 healthy men who were dehydrated to 3% body mass and then were given fluid *ad libitum* for the first 2 h of rehydration period and were observed up to 6 h. The results show that the level of rehydration did not correlate with urine colour and osmolality and therefore suggest that urine markers are poor index of hydration status during post-exercise rehydration.

Perception of thirst has been used to assess hydration status (McGarvey et al., 2008) but not as extensively as objective markers. Most data available suggest that thirst is a poor indicator of hydration because it lags behind the body mass loss, and only occurs when total body water reaches 1 to 2 % of body mass (Greenleaf & Harrison, 1986; Hubbard, Szlyk, & Armstrong, 1990). However, perception of thirst may potentially be used in situations where instrumentations or technical expertises are unavailable (Armstrong, 2005), while at the same time provides the non-invasive alternative to individuals who may not like the invasive methods such as blood samples.

Thirst and fluid intake

Keeping body water at an appropriate volume is essential for life. Fluid balance depends on two primary mechanisms; one mechanism involves fluid-regulating hormones that control renal water retention and loss and another mechanism stimulates thirst drive leading to voluntary fluid intake.

A proper definition of thirst has become the subject of debate whether it is local (pharyngeal), general sensation or central (Fitzsimons, 1972).

Dry mouth has long been associated with thirst sensation (Cannon, 1919). Essentially, the 'dry mouth' theory proposes that impaired salivary gland over time leading to unpleasant dry feeling in the mouth and throat and if the feelings are not removed adequately, thirst may result as an indicator to lessen that unpleasant feeling. Engell et al. (1987) support this manifestation of thirst and subjective feeling of mouth dryness and further included other subjective feelings associated with thirst such as a dry, scratchy mouth and throat, chapped and dry lips, tiredness, light-headedness, dizziness, irritability and loss of appetite. However, Wolf (1950a) suggested that dry mouth is not necessarily accompanied by thirst sensations. He conducted a study whereby an intravenous infusion of hypertonic sodium chloride was used to create eudipsia and hyperdipsia in human participants and found that the participants reported dry mouth before the desire to drink developed. Conversely, when thirst was relieved by infusion of 5% glucose, the participants reported moist mouth before the desire to drink subsided.

Following the work of Wolf (1950a), another theory of thirst emerged where thirst is said to be a general sensation. Generally, thirst is a conscious sensation that provides desire to drink fluids in human and animals (McKinley & Johnson, 2004) to maintain fluid homeostasis. Thirst is stimulated by either cellular or extracellular dehydration. Cellular dehydration that causes osmometric thirst occurs when the osmotic pressure of the extracellular fluid increases relative to the intracellular fluid and the changes in osmotic pressure are sensed by the osmoreceptor. Extracellular dehydration caused by a reduction in the volume of fluid between cells results in hypovolemic thirst. A decrease in blood volume activates baroreceptors in large vessels and renal mechanism to correct the dehydration situation. In resting humans, thirst is stimulated when the plasma osmolality is increased up to 1-2% from normal physiological range of 280-292 mOsm kg^{-1} by increasing the concentration of solutes that are not readily permeable across membrane such as sodium chloride and sucrose (McKinley & Johnson, 2004) or when the body water loss is approximately 0.8% of body mass (Wolf, 1950b) or both.

Another theory of thirst shows that thirst may result from a direct stimulation of drinking centre located in some part of the brain or central nervous system. The anterior hypothalamus in the brain is the established location of the thirst centre as proved by Andersson (1952) using animal models. He injected about 0.1 ml of 1.5 to 2% sodium chloride into the hypothalamus of the unanaesthetised goats and after one to one-half min of injection, the goats started to drink and consumed about 500 to 2500 ml of water in 5 min.

Conversely, some literature suggests that thirst may not be stimulated by only one stimulus at a time, but actually arises from multiple factors that act concurrently to elicit thirst and subsequent fluid intake. Few investigators have presented the roles of the brain in concert with hypertonicity and hypovolemia to induce thirst-driven drinking (Andersson, 1978; Johnson, Cunningham, & Thunhorst, 1996). Others have investigated the role of hormones such as argininine vasopressin and aldosterone that might be responsible for the subjective feeling of thirst and subsequent drinking behaviour in different settings but with inconclusive results (Greenleaf, Brock, Keil, & Morse, 1983; Nose, Mack, Shi, & Nadel, 1988; Maresh et al., 2004). Indeed, this multiple factors of thirst was initially described by Adolph, Barker, and Hoy (1954) which has not been refuted to date, which then make the search for the mechanisms of thirst and drinking continues to be a challenge.

Factors affecting thirst and fluid intake

Thirst may be triggered from physiological or behavioural factors or combinations of both that often, but not always results in fluid intake. As mentioned previously, fluid deprivation leading to dehydration is a potent stimulus that triggers physiological thirst and subsequent fluid intake (Fitzsimons, 1972), meanwhile behavioural factors include, but are not limited to heat stress, exercise, beverage availability and palatability (Kenney & Chiu, 2001), nature of foods such as saltiness, sweetness and spiciness (Fitzsimons, 1972).

As described above, two main physiological factors that cause thirst and drinking are cellular dehydration and hypovolemia. Most everyday situations such as exercise, heat stress and fluid deprivation may increase cellular tonicity or decrease extracellular fluid volume or both. Of the two, cellular dehydration is the stronger stimulus for thirst, which constitutes approximately 64-85% of fluid intake behaviour following water deprivation in humans (Rolls et al., 1980). Drinking triggered by cellular dehydration is regulated by hormone arginine vasopressin, which is very sensitive to the change in plasma osmolality. It has been shown that a change in plasma osmolality of 1-2% results in vasopressin change but requires approximately 7-10% of blood volume reduction to produce similar effect on vasopressin (Star, 1990). Hypovolemia, on the other hand is regulated by renin-angiotensin-aldosterone system. As dehydration reduces the blood volume, the kidney stimulates the release of renin that results in an increase level of circulating angiotensin II that stimulates fluid intake and aldosterone that enhances the retention of sodium and water.

The effect of dehydration on thirst and fluid intake has been reported in the literature. In a study by Engell and colleagues (1987), 7 male subjects were hypohydrated to differential levels of 3, 5, and 7% of body mass loss by food and 24 to 48 h food/fluid restriction as well as by performing light exercise in the heat (T=38°C, rh=20%) prior to the trial. Then, the subjects underwent a series of heat stress tests in a hot dry environment (T=49°C, rh=20%). Thirst sensation and fluid intake on the dehydrated trials were compared with the euhydrated trial. The results show that thirst sensation and fluid intake increased as the level of hypohydration increased when the participants were exposed to the heat stress.

Thirst and fluid intake may also be affected by heat stress. Maresh et al. (2004) investigated the relationship between thirst, drinking and hormonal responses during low intensity exercise in the heat. Ten male subjects performed treadmill walking at 5.6 km h^{-1} for 90 min in hot conditions (T= 33 ± 0°C and 56 ± 3% rh) in four experimental trials differing in pre-exercise hydration status (euhydrated or hypohydrated) and access to water during exercise (water *ad libitum* or no water). Perceived thirst based on 9-point scale and fluid intake were higher in dehydrated trial (perceived thirst = 6.65 ± 0.65, fluid intake = 1.65 ± 0.18 L) compared to euhydrated trial (perceived thirst = 1.59 ± 0.41, fluid intake = 0.31 ± 0.11 L) when the participants exercised in the heat. Likewise, thirst sensation was rated higher on dehydrated trial without water (5.47 ± 0.76) than euhydrated trial without water (2.00 ± 0.35). This study concludes that there is a direct relationship between perceived thirst, fluid intake and the degree of hypohydration in individuals exercising in the heat.

While many studies have been devoted to thirst response and fluid intake in humans exposed to heat stress, particularly in exercising individuals, there is limited information available for people in the cold ambient temperature. Many data regarding suppressed thirst and reduced fluid intake in the cold were reported in animal studies (Fregly, 1982; Sobocinska & Kozlowski, 1987), but in humans, this view lacks considerable scientific evidence. Research has shown that people who are working in the cold also prone to body fluid deficit through cold-induced diuresis, decreased fluid intake and increased insensible sweat loss and respiratory fluid loss (Freund & Sawka, 1995; O’Brien, Young, & Sawka, 1998, Maughan, Shirreffs, Merson, & Horswill, 2005). However, in these studies, data on perceived thirst was not available.

Dann, Gillis and Burstein (1990) conducted a study of 21 male adults who walked in mountains at an altitude between 1480 m and 1730 m for 4.5 h in the cold (T=0°C, rh=87%). The participants were divided into two groups based on drinking pattern: group 1 was instructed to drink 250 ml every 30 min while group 2 was instructed to drink *ad libitum*. The findings show that the participants in group 2 consumed a smaller volume of fluids, and the highest volume reported was 240 ml compared to the participants in group 1 who drank on average 900 ml during the walk. The authors concluded that cold exposure decreases fluid intake in exercising humans, but this study did not report thirst response. Therefore, the association between reduced fluid intake and thirst suppression could not be established.

Another investigation about the effect of cold exposure on thirst sensations was carried out by Kenefick, Hazzard, Mahood and Castellani (2004). Eight adult men performed treadmill exercise at 50% VO_{2max} for 60 min in cold (T=4°C, rh=74%) and temperate (T=27°C, rh=39%) conditions and in a state or euhydrated or hypohydrated by 3.8% of body mass. Thirst sensations were measured pre-exercise and at 20 min intervals. The results show that thirst sensations were rated lower in the cold compared with the temperate trial regardless of hydration status. However, this study did not include the measure of fluid intake simultaneously, therefore a relationship between the intensity of thirst sensation and fluid intake in the cold could not be justified.

Some discrepancies exist in the literature regarding the effect of aging on thirst and fluid intake. Phillips and colleagues (1984b) restricted the water intake in 7 healthy young (20-31 y) and older (67-75 y) men for 24 h. Both groups had the same amount of weight loss and thus assumed the same body water loss. It was found that the older group was less thirsty and drank less during a subsequent 2 h rehydration period compared to the younger controls. Another study by Miescher and Fortney (1989) supports this observation. Five older men (61-67 y) and 6 younger men (21-29 y) were thermally dehydrated by 1.55 ± 0.22% and 1.52 ± 0.11% of body mass, respectively for 180 min followed by 60 min rehydration and the results show that the older men rated themselves as less thirsty despite having a higher plasma osmolality and lower plasma volume than the younger counterparts. However, Mack et al. (1994) reported that thirst and subsequent drinking are not affected by aging. Ten healthy older adult (65-78 y) and 6 younger men (18-28 y) were dehydrated by 2.2 and 2.5 % of body mass respectively, by exercising in the heat and given fluid *ad libitum* during 180 min rehydration period, whereby the thirst rating and fluid intake were measured together with collection of blood and urine samples. The results show that thirst ratings were similar between groups and that fluid intake matches the thirst ratings in both groups. Stachenfeld, Mack, Takamata, Dipietro and Nadel (1996) infused hypertonic saline solution into 6 older men (72 ± 2 y) and 6 younger men (26 ± 2 y) and reported that the thirst rating and fluid intake were similar between groups, further supports the view that thirst and fluid intake are unaffected by aging. Since there is inconclusive evidence regarding the effect of aging on thirst and fluid intake, this aspect of thirst remained to be investigated.

It is often assumed that thirst and fluid intake are the same especially in studies investigating thirst and fluid intake using animal models (Fregly, 1982; Sobocinska & Kozlowski, 1987). However, at least in the human, thirst and drinking behaviour are possibly a separate entity because in some conditions, fluid intake may not be stimulated by thirst, but probably due to psychological, environmental, sensory, social, cultural and other stimuli.

Subjective feelings related to thirst

A subjective feeling of thirst has long been associated with fluid intake behaviour. However, an attempt has been made to explain fluid intake with other subjective feelings associated with dehydration such as tiredness or headache (Rolls et al., 1980; Engell et al., 1987). A visual analog scale has been used to quantify the intensity of thirst related sensations in different occasions. Rolls and colleagues (1980) conducted the study on the effect of 24 h water deprivation on the physiological and subjective responses as well as fluid consumption in 5 healthy adult. The results show that the subjective sensations of thirst, mouth dryness, unpleasantness of taste in mouth and pleasantness of drinking water were significantly increased following 24 h water deprivation and that changes in these sensations were significantly correlated with fluid intake. A similar result was reported by Engell et al. (1987) whereby 7 healthy participants were hypohydrated by 0, 3, 5 and 7% of their body mass via fluid and food restriction and moderate heat-exercise stress followed by 1 h rehydration. The findings show that 18 out 37 subjective feelings related to thirst such as dry, scratchy mouth and throat, chapped and dry lips, tiredness, light-headedness, dizziness, irritability and loss of appetite ratings increased with increasing level of hypohydration. Besides, fluid intake was found to be correlated with the subjective feelings of thirst. Therefore, these studies show that subjective feelings other than thirst may contribute to fluid intake in dehydrated individuals.

Mood states

The effect of mood states on fluid intake has not been studied as extensively as food intake, hence the evidence regarding the effect of mood on fluid intake is limited. The only available data was provided by Yannakoulia et al. (2008) who observed a positive correlation between an elevated depression level and increased intake of meat, soft drinks and coffee.

On the other hand, much of the information available has focused on the extent of fluid intake consumption on mood (Steptoe & Wardle, 1999; Quinlan et al., 2000; Smit & Rogers, 2002). The results from these studies are variable whereby ingestions of tea and coffee did not affect mood states (Steptoe & Wardle, 1999) but in others, caffeinated beverages and energy drinks had been shown to exert a positive improvement in mood particularly alertness scores (Quinlan et al., 2000; Smit & Rogers, 2002). Even though these studies provide information on the relationship between fluid intake and mood states, the results cannot be used to conclude the reciprocal relationship between mood states and fluid intake.

Studies investigating the effect of mood states on food intake show that increased feelings of depression, anxiety, boredom (Cooper & Bowskill, 1986) and high work-related stress (Wardle, Steptoe, Oliver, & Lipsey, 2000) contribute to increase eating. However, even though negative mood states have been shown to be related to increased food intake, other studies found that positive mood states may also increase food intake. Yeomans and Coughlan (2009) induced a positive and negative mood by film viewing and examined the mood states on snacking in 96 women. The results showed that positive and neutral mood induced higher food intake compared to negative mood in those participants. Patel and Schlundt (2001) also reported similar findings whereby both positive (696 ± 256 kcal) and negative (629 ± 208 kcal) moods induced higher food intake compared to neutral (590 ± 177 kcal) mood in obese women.

Coupled with the limited data on the effect of mood on fluid intake and the extensive, but inconclusive evidence regarding the effect of mood states on food intake, it is of interest to see whether the similar findings would be applied with respect to the fluid consumption. Therefore, a study into the influence of mood states on fluid intake warrants further considerations.

Voluntary dehydration

Under resting conditions, a stimulation of thirst sensation that often results in voluntary fluid intake is capable of maintaining hydration status in human (Greenleaf, 1992). However, stress factors such as heat exposure and exercise may not be sufficient to induce thirst-driven drinking, leading to voluntary dehydration (Greenleaf, 1992) because of incomplete restoration of body water loss. Research has demonstrated that despite being offered fluid *ad libitum*, some individuals may not fully replace their body water loss. In this case, thirst is not perceived until body mass loss of 1-2% occurs (Greenleaf & Harrison, 1986). This condition increases the risk of dehydration in groups of people who are exposed to extreme environmental conditions and athletes performing under heat stress. One of the earliest documented cases of voluntary dehydration was showed by

Rothstein, Adolph and Wills (1947) observed that subjects became dehydrated while working in the desert, regardless of unlimited access to water. A recent study by Passe, Horn, Stofan, Horswill and Murray (2007) in 18 marathon athletes on their perceptions of thirst and fluid intake during running in the heat showed that the participants only replaced 30 ± 18% of their sweat loss, leading to voluntary dehydration. Similarly, Miescher and Fortney (1989) who conducted the study on the responses to prolonged passive heat exposure in resting young and older men show that both young and old groups only replaced 49.0 ± 3.1% and 46.6 ± 4.9% of their body mass loss, respectively. These studies show that under stress conditions, thirst is not capable to stimulate fluid intake to the amount that could prevent dehydration.

However, some body of evidence has challenged this view and suggested that thirst may actually be a good indicator of when to drink during prolonged strenuous exercise (Sharwood, Collins, Goedecke, Wilson, & Noakes, 2004; Noakes, 2007a, Noakes, 2007b). Indeed, if endurance athletes ignore the thirst sensations and drink the amount that exceeds sweat losses, this may lead to voluntary hyperhydration and may cause exercise hyponaetremia, a condition of diluted sodium concentration in the blood (Frizzell, Lang, Lowance, & Lathan, 1986; Noakes et al., 1990).

Since the debates on this conflicting opinion of the role of thirst in stimulating fluid intake under stress conditions continue, it remained to be resolved.

Beverage palatability

It has been shown that beverage palatability plays an important role in stimulating fluid intake. Drink palatability is influenced by temperature of the drink, taste and composition. The effect of drink temperature on fluid intake has been shown by Hubbard et al. (1984) who conducted the marching of 29 males on the treadmill for 14.5 km at 40°C and the participants received the beverages kept at temperatures 15°C or 40°C. The findings show that consumption of cool water was significantly higher ($P<0.05$) than warm water, indicating the preference for cool beverages during exercise in the heat. In a more recent study by Mundel, King, Collacott and Jones (2006), 8 healthy males cycled to exhaustion at 34ºC, consuming a drink that was kept at either 19ºC or 4ºC. The results show that 4ºC drink was consumed in a significantly greater ($P<0.05$) volume than the 19ºC drink, suggesting that cold beverage is more palatable than the beverage at neutral temperature when working in the heat. Much of the evidence regarding beverage temperature on fluid intake during heat stress and exercise suggests that fluid intake decreases with increasing fluid temperature (Rothstein et al., 1947; Armstrong, Hubbard, Szlyk, Matthew, & Sils, 1985; Szlyk, Sils, Francesconi, Hubbard, & Armstrong, 1989), but the effect of water temperature in individuals exposed to cold environment is lacking, which warrants further investigations.

Extensive evidence of the relationship between flavour and fluid intake show that fluid intake is improved with appropriate flavouring beverages (Rothstein et al., 1947; Engell & Hirsch, 1991; Rolls, 1991). In fact, consumption of flavoured beverages under stress conditions such as exercise or working in the heat may help to lessen or prevent dehydration, hence maintain fluid balance. Passe, Horn and Murray (1999) compared the consumption of 4 different beverages in 50 athletes who exercised for 75 min and were given fluid *ad libitum* during exercise. Four different beverages were water, diluted fruit juice, a homemade 6% CHO-electrolyte drink and a commercially 6% CHO-electrolyte sport drink which were orange flavoured except for water. The results show that commercial sports drink scored significantly higher ($P<0.05$) in term of beverage acceptance, consumed in significantly greater ($P<0.05$) volume and resulted in significantly lower ($P<0.05$) dehydration compared to other drinks. A more recent investigation by Bergeron, Waller and Marinik (2006) supports the previous observation. They compared the effect of a 6% CHO-electrolyte drink versus water on voluntary fluid intake and core temperature in 14 adolescent tennis players during 120 min training session in the heat. The results show that changes in body mass were significantly higher ($P<0.05$) in a water ($-0.9 \pm 0.6\%$) than a CHO-electrolyte drink ($-0.5 \pm 0.7\%$) trial. Similarly, core body temperature was also significantly higher in water (38.20 ±

0.31ºC) compared to CHO-electrolyte drink (37.97 ± 0.24ºC) trial. They concluded that the consumption of CHO-electrolyte drink might be more effective than water in minimising dehydration and thermal strain during exercise in the heat because of the greater volume consumed.

Flavoured beverages containing an appropriate amount of sodium have been showed to stimulate fluid intake and fluid balance under stress conditions (Sawka et al., 2007). Gonzalez – Alonso, Heaps and Coyle (1992) investigated the effect of caffeinated diet cola, a 6% carbohydrate-electrolyte drink and water on whole body water rehydration after exercise-induced dehydration in 10 healthy adults. The findings showed that a 6% CHO-electrolyte drink provides greater rehydration than cola and water. In another study, Maughan, Owen, Shirreffs and Leiper (1994) examined the effects of sodium and potassium addition to ingested beverages on water balance in 8 healthy males. The participants were dehydrated by approximately 2% of body mass and test drinks containing either 90 mmol L^{-1} glucose, 60 mmol L^{-1} sodium chloride, 25 mmol L^{-1} potassium chloride or combinations of glucose, sodium and potassium were offered during rehydration period. The results of this study suggested that the ingestion of electrolyte containing drinks improves the whole body water and electrolyte balance better than the drinks without addition of electrolyte.

Food intake

Some literature suggests that in everyday life, physiological thirst does not necessarily elicit drinking, rather it may cause by food intake (Phillip, Rolls, Ledingham, & Morton, 1984a; De Castro, 1988; Engell, 1988). Phillips and colleagues (1984a) investigated thirst and drinking responses in 5 healthy men during a normal working day and reported that despite the significant increases in the ratings of thirst, mouth dryness and unpleasantness of taste in the mouth, there were no significant changes in hydration indicators such as haematocrit, plasma osmolality, plasma sodium, potassium and angiotensin II during the experimental period. Therefore, they concluded that during free access to water, the participants drank not because they were thirsty, but due to eating and anticipation that they were going to be thirsty. De Castro (1988) also reported a similar observation in which he investigated the characteristics of fluid intake in 36 participants using 7-day self-reported diary. The results show that fluid intake was significantly correlated with eating episodes, but only slightly related with the thirst rating and concluded that in a natural environment, *ad libitum* drinking is mainly associated with food intake.

Aim of the thesis

The aim of this thesis is to examine selected aspects of hydration, thirst and fluid balance in resting and exercising individuals in different ambient temperatures. Specifically, the study aimed to investigate the perception of hydration, thirst and fluid intake in free-living population, to examine the feasibility of thirst as a marker of hydration status and also to examine the thirst related sensations and fluid balance in resting and exercising individuals in different ambient temperatures.

Objectives of the thesis

1) To examine the extent of hydration knowledge and fluid intake habits in people who work within fitness industry.
2) To examine whether thirst related sensations can be used to signify hydration status in young healthy population.
3) To examine the factors that influence thirst and fluid balance in resting and exercising individuals under cool and warm temperatures.

Chapter 2

General Methods

Ethical Approval

All of the studies described in this thesis were approved by the Ethical Advisory Committee of Loughborough University. Prior to the start of the experimental trials, all potential participants received both written and verbal details of the study. They were free to discuss the details of testing with their families, physicians and anyone else before taking part in the study. Once they agreed to proceed with the trial, written informed consent was obtained from them and they completed a health-screening questionnaire. Participants were informed about their right to withdraw at any stage of the study with no obligation to give any reason for doing so. For studies that involve more than one trial, the participants also gave their verbal and written consent at the start of each trial.

Participants

The participants' characteristics are described in detail in each experimental chapter. The participants in the study reported in chapter 3 were healthy adults aged between 20 to 55 years. For studies reported in chapters 4, 5, 6 and 7, the age was between 20 to 40 years. They did not take any prescribed medication with the exception of females taking contraceptive pills. They had no history of renal or heart diseases and females who were pregnant or lactating were excluded.

Pre-trial standardisation

Participants were instructed to undertake similar diets and physical activities prior to all testing that involved randomised crossover designs (chapters 5, 6 and 7). They were also asked to avoid alcohol consumption and strenuous physical activity 24 h prior to testing.

The participants were instructed to record all food and drinks they consumed and physical activity they undertook for the 24 h before commencing the first trial. The data obtained were not analysed for dietary contents but served only as a record so that the participants could repeat the similar diets and physical activities before the subsequent trials.

Familiarisation prior to main experimental trials

Prior to the main experimental trials, the participants were asked to visit the laboratory to perform preliminary testing. This protocol was performed in studies reported in chapters 5 and 7 and served to familiarise the participants with the experimental procedures. The familiarisation protocol conducted was similar to the experimental trial. In addition, in chapter 7, the protocol was also performed to measure the participants' maximum oxygen uptake. Details of the protocols are explained in the material and methods section of the appropriate chapters.

Urine collection and analysis

Details of the urine collection timing are explained in each experimental chapter. Urine sampling was done in all studies except in the study reported in chapter 3.

In chapter 4, the participants collected complete 24 h urine samples and full details are given in that chapter.

Urine samples in chapters 5, 6 and 7 were collected by asking the participants to urinate into a plastic jug. The total volume was measured using a measuring cylinder and a 5 ml aliquot was retained for analysis. The urine specimens collected in chapter 5 were then analysed for osmolality, colour and specific gravity. Meanwhile urine samples collected in chapters 6 and 7 were analysed for urine specific gravity only. Urine osmolality was determined by freezing-point depression (Gonotec Osmomat 030 Cryoscopic Osmometer, Gonotec, Berlin, Germany). Urine colour was measured using the Armstrong (1994) eight-colour scale. Urine specific gravity was obtained via a hand-held refractometer (DIGIT-012, Ceti, Belgium).

Blood sampling and analysis

Blood sampling was performed in the study reported in chapter 5 only. Detailed information regarding the timing, volume and method of collection are explained in the materials and methods section of that chapter.

Questionnaires

A questionnaire was specifically developed to obtain the descriptive data about hydration and fluid intake in fitness professionals as reported in chapter 3. Details of the questionnaire are explained in the materials and methods section of that chapter.

A 100 mm visual analog scale (VAS) questionnaire (Engell et al., 1987) was used to measure the subjective feelings related to thirst in all studies reported in this thesis. The rating ranges from 0 (not at all) to 100 (very). In the study reported in chapter 4, the questionnaire consisted of 24 items and took approximately 15 min to complete. In the studies reported in chapters 5 and 6, there were 18 items asked and took about 10 min to complete, whereas questionnaires used in the studies reported in chapters 7 consisted of 14 items and took approximately 5 min to complete. The questionnaire used for each experimental chapter is shown in Appendix A.

A 65-item Profile of Mood State (POMS) questionnaire (McNair, Lorr, & Droppleman, 1971) was used to measure the mood changes of the participants in studies reported in chapters 4 and 6. Six components of POMS assessed were tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment. The participants were asked to rate how they were feeling at "the moment" on a scale of 0 (not at all) to 4 (extremely).

Thermal sensation scale (Parsons, 2003) was used to assess the extent of thermal sensation in studies reported in chapters 6 and 7. The participants were asked to rate how they were feeling regarding thermal sensation on 21-point scale ranging from -10 (cold impossible to bear) to 10 (heat impossible to bear) with the midpoint of 0 (neutral) in between the scale. The scale is shown in Appendix B.

Rating of Perceived Exertion (RPE) scale (Borg, 1998) was used in chapter 7 to determine how hard the participants felt they were working during exercise. The participants were asked to rate their perceived effort on 15-point scale ranging from 6 (very, very light) to 20 (exhaustion). The RPE is shown in Appendix C.

Measurement of core body temperature and heart rate

Continuous core body temperature was recorded using a MP100 data acquisition system (Biopac System Inc, USA) and heart rate was recorded using Polar team system (Polar Electro Oy, Kempele, Finland) in the study reported in chapter 6. In chapter 7, core body temperature and heart rate were measured using an ingestible temperature pill telemetry system (CorTemp, HQ Inc, Palmetto, FL) and short range telemetry (Polar Vantage, Polar Electro Oy, Kempele, Finland), respectively.

Anthropometry

All body mass measurements described in this thesis was performed nude but out of sight behind a screen except in chapter 3 whereby the participants recorded their weight in the questionnaire. The body mass was measured nearest to 20 g using an electronic scale (AFW-120K, Adams Equipment Co., Milton Keynes, UK). Stature was measured nearest to 0.1 cm using a wall fixed stadiometer (Seca 240, Germany) in all studies except in chapter 3 whereby the participants recorded their height in the questionnaire.

Coefficient of variation for analytical assays

Coefficient of variation (CV) for the assays used in this thesis is shown in Table 2.1 below. The CV was calculated as the ratio of standard deviation to the mean of the difference between duplicates of 30 samples and expressed as percentage.

Table 2.1: The CV, mean and SD of samples used for calculation the coefficient of variation of analytical methods.

Assay	N	%CV	Mean	SD
Urine osmolality (mOsm kg^{-1})	30	1.2	552	217
Urine Na^+ concentration (mmol L^{-1})	30	0.9	141	48
Urine K^+ concentration (mmol L^{-1})	30	2.3	50	25
Urine Cl^- concentration (mmol L^{-1})	30	1.1	88	28
Urine creatinine (mg dL^{-1})	30	2.0	1.4	0.4
Urine deuterium (ppm)	30	2.1	397	62
Haemoglobin concentration (g 100 ml^{-1})	30	1.0	15.0	1.3
Haematocrit (%)	30	0.6	43	3
Serum osmolality (mOsm kg^{-1})	30	0.5	281	5

Coefficient of variation of thirst related sensation questionnaire and POMS questionnaire

Coefficient of variation (CV) for the thirst related sensations questionnaire and POMS used in this thesis is shown in tables 2.2 and 2.3, respectively. The CV was calculated as the ratio of standard deviation to the mean of the difference between duplicates of 30 samples and expressed as percentage.

Table 2.2: The CV, mean and SD of samples used for calculation the coefficient of variation of thirst sensation questionnaire.

Subjective feeling	N	%CV	Mean	SD
Dry mouth	30	9.3	51	22
Mouth feels irritated	30	11.4	44	26
Mouth feels pleasant	30	2.8	67	19
Bad taste in mouth	30	11.7	32	22
Chalk like taste	30	15.0	29	22
Dry throat	30	10.2	42	24
Scratchy throat	30	12.4	46	23
Warm throat	30	6.2	51	14
Chapped lips	30	10.7	43	24
Swollen tongue	30	10.2	38	22
Feel weary	30	15.9	51	14
Feel dizzy	30	17.3	25	20
Feel lightheaded	30	13.9	27	21
Feel sleepy	30	12.9	55	16
Feel tired	30	10.6	50	19
Feel irritable	30	10.8	48	22
Feel thirsty	30	4.4	59	21
Feel alert	30	3.5	56	18
Concentration	30	5.2	68	18
Trembling hands	30	14.9	30	23
Sorehead	30	6.1	42	24
Drink pleasantness	30	3.9	62	15
Hungry	30	9.7	33	24
Full stomach	30	5.6	56	18
Full of energy	30	4.7	61	16
Feel bloated	30	7.5	44	21
Nauseous	30	10.8	37	27

Table 2.3: The CV, mean and SD of samples used for calculation the coefficient of variation of POMS questionnaire.

POMS component	N	%CV	Mean	SD
Vigour	30	8.7	14	5
Anger	30	14.2	11	4
Fatigue	30	11.7	10	2
Depression	30	10.6	12	6
Tension	30	9.0	15	4
Confusion	30	8.1	11	3

Statistical analysis

Statistical analysis described in this thesis was performed using the Statistical Program for Social Sciences (SPSS) version 16.0.

Initially the data were tested for normal distribution using a Kolomogorov-Smirnov test. Mean ± SD was used to report normally distributed data and any data that was not normally distributed is reported as median (range).

Inferential statistics are described in detail in each experimental chapter where appropriate. The level of significant was set at $p<0.05$.

Chapter 3

Hydration and fluid intake habits in fitness professionals

Introduction

A fluid replacement strategy may in some circumstances be important to ensure adequate hydration when exercising. Nowadays, with easy access to either printed or online information, people are overloaded with all kinds of information regarding hydration strategies which are not always backed up with scientific evidence. Therefore, sound knowledge may be needed to determine appropriate fluid intake behaviour.

Much of the data available on hydration knowledge and habits are from studies conducted on general nutrition knowledge and behaviour, which hydration section only contributes to a very small portion of the studies (Chapman, Toma, Tuveson, & Jacob, 1997; Felder et al., 1998; Shifflett, Timm, & Kahanov, 2002; Zawila et al., 2003). Hence, these studies do not convey the information that represents the specific knowledge and habit on hydration itself.

At present, little information regarding fluid intake habits among recreationally active people is documented. Much information about hydration and fluid intake habits has been obtained from collegiate athletes (Rosenbloom et al., 2002; Nichols et. al., 2005) and high performance athletes (Felder et al., 1998; Zawila et al., 2003; Soo & Naughton, 2007). These studies suggest that these populations generally exhibit insufficient knowledge and poor hydration habits and therefore are susceptible to risks of dehydration and performance deterioration.

Thus, this descriptive study was undertaken to gain an insight into hydration knowledge, opinions and fluid intake behaviour in individuals who work within the fitness industry.

Materials and methods

Participants

The participants were approached during the Fitness Professionals Convention (http://www.fitpro.com) held at Loughborough University in April 2008. After being given verbal and written information about the study, 40 people agreed to participate, giving their written informed consent. The study protocol was approved by the Loughborough University Ethical Advisory Committee (R08-P46).

Instrument and Data Analysis

In this descriptive study, the participants were asked to complete a questionnaire which consisted of two parts. Part A contained 6 questions on demographic information and Part B contained 10 questions to find out about their perspectives on hydration and fluid intake behaviour. The questionnaire is shown in Table 3.1. The items asked were combinations of open-ended and closed-ended type questionnaire. Statements for closed-ended question were either “yes” or “no”. For a 5-point Likert scale question, the responses range from 1 (excellent) to 5 (poor). Responses for open-ended questions were classified into different main themes such as “hydration”, “performance” and so forth. Similar responses for each question then were grouped together under the same themes and transferred into nominal scale for statistical analysis. For instance, answers such as “to maintain performance” and “to ensure good performance” were placed under “performance” theme and given a nominal code to calculate the statistics. This questionnaire took approximately 15-20 min to complete.

Before administering to the participants, the questionnaires were assessed for content validity by graduate students and lecturers of sport and exercise sciences at Loughborough University. The pilot test was conducted using 10 participants at Loughborough University. The internal reliability for the questionnaires was 0.74

as calculated using Cronbach alpha (SPSS 16.0). When the value is closer to 1, the more reliable the instruments are (Cronbach, 1951). Hence, the value obtained in the present study suggests that the questionnaire had high internal reliability.

Statistical analysis

All data sets were tested for normal distribution using a Kolomogorov-Smirnov test. Parametric data are reported as mean ± SD. Responses to the open-ended questionnaires were treated as non-parametric data and the results were expressed as frequency, percentages and median (range).

Data analysis was conducted using the Statistical Program for Social Sciences (SPSS) version 16.0.

Table 3.1: Questionnaire on perceptions on hydration and fluid intake behaviour.

Question	Question
Part A (Demographic information)	2. What, in your opinion, is the best drink for the human body
Please describe yourself.	a) for before exercise? Why?
Sex:	b) for during exercise? Why?
Height (in m, cm, or ft and in):	c) for after exercise? Why?
Weight (in kg or stone/lb):	d) generally during the day? Why?
Age:	3. What is your preferred/favourite drink
Occupation:	a) for before exercise?
Typical weekly exercise routine:	b) for during exercise?
	c) for after exercise?
Part B (perception on hydration and fluid intake habits)	d) generally during the day?
1. In your opinion, should you	4. How do you decide when you need to drink
a) drink before exercise (please circle one) ALWAYS SOMETIMES NEVER	a) when exercising?
Why?	b) in general during the day?
b) drink when exercising or during short breaks in exercise(please circle one)	
ALWAYS SOMETIMES NEVER	
Why?	
c) Drink after exercising (please circle one) ALWAYS SOMETIMES NEVER	
Why?	

5. Rate the following drinks in terms of how good they are at providing water to the body? (1=excellent, 5=poor)

a) plain tap water 1 2 3 4 5

b) plain bottled mineral water 1 2 3 4 5

c) sparkling water 1 2 3 4 5

d) flavoured water 1 2 3 4 5

e) fruit juices 1 2 3 4 5

f) vegetable juices 1 2 3 4 5

g) milk 1 2 3 4 5

h) colas/lemonades etc 1 2 3 4 5

i) sports drinks 1 2 3 4 5

j) energy drinks 1 2 3 4 5

k) tea 1 2 3 4 5

l) coffee 1 2 3 4 5

m) chocolate 1 2 3 4 5

n) soup 1 2 3 4 5

o) any other drinks…… 1 2 3 4 5

6. What problems, if any, can occur if we have too little water in our diet?

7. What problems if any, can occur if we have too much water in our diet?

8a) Are you aware of how hydrated you are just now? YES NO

8b) If yes, how do you know?

9. How much do you typically drink in an average day? (give the amounts in pints, litres, glasses or any other way)

a) plain tap water

b) plain bottled mineral water

c) sparkling water

d) flavoured water

e) fruit juices

f) vegetable juices

g) milk

h) colas/lemonades etc

i) sports drinks

j) energy drinks

k) tea

l) coffee

m) chocolate

n) soup

o) any other drinks……

10) From your observation of people attending your class or the same classes as you, how often do you think they drink

a) before exercising? ALWAYS SOMETIMES NEVER

b) during exercising? ALWAYS SOMETIMES NEVER

c) after exercising? ALWAYS SOMETIMES NEVER

d) do you think they drink the right amount? YES NO

Results

Characteristics of the participants

Table 3.2 shows the demographic and occupation data of the participants. Twenty-three males and 17 females participated in this study. They had a mean age of 35 ± 10 years, and self-reported height and body mass of 1.72 ± 0.10 m and 73 ± 13 kg, respectively. A total of 27 out of 40 (67.5%) participants were working full time as personal trainers and fitness instructors. The remainder were part-time fitness instructors.

Table 3.2: Demographic and employment data of study participants (N=40).

Variable	Mean ± SD	Frequency (N)	Percentage (%)
Age (y)	35 ± 10		
Height (m)	1.72 ± 0.10		
Body mass (kg)	73 ± 13		
Gender			
Male		23	57.5
Female		17	42.5
Occupation			
Personal trainer/fitness instructor		27	67.5
Lecturer		4	10.0
Marketing director		3	7.5
Administration assistant		2	5.0
General practitioner		2	5.0
Computer analyst		1	2.5
Student		1	2.5

In terms of the types of weekly exercise undertaken by the participants, strength training (31.2%) was performed the most by the participants, followed by cardio session (14.5%) and other activities as shown in Table 3.3.

Table 3.3: Typical weekly exercise routine of the participants (N=40).

Exercises	Number of responses (N=96)	
	N	%
1. Strength training	30	31.2
2. Cardio session	14	14.5
3. Cycling	12	12.5
4. Running	12	12.5
5. Body balance (pilate, yoga)	6	6.2
6. Teaching exercise classes	6	6.2
7. Walking	5	5.2
8. Body combat	3	3.1
9. Body pump	3	3.1
10. Aquafit	2	2.1
11. Basketball	2	2.1
12. Jogging	2	2.1
13. Swimming	1	1.0
14. Football	1	1.0

Table 3.4 shows that most of the participants (47.5%) performed exercise 2 times/week, followed by 3 times/week (22.5%) and so forth.

Table 3.4: Frequency of weekly routine exercise performed by the participants (N=40).

Frequency of exercise per week	N	%
1. 1 time/week	5	12.5
2. 2 times/week	19	47.5
3. 3 times/week	9	22.5
4. 4 times/week	5	12.5
5. 5 times/week	1	2.5
6. 6 times/week	1	2.5

Perception on hydration and fluid intake behaviour

Participants were asked whether in their opinion they should drink before, during and after exercise with three options of "always", "sometimes" and "never". Eighty percent of participants stated that they should always drink before exercising. Likewise, 78.0% and 90.0% participants chose the option should "always" drink during and after exercise, respectively. Meanwhile, only the respective 20.0%, 22.0% and 10.0% of the participants answered that they should "sometimes" drink before, during and after exercise. None of the participants chose the option "never" for these questions.

Table 3.5 presents the reasons given by the participants as to why they should drink before, during and after exercising. The reason "to stay hydrated" was given by most of the participants as to why they should drink before and during exercise (40.0% and 67.5% respectively), and 62.5% of the participants answered "to replace fluid loss or recovery" as the reason for why they should drink after exercise.

Table 3.5: Reasons for why the participants should drink before, during and after exercising (N=40).

Reasons	Before exercise		During exercise		After exercise	
	N	%	N	%	N	%
1. To stay hydrated	16	40.0	27	67.5	9	22.5
2. To ensure optimum performance	8	20.0	5	12.5	1	2.5
3. Don’t know	6	15.0	5	12.5	5	12.5
4. Pre hydration	6	15.0	na	na	na	na
5. If feel thirsty	2	5.0	na	na	na	na
6. To replace fluid loss/recovery	2	5.0	3	7.5	25	62.5

na denotes not applied

Responses to the question on the best drink for the human body for before, during and after exercise as well as in general during the day are shown in Table 3.6. For all occasions, most of the participants believed that water is the best drink for human body (85.0% for before exercise, 62.5% for during exercise, 50.0% for after exercise and 95.0% for general during the day).

The rationales for their opinion on the best drink for human body are shown in Table 3.7. The reason “to stay hydrated” was given by the majority of the participants for before (50.0%) and during (50.0%) exercise as well as in general during the day (55.0%), whereas the reason “to replace fluid, energy and salt loss or recovery” was cited by the majority of the participants as regard to why they think their chosen drink is the best for human body after exercise (52.5%).

Table 3.6: Participants' opinion on the best drink for human body for before, during and after exercise and in general during the day (N=40).

Types of drink	Before exercise		During exercise		After exercise		General during the day	
	N	%	N	%	N	%	N	%
1. Water	34	85.0	25	62.5	20	50.0	38	95.0
2. Sports drink	1	2.5	11	27.5	12	30.0	na	na
3. Carbohydrate protein mix drink	1	2.5	na	na	1	2.5	na	na
4. Tea/ coffee	1	2.5	na	na	na	na	na	na
5. Squash/cordial	1	2.5	na	na	na	na	1	2.5
6. Mixture of water and fruit juice	2	5.0	1	2.5	1	2.5	1	2.5
7. Water with carbohydrate	na	na	na	na	2	5.0	na	na
8. Flavoured water	na	na	2	5.0	1	2.5	na	na
9. Water with added salt	na	na	1	2.5	1	2.5	na	na
10. Fruit juice	na	na	na	na	1	2.5	na	na
11. Meal replacement drink	na	na	na	na	1	2.5	na	na

na denotes not applied

Table 3.7: Rationales for the best drink for human body before, during and after exercise and in general during the day (N=40).

Rationales	Before exercise		During exercise		After exercise		General during the day	
	N	%	N	%	N	%	N	%
1. Don't know	2	5.0	2	5.0	3	7.5	5	12.5
2. To stay hydrated	20	50.0	20	50.0	6	15.0	22	55.0
3. It is quickly absorbed into the body	5	12.5	4	10.0	3	7.5	na	na
4. It is natural, pure with no additives	2	5.0	1	2.5	1	2.5	2	5.0
5. Provide hydration and fuel for activity	2	5.0	1	2.5	na	na	na	na
6. Recommended guidelines	2	5.0	1	2.5	1	2.5	na	na
7. Not too heavy	1	2.5	na	na	na	na	na	na
8. It is the only thing I like	2	5.0	2	5.0	1	2.5	1	2.5
9. Water is the body basic's nutritional requirement	4	10.0	3	7.5	3	7.5	7	17.5
10. To replace fluid, energy and salt loss/rehydration	na	na	5	12.5	21	52.5	na	na
11. Easy to drink	na	na	1	2.5	na	na	na	na
12. Thirst quencher	na	na	na	na	1	2.5	na	na
13. Detox	na	na	na	na	na	na	1	2.5
14. Taste nicer than water	na	na	na	na	na	na	1	2.5
15. Coffee helps with my concentration	na	na	na	na	na	na	1	2.5

na denotes not applied

In response to the question asking the participants to indicate their preferred drink, water tops the list for before exercise (75.0%), during exercise (72.5%) and after exercise (57.5%) as well as generally during the day (75.0%) (Table 3.8).

Table 3.8: Participants' preferred drink before, during and after exercise and generally during the day (N=40).

Types of drink	Before exercise		During exercise		After exercise		General during the day	
	N	%	N	%	N	%	N	%
1. Water	30	75.0	29	72.5	24	60.0	30	75.0
2. Squash/cordial	3	7.5	3	7.5	2	5.0	na	na
3. Sports drink	2	5.0	8	20.0	6	15.0	1	2.5
4. Coffee/tea	2	5.0	na	na	na	na	6	15.0
5. Carbohydrate/protein mix drink	2	5.0	na	na	6	15.0	na	na
6. Energy drink	1	2.5	na	na	na	na	na	na
7. Fruit juice	na	na	na	na	1	2.5	2	5.0
8. Skimmed milk	na	na	na	na	1	2.5	na	na
9. Carbonated drink	na	na	na	na	na	na	1	2.5

na denotes not applied

When asked how the participants decide when to drink during exercise and in general during the day, the response "feel thirsty" tops the list that lead them to drink both during exercise (32.5%) and in general during the day (30.0%) (Table 3.9).

Table 3.9: Participants' responses to the question "how do you decide when to drink?" during exercise and in general (N=40).

How do you decide when to drink?	During exercise		In general	
	N	%	N	%
1. Feel thirsty	13	32.5	12	30.0
2. Between sets and reps/ regular interval	12	30.0	na	na
3. Habitual/ time increment	3	7.5	1	2.5
4. Conscious of hydration/drink before getting thirsty	3	7.5	5	12.5
5. Feel dry mouth	2	5.0	2	5.0
6. Intuitive	2	5.0	4	10.0
7. When get opportunity/time break	2	5.0	2	5.0
8. Drink regularly or sip continuously	1	2.5	12	30.0
9. If sweating a lot	1	2.5	na	na
10. When I stop exercising	1	2.5	na	na
11. Watch urine colour	na	na	2	5.0

na denotes not applied

Table 3.10 shows the median scores of the participants' rating on how good they feel the drinks provide water to the body. The participants rated on five-point scale ranges from 1 (excellent) to 5 (poor). More than half of the participants rated plain tap water (60.0%) and plain bottled mineral water (70.0%) as excellent source of water for human body. On the other hand, colas or lemonade and chocolate drinks were rated as a poor source of water for human body by the respective 50.0% and 55.0% of the participants.

Table 3.10: Median rating score on types of drink that provide water to the body, frequency and percentage of participants for the median score (N=40).

Types of drink	Median (range)	Frequency (N)	Percentage (%)
1. Plain tap water	1 (1-5)	24	60.0
2. Plain bottled mineral water	1 (1-5)	28	70.0
3. Sparkling water	3 (1-5)	12	30.0
4. Flavoured water	2 (1-5)	14	35.0
5. Fruit juices	3 (1-4)	17	42.5
6. Vegetable juices	3 (1-5)	15	37.5
7. Milk	3 (1-5)	18	45.0
8. Colas/lemonades etc	5 (1-5)	20	50.0
9. Sports drinks	2 (1-5)	17	42.5
10. Energy drinks	3 (2-5)	15	37.5
11. Tea	3 (2-5)	17	42.5
12. Coffee	4 (1-5)	19	47.5
13. Chocolate	5 (1-5)	22	55.0
14. Soup	4 (1-5)	14	35.0

Table 3.11 shows the response to an item asking participants to list any potential problems that may arise if we have too little water in our diet. The majority of responses given by the participants were “poor bowel movement or constipation” (18.2%), followed by “fatigue” (14.1%), “headache” (13.1%) and so forth.

Table 3.11: Participants’ opinion on problems that may occur for having little water in the diet (N=40).

Responses	Number of responses (N=99)	
	N	%
1. Poor bowel movement/constipation	18	18.2
2. Fatigue/tiredness	14	14.1
3. Headache	13	13.1
4. Dehydration	10	10.1
5. Poor skin tone	6	6.1
6. Poor performance	7	7.1
7. Poor/lack of concentration	10	10.1
8. Dizziness	7	7.1
9. Irritated	4	4.0
10. Electrolyte imbalance	2	2.0
11. Cramp	3	3.0
12. Dark and smelly urine	2	2.0
13. Lethargy	3	3.0

Conversely, Table 3.12 shows the response to an item asking the participants to list any potential problems that may arise if we have too much water in our diet. The most popular responses given by the participants were “flush out mineral/salt dilution/hyponatremia” (24.2%), followed by “increase urination/toilet visit or water incontinence” (21.2%), “feel bloated” (18.2%) and other responses.

Table 3.12: Participants’ opinion on problems that may occur from having too much water in the diet (N=40).

Responses	Number of responses(N=33)	
	N	%
1. Flush out of minerals/salt dilution/hyponatremia	8	24.2
2. Increase urination/increase toilet visit	7	21.2
3. Feel bloated	6	18.2
4. Death	4	12.1
5. Poor performance	3	9.1
6. Swollen tissue	2	6.1
7. Kidney problem	1	3.0
8. Addiction to certain substances i.e. caffeine	1	3.0
9. None if diet is correct	1	3.0

When asked whether they were aware about their hydration status, 80.0% of the participants responded “yes”, meanwhile 20.0% of them said they were not aware how hydrated they were at the moment they answered the questionnaire. For those who said they were aware of their hydration status, they were asked to list how they knew about it. “Watching urine colour” tops the list of responses given by the participants which was 32.6%, followed by “I know how much I drink” (30.2%), “feel thirsty” (14%), “feel OK” (11.6%), “having headache” (4.7%) and “dry mouth”, “enough saliva” and “energetic” which had a response of 2.3% respectively.

Table 3.13 shows the mean volume of different type of beverages reported to be consumed by participants in an average day. The participants stated the amount of drink in different units such as litre, millilitre, pint, glasses, and so forth, which were converted and standardised in litres. The results show that plain tap water accounts for the highest volume reported to be consumed by the participants that was about 1.5 ± 1.2 L per day (Table 3.13).

Table 3.13: Mean volume of drink in an average day (N=40).

Types of drink	Volume of drink (L) Mean ± SD
1. Plain tap water	1.5 ± 1.2
2. plain bottled mineral water	0.5 ± 0.9
3. sparkling water	0.1 ± 0.3
4. flavoured water	0.1 ± 0.5
5. fruit juices	0.1 ± 0.2
6. vegetable juices	0.0 ± 0.1
7. milk	0.2 ± 0.4
8. colas/lemonades etc	0.1 ± 0.2
9. sports drinks	0.0 ± 0.2
10. energy drinks	0.0 ± 0.1
11. tea	0.4 ± 0.5
12. coffee	0.2 ± 0.3
13. chocolate	0.0 ± 0.1
14. soup	0.0 ± 0.1
15. any other drink	0.0 ± 0.2

When asked about their opinion of other people's drinking behaviour with regard to exercise, 17.5%, 27.5% and 45.0% of the participants said the people attending their class or the same class as them always drink before, during and after exercising, respectively. Meanwhile, 77.5%, 72.5% and 52.5% of the participants believed that the people around them only sometimes drink before, during and after exercise, respectively. Approximately 5.0% and 2.5% of the participants said that they noticed people around them never had a drink before and after exercise. Overall, 72.5% of the participants believed that other people do not drink the right amount for exercise, meanwhile 27.5% agreed that those people drink sufficiently.

Discussion

The main findings of the study showed that the participants had some knowledge but also some misunderstanding with respect to hydration and fluid intake.

Most participants (>50.0%) in the present study believed that they should drink before, during and after exercise to stay hydrated and to replace fluid loss. This result was similar to the findings of the survey conducted by Decher et al. (2008) on hydration status, knowledge and behaviour in youths at summer sports camps in Connecticut and Pennsylvania. On scale 1 (never) to 10 (always), the adolescent participants agreed it is important to drink to stay hydrated before (9.7 ± 0.5), during (9.8 ± 0.5) and after (9.1 ± 1.3) practices and games.

Despite the suggestion made by American College of Sports Medicine (Sawka et al., 2007) and National Athletic Trainers' Association (Casa et al., 2000) on sports drink consumption during high intensity exercise for more than 1 h and less intense exercise sustained for longer period, water was regarded as the best drink by these individuals and was the most preferred beverage to be consumed by the participants during exercise. This result is supported by the work of Schroder et al. (2004) who conducted a survey on dietary habits and fluid intake of the elite Spanish basketball players and observed that water was the preferred beverage consumed by the participants during training (92%) and competition (88%).

If people regularly exercise for more than 1 h at high intensity or less intense but sustained for a longer period, sports drinks may be a better option for fluid and fuel replacement during exercise because sodium and carbohydrate contents help to increase physiological drive to drink to replace sweat losses and to maintain blood glucose level, respectively (Sawka et al., 2007). This result from the present study was comparable to the previous works reported by Felder et al. (1998), Rosenbloom et al. (2002) and Nichols et al. (2005), whereby the athletes showed little understanding about the positive effect of carbohydrate electrolyte drinks in maintaining proper hydration status and exercise performance.

Surprisingly, the present study also reveals that most of the participants believed that plain water is the excellent source of fluid for human body, whereas cola and chocolate drinks as poor sources of hydration. However, some evidence suggest that other beverages such as caffeinated soft drinks (Grandjean et al., 2000), orange juice and coffee (Grandjean et al., 2003) are as good as plain water in providing fluid to the body. Based on this scientific evidence, the results of the present study show that the participants held a misperception about the type of beverages that contribute water to human body.

Furthermore, the results show that "feels thirsty" was the main trigger for drinking during exercise and in general during the day. Under resting conditions, thirst is adequate to stimulate fluid intake in order to prevent hypohydration (Greenleaf, 1992). However, available data suggest that thirst is insufficient to promote fluid intake to maintain normal hydration during exercise (Greenleaf, 1992; Passe et al., 2007) which may lead to involuntary dehydration. This concern results in ACSM fluid replacement guideline that recommends that during exercise, people should drink fluid to limit body mass loss to less than 2% body mass to maintain the exercise performance (Sawka et al., 2007). However, other investigators have challenged this view and recommend that exercising man should only drink as dictated by thirst because drinking *ad libitum* results in similar performance outcome as drinking to replace the body mass loss during exercise (Daries, Noakes, & Dennis, 2000; Noakes, 2007a). Since the view remains inconclusive, further work in the area of hydration, thirst and fluid intake are warranted.

The present study also investigated the issue of health concerns regarding under or over consumption of water. Among the popular responses given by the participants regarding the effect of little water consumption on health were “constipation”, “fatigue” and “headache”. The role of water together with fibre in maintaining bowel function is well recognized (Williams, 2007). Lack of fluid during exercise may lead to dehydration, which then increases core body temperature and also disturbs electrolyte balance and eventually fatigue will occur (Williams, 2007). Headache is also often attributed to water deprivation, but the view remains inconclusive (Negoianu & Goldfarb, 2008). Work conducted by Spigt et al. (2005) showed that there was no significant difference found in terms of experiencing headache between participants who increased their fluid intake compared to those who did not. On the other hand, Shirreffs, Merson, Fraser and Archer (2004) who conducted the study on the effects of fluid restriction on hydration status and subjective feelings in adult males and females showed that the participants reported to have significantly greater feeling of headache at 24 and 37 h of fluid restriction trial compared to euhydrated trial.

For the question on the effect of too much water consumption on the human body, the responses “flush out of mineral/hyponatraemia” and “increased urination” were among the most popular answers given by the participants. Overhydration is often related to hyponatraemia, a condition of diluted sodium caused by excessive water intake over a short period of time, which has been reported in a small number of recreational and endurance-exercising individuals (Noakes & Speedy, 2006; Flinn & Sherer, 2000) and also in resting individuals (Flear, Gill, & Burn, 1981). Increased urination is the result of decreased antidiuretic hormone secretion in order to maintain normal body fluid volume (Williams, 2007). From the results of the present study (Tables 3.11 and 3.12), it could be concluded that most participants were knowledgeable and aware about the issue of general health and amount of water consumption.

This study also reveals that 80.0% of the participants reported to be aware of their hydration status based on their urine colour and their subjective feelings such as “feel thirsty” and “headache”. According to Armstrong et al. (1994), urine colour may be used as a reliable tool to assess hydration status when other laboratory-based indices such as urine specific gravity and urine osmolality are unavailable. However the use of subjective feelings to measure hydration status still lacks strength of evidence (Armstrong, 2007) but has since been further investigated and is reported later in this thesis (chapter 5).

Furthermore, the results show that plain tap water was reported to be consumed the most (1.5 ± 1.2 L) during an average day compared to other beverages. This finding was in agreement with Heller, Sohn, Burt and Eklund (1999) who conducted the survey on water consumption in the United States from 1994 - 1996 and reported that plain water made up approximately one-third of total beverage intake among individuals aged 20-64 years old. In addition, the results in the present study also parallel to the participants’ belief that water is the most hydrating (Table 3.10) and most preferred beverage during exercise and in general during the day (Table 3.8).

Concerning the participants’ opinion on other people hydration habits, most of the participants believed that people around them did not always drink during exercise and did not drink sufficiently for exercise. At present, no published studies that the investigator is aware of are available to either support or refute this view.

It is difficult to determine the quantity that is deemed a sufficient amount to drink for exercising individuals because it depends on many factors such as duration and types of exercise and environmental conditions . However, established guidelines of fluid replacement for exercise by ACSM recommends that before exercise, exercising individuals are to drink slowly approximately 5-7 ml kg^{-1} per body weight of fluid 4 h, only if they have not consumed sufficient beverages with meals and there has been insufficient recovery period since the last exercise session, to drink fluid to limit body mass loss to less than 2% body mass during exercise and to consume normal meals and beverages after exercise in order to restore euhydration (Sawka et al., 2007).

In general, the fitness professionals in this study demonstrated many acceptable perceptions that are in line with scientific evidence in the area of hydration, thirst and fluid intake. However, the present study also identifies the need for fitness professionals to accurately pick information based on sound evidence regarding their perspectives on hydration and fluid intake habits.

Chapter 4

Water turnover and thirst response in free-living people

Introduction

In humans, fluid balance is maintained within a narrow range by matching water intake with water output. Water intake is mainly from fluid ingestion, which is triggered by thirst sensation while urine loss controlled by hormonal action is the main contributor to regulation of water excretion. Other components of water intake include water from foods and metabolic water and components of water output are water from faeces, respiratory and skin losses (Williams, 2007).

One of the main findings of the study reported in chapter 3 was that "feel thirsty" was the main reason that the participants reported as their stimulus for drinking during exercise and in general during the day. Available data have shown that thirst induced drinking is usually associated with the response to body fluid deficit due to exercise (Maresh et al., 2004) or water deprivation (Engell et al., 1987). However other studies suggest that in everyday life, spontaneous drinking may not respond to significant fluid deficit but due to other factors such as to drink accompanying food intake (Saltmarsh, 2001) or due to anticipation of fluid deficit (Phillips et al., 1984a).

Body fluid balance can be assessed by water turnover rate, which is the replacement of body water that is lost over a specific period of time. Few studies have reported water intake and excretion in different population such in exercising adults (Shimamoto & Komiya, 2003), elderly group (Leiper et al., 2005) and in healthy children (Fusch, Hungerland, Scharrer, & Moeller, 1993). However, an assessment of subjective feelings of thirst was not reported in these literatures. Only one study conducted by Bossingham, Carnell and Campbell (2005) provides the comprehensive data on water turnover and perceived thirst in younger and older adults. However, the participants in the study were given a controlled diet and beverages with the exception of only water that was given *ad libitum*. Hence, the study may not reflect the true picture of the effect of perceived thirst on the water balance components, in particular, the fluid intake on day-to-day basis.

Therefore, the aim of this study was to examine fluid balance components in free-living healthy adults by assessing their body water turnover rate. This study also aimed to investigate whether the degree of thirst sensation and mood states may play a role for their fluid intake behaviour.

Materials and methods

Twenty-six healthy adults aged between 20 and 40 years old participated in this study. The participants consisted of 13 males (mean ± SD: age= 28 ± 6 y; height= 172 ± 7 cm; body mass= 77 ± 12 kg; BMI= 26 ± 4 kg m^{-2}) and 13 females (mean ± SD: age= 26 ± 6 y; height= 165 ± 9 cm; body mass= 64 ± 13 kg; BMI= 23 ± 5 kg m^{-2}). All participants received both written and verbal details of the study, prior to the start of the trials. Once they agreed to proceed with the trial, written informed consent was obtained from them. This study protocol was reviewed and approved by Loughborough University Ethical Advisory Committee (R07-P140).

On the evening the participants were to start the trial (day 0), they emptied their bladder and provided a baseline urine sample in one of the small tubes provided. Then, before retiring to bed, they consumed an accurately weighed 10.01 ± 0.01 g of deuterium oxide (^2H_2O) (99.9 atom%, Sigma, London, U.K). They were asked to rinse the container of ^2H_2O several times, drinking the water (or other drink) they rinsed it with, to ensure that the entire contents of ^2H_2O were ingested. The next morning, the participants collected the first morning urine they passed in a container provided and retained the aliquot in a small tube provided. The remainder of this urine was placed into the specimen container. Then, before having anything to eat or drink, they were asked to come into the laboratory to have their nude body mass and height measured. Throughout the day, the participants collected all the passed urine in the container provided. Before retiring to bed, they completed 2 sets of questionnaires, which were to measure thirst sensations assessed by 100 mm VAS questionnaire (Engell et al., 1987) and mood states by POMS questionnaire (McNair et al., 1971). The thirst sensation questionnaire consists of 24 items, which were combinations of general and specific symptoms related to thirst. The participants repeated the collection of first morning urine samples for tracer analysis, 24 h urine collections and completion of questionnaires for seven consecutive days.

In addition to the above tasks, from day 3 to day 5 of the trial periods, the participants kept a record of all the food and drink they had and the activities they did in a diary record. They were provided with a set of portable electronic kitchen scale, accurate to 1 g for diet recording (Ohaus LS2000, US). On the last day of the trial periods (day 8), the participants reported to the laboratory again, to have their body mass measured after an overnight fast. During the study, the participants were asked to maintain their normal routine, eat, drink and exercise, as they normally would do. The dietary data obtained was analysed using computerised dietary analysis software (CompEat Pro version 5.8.0, Nutrition Systems, U.K.) to estimate energy intake and water intake. Calculated energy expenditure was determined using methods described by Ainsworth et al. (1993).

The daily environmental temperature and humidity recordings during the study period were obtained from the local meteorological office (Castle Donington, UK).

Urine specimen handling and analysis

Twenty-four hour urine samples were weighed using an electronic balance to the nearest 0.01 g (Mettler PC 2200, Switzerland). Assuming that 1 g = 1 ml, the weight of urine samples was converted to get the volume of urine output. The urine volume was not corrected for specific gravity because it contained deuterium oxide, which may raise the urine specific gravity, hence might consequently underestimate the volume of the urine samples. Of this, 5 ml aliquot was retained for further analysis of urine osmolality and chloride, sodium, potassium and creatinine concentrations. Urine osmolality was determined by freezing-point depression (Gonotec Osmomat 030 Cryoscopic Osmometer, Gonotec, Berlin, Germany). Urine chloride was measured by coulometric titration

(Jenway Chloride Meter PCLM3, Jenway Ltd., Dunmow, Essex, UK). Sodium and potassium concentrations were determined by flame photometry (Corning Clinical Flame Photometer 410C, Corning Ltd., Halstead, Essex, UK). Urine creatinine concentrations were obtained to confirm a full 24 h urine volume. This was done using the creatinine assay as described by Owen, Iggo, Scandrett and Stewart (1954) which is based on Jaffe reaction. All analyses were performed in duplicate.

The first morning urine samples, which were parts of a 24 h urine collection, were also weighed using the same analytical balance as above. Of this, a 2 ml aliquot, in duplicate were retained in sealed small tubes and kept in -20°C freezer for analysis of the deuterium oxide ($^2H_20$) content. Deuterium oxide concentration was measured using an infrared spectrometry (Miran-1a, the Foxboro Company, Connecticut, USA) as described by Lukaski and Johnson (1985). When the deuterium (^2H) concentration is determined, total body water (TBW) volume was calculated using the formula described by Schoeller (1996). From here, water turnover (WT) of the participants was determined using the formula by Lifson and McClintock (1966) as follows:

WT=TBW.k

where k is the slope of regression line of natural logarithm of the ^2H readings over the baseline measure of the morning urine sample (Leiper et al., 2001).

Meanwhile, data of total 24 h urine output was used to estimate the daily non-renal water loss in those participants. The non-renal water loss was determined by subtracting the average of water turnover of the particular participant with the mean volume of 24 h urine output excreted over the trial periods.

Statistical analysis

All data sets were checked for normative distribution using the Kolmogorov-Smirnov test. Parametric data are reported as mean ± standard deviation. Non-parametric data are presented as median (range).

For parametric data sets, Independent t-tests and within group one-way analysis of variance (ANOVA) were used to evaluate differences between gender and consecutive testing days, respectively. For equivalent non-parametric data, Mann Whitney and Friedman tests were used. Spearman correlation coefficient test was used to determine the relationship between subjective feelings related to thirst with fluid intake and WT. The level of significant was set at $P<0.05$. Statistical analysis was performed using SPSS version 16.0. for Windows.

Results

The average daytime temperature and humidity during the study period was 8 ± 5 °C and 86 ± 14%, respectively. Body mass (mean ± SD) was the same at the start and at the end of the study period in both male (77.5 ± 12.0 kg and 77.3 ± 11.7 kg, P=0.168) and female (63.6 ± 12.9 kg and 63.5 ± 12.7 kg, P=0.405) participants.

Urine volume was significantly higher ($P<0.05$) in females than male participants. On the other hand, urine osmolality, urine sodium, potassium and chloride concentrations were significantly higher ($P<0.05$) in males than female participants (Table 4.1).

There were no significant changes ($P>0.05$) observed for urine volume, urine osmolality, urine sodium, potassium and chloride over 7 consecutive days of trial periods in male (Table 4.2) and female (Table 4.3) participants.

Total body water was significantly higher ($P<0.05$) in males than female participants. However, when TBW was expressed relative to percentage body mass, the difference was not significant between genders. Water turnover expressed as an absolute value or relative to TBW and body mass were also significantly greater ($P<0.05$) in males compared to females. Non-renal loss was also significantly higher in males than female groups (Table 4.4).

Urine creatinine which is used to assess the completeness of 24 h urine volume was also significantly higher ($P<0.05$, $r=0.549$) in male compared to female participants. There was no significant differences ($P>0.05$) detected for urine creatinine concentration over 7 days of testing in both groups (Figure 4.1).

Table 4.1: Urine volume, osmolality and electrolytes of the 24 h urine in male and female participants. Values are median (range).

Variables	Male (N=13)	Female (N=13)	P value	Effect size (*g*)
Urine volume (L)	1.3 (0.5-4.1)	1.6 (0.4-5.8)	0.046*	-0.411
Urine osmolality (mOsm kg^{-1})	648 (271-1124)	438 (145-1041)	<0.001*	-1.251
Urine Na^+ concentration (mmol L^{-1})	137 (37-278)	98 (32-275)	<0.001*	-0.779
Urine K^+ concentration (mmol L^{-1})	60 (23-116)	46 (11-129)	<0.001*	-0.962
Urine Cl^- concentration (mmol L^{-1})	101 (30-252)	77 (24-224)	0.001*	-0.679

* significant at P<0.05

Table 4.2: Urine osmolality and electrolyte excretion over 7 days of testing in male participants. Values are median (range).

Time (d)	Urine volume (L)	Urine osmolality (mOsm kg^{-1})	Urine Na^{+} concentration (mmol L^{-1})	Urine K^{+} concentration (mmol L^{-1})	Urine Cl^{-} concentration (mmol L^{-1})
1	1.4 (0.5-3.0)	674 (366-1076)	148 (57-217)	62 (43-105)	129 (46-221)
2	1.2 (0.8-4.1)	643 (348-1055)	137 (40-219)	62 (29-109)	117 (41-201)
3	1.6 (0.6-3.2)	611 (320-1082)	130 (50-229)	60 (32-108)	103 (47-185)
4	1.3 (0.7-3.6)	652 (327-1108)	127 (37-217)	60 (23-96)	102 (30-179)
5	1.4 (0.5-2.7)	777 (389-1025)	179 (95-241)	78 (34-116)	151 (73-211)
6	1.3 (0.9-3.7)	668 (271-1055)	146 (81-235)	58 (33-100)	120 (56-244)
7	1.2 (0.5-2.4)	740 (380-1124)	160 (53-278)	71 (41-112)	132 (41-252)
P value	0.705	0.600	0.332	0.347	0.350

Table 4.3: Urine osmolality and electrolyte excretion over 7 days of testing in female participants. Values are median (range).

Time (d)	Urine volume (L)	Urine osmolality (mOsm kg^{-1})	Urine Na^+ concentration (mmol L^{-1})	Urine K^+ concentration (mmol L^{-1})	Urine Cl^- concentration (mmol L^{-1})
1	1.4 (0.5-5.0)	445 (151-878)	107 (42-234)	51 (29-84)	83 (25-173)
2	1.8 (0.7-5.0)	457 (168-964)	115 (54-275)	50 (24-105)	91 (29-209)
3	1.9 (0.4-4.6)	445 (168-1041)	110 (39-223)	47 (22-101)	90 (26-215)
4	2.0 (0.8-4.7)	394 (156-1017)	94 (32-240)	45 (21-71)	75 (26-224)
5	1.8 (0.4-5.8)	472 (145-930)	109 (38-223)	51 (25-129)	89 (24-192)
6	1.5 (0.5-4.2)	443 (164-1010)	110 (41-192)	43 (11-78)	87 (32-156)
7	1.8 (0.4-4.3)	413 (159-1005)	93 (44-185)	48 (26-88)	78 (29-192)
P value	0.590	0.413	0.315	0.763	0.650

Table 4.4: Total body water, water turnover rate and non-renal water loss in male and female participants. Values are mean ± SD.

Variables	Male (N=13)	Female (N=13)	P value	Effect size (*r*)
Total body water (L)	43.9 ± 4.6	31.1 ± 3.9	<0.001*	0.832
Total body water (%)	56.8 ± 8.1	50.6 ± 8.7	0.070	0.346
Water turnover rate (L d^{-1})	2.9 ± 0.7	2.1 ± 0.9	0.010*	0.444
Water turnover rate (%)	6.8 ± 1.3	4.9 ± 1.6	0.005*	0.546
Water turnover rate (ml kg d^{-1})	41.7 ± 8.1	34.2 ± 9.1	0.023*	0.399
Non-renal water loss (L d^{-1})	1.5 ± 0.6	0.6 ± 0.9	0.010*	0.507

*significant at P<0.05

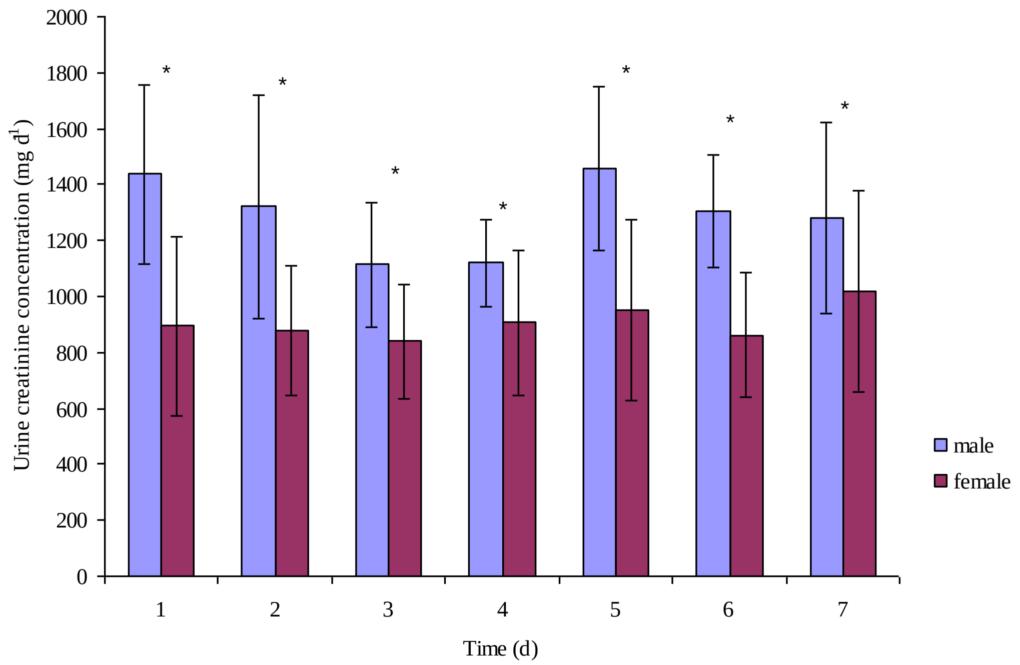


Figure 4.1: Urine creatinine concentration in male and female participants over 7 days of testing. Values are mean ± SD. * indicates values are significant at P<0.05 between gender.

Calculated basal metabolic rate (BMR), activity energy expenditure (AEE) and total energy expenditure (TEE) were significantly higher (P<0.05) in male than female participants. Energy intake was the same in males and females. There was no difference in water intake in the females compared to the males (Table 4.5).

Table 4.5: Calculated energy expenditure variables, energy intake and water intake in male and female participants. Values are mean ± SD.

Variables	Male (N=13)	Female (N=13)	P value	Effect size (*r*)
Basal metabolic rate (MJ d^{-1})	6.9 ± 0.6	6.1 ± 0.7	0.040*	0.523
Activity energy expenditure (MJ d^{-1})	3.9 ± 1.1	3.3 ± 0.5	0.047*	0.331
Total energy expenditure (MJ d^{-1})	12.1 ± 1.1	10.4 ± 1.1	0.031*	0.611
Energy intake (MJ d^{-1})	10.4 ± 3.2	9.1 ± 2.6	0.299	
Water intake (L d^{-1})	2.4 ± 1.2	2.8 ± 1.4	0.433	

*significant at P<0.05

Table 4.6 shows the frequency and percentage of drinking episode accompanying food intake or drinking without food consumption. The results show that 69% of fluid intake occurred accompanying food intake while 31% of drinking was without eating.

Table 4.6: Frequency of fluid consumption episodes with and without food intake during 3 consecutive days.

Type of drinking episode	Number (N)	Percentage (%)
Drinking with food	262	69
Breakfast	48	13
Brunch	27	7
Lunch	74	19
Snack	27	7
Dinner	75	20
Supper	11	3
Drinking without food	117	31
Breakfast	12	3
Brunch	22	6
Lunch	4	1
Snack	35	9
Dinner	2	1
Supper	42	11
Total episode	379	100

Figure 4.2 shows the results for the rating of 24 thirst related sensations between sex during the 7 days of trial periods. Females participants rated significantly higher for "tiredness" (P=0.018, *g*=-2.371), "dry mouth" (P=0.030, *g*=-0.427) and "feel thirsty" (P=0.002, *g*=-0.596) compared to males participants. For other feeling ratings, there were no significant difference (P>0.05) detected between gender.

Tables 4.7 and 4.8 show that there was no significant difference ($P>0.05$) for all 24 subjective feelings ratings over 7 days of testing in male and female participants, respectively.

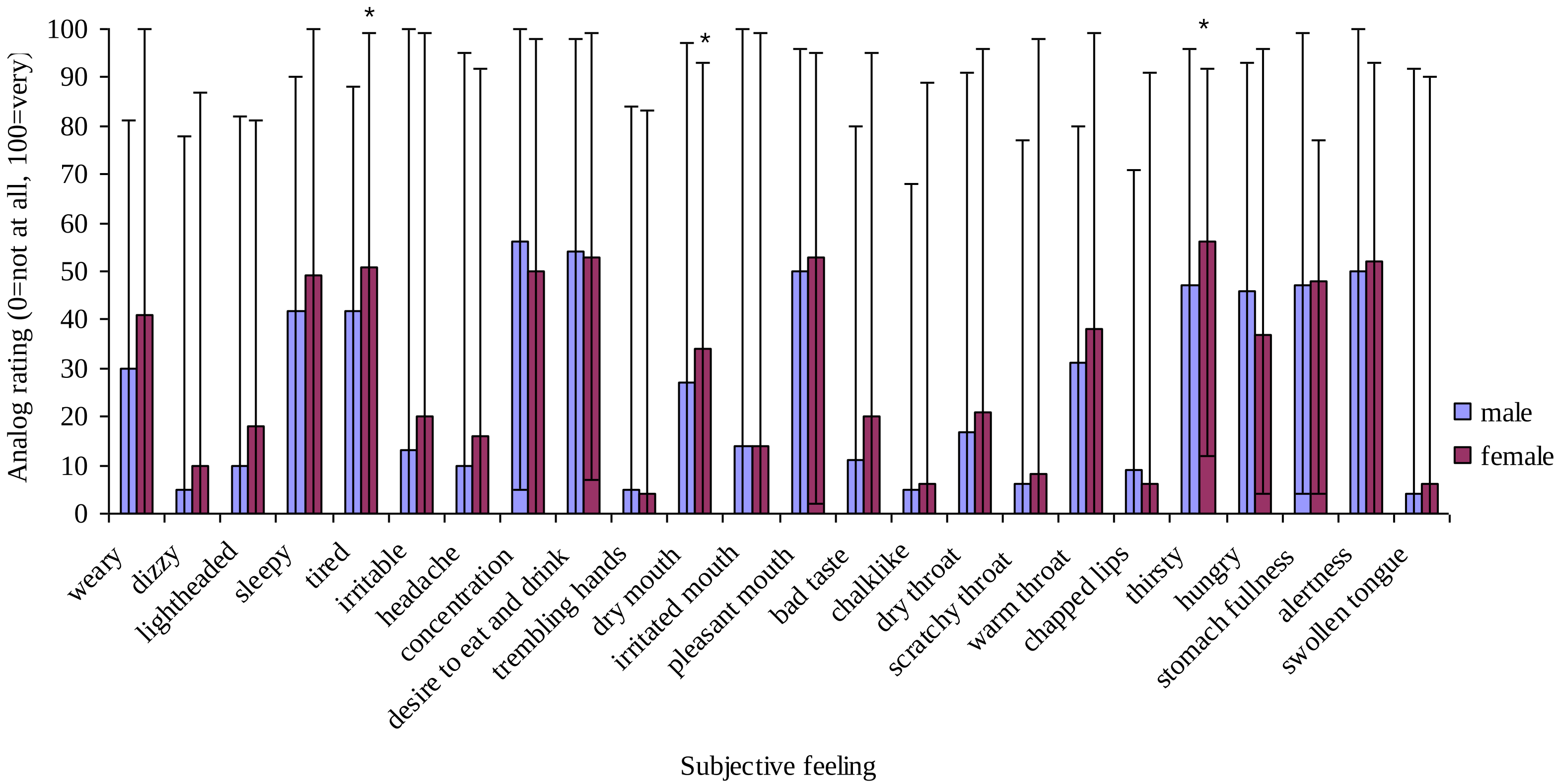


Figure 4.2: Thirst related sensations in male and female participants. Values are median (range).* indicates values are significant at P<0.05 between gender.

Table 4.7: Thirst related sensations over 7 days of testing in male participants. Values are median (range).

Variables	1	2	3	4	5	6	7	P value
Weary	35 (0-71)	50 (3-81)	30 (2-76)	36 (6-56)	35 (0-70)	34 (0-80)	23 (0-65)	0.899
Dizzy	5 (0-35)	3 (0-69)	4 (0-40)	6 (0-40)	5 (0-66)	6 (0-78)	9 (0-69)	0.995
Lightheaded	30 (0-78)	17 (2-67)	7 (0-52)	10 (0-64)	10 (0-63)	6 (0-82)	10 (0-71)	0.863
Sleepy	63 (0-90)	48 (4-76)	46 (4-87)	38 (3-81)	44 (0-71)	30 (0-61)	26 (4-71)	0.183
Tired	54 (4-77)	48 (5-87)	41 (5-74)	34 (3-76)	27 (0-85)	35 (0-88)	21 (4-80)	0.670
Irritable	13 (0-63)	20 (0-100)	11 (0-82)	13 (0-62)	18 (1-65)	4 (0-70)	14 (0-62)	0.813
Headache	15 (0-69)	5 (0-85)	5 (0-66)	5 (0-70)	20 (0-95)	11 (0-69)	10 (0-68)	0.989
Concentration	59 (20-100)	61 (5-97)	66 (11-91)	56 (31-95)	59 (22-100)	60 (32-95)	49 (16-95)	0.809
Desire to eat and drink	58 (5-78)	52 (0-92)	69 (23-91)	50 (2-90)	52 (3-98)	58 (2-78)	46 (1-89)	0.278
Trembling hands	6 (0-64)	8 (0-36)	5 (0-70)	2 (0-67)	3 (0-54)	7 (0-84)	9 (0-44)	0.977
Dry mouth	22 (0-73)	21 (0-97)	41 (1-78)	30 (0-79)	48 (0-82)	32 (0-81)	24 (0-80)	0.965
Irritated mouth	8 (0-68)	13 (0-97)	5 (0-78)	19 (0-80)	33 (0-100)	10 (0-63)	10 (0-79)	0.878
Pleasant mouth	42 (1-85)	55 (4-93)	53 (0-88)	46 (13-96)	51 (1-84)	51 (0-83)	50 (0-85)	0.984
Bad taste	18 (1-71)	9 (0-80)	9 (0-80)	16 (0-73)	9 (0-69)	9 (0-60)	28 (0-75)	0.986
Chalklike	6 (0-41)	4 (0-46)	0 (0-41)	13 (0-57)	5 (0-49)	6 (0-68)	5 (0-57)	0.953
Dry throat	19 (0-74)	12 (4-85)	12 (0-79)	13 (0-85)	25 (0-81)	23 (1-91)	26 (0-70)	0.998
Scratchy throat	7 (0-59)	6 (0-62)	4 (0-77)	7 (0-60)	5 (0-60)	9 (0-51)	6 (0-71)	0.988
Warm throat	22 (0-62)	31 (0-62)	41 (0-80)	28 (0-56)	39 (0-67)	29 (1-62)	31 (0-73)	0.994

Chapped lips	10 (0-60)	7 (0-57)	12 (0-63)	12 (0-57)	7 (0-57)	7 (0-71)	9 (0-62)	0.976
Thirsty	43 (3-74)	49 (15-96)	54 (1-76)	45 (1-95)	49 (0-85)	47 (0-92)	43 (1-78)	0.999
Hungry	46 (2-66)	49 (5-93)	57 (8-88)	56 (4-87)	36 (0-58)	43 (0-85)	45 (1-83)	0.358
Stomach fullness	48 (10-64)	42 (5-65)	52 (16-79)	38 (17-96)	47 (19-99)	46 (4-85)	52 (20-79)	0.677
Alertness	54 (0-78)	62 (0-99)	49 (0-100)	46 (1-94)	47 (0-98)	50 (1-91)	49 (0-86)	0.938
Swollen tongue	3 (0-65)	6 (0-92)	1 (0-61)	5 (0-82)	2 (0-61)	5 (0-63)	4 (0-59)	0.993

Table 4.8: Thirst related sensations over 7 days of testing in female participants. Values are median (range).

Variables	1	2	3	4	5	6	7	P value
Weary	62 (1-80)	41 (0-76)	50 (0-100)	50 (1-67)	32 (5-84)	20 (0-55)	47 (4-73)	0.280
Dizzy	12 (0-87)	4 (0-52)	12 (0-70)	9 (0-57)	12 (0-72)	10 (0-53)	9 (0-69)	0.882
Lightheaded	18 (3-73)	22 (0-81)	17 (0-80)	17 (1-68)	41 (0-81)	12 (0-78)	24 (0-75)	0.895
Sleepy	64 (0-78)	36 (2-81)	46 (4-98)	51 (4-97)	44 (0-100)	34 (9-81)	51 (4-94)	0.470
Tired	59 (20-90)	38 (3-68)	59 (11-99)	56 (24-81)	65 (0-85)	25 (0-60)	51 (7-70)	0.065
Irritable	21 (8-88)	14 (0-41)	26 (0-99)	26 (0-56)	16 (2-75)	10 (0-52)	15 (4-57)	0.243
Headache	16 (0-82)	22 (0-70)	30 (0-92)	9 (0-51)	9 (0-78)	10 (0-53)	17 (5-46)	0.489
Concentration	49 (26-98)	49 (26-95)	53 (16-73)	51 (7-90)	48 (0-78)	48 (0-91)	51 (2-92)	0.983
Desire to eat and drink	46 (12-98)	65 (10-74)	56 (10-99)	54 (33-81)	59 (18-82)	38 (18-83)	49 (7-69)	0.398
Trembling hands	5 (0-25)	3 (0-57)	6 (0-83)	6 (0-62)	7 (0-72)	4 (0-41)	4 (0-39)	0.991
Dry mouth	22 (8-73)	49 (14-74)	31 (0-87)	46 (0-90)	49 (2-93)	32 (5-85)	35 (2-85)	0.670
Irritated mouth	16 (0-94)	17 (0-69)	6 (0-99)	25 (0-80)	14 (0-94)	12 (0-79)	11 (0-87)	0.957
Pleasant mouth	53 (2-91)	47 (6-95)	54 (17-89)	53 (29-75)	58 (4-83)	51 (15-89)	61 (14-88)	0.838
Bad taste	17 (0-83)	13 (0-56)	13 (0-51)	22 (3-73)	21 (2-95)	22 (7-73)	21 (5-87)	0.439
Chalklike	12 (0-73)	5 (0-32)	4 (0-45)	5 (0-72)	7 (0-89)	9 (0-72)	7 (0-74)	0.794
Dry throat	9 (0-80)	26 (1-74)	17 (0-96)	27 (0-79)	33 (0-94)	20 (0-80)	21 (1-90)	0.970
Scratchy throat	4 (0-77)	10 (0-95)	7 (0-97)	8 (0-78)	13 (0-94)	9 (0-80)	7 (1-98)	0.989
Warm throat	47 (0-75)	47 (0-69)	50 (0-99)	31 (0-54)	25 (0-84)	35 (0-79)	27 (0-94)	0.826

Chapped lips	5 (0-65)	6 (0-54)	5 (0-59)	9 (0-72)	15 (0-91)	7 (0-79)	14 (0-87)	0.955
Thirsty	47 (12-80)	61 (16-85)	47 (13-80)	63 (15-83)	69 (47-92)	52 (13-78)	56 (14-90)	0.213
Hungry	30 (7-76)	49 (10-68)	32 (12-96)	41 (19-67)	47 (7-78)	31 (9-79)	47 (4-83)	0.896
Stomach fullness	50 (8-77)	45 (15-77)	49 (15-68)	49 (24-70)	46 (25-61)	53 (4-70)	46 (14-70)	0.942
Alertness	45 (19-85)	47 (27-92)	48 (0-92)	66 (1-90)	56 (11-93)	48 (38-88)	56 (7-92)	0.862
Swollen tongue	7 (0-47)	5 (0-48)	5 (0-90)	8 (0-52)	8 (0-77)	6 (0-65)	8 (0-85)	0.999

For POMS assessment, male participants had significantly higher score for vigour (P<0.001,*g*=0.459) and significantly lower score for depression (P=0.018, *g*=0.200) compared to female participants. There was no significant difference in terms of tension, anger, fatigue and confusion scores between genders (Figure 4.3).

There was no significant difference observed for all POMS components during 7 days of testing for both groups (Figure 4.4)

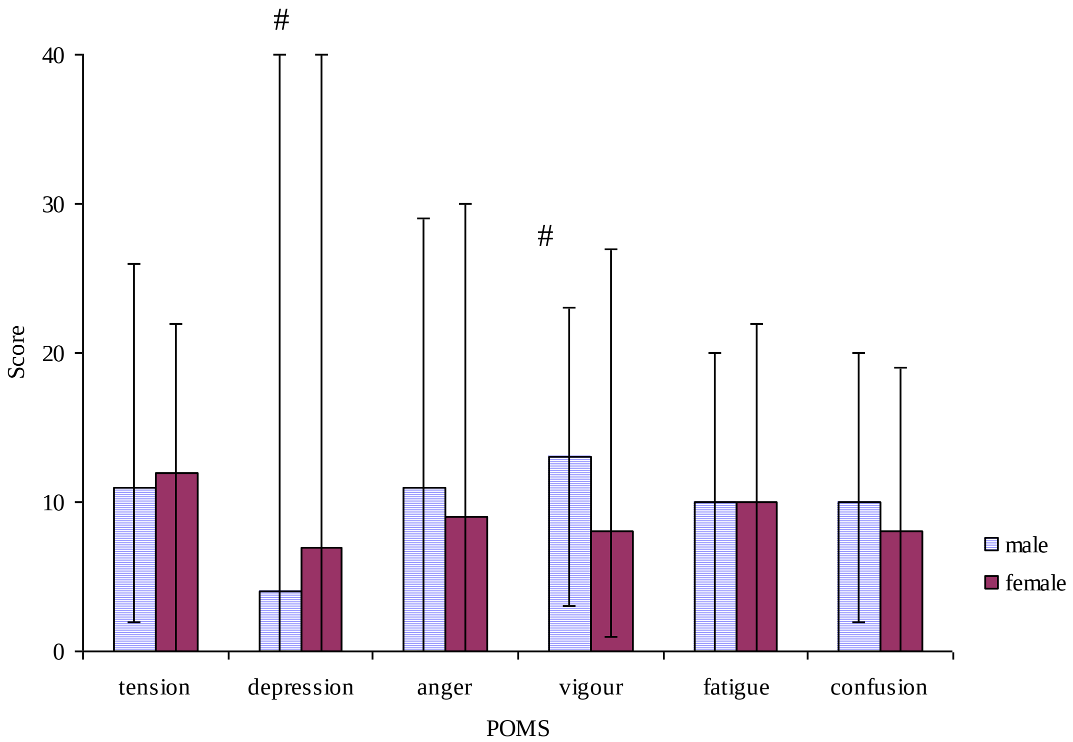


Figure 4.3: POMS score between male and female participants. Values are median (range). # indicates significant different at P<0.05.

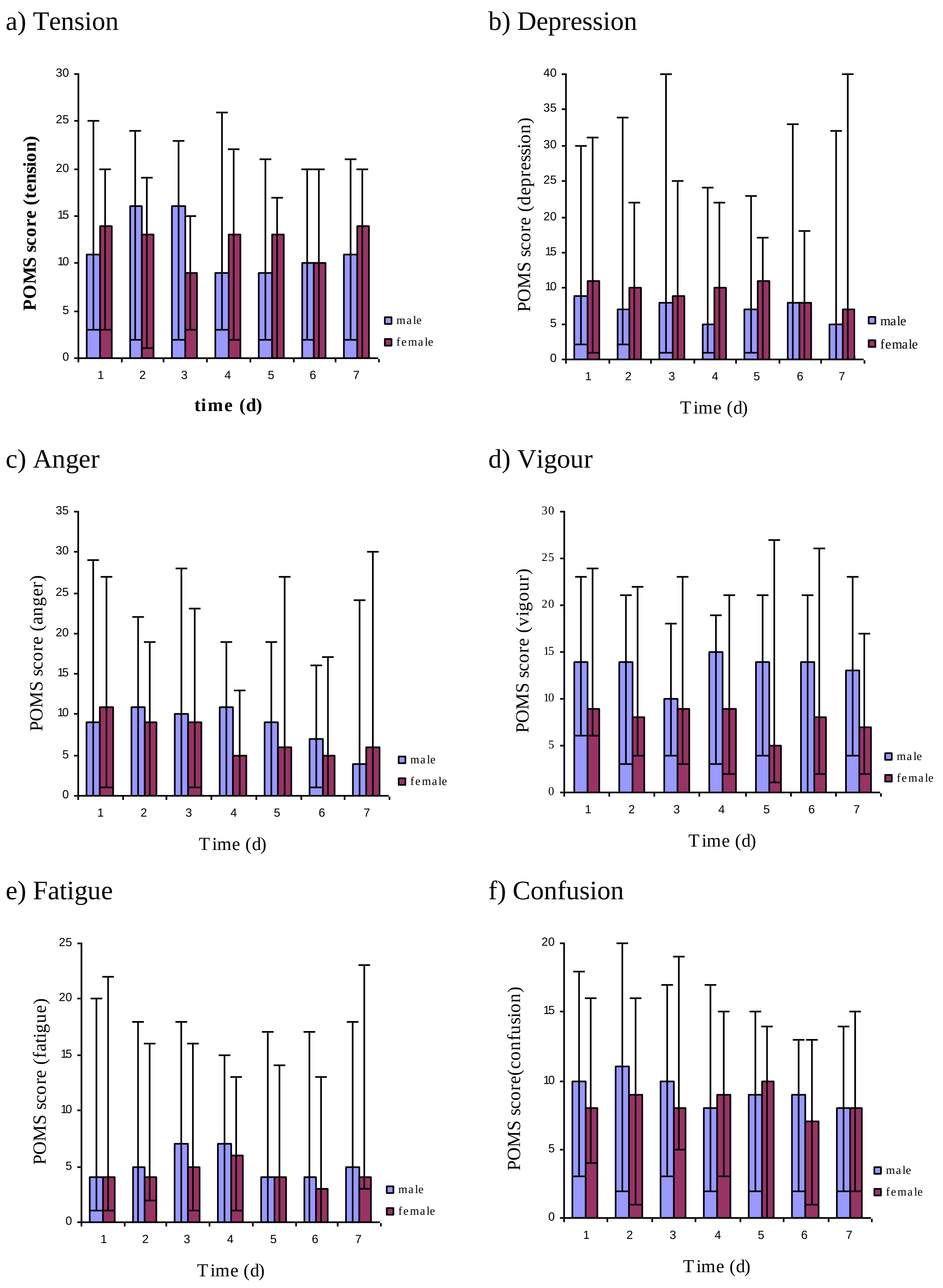


Figure 4.4: POMS score of (a) tension (b) depression (c) anger (d) vigour (e) fatigue (f) confusion over 7 days of testing. Values are median (range).

Spearman correlation coefficient test between the 24 items of thirst related sensations and WT show that there was no significant correlation between subjective feelings and WT in male participants. In females, only “dry mouth” was found to be significantly associated with WT (rho=0.656, P=0.015) (Table 4.9).

There was no correlation detected between all POMS components and WT in both male and female participants (Table 4.10).

Table 4.9: Spearman correlation between water turnover rate and subjective feelings related to thirst in male and female participants.

Water turnover rate vs.	Male (N=13)			Female (N=13)		
	rho	P value	CI	rho	P value	CI
Weary	0.402	0.174		-0.027	0.929	
Dizzy	-0.138	0.652		-0.050	0.871	
Lightheaded	0.181	0.554		-0.206	0.500	
Sleepy	0.058	0.850		-0.088	0.774	
Tired	0.293	0.325		0.347	0.245	
Irritable	0.299	0.321		-0.224	0.462	
Headache	-0.038	0.903		-0.055	0.858	
Concentration	0.412	0.161		0.061	0.844	
Desire to eat and drink	0.433	0.140		-0.039	0.901	
Trembling hands	0.247	0.415		0.014	0.964	
Dry mouth	0.640	0.064	0.138 to 0.880	0.656	0.015*	0.165 to 0.886
Irritated mouth	0.030	0.922		0.204	0.504	
Pleasant mouth	-0.046	0.882		-0.061	0.844	
Bad taste	0.281	0.352		0.017	0.957	
Chalklike	0.138	0.652		0.045	0.885	
Dry throat	0.538	0.158		0.410	0.164	
Scratchy throat	0.027	0.931		0.165	0.591	
Warm throat	0.140	0.652		-0.082	0.789	
Chapped lips	0.055	0.931		0.254	0.402	
Thirsty	0.062	0.648		0.188	0.538	
Hungry	0.107	0.858		-0.545	0.064	-0.842 to 0.008
Stomach fullness	-0.232	0.839		-0.559	0.077	0.848 to -0.012
Alertness	0.217	0.729		-0.308	0.306	
Swollen tongue	0.382	0.446		-0.168	0.582	

* correlation is significant at P<0.05 (2-tailed)

Table 4.10: Spearman correlation between water turnover rate and POMS items in male and female participants.

Water turnover rate vs.	Male (N=13)		Female (N=13)	
	rho	P value	rho	P value
Tension	0.172	0.575	-0.050	0.872
Depression	-0.162	0.598	0.042	0.892
Anger	0.044	0.885	-0.297	0.325
Vigour	0.322	0.284	-0.116	0.706
Fatigue	-0.109	0.723	-0.190	0.534
Confusion	-0.093	0.762	0.056	0.857

Spearman correlation coefficient test between 24 items of thirst related sensations and fluid intake show that there was no correlation between the subjective feelings and fluid intake in males. However, in females, “dry mouth” was positively correlated with fluid intake (rho=0.600, P=0.030, CI=0.074 to 0.865) (Table 4.11).

Similarly, there was no correlation detected in male participants between all POMS components and fluid intake, but in females, depression was significantly correlated with fluid intake (rho=0.612, P=0.026, CI=0.093 to 0.869) (Table 4.12).

Table 4.11: Spearman correlation between subjective feelings related to thirst and fluid intake in male and female participants.

Fluid intake vs.	Male (N=13)			Female (N=13)		
	rho	P	CI	rho	P	CI
Weary	-0.544	0.075	-0.842 to 0.009	-0.776	0.080	-0.929 to -0.394
Dizzy	-0.131	0.670		-0.015	0.960	
Lightheaded	-0.039	0.900		0.209	0.493	
Sleepy	-0.179	0.558		-0.397	0.179	
Tired	-0.291	0.335		-0.399	0.177	
Irritable	-0.016	0.958		-0.387	0.192	
Headache	-0.134	0.663		0.406	0.169	
Concentration	-0.164	0.593		-0.286	0.344	
Desire to eat and drink	0.200	0.512		0.128	0.676	
Trembling hands	0.255	0.069	-0.344 to 0.706	0.381	0.1990	
Dry mouth	0.042	0.892		0.600	0.030*	0.074 to 0.865
Irritated mouth	0.109	0.723		0.317	0.292	
Pleasant mouth	-0.308	0.306		-0.117	0.703	
Bad taste	0.052	0.865		0.328	0.274	
Chalklike	0.071	0.785		0.247	0.415	
Dry throat	0.098	0.750		0.364	0.221	
Scratchy throat	0.142	0.642		0.041	0.895	
Warm throat	0.110	0.729		0.426	0.147	
Chapped lips	0.160	0.601		-0.417	0.156	
Thirsty	0.006	0.986		0.364	0.221	
Hungry	-0.359	0.229		0.210	0.492	
Stomach fullness	-0.106	0.730		-0.056	0.857	
Alertness	0.171	0.577		0.351	0.240	
Swollen tongue	0.178	0.562		-0.040	0.897	

* correlation is significant at $P<0.05$ (2-tailed)

Table 4.12: Spearman correlation between fluid intake and POMS items in male and female participants.

Fluid intake vs.	Male (N=13)			Female (N=13)		
	rho	P	CI	rho	P	CI
Tension	0.078	0.801		-0.315	0.294	
Depression	-0.120	0.697		0.612	0.026*	0.093 to 0.869
Anger	-0.043	0.889		-0.153	0.100	
Vigour	0.171	0.576		0.092	0.089	-0.483 to 0.611
Fatigue	0.014	0.064	-0.541 to 0.560	-0.476	0.764	
Confusion	-0.211	0.488		-0.463	0.111	

* correlation is significant at P<0.05 (2-tailed)

Discussion

The main findings of this study suggest that there is variability in terms of fluid balance components across gender but these are constant during the study period. Subjective feelings related to thirst also vary between gender but similar throughout the study period.

In the present study, body water turnover rate was faster in males than females whether expressed relative to TBW or body mass. The average water turnover in males was 2.9 ± 0.7 L d^{-1} and 2.1 ± 0.9 L d^{-1} in females, equivalent to 6.8 ± 1.3% and 4.9 ± 1.6%, respectively. This result was opposite to the study conducted by Schloerb et al. (1950) whereby the turnover rate for females (8.1 ± 0.6%) was faster than males (7.6 ± 1.2%). This was different probably because in Schloerb et al. (1950), they had different number of participants which were 18 males and 3 females, but in the present study, the same number of participants between groups was used (13 males and 13 females).

The urine output in the present study was significantly higher in females (1.6 [0.4-5.8] L) than males (1.3 [0.5-4.1] L) with faster water turnover in male participants due to higher non-renal loss which were calculated to be 1.5 ± 0.6 L compared to 0.6 ± 0.9 L in female group. The male to female ratio of urine loss was approximately 1.2 as compared to ratio of non-renal loss, which was 2.5. It could be speculated that larger non-renal water loss in male participant in this study was probably due to increased amount of sweat and respiratory losses resulted from increased level of physical activity. There was no direct measure conducted on the sweat and respiratory losses during the study, however, essentially men subjected to similar intensity of physical exercise under similar environmental conditions are reported to sweat 30% to 40% more compared to women (Giacomoni, Mammone, & Matthew, 2009). Based on self-reported activities, the results in the present study showed that calculated BMR, AEE and TEE were significantly higher ($P<0.05$) in male compared to female participants. Higher AEE value in male (3.9 ± 1.1 MJ d^{-1}) than female (3.3 ± 0.5 MJ d^{-1}) participants shows that males had higher level of physical activity, hence may contribute to the higher amount of sweat and respiratory losses than the female counterparts and further support the above speculation.

Leiper and colleagues (2001) studied the water turnover in cyclists (CG) and sedentary men (SG) and found that water turnover rate was faster in exercising individuals (3.5 [2.9-4.9] L d^{-1}) than the sedentary group (2.3 [2.1-3.5] L d^{-1}). The finding in this study revealed that the average urine loss was similar in both groups (CG= 2.0 [1.8-2.4] L d^{-1}, SG= 1.9 [1.8-2.0] L d^{-1}, P=0.47) but the average non-renal water loss was significantly higher in CG (1.4 [1.1-3.0] L d^{-1}) than SG (0.4 [0.3-1.5] L d^{-1}). Based on this result, they concluded that the faster water turnover rate in exercising group was due to 3 times greater non-renal water loss in CG than the SG.

Other studies carried out to compare water turnover rate in physically active subjects versus sedentary subjects also produced the similar findings, which showed higher water turnover rate with increased level of physical activity (Leiper et al., 1996; Shimamoto & Komiya, 2003). Therefore, it can be concluded that these previous studies support the findings in the present study that faster WT was due to higher non-renal water losses in male participants.

Even though the results showed that calculated water intake was higher in females (2.8 ± 1.4 L d^{-1}) compared to males (2.4 ± 1.2 L d^{-1}), the water turnover rate was significantly faster in male (2.9 ± 0.7 L d^{-1}) compared to female (2.1 ± 0.9 L d^{-1}) participants. Calculated water intake based on self–reported intake may be less accurate for the assessment water turnover rate due to over reporting or underreporting (Johansson, Callmer, & Gustafsson, 1992; Goris & Westerterp, 1999). On the other hand, tracer dilution technique that was used to assess the WT in this study may provide more objective measure of water turnover rate in humans (Lifson & McClintock, 1966).

This study also aimed to determine whether any correlation occurs between subjective feelings related to thirst and mood states on components of fluid balance, particularly water turnover and fluid intake. Despite female participants giving a significantly higher median rating for "tiredness", "dry mouth" and "thirsty" during the day compared to male group, the Spearman test showed that only "dry mouth" had a significant relationship with fluid intake. There was no association between all subjective feelings and fluid intake in male participants. Similarly, Spearman tests showed that no significant association occurs between subjective feelings and WT in males. In female group, only “dry mouth” was found to be significantly correlated with WT.

Thirst is often associated with the stimulation of fluid intake in order to meet hydration needs (Rolls & Rolls, 1982) but it may not be necessarily true in all occasion. Previous studies have shown that when food and drink are consumed in *ad libitum* situations, most of the spontaneous drinking is related to eating episodes (Engell, 1988; de Castro, 1988). In the present study, the participants were free-living individuals who had unrestricted access to food and drink. Since thirst related sensations did not associate with fluid intake, it could be inferred that eating might induce their fluid intake consumption in the present study. In deed, the result from 3 d diet record in the present study showed that drinking episode accompanying food intake was higher (69%) than without food intake (31%) (Table 4.6), which could support the previous assumption.

Higher rating for dry mouth by female participants in the present study may account for higher mean fluid intake during the study period compared to male participants. This finding is supported by the previous studies which showed that dry mouth sensation is often associated with thirst induced drinking (Holmes & Gregersen, 1947) and produced longer drinking episode, greater number of drinking bouts and greater volume of water compared to control condition (Brunstrom, Tribbeck, & MacRae, 2000).

To date, there is no published data available on the relationship between dry mouth and WT. WT is the measure of turnover of water intake and water output within a certain period of time. In this study, female participants had significantly higher urine volume and also higher calculated water intake although did not reach significance compared to male participants. It is speculated that dry mouth may stimulate higher amount of water intake, hence may explain the higher urine volume in female participants.

Furthermore, in the present study, males had significantly higher score for vigour and lower score for depression than female participants but no components of POMS score in males correlated with fluid intake. In female, only depression was found to correlate well with fluid intake.

There is no data available to support that depression increases water intake *per se*. Nonetheless, previous investigations conducted on the effect of mood states on the amount of food intake show that boredom, depression and fatigue were associated with higher food consumption (Mehrabian, 1980) and positive correlation was observed between the level of depression and increased intake of meat, soft drinks and coffee (Yannakoulia et al., 2008). Therefore, it could be indirectly inferred that a higher level of depression scores in females may probably contribute to the higher intake of fluid compared to males in this study.

In summary, this study demonstrates that despite higher urine output and fluid intake in female participants, water turnover was faster in males due to a much higher non-renal water loss. Furthermore, this study also suggests that thirst sensation did not correlate with fluid intake in both male and female participants but dry mouth and depression may explain the higher amount of fluid intake in female compared to male group.

Chapter 5

Thirst as a marker of hydration status

Introduction

Adequate hydration status is important for general well being and sports performance. At present, assessment of hydration status in free-living individuals is typically estimated by body mass and analyses on urine and blood samples. Changes in body mass is often used to quantify water loss over a short period of time (Shirreffs, 2003), but less practical to assess hydration status over a longer period of time because other factors such as food and fluid intake or changes in body composition may mask or exacerbate the changes in body weight (O'Brien et al., 2002). Urine markers such as urine colour, urine specific gravity and urine osmolality are commonly used to measure hydration status in different settings with varied success (Francesconi et al., 1987; Armstrong et al., 1994). Blood indices such as plasma osmolality has been assessed as a marker of hydration status, particularly during exercise-induced dehydration (Popowski et al., 2001), nevertheless the use of this invasive method may not appeal to some individuals and its use in different settings also produces inconsistent results (Armstrong, 2007).

Subjective measures of hydration status such as thirst response has been investigated as potential markers (McGarvey et al., 2008), but not as extensively as the objective markers. Given the limitations of the objective measures of hydration status, an individual's perceived level of thirst may provide an alternative method to assess hydration status. This would have benefits from being non-invasive, require little technical expertise and less costly.

Therefore, the objective of this study was to investigate the feasibility of subjective feelings of thirst as markers of hydration status in free-living humans.

Materials and methods

Twenty-eight healthy participants (17 males and 11 females) with a mean age of 20 ± 1 year, height of 176 ± 9 cm and body mass of 65 ± 8 kg took part in this study. All participants received both written and verbal details of the study prior to the start of the trials. Once they agreed to proceed with the trial, written informed consent was obtained from them. This study protocol was reviewed and approved by Loughborough University Ethical Advisory Committee (R08-P117).

The participants were asked to visit the laboratory 4 times, which involved 2 sessions of familiarisation trials and 2 experimental trials. The familiarisation trials were identical to the experimental trials served to familiarise the participants with the experimental procedures and to reduce any potential learning effect. They completed 2 trials either after an overnight fast (fasted) or in the morning after having had a meal and drink 2 h earlier (non-fasted), in random order separated by one week. The meal and drink was selected by the participants to represent their typical breakfast. Upon arrival at the laboratory, they had their nude body mass and height measured. Then, they were asked to empty their bladder and collect the urine sample in the container provided. After that, the participants were asked to sit in an upright position for 15 min before a 5 ml blood sample was collected by venupuncture of an anticubital vein. Finally, they were asked to fill in the 18-item thirst related sensations questionnaire assessed by a 100 mm visual analog scale questionnaire. This completed the trial and the participants free to leave the laboratory.

Sample analysis

Urine samples were analysed for urine specific gravity, urine colour and urine osmolality. All analysis was performed as described in the general methods reported in chapter 2.

Whole blood samples were collected in a syringe and dispensed into a tube containing K_2EDTA (2.5 ml) for analysis of haemoglobin concentration and haematocrit and into a plain tube (2.5 ml) for analysis of serum osmolality. To extract the serum, whole blood was centrifuged at 1500 g for 15 min at 4ºC (ALC multispeed refrigerated centrifuge, UK). Haemoglobin concentration was determined by the cyanmethaemoglobin method and haematocrit was measured by microcentrifugation (Hawksley micro-haematocrit centrifuge, UK). Serum osmolality was obtained via freezing-point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany). Measurement of haemoglobin concentration and serum osmolality were carried out in duplicate while haematocrit was performed in triplicate.

Statistical analysis

All data sets were checked for normative distribution using the Kolmogorov-Smirnov test. Parametric data are reported as mean ± standard deviation. Non-parametric data are presented as median (range).

For parametric data sets, an independent t-test and paired t-test were used to evaluate differences between gender and trial, respectively. For not normally distributed data, non-parametric tests of Mann Whitney and Wilcoxon tests were used to assess differences between gender and trial, respectively. Spearman correlation coefficient test was used to determine the relationship between physiological indices of hydration status and subjective feelings related to thirst. The level of significance was set at $p<0.05$.

Receiver operating characteristics (ROC) curve analysis was carried out to determine the cut off values for the subjective feelings that could potentially be used to distinguish between euhydration and hypohydration. The ROC curve was generated by plotting the true-positive rate (sensitivity) versus the false-positive rate (specificity) of different cut off points. The area under curve (AUC) was calculated to establish whether any discrimination exists between 2 hydration states. The AUC value above 0.5 suggests the ability of the subjective feelings to distinguish between euhydration and hypohydration. The cut off point associated with the lowest false-negative and false-positive rate and sufficient to detect hypohydration based on satisfactory sensitivity and specificity rate (>85%) was established. Statistical analysis was performed using SPSS version 16.0. for Windows.

Results

Physiological indices of hydration status

Body mass was significantly lower (P<0.05) in fasted than non-fasted trial, equivalent to the average body mass difference of 0.67 ± 0.78 kg or 1.04 ± 1.17%. Urine colour was significantly lighter (P<0.05) in non-fasted than fasted trial. There were no significant differences detected between trials for urine specific gravity, urine osmolality and haemoglobin concentration. In contrast, haematocrit and serum osmolality were found to be significantly lower (P<0.05) in non-fasted than fasted trial (Table 5.1). When the data were analysed only on those participants who had lighter body mass on the fasted trial, the similar results were obtained for all parameter.

Table 5.1: Body mass, urine colour, urine specific gravity, haemoglobin concentration, haematocrit and serum osmolality in non-fasted and fasted trial. Values are mean ± SD.

Parameter	Non-fasted trial (N=28)	Fasted trial (N=28)	P value	Effect size (*r*)
Body mass (kg)	65.11 ± 7.86	64.44 ± 7.83	<0.001*	0.697
Urine colour	4 ± 1	6 ± 1	<0.001*	0.707
Urine specific gravity	1.019 ± 0.009	1.021 ± 0.006	0.314	
Urine osmolality (mOsm kg^{-1})	611 ± 292	657 ± 196	0.389	
Haemoglobin conc.(g 100 ml^{-1})	15.3 ± 1.5	15.4 ± 1.2	0.814	
Haematocrit (%)	43.2 ± 2.8	43.8 ± 2.6	0.013*	0.135
Serum osmolality (mOsm kg^{-1})	284 ± 3	287 ± 4	0.001*	0.248

* significant at P<0.05.

When the data from both trials were pooled together, body mass, haemoglobin concentration, haematocrit and serum osmolality were significantly lower (P<0.05) in female compared to male participants. However, urine colour, urine specific gravity and urine osmolality were not significantly different between genders (Table 5.2). Similar results were obtained when the data were analysed separately for non-fasted and fasted trial.

Table 5.2: Body mass, urine colour, urine specific gravity, haemoglobin concentration, haematocrit and serum osmolality in male and female participants. Values are mean ± SD.

Parameter	Male (N=17)	Female (N=11)	P value	Effect size (*r*)
Body mass (kg)	67.83 ± 5.78	60.05 ± 8.23	<0.001*	0.430
Urine colour	5 ± 2	4 ± 2	0.321	
Urine specific gravity	1.020 ± 0.007	1.019 ± 0.009	0.669	
Urine osmolality (mOsm kg^{-1})	659 ± 225	595 ± 279	0.373	
Haemoglobin conc.(g 100 ml^{-1})	15.9 ± 1.1	14.4 ± 1.1	<0.001*	0.563
Haematocrit (%)	44.8 ± 2.3	41.6 ± 2.0	<0.001*	0.596
Serum osmolality (mOsm kg^{-1})	286 ± 4	284 ± 3	0.016*	0.565

* significant at P<0.05.

Subjective feelings questionnaires

Figure 5.1 shows the results for the rating of 18 items of thirst related sensations rated by the participants in different trials. Participants' rating on all items were significantly different between trials except for warm throat. Participants gave a significantly higher rating (P<0.05) during fasted than non-fasted for the following items; dry mouth (P<0.001, *r*=0.608), irritated mouth (P<0.001, *r*=0.563), bad taste in mouth (P<0.001, *r*=0.517), chalk like (P<0.001, *r*=0.601), dry throat (P<0.001, *r*=0.589), scratchy throat (P<0.001, *r*=0.602), chapped lips

(P<0.001, *r*=0.601), thirsty (P<0.001, *r*=0.531), water pleasantness (P<0.001, *r*=0.603), hungry (P<0.001, *r*=0.588), swollen tongue (P<0.001, *r*=0.615), weary (P<0.001, *r*=0.596), sleepy (P=0.013, *r*=0.333) and sorehead (P=<0.001, *r*=0.519). On the other hand, the following items were rated significantly lower (P<0.05) during fasted than non-fasted trial; pleasant mouth (P<0.001, *r*=0.487), full stomach (P<0.001, *r*=0.408) and alert (P=0.007, *r*=0.359).

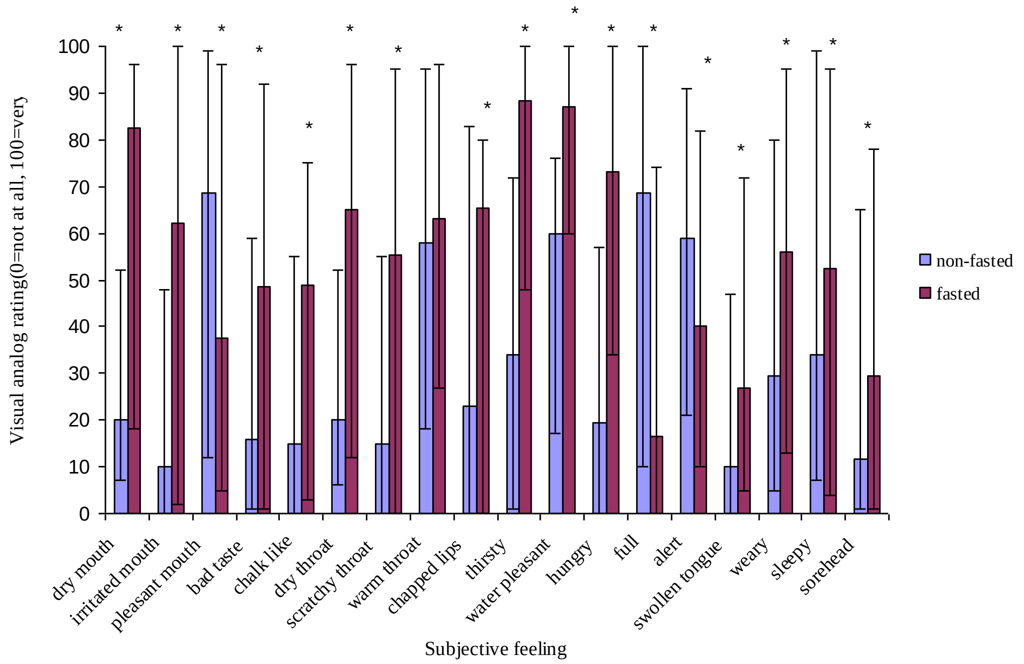


Figure 5.1: Subjective feelings rating in non-fasted and fasted trial. Values are median (range). * indicates values are significant at P<0.05 between trials.

Figure 5.2 shows the results for the rating of 18 items of subjective feelings related to thirst in male and female participants. No significant difference (P>0.05) were observed for any subjective feelings ratings between male and female participants. When the data were analysed separately for non-fasted and fasted trial, similar results were obtained.

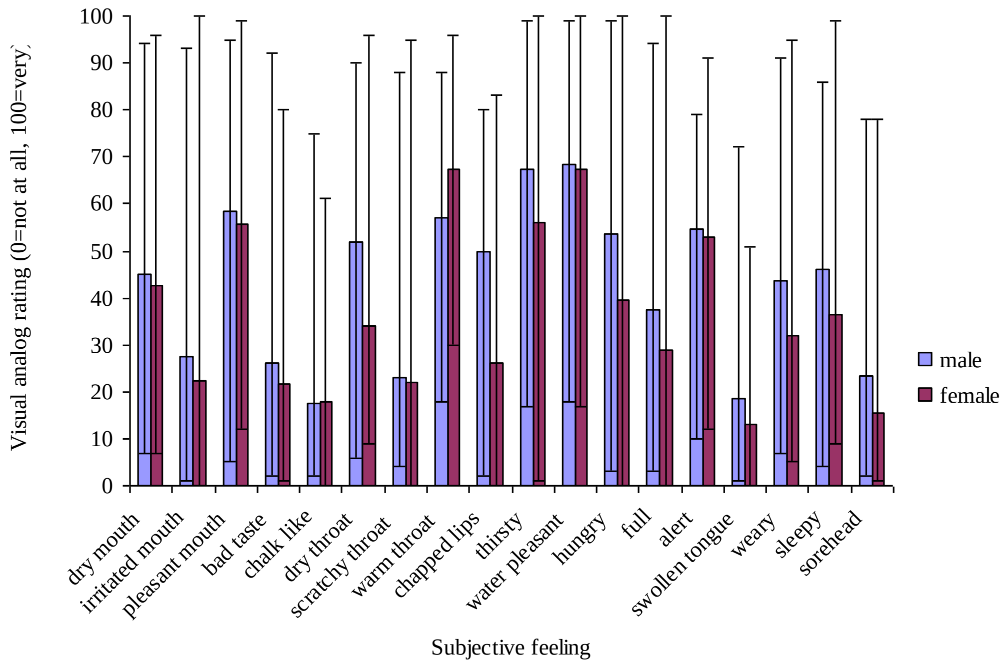


Figure 5.2: Subjective feelings rating in male and female participants when the data for both trials were pooled together. Values are median (range).

Spearman coefficient correlation test between thirst rating and physiological markers of hydration status showed that thirst rating was significantly correlated with urine osmolality in non-fasted trial and with urine colour in fasted trial. There were no significant association detected between thirst and other markers such as urine specific gravity, haemoglobin concentration, haematocrit and serum osmolality in both trials (Table 5.3).

Table 5.3: Spearman correlation between thirst and physiological indices of hydration status in non-fasted and fasted trial.

	Non-fasted (N=28)			Fasted (N=28)		
Thirst vs.	rho	P value	CI	rho	P value	CI
Urine colour	0.356	0.063	-0.019 to 0.643	0.461	0.014*	0.107 to 0.711
Urine specific gravity	0.332	0.084	-0.046 to 0.627	0.108	0.586	
Urine osmolality ($mOsm\ kg^{-1}$)	0.385	0.043*	0.014 to 0.662	0.009	0.962	
Haemoglobin conc. ($g\ 100\ ml^{-1}$)	0.180	0.358		0.336	0.091	-0.042 to 0.630
Haematocrit (%)	0.135	0.493		0.343	0.074	-0.034 to 0.634
Serum osmolality ($mOsm\ kg^{-1}$)	0.008	0.969		0.081	0.682	

* correlation is significant at P<0.05 (2-tailed)

Table 5.4 shows the results of Spearman coefficient correlation test between thirst and physiological indices of hydration status for males and females. Thirst rating was only significantly associated with urine colour both in male and female participants. No correlation was observed between thirst rating with body mass change, urine specific gravity, urine osmolality, urine specific gravity, haemoglobin concentration, haematocrit and serum osmolality in either gender.

Table 5.4: Spearman correlation between thirst and physiological indices of hydration status in male and female participants.

	Male (N=17)			Female (N=11)		
Thirst vs.	rho	P value	CI	rho	P value	CI
Body mass changes (%)	0.197	0.448		-0.179	0.599	
Urine colour	0.460	0.006**	-0.188 to 0.829	0.466	0.029*	-0.185 to 0.833
Urine specific gravity	0.159	0.370		0.236	0.291	
Urine osmolality (mOsm kg^{-1})	0.243	0.165		0.201	0.370	
Haemoglobin conc. (g 100 ml^{-1})	-0.067	0.707		-0.090	0.689	
Haematocrit (%)	-0.014	0.938		-0.088	0.698	
Serum osmolality (mOsm kg^{-1})	0.297	0.088	-0.214 to 0.680	0.335	0.128	

* correlation is significant at P<0.05 (2-tailed)

** correlation is significant at P<0.01 (2-tailed)

Table 5.5 shows the results for Spearman correlation test between thirst rating and other subjective feelings related to thirst in different trials. Thirst was positively associated with dry mouth, bad taste in mouth, pleasantness of drinking water and negatively correlated with full stomach and alert in non-fasted trial. In fasted trial, the thirst rating was positively correlated with dry mouth, irritated mouth, pleasantness of drinking water and hungry, and inversely correlated with full stomach.

Table 5.5: Spearman correlation between thirst and other subjective feelings related to thirst in non-fasted and fasted trial.

	Non-fasted (N=28)			Fasted (N=28)		
Thirst vs.	rho	P value	CI	rho	P value	CI
Dry mouth	0.473	0.011**	-0.001 to 0.773	0.843	<0.001**	0.615 to 0.940
Irritated mouth	0.287	0.139		0.399	0.035*	0.031 to 0.672
Pleasant mouth	-0.322	0.094	-0.620 to 0.058	-0.158	0.423	
Bad taste in mouth	0.433	0.021*	0.072 to 0.693	0.294	0.129	
Chalk like taste	0.112	0.570		0.094	0.635	
Dry throat	0.282	0.146		0.276	0.156	
Scratchy throat	0.085	0.666		0.105	0.596	
Warm throat	-0.427	0.023	-0.690 to -0.065	0.395	0.068	0.026 to 0.935
Chapped lips	0.211	0.281		-0.152	0.439	
Water pleasantness	0.544	0.003**	0.095 to 0.809	0.828	<0.001**	0.583 to 0.935
Hungry	0.331-	0.085	-0.048 to 0.626	0.440	0.019*	0.081 to 0.698
Full stomach	0.376	0.048*	-0.656 to -0.004	-0.427	0.023**	-0.749 to 0.058
Alert	-0.452	0.016*	-0.706 to -0.095	-0.145	0.463	
Swollen tongue	-0.055	0.782		0.478	0.070	0.128 to 0.772
Weary	0.045	0.819		0.191	0.330	
Sleepy	0.201	0.304		0.079	0.690	
Sorehead	-0.066	0.737		-0.089	0.768	

* correlation is significant at P<0.05 (2-tailed)

** correlation is significant at P<0.01 (2-tailed)

Spearman correlation tests show that in both male and female participants, thirst rating was significantly and positively associated with dry mouth, irritated mouth, bad taste in mouth, chalk like taste, dry throat, scratchy throat, chapped lips, pleasantness of drinking water, hungry and weary. On the other hand, thirst rating was negatively associated with pleasant mouth and full stomach in both male and female participants and also negatively correlated with rating of alertness in female participants (Table 5.6).

Table 5.6: Spearman correlation between thirst and other subjective feelings related to thirst in male and female participants.

Thirst vs.	Male (N=17)			Female (N=11)		
	rho	P value	CI	rho	P value	CI
Dry mouth	0.858	<0.001**	0.536 to 0.962	0.938	<0.001**	0.670 to 0.989
Irritated mouth	0.604	<0.001**	0.012 to 0.882	0.738	<0.001**	0.036 to 0.952
Pleasant mouth	-0.531	0.001**	-0.856 to 0.096	-0.514	0.014*	-0.851 to 0.124
Bad taste in mouth	0.616	<0.001**	0.031 to 0.886	0.669	0.001**	-0.101 to 0.937
Chalk like taste	0.445	0.008**	-0.206 to 0.823	0.583	0.004**	-0.238 to 0.918
Dry throat	0.739	<0.001**	0.255 to 0.927	0.536	0.010**	-0.302 to 0.906
Scratchy throat	0.565	<0.001**	-0.048 to 0.868	0.462	0.030*	-0.190 to 0.831
Warm throat	0.101	0.571		-0.128	0.569	
Chapped lips	0.458	0.007**	-0.191 to 0.828	0.445	0.038*	-0.211 to 0.824
Water pleasantness	0.828	<0.001**	0.457 to 0.953	0.885	<0.001**	0.453 to 0.980
Hungry	0.767	<0.001**	0.314 to 0.935	0.852	<0.001**	0.339 to 0.974
Full stomach	-0.721	<0.001**	-0.921 to -0.218	-0.849	<0.001*	-0.959 to -0.508
Alert	-0.194	0.273		-0.609	0.003**	-0.924 to 0.200
Swollen tongue	0.214	0.224		0.452	0.055	-0.202 to 0.827
Weary	0.342	0.047**	-0.320 to 0.779	0.649	0.001**	-0.136 to 0.993
Sleepy	0.309	0.076	-0.201 to 0.687	0.404	0.062	-0.258 to 0.808
Sorehead	0.328	0.058	-0.181 to 0.698	0.200	0.373	

* correlation is significant at P<0.05 (2-tailed)

** correlation is significant at P<0.01 (2-tailed)

Table 5.7 depicts the results of ROC analysis for subjective feelings related to thirst. Of 18 variables, 12 could discriminate between fasted and non-fasted rating; dry mouth, irritated mouth, bad taste in mouth, chalk like taste, dry throat, scratchy throat, chapped lips, thirsty, water pleasantness, hungry, swollen tongue, and weary. Of these items, only dry mouth, thirsty, water pleasantness and hungry showed sensitivity and specificity values above 85% at the particular chosen cut off point.

Table 5.7: Receiver operating characteristic (ROC) analysis for subjective feelings related to thirst.

	AUC+	Criterion	Sensitivity (%)	Specificity (%)
Dry mouth	0.96*	52#	86	100
Irritated mouth	0.62*	48	68	100
Pleasant mouth	0.21	12	4	86
Bad taste in mouth	0.85*	59	39	100
Chalk like taste	0.79*	55	39	100
Dry throat	0.87*	52	71	100
Scratchy throat	0.82*	55	54	100
Warm throat	0.50	27	100	4
Chapped lips	0.79*	83	100	0
Thirsty	0.96*	72#	86	100
Water pleasantness	0.95*	74#	89	100
Hungry	0.99*	57#	89	100
Full	0.06	10	39	100
Alert	0.33	21	18	100
Swollen tongue	0.77*	47	29	100
Weary	0.78*	80	11	100
Sleepy	0.32	99	100	0
Sorehead	0.30	65	21	100

+ AUC is the calculation of area under the ROC curve. * Value above 0.50 indicates that discrimination exists for subjective feelings rating between fasted and non-fasted conditions. # indicates the criterion (cut off) value of the subjective feeling ratings that has the lowest false-negative and false-positive rate and is adequate to detect hypohydration based on satisfactory sensitivity and specificity rate (>85%).

Discussion

The main findings of the present study show that subjective feelings related to thirst such as dry mouth, thirst perception, desire to drink (water pleasantness) and hunger rating could be used as a marker of hydration status to signify at least a 1% body mass loss due to food and fluid restriction in free living individuals.

The overnight food and fluid restriction protocol used in this study achieved a significant body mass difference of 1.04 ± 1.17% between non-fasted and fasted states. According to Casa et al. (2000), reduction of approximately 1% body mass from baseline suggesting mild hypohydration. Mild hypohydration may or may not affect the physiological function in human as some evidence suggest that dehydration level of 1% body mass may affect cognitive function (Sharma, Sridharan, Pichan, & Panwar, 1986) but others reported that the decrement in cognitive function (Lieberman, 2007; Wilson & Morley, 2003) and exercise performance (Cheuvront, Carter, & Sawka, 2003) are noticeable when the body mass loss is more than 2%. In addition, Cheuvront et al., (2004) who conducted a 15 d study on 65 healthy men undergoing normal free-living lifestyle following moderate intensity walking in the heat for 2-3 h suggest that fluctuation in body mass of 1.1% or less is the normal daily variation of body mass that may be experienced by any individual under similar circumstances. Therefore, in this study, a reduction of 1% body mass either could indicate a mild dehydration or could simply represent a normal variation of body mass on day-to-day basis.

The protocol used also induced significant differences for urine colour, haematocrit and serum osmolality between trials. Urine colour in non-fasted was significantly lighter than in fasted trial which were 4 ± 1 and 6 ± 1, respectively. According to Armstrong et al. (1994), urine colour between 3 to 7 indicates euhydration. Even though the urine colour in this study was significantly different between trials, if the values are based on Armstrong et al. (1994) then it suggests the participants in both trials were euhydrated. This result was in agreement with the study by Armstrong et al. (1998) whereby the participants had a urine colour of 5 ± 1 even though they were undertook 2 h moderate intensity exercise to a level of 2.5 ± 0.9 % body mass loss from the initial body mass. Urine colour may not always be accurate at detecting hydration status because other confounding factors may alter the colour such as illness, vitamin supplements, medications (Graf, 1983) and ingestion of large volumes of hypotonic fluid (Shirreffs, 2003). To the author's knowledge, none of these factors could affect the urine colour during both trials except for breakfast taken during non-fasted trial that may confound the results in the present study.

Haematocrit was also significantly higher in the fasted than non-fasted trial. It is expected that haematocrit will increase with the increasing body mass loss due to water loss because a reduction in plasma volume concentrates the red blood cells, hence increases the haematocrit level. In this study, a significantly higher haematocrit during the fasted trial indicates that the participants may be hypohydrated. Whiting, Maughan and Miller (1984) assessed 90 marathon runners and found that haematocrit was significantly higher after the race (44.4 ± 2.8%) than in pre-race (42.5 ± 2.1%) with a range of 1-5% body mass loss during 219 ± 36 min race. This study therefore supports the findings of the present study that significant increases in haematocrit may reflect hypohydration.

Similarly, serum osmolality in this study was significantly higher in the fasted (287 ± 4 mOsm kg^{-1}) than non-fasted (284 ± 3 mOsm kg^{-1}) trial. According to Sawka et al. (2007), plasma osmolality less than 290 mOsm kg^{-1} represents euhydration. Therefore, the serum osmolality from this study showed that all participants in both non-fasted and fasted trial were indeed in a similar hydration state. Some studies have shown that plasma osmolality is a reliable indicator of hydration status when the body mass loss is more than 3% from the baseline (Armstrong et al., 1998; Popowski et al., 2001). Nevertheless, the use of plasma osmolality to detect hypohydration below 3% body mass loss is disputable (Oppliger & Bartock, 2002). Francesconi et al. (1987) who conducted a field study to examine urinary and hematologic indexes of hypohydation in the US army personnel found that serum osmolality when body mass loss less than 3%

(Sosm= 290 ± 3 mOsmol kg^{-1}) was similar (P=0.46) to the serum osmolality when body mass loss was above 3% (Sosm= 289 ± 5 mOsmol kg^{-1}). In contrast, Popowski et al. (2001) who conducted a study in 12 male participants who were subjected to 1-5% progressive dehydration showed that a reduction of body mass of as low as 1% from the baseline had a marked effect on the plasma osmolality level. Similar to Francesconi et al. (1987), 1% the body mass loss in the present study may not be sufficient to produce an effect on serum osmolality, and it was not able to distinguish hydration status between the non-fasted and fasted trial.

The participants rated significantly higher for the 14 negative feelings associated with thirst in the fasted than non-fasted trial except for warm throat (Figure 5.1), but no significant differences were found between male and female participants (Figure 5.2). Since 1% body mass loss during fasted trial suggests that the participants were mildly hypohydrated, the higher rating of subjective feelings in the fasted trial was expected. This result was similar to the study conducted by Engell et al. (1987) who investigated the relationship between thirst and fluid intake following 0-7% hypohydration in humans. They reported that the rating for most of sensations related to thirst were significantly increased accompanying elevated level of hypohydration.

Spearman correlation tests show that of the 18 thirst related sensations, thirst rating was only significantly and positively correlated with dry mouth, irritated mouth, bad taste in mouth, water pleasantness, and hungry and inversely correlated with full stomach and alert. This result was similar to the work of Engell et al. (1987) who reported that most of 37 items of thirst related sensations were significantly correlated with each other at the similar hypohydration level.

Furthermore, Spearman correlation test was conducted to determine the relationship between thirst and physiological markers of hydration status. Thirst rating was chosen for this test because preliminary correlation test among subjective feelings showed that the thirst rating had more of strong and significant association (rho $\geq$ 0.7) with other subjective feelings being measured (see

Appendix E). The results of this study showed that thirst rating was significantly correlated with some physiological markers of hydration status such as urine colour and urine osmolality. At present, there are no studies available to either refute or support this finding. However, Shirreffs et al. (2004) investigated the effects of 24 and 37 h of fluid restriction on hydration status and subjective feelings in 15 healthy participants and found that there was no correlation between thirst rating and serum osmolality (R^2=0.10) or changes in plasma volume (R^2=0.09) during the fluid restriction trial. Some studies suggest that urinary indices are more sensitive than blood markers to detect mild hypohydration (Kovacs et al., 1999; Oppliger & Bartock, 2002). Therefore, when there is correlation between thirst rating and urine markers, it is most likely that thirst rating may have potential to detect hydration status.

To consider the possibility of using subjective feelings as a marker of hydration status, ROC analysis was carried out to establish the cut off value that distinguishes between euhydration and hypohydration. Of the 18 subjective feelings, dry mouth, irritated mouth, bad taste in mouth, chalk like taste, dry throat, scratchy throat, chapped lips, thirsty, water pleasantness, hungry, swollen tongue, and weary had an AUC value above 0.5 which means that they can be used to discriminate between euhydration and hypohydration. However, only dry mouth, thirsty, desire to drink (water pleasantness) and hungry exhibited satisfactory (>85%) sensitivity (correct identification of hypohydration) and specificity (identification of non-hypohydration) at the chosen cut off point. The cut off point associated with the lowest false-negative and false-positive rate and sufficient to detect hypohydration based on satisfactory sensitivity and specificity rate for dry mouth, thirsty, water pleasantness and hungry were 52 (sensitivity=86%, specificity=100%), 72 (sensitivity=86%, specificity=100%), 74 (sensitivity=89%, specificity=100%) and 57 (sensitivity=89%, specificity=100%), respectively. The cut off points for dry mouth and thirst ratings in this study were similar to the results reported in Shirreffs et al. (2004), whereby the participants gave a mean rating (on visual analog scale of 0-100 mm) above 60 for dry mouth and above 70 for thirst during 13 h of fluid restriction trial. However, no data is available to either support or refute the cut off points for pleasantness of drinking water and hungry ratings established in this study.

To conclude, this study provides a promising start for thirst related sensations to be considered as hydration status marker but further works are warranted.

Chapter 6

Thirst and drinking at rest in different environmental conditions

Introduction

Body water balance can be disturbed by stressful conditions such as heat exposure and exercise. When dehydration has occurred, the sensation of thirst plays an important role to initiate the act of drinking, and thus to increase fluid intake.

There are extensive data that show that people increase their fluid intake to replace sweat water losses when subjected to hot and temperate ambient temperatures (Engell et al., 1987; Maresh et al., 2004). On the other hand, it is generally assumed that in cold environment people are less dehydrated therefore feel less thirsty and drink less but not much data are available to support such assumption. Maughan and colleagues (2005) compared the drinking behaviour and sweat loss in 17 elite football players during training in the cool (5°C, 81% rh) versus temperate (27°C, 55% rh) and warm (32°C, 20% rh) reported in studies by Maughan, Merson, Broad, and Shirreffs (2004) and Shirreffs et al. (2005), respectively. They found that despite the similar extent of dehydration in three environment conditions, the players who trained in the cool consumed lower volume (423 ± 215 ml) than in the temperate (971 ± 303 ml) and warm (972 ± 335 ml) environments. Maughan et al. (2005) speculated that the lower fluid intake during training in the cool may be due to suppression of thirst sensation but no data on perceived thirst was available to support their assumption. In addition, Dann et al. (1990) conducted a study in which 21 male subjects walked in a mountain at an altitude between 1480 m and 1730 m for 4.5 hours in the cold (0^{o}C, 87% rh) with drinks either offered *ad libitum* or scheduled drinking every 30 min. The authors found a smaller volume was consumed with *ad libitum* drinking, but this study did not report thirst response. Another recent study by Kenefick et al. (2004) suggests that when either euhydrated or hypohydrated, thirst sensation was blunted by 40% when resting and exercising individuals were exposed to acute cold temperature, but in this study fluid intake data was not available. With the absence of either thirst sensation or fluid intake in these two studies, the effect of thirst sensation on fluid intake in the cold could not be properly justified.

Therefore, the present study aimed to investigate the subjective feelings of thirst and other related sensations and fluid intake in resting humans exposed to different temperatures.

Materials and methods

Ten healthy males (mean ± SD: age= 20 ± 7 years, height= 179 ± 7 cm, body mass (BM) = 75.7 ± 6.8 kg) took part in this study. All participants received both written and verbal details of the study, prior to the start of the trials. Once they agreed to proceed with the trial, written informed consent was obtained from them. This study protocol was reviewed and approved by Loughborough University Ethical Advisory Committee (R08-P103).

On the day of the trial, two hours before coming to the laboratory, the participants were asked to take a light breakfast and consume at least 500 ml of fluid in an attempt to ensure euhydration. They were asked to consume the same meal prior to second trial to ensure that they were in similar hydration and post-prandial status for all trials. When the participants arrived at the laboratory, they were asked to empty their bladder in a container provided. Then, they had their nude body mass and height measured. They were asked to change into a long sleeve cotton shirt and long legged pant with their own undergarments on. After that, they were asked to insert a rectal thermistor for core body temperature measurement and to put on a Polar heart rate monitor. The participants were asked to enter the climatic test chamber (Weiss Gallenkamp, Loughborough, UK) where the conditions was set either cool (T= 20.0ºC, 60.0% rh) or warm (T= 33.0ºC, 60.0% rh) and sit at rest for the next 180 min. They were not given access to anything to eat or drink during the first 120 min, but water was offered *ad libitum* at 120 min for 1 h. The water bottles were weighed before and after each trial using an electronic balance (Sartorius portable, Germany) to the nearest 0.01 g to determine the volume of water consumed by the participants. Subjective feelings questionnaires based on 100 mm visual analog scale, profile of mood state (POMS) questionnaire and thermal sensation scale (TSS) were assessed every 30 min, whereas heart rate and rectal temperature were recorded every 5 min throughout the trial. Body mass (with clothing and apparatus) was measured again at 120 min, corrected to nude body mass.

After the exposure period, the participants were asked to exit the chamber. They were asked to remove the thermistor and heart rate monitor. A final urine sample was collected and post trial nude body mass was measured. After that, the participants were free to leave the laboratory.

In all trials, the participants were asked to refrain from exercise and to consume their regular composition and amount of food and fluid the day before to ensure euhydration.

Sample analysis

Urine samples were analysed for specific gravity to assess hydration status before and after each trial. Analysis was performed as described in the general methods reported in chapter 2.

Statistical analysis

All data sets were checked for normative distribution using the Kolmogorov-Smirnov test. Parametric data are reported as mean ± standard deviation. Non-parametric data are presented as median (range).

A Student's paired t test was used to evaluate differences between trials for the physiological variables measured at a single time point. For parametric data, a two factors repeated measure ANOVA was used to evaluate differences of time and trial and an interaction effect of time and trial. A Student's paired t test followed by Bonferoni pairwise comparisons was used to locate the differences. For not normally distributed data, the non-parametic Friedman's ANOVA was used to assess differences between trial and time. Wilcoxon's rank test followed by Bonferoni pairwise comparisons was then used to locate the differences. The level of significant was set at $p<0.05$.

Statistical analysis was performed using SPSS version 16.0. for Windows.

Results

Fluid balance

There were no significant differences ($P>0.05$) in body mass (BM) and pre-trial urine specific gravity (Usg) between trials, indicating that the participants started the trials with similar hydration status. According to Cheuvront et al. (2004) and Casa et al. (2000), daily BM fluctuation ≤ 1% and Usg ≤ 1.020 respectively, indicate that the individuals are euhydrated. Therefore, it was concluded that the participants began the trial euhydrated because all the Usg values were less than 1.020 and the body mass variation was 0.18 ± 1.06 kg or 0.19 ± 1.36%. The amount of water loss as expressed by body mass changes during 120 min exposure was significantly higher ($P<0.05$) in warm than cold trial. Water intake at 120 min and onwards of exposure was significantly higher ($P<0.05$) in warm than cool trial. The total water loss during 180 min exposure was also significantly higher in warm than cool trial (Table 6.1).

Table 6.1: Body mass, urine specific gravity, water lost and water replaced during cool and warm trial. Values are mean ± SD.

Parameter	Cool (N=10)	Warm (N=10)	P value	Effect size (*r*)
Pre-trial body mass (kg)	75.16 ± 10.00	75.35 ± 10.35	0.604	
Pre-trial urine specific gravity	1.015 ± 0.004	1.016 ± 0.003	0.275	
Water lost by120 min (L)	0.06 ± 0.04	0.33 ± 0.22	0.002*	0.500
Water lost by 120 min (%)	0.08 ± 0.05	0.43 ± 0.29	0.003*	0.565
Total water lost by 180 min (L)	0.15 ± 0.09	0.51 ± 0.23	0.001*	0.399
Total water lost by 180 min (%)	0.25 ± 0.15	0.69 ± 0.32	0.004*	0.182
Water replaced from120 min onwards (L)	0.07 ± 0.05	0.41 ± 0.22	0.001*	0.400
Water replaced from 120 min onwards (%)	43.3 ± 35.3	84.3 ± 43.4	0.038*	0.624

Net fluid balance

Net fluid balance was calculated from the change in body mass estimated from sweat loss, the volume of water consumption and urine output throughout the duration of the trial. There was no difference when the participants started the trial in both cool and warm trial. After 120 min of exposure, the participants were in negative fluid balance in both trials but they were less negative in cool than warm trial. At the end of rehydration period, the net fluid balance returned to positive in both cool and warm trials with more positive fluid balance observed in warm trial.

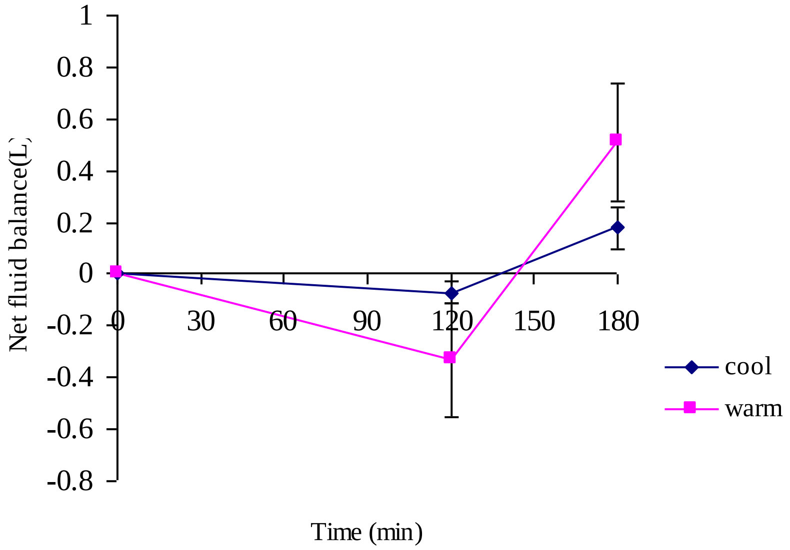


Figure 6.1: Net fluid balance during cool and warm trial. Values are mean ± SD.

Thermal sensation rating

Statistical analysis on thermal sensation rating showed a significant effect of trial ($P<0.001$, $\eta^2=0.928$) time ($P<0.001$, $\eta^2=0.716$) and interaction ($P<0.001$, $\eta^2=0.774$). The participants' rating of thermal sensation was lower on the cool trial than the warm trial upon entry into the environmental chamber and this difference remained until the end of the exposure period. There was no change in rating over the duration of the exposure on the warm trial, but the rating was lower ($P<0.001$, $\eta^2=0.800$) than the 0 min rating from 60 min onwards on the cool trial (Figure 6.2).

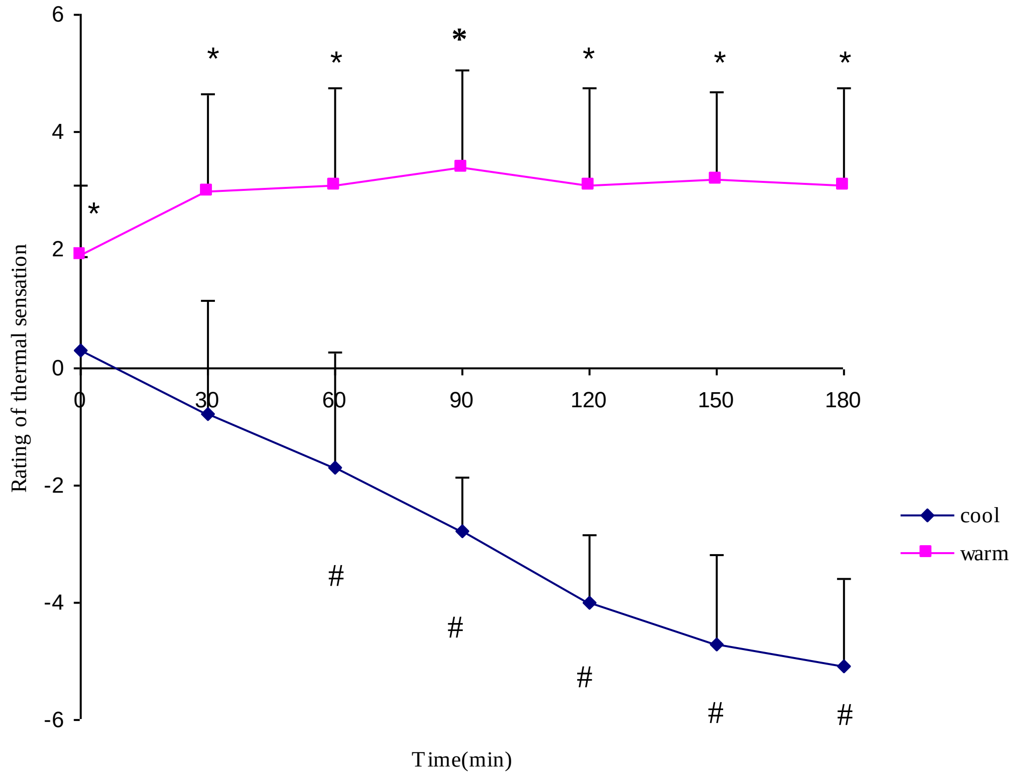


Figure 6.2: Thermal sensation ratings during cool and warm trial. Values are mean ± SD. * denotes significant different between trials (P<0.05). # indicates TSS scores of cool trial time point significantly different (P<0.001) from the baseline value.

Heart rate

Statistical analysis on heart rate showed a significant effect of trial (P=0.046, η^2=0.844), time (P<0.001, η^2=0.201,) and interaction (P<0.001, η^2=0.221,). Heart rate was lower on the cool trial than the warm trial upon entry into the environmental chamber and this difference remained until the end of the exposure period. There was no change (P>0.05) in heart rate over the duration of the exposure on the warm trial, but the heart rate was lower (P<0.001, η^2=0.338) than the 0 min from 140 min onwards on the cool trial (Figure 6.3).

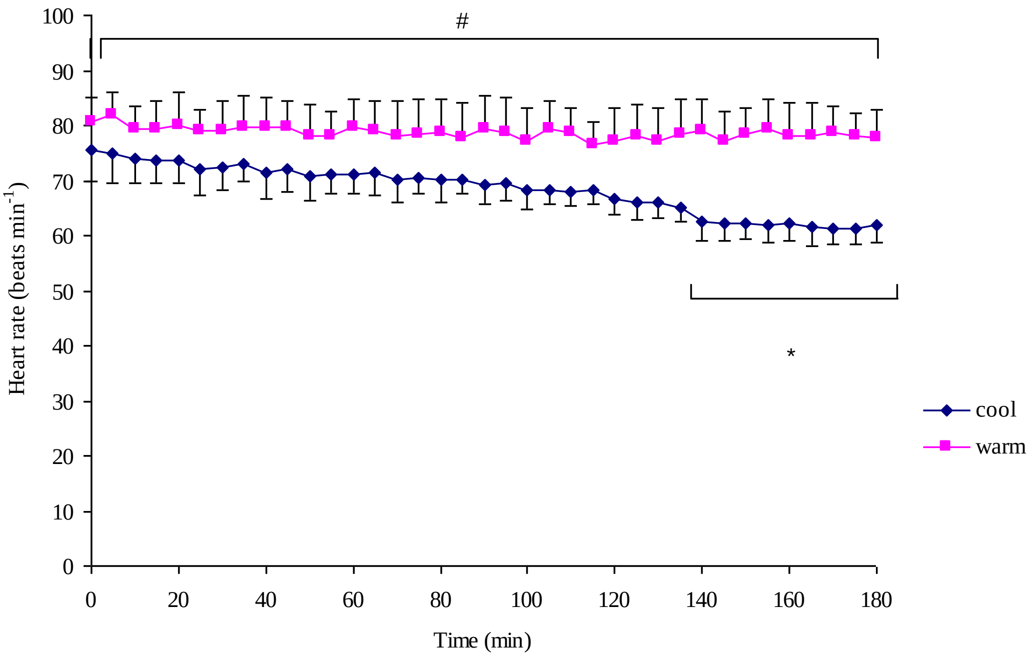


Figure 6.3: Heart rate during cool and warm trial. Values are mean ± SD. # denotes significant different between trials (P<0.05). * indicates heart rate of cool trial time point significantly different (P<0.001) from the baseline value.

Rectal temperature

Statistical analysis on rectal temperature showed no effect of trial (P=0.202), but a significant effect of time (P<0.001, η^2=0.865) and interaction (P<0.001 η^2=0.770,). Rectal temperature was lower (P<0.001, η^2=0.361) than the 0 min from 65 min onwards during cool trial, but no changes (P>0.05) from the baseline value were observed during warm trial (Figure 6.4).

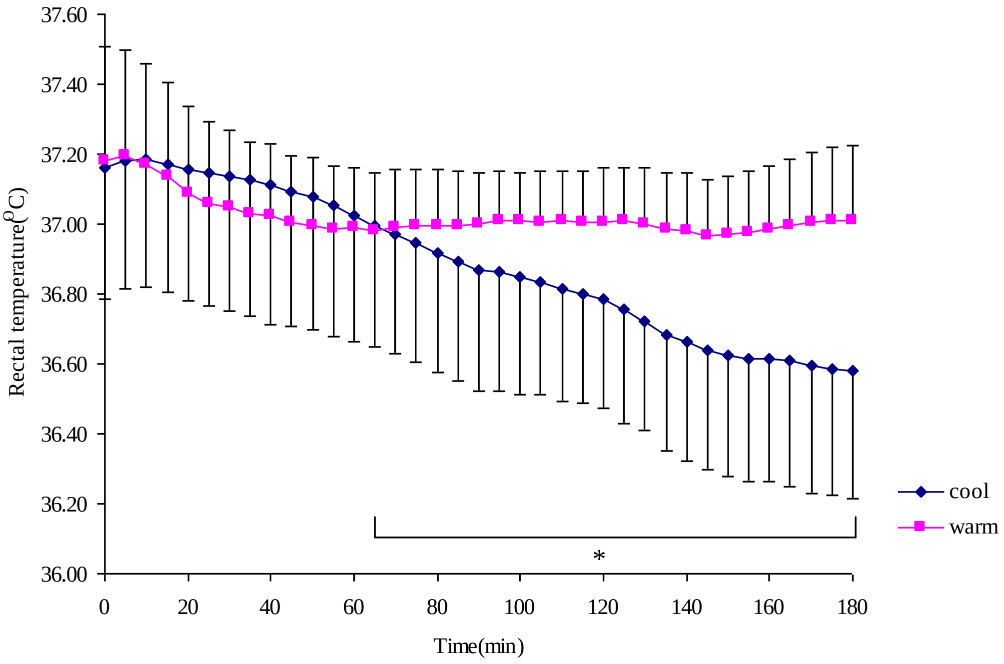


Figure 6.4: Rectal temperature during cool and warm trial. Values are mean ± SD. * indicates rectal temperature of cool trial time point significantly different (P<0.001) from the baseline value.

Subjective feelings related to thirst

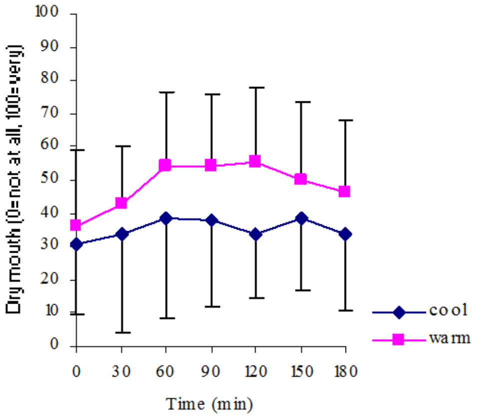
A summary of the statistical analysis for the subjective feelings related to thirst data is given in Table 6.2. Of the 12 subjective feelings, none of them showed a significant interaction between trial and time (P>0.05). Only mouth dryness and thirst was significantly difference (P<0.05) between trials. Ratings for both mouth dryness (Figure 6.5 (a)) and thirst (Figure 6.5 (f)) were higher on the warm trial than the cool trial. There were significant changes (P<0.05) over time detected in the ratings of the subjective feelings except for mouth pleasantness, thirst and weariness (Figure 6.5 (a) to 6.5 (l)).

Table 6.2: A summary of statistical analysis on subjective feelings related to thirst.

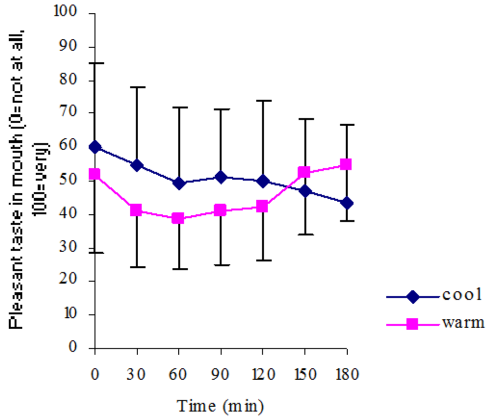
Subjective feeling	Trial effect (P value)	Effect size (η^2)	Time effect (P value)	Effect size (η^2)	Trial x Time interaction effect (P value)	Figure
Mouth dryness	0.048*	0.368	0.001*	0.331	0.498	6.5 (a)
Mouth pleasantness	0.086	0.293	0.052	0.254	0.121	6.5 (b)
Bad taste in mouth	0.299		0.034*	0.282	0.314	6.5 (c)
Throat dryness	0.223		0.001*	0.341	0.708	6.5 (d)
Throat warmness	0.104		0.002*	0.467	0.161	6.5 (e)
Thirst	0.035*	0.407	0.504		0.401	6.5 (f)
Drinking pleasantness	0.114		0.001*	0.508	0.269	6.5 (g)
Hunger	0.082	0.268	0.022*	0.376	0.440	6.5 (h)
Stomach fullness	0.790		0.018*	0.351	0.224	6.5 (i)
Alertness	0.112		0.009*	0.264	0.291	6.5 (j)
Weariness	0.232		0.388		0.388	6.5 (k)
Sleepiness	0.997		0.012*	0.358	0.353	6.5 (l)

*significant at P<0.05

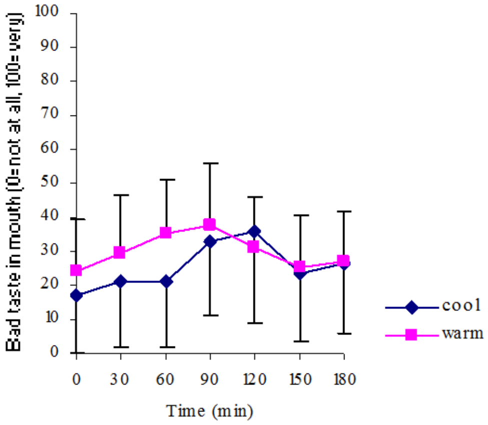
a) Dry mouth



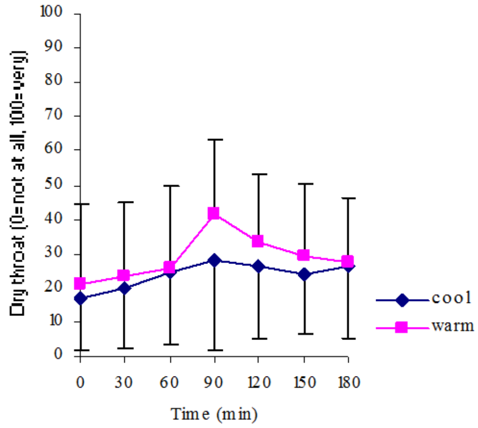
b) Pleasant taste in mouth



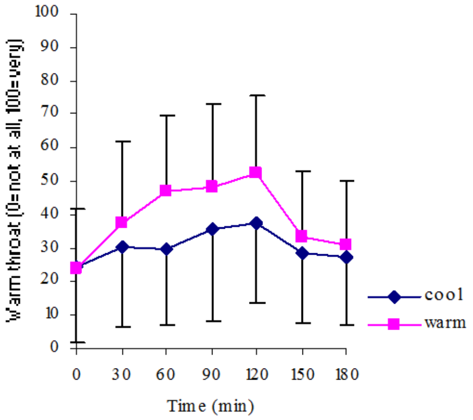
c) Bad taste in mouth



d) Dry throat



e) Warm throat



f) Thirsty

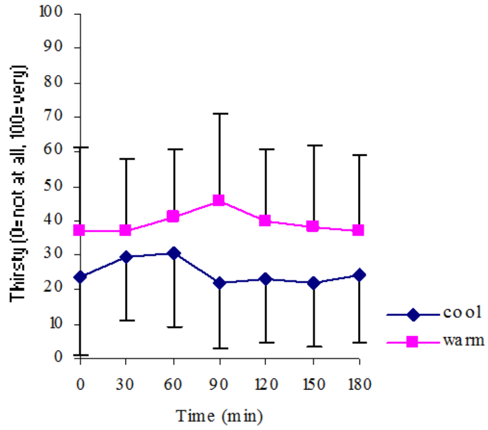


Figure 6.5: Ratings of subjective feelings related to thirst over 180 min of exposure during cool and warm trial. Values are mean ± SD.

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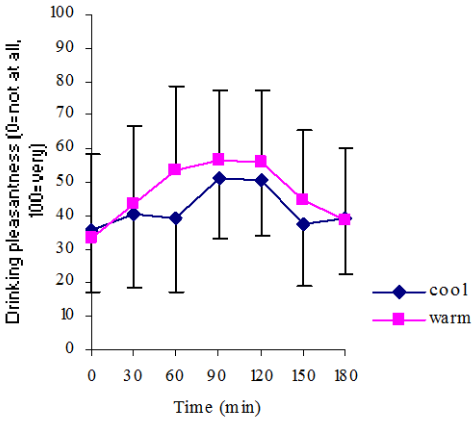
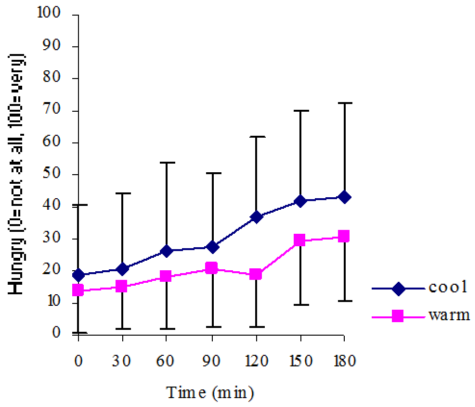
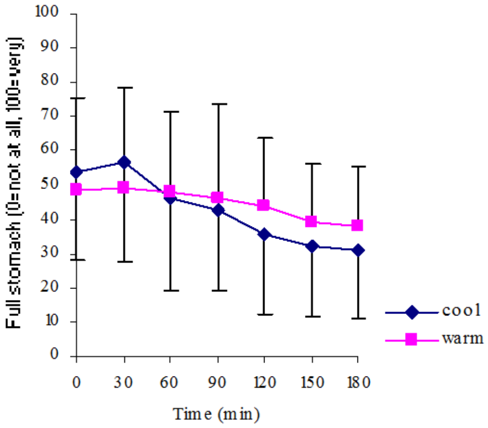
g) Drinking pleasantness



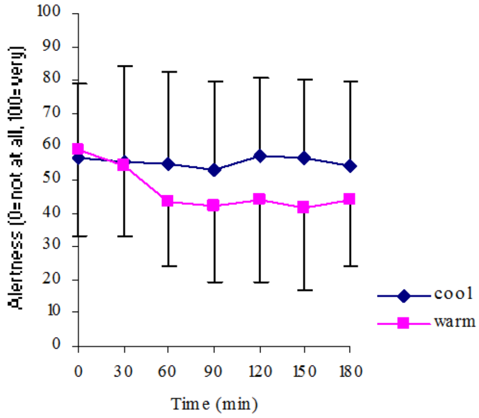
h) Hungry



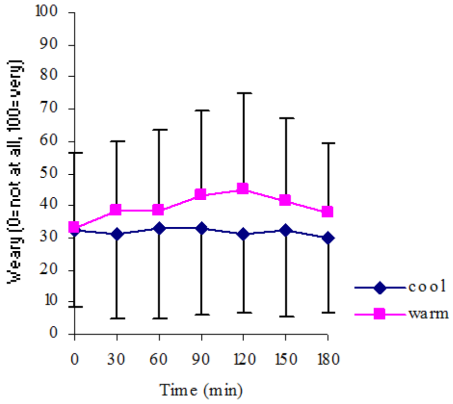
i) Full stomach



j) Alert



k) Weary



l) Sleepy

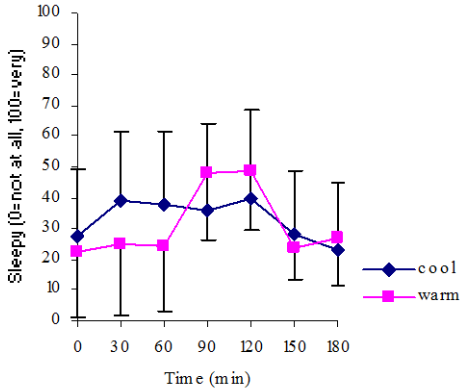


Figure 6.5: Ratings of subjective feelings related to thirst over 180 min of exposure during cool and warm trial. Values are mean ± SD.

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Non parametric test on the following subjective feelings rating; irritated mouth, mouth chalkiness, throat scratchiness, chapped lips, swollen tongue and head soreness showed that there were no significant differences ($P>0.05$) between cool and warm trial. Similarly, there were also no significant changes ($P>0.05$) over time detected in the ratings of these subjective feelings except for throat scratchiness (Figure 6.5 (m) to 6.5 (r)).

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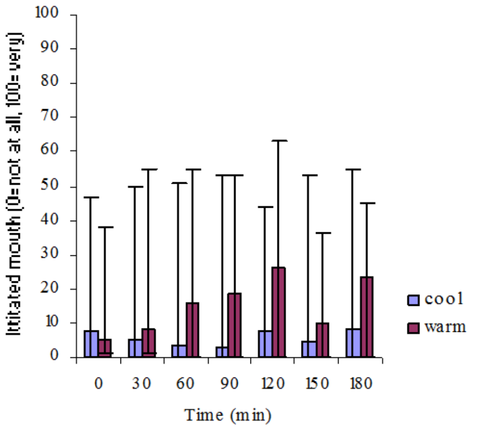
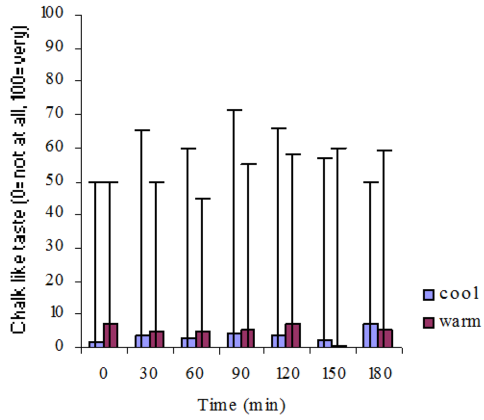
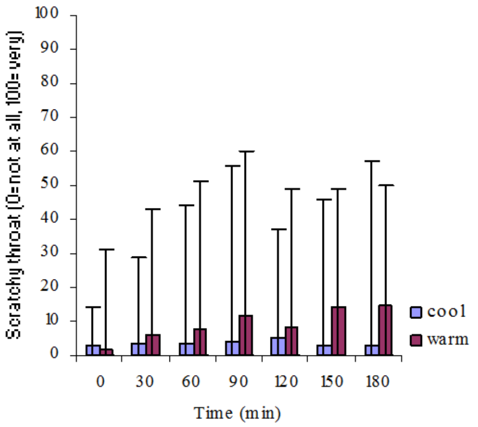
m) Irritated mouth



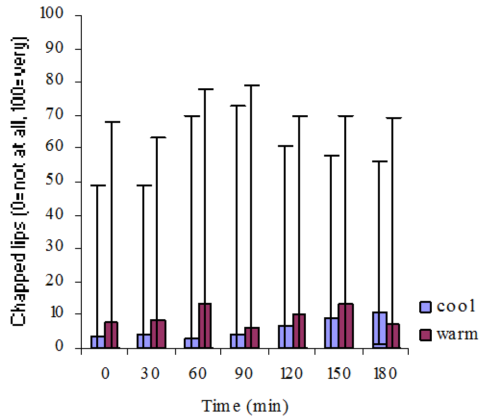
n) Chalk like taste



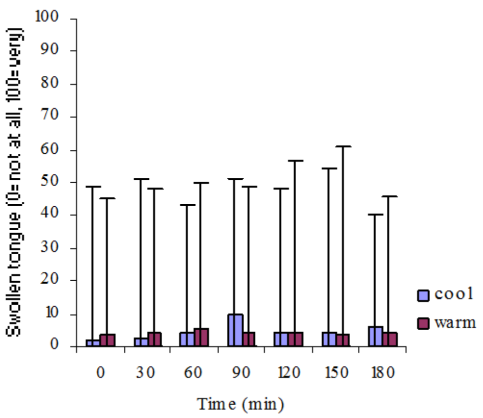
o) Scratchy throat



p) Chapped lips



q) Swollen tongue



r) Sorehead

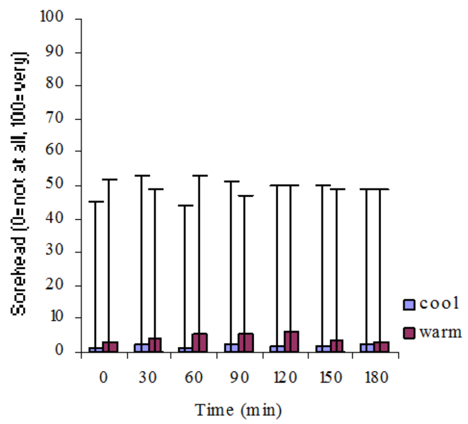


Figure 6.5: Ratings of subjective feelings related to thirst over 180 min of exposure in cool and warm trial. Values are median (range).

A summary of the statistical analysis for the POMS data is given in Table 6.3. There were no significant interaction of trial and time observed for all components (P>0.05). No significant differences (P>0.05) between trials were observed for any of the components of POMS. Of the 6 components, vigour (Figure 6.6 (d), fatigue (Figure 6.6 (e) and confusion (Figure 6.6 (f)) scores significantly changed (P<0.05) over time.

Table 6.3: A summary of statistical analysis on POMS data.

POMS	Trial effect (P value)	Time effect (P value)	Effect size (η^2)	Trial x Time interaction effect (P value)	Effect size (η^2)	Figure
Tension	0.131	0.585		0.110		6.6 (a)
Depression	0.807	0.331		0.135		6.6 (b)
Anger	0.133	0.460		0.376		6.6 (c)
Vigour	0.394	0.011*	0.333	0.165		6.6 (d)
Fatigue	0.160	0.001*	0.570	0.059	0.252	6.6 (e)
Confusion	0.870	0.001*	0.426	0.157		6.6 (f)

*significant at P<0.05

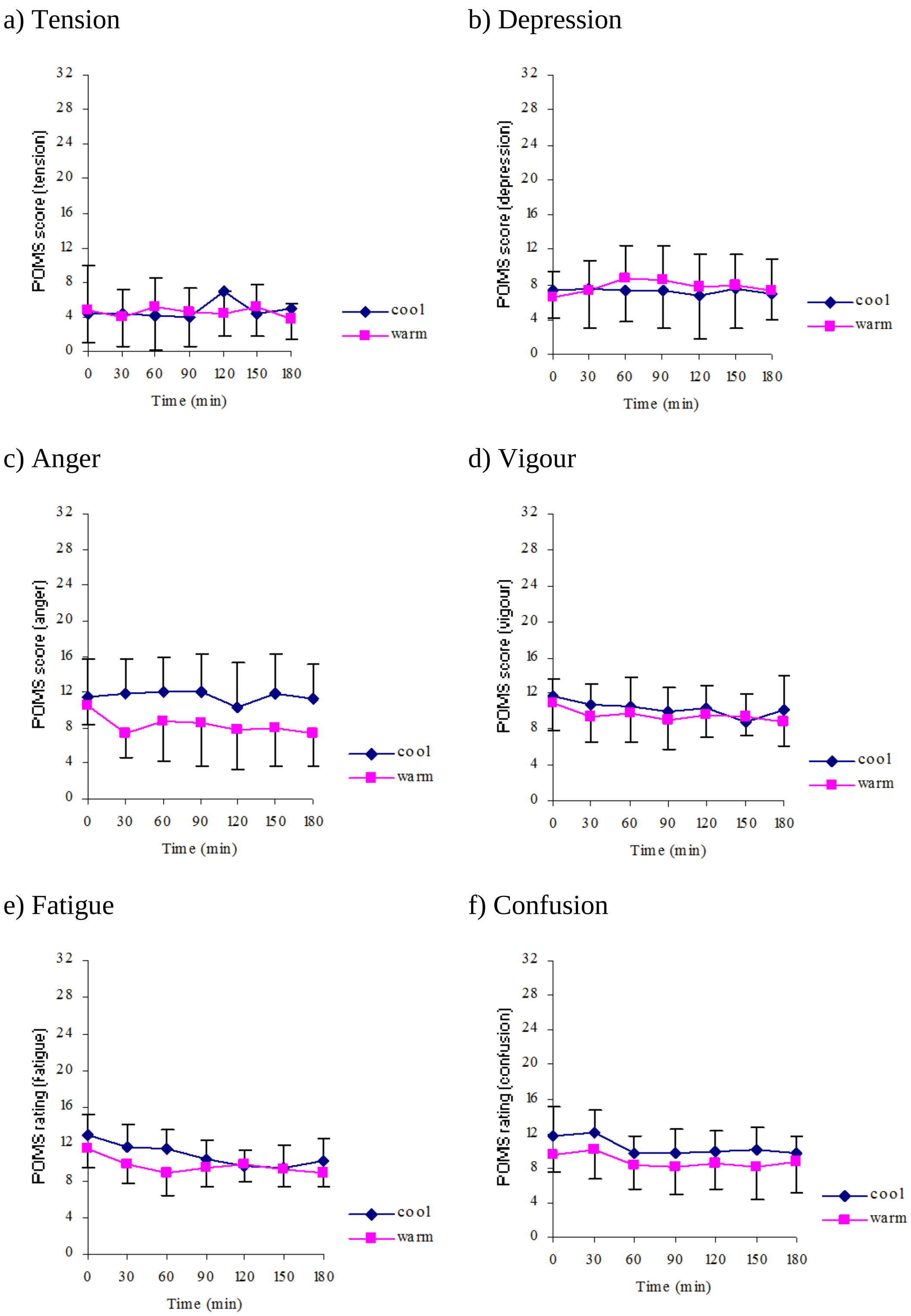


Figure 6.6: POMS scores over 180 min of exposure in cool and warm trial. Values are mean ± SD.

Discussion

The main findings from this study were that the participants consumed more fluid in both absolute and relative terms to replace fluid loss from sweating during warm compared to cool exposure. In addition, subjective feelings of thirst ratings and mood states were not significantly different during 180 min exposure in both warm and cool temperature. Other factors such as increases in central blood volume due to vasoconstriction or simply the absence of dehydration may explain the lower water consumption during the cool trial.

After 120 min exposure in the environmental chamber, the participants lost a significantly higher ($P<0.05$) body fluid through sweating in the warm than cool trial which was 0.43 ± 0.29% and 0.08 ± 0.05% from their pre-trial body mass, respectively. By the end of 180 min exposure, body fluid loss on the warm trial (0.51 ± 0.23%) was significantly higher ($P<0.05$) than on the cool trial (0.15 ± 0.09%). Bates and Miller (2008) who conducted the study on the sweat rate and sodium loss in 29 healthy males during work with similar intensity and duration in the summer (30-35°C) and winter (15-20℃) showed that sweat rate was significantly greater ($P=0.029$) during summer (0.47 L h^{-1}) than winter (0.41 L h^{-1}). This study supports the findings of the present study that sweat loss was greater on the warm (33°C) trial compared to the cool (20℃) trial.

In term of fluid replacement, the participants replaced significantly higher ($P<0.05$) fluid in the warm compared to cool trial with 0.41 ± 0.22 L and 0.07 ± 0.05 L equivalent to 84.3 ± 43.4% and 43.3 ± 35.3% of their body mass loss, respectively. Greenleaf, Averkin and Sargent II (1966) reported the similar result in which the participants voluntarily consumed 146% greater volume in the hot (49°C) than temperate (24°C) environment. In spite of the higher fluid replacement observed during warm trial, the participants in this study did not match sweat losses during the exposure. Similarly, Miescher and Fortney (1989) who conducted the study on the effect of dehydration and rehydration during 240 min heat exposure (45 ± 1.5°C and 25 ± 4% relative humidity) in young and older men showed that both young and old participants in their study only replaced 49.0 ± 3.1% and 46.6 ± 4.9% of their body mass loss, respectively which may lead to involuntary dehydration. However, in the present study, the unmatched sweat loss did not lead to dehydration because at the end of the 180 min of heat exposure, the participants were found to be in positive fluid balance (Figure 6.1). It shows that even though the volume of ingested water during rehydration period did not match the sweat loss, but it was sufficient to restore normal fluid balance in the participants of the present study.

This study also investigated the effect of environmental temperature on the ratings of subjective feelings of thirst and mood states over the 180 min of exposure. The findings show that no significant interaction effect of trial and time ($P>0.05$) observed for all 18 subjective feelings related to thirst ratings (Figure 6.5 (a) to 6.5 (r)) and 6 components of POMS scores (Figure 6.6). It could be concluded that 180 min of cool and warm exposure did not significantly affect the subjective feelings among the participants in this study. This possibly because the participants were not dehydrated during the course of the trial. D'Anci, Vibhakar, Kanter, Mahoney and Taylor (2009) examined the voluntary dehydration in 31 rowing and lacrosse university athletes whereby the participants in euhydrated trial were given fluids during practice but no fluids were provided in dehydration trial, and the thirst sensations, mood states and cognitive function were assessed after the practices. The authors found that perceived thirst and negative mood components such as anger, depression, fatigue, tension and confusion were significantly higher ($P<0.05$) in dehydrated than euhydrated trial. Therefore, the authors concluded that mild dehydration (1.5-2.0% of body mass loss) was associated with higher thirst and negative mood ratings. In the present study, the participants started the trial with euhydrated state and went to negative fluid balance for 120 min of exposure but returned to positive fluid balance at the end of 180 min exposure both in cool and warm trials (Figure 6.1). Even though the participants were in negative balance for the first 120 min of exposure, but the sweat loss was less than 1% of body mass (Table 6.1). Therefore, it could be concluded that in the present study, the participants were not dehydrated to a level that could affect the subjective feelings of thirst and mood states.

On the other hand, when the data is judged based on the trial alone, the results showed that the participants gave a significantly lower rating ($P<0.05$) for mouth dryness (Figure 6.5 (a)) and thirst (Figure 6.5 (f)) on the cool trial than in the warm trial. If based on this finding, the present study shows that mouth dryness and thirst ratings were affected by the environmental temperature. This result was similar to Kenefick et al. (2004) who investigated the effect of cold exposure on thirst sensations in eight adult men. The participants were instructed to stand at rest for 30 min, followed by 30 min walking exercise at 50% VO_{2max} in the cold (T=4°C, rh=74%) and temperate (T=27°C, rh=38.5%) conditions and thirst sensations were measured at 30 min intervals. The authors concluded that when either euhydrated or hypohydrated, cold exposure reduces thirst sensation during rest and moderate exercise by up to 40% attributed to increased central blood volume induced by peripheral vasoconstriction occurs during cold exposure.

An increased central blood volume induced by vasoconstriction when individuals are subjected to cold exposure usually results in decreased heart rate (Kenefick, St. Pierre, Riel, Cheuvront, & Castellani, 2008). In the present study, a significant decrease ($P<0.001$) in heart rate was observed after 140 min of exposure in the cool trial, whereas no significant different ($P>0.05$) was observed in heart rate during 180 min of warm exposure. It is worth noting that at 120 min onwards, the participants were offered water (15°C) *ad libitum* and if the significant decrease in heart rate was actually due to the increased central blood volume, it may explain the lower rating of the thirst sensation and hence the fluid intake during cool trial. In Kenefick et al. (2004), heart rate during 30 min standing rest was significantly lower ($P<0.05$) in euhydrated-cold (75 ± 4 beats min^{-1}) than euhydrated-temperate (78 ± 3 beats min^{-1}) which supports their conclusion about the role of central blood volume in attenuating thirst sensation during cold exposure.

Another possibility for the lower fluid intake during cool exposure in this study could be that the participants were not dehydrated to a level that stimulates thirst response and fluid intake. Wolf (1950b) proposed that the threshold for stimulation of thirst in individuals at rest to occur at 0.8% of body mass loss or at P_{osm} of approximately 290 mOsm kg^{-1} (Phillips, Rolls, Ledingham, Forsling, & Morton, 1985). In this study, P_{osm} was not measured but the total water loss over 180 min of exposure in cool temperature was 0.25 ± 0.15% which was far less than the threshold value of 0.8% of body mass loss. It could be concluded that the participants in this study were not dehydrated, hence thirst sensation is not stimulated which may explain the lower fluid intake during the exposure.

To conclude, even though *ad libitum* drinking fails to stimulate fluid intake that matches sweat loss in the resting participants exposed to either cool and warm conditions, but the volume ingested was sufficient to restore positive fluid balance. In addition, central blood volume due to vasoconstriction or the absence of dehydration may explain the lower water intake during cool exposure compared to warm exposure, but the evidence remains to be fully demonstrated.

Chapter 7

Thirst and drinking during exercise in the cool and warm conditions

Introduction

Prolonged endurance exercise in hot environment has been shown to produce high sweat rate and if fluid intake does not match fluid loss, dehydration of at least 2% of body mass may result which eventually impairs performance (Walsh, Noakes, Hawley, & Dennis, 1994; Below, Mora-Rodriguez, Gonzalez-Alonso, & Coyle, 1995). Most of the data suggest that ingestion of an appropriate amount of beverage particularly carbohydrate electrolyte drink before (Sherman, Peden, & Wright, 1991) and during (Coyle, 2004) exercise in the heat improves physical performance and maintains thermoregulation (Bergeron et al., 2006; Horswill, Stofan, Lovett, & Hannasch, 2008).

Similarly, there have been studies conducted to examine the beneficial effect of ingesting carbohydrate drink on exercise capacity during exercise in the cold with varying outcomes (Febbraio et al., 1996; Galloway & Maughan, 1998; Galloway, Wooton, Murphy, & Maughan, 2001). However, subjective feelings associated with fluid ingestion during exercise are limited. A study by Backhouse, Biddle and Williams (2007) on the effect of water ingestion during prolonged exercise in the heat showed that thirst rating was higher during non-fluid compared to fluid replacement trial, suggesting the beneficial effect of fluid ingestion on the subjective feeling. Another investigation by Kenefick and colleagues (2004) suggest that when either euhydrated or hypohydrated, thirst sensation was attenuated by 40% when resting and exercising individuals were exposed to acute cold temperature. Nevertheless, in this particular study, the participants were not given any fluid during exercise, therefore the extent of fluid intake affecting the subjective feeling of thirst was not available.

Therefore, this study aims to assess subjective feelings of thirst related sensations when ingesting carbohydrate-containing beverages during exercise in cool and warm temperatures.

Materials and methods

This study consisted of two parallel experiments, one carried out at an intensity equivalent to 70% of VO_{2max} at an ambient temperature of 10ºC (study A) and another was carried out at an intensity equivalent to 60% VO_{2max} at an ambient temperature of 30ºC (study B). All other aspects of the experiments were similar. Twelve healthy male adults (mean ± SD: age= 22 ± 2 years, height= 181 ± 7 cm, body mass= 73.5 ± 8.1 kg, VO_{2max}=54.3 ± 5.4 ml $kg^{-1}min^{-1}$) took part in study A and another 12 healthy male adults (mean ± SD: age= 21 ± 2 years, height= 178 ± 8 cm, body mass= 80.2 ± 7.1 kg, VO_{2max}=52.6 ± 6.1 ml $kg^{-1}min^{-1}$) took part in study B. All participants received both written and verbal details of the study, prior to the start of the trials. Once they agreed to proceed with the trial, written informed consent was obtained from them. This study protocol was reviewed and approved by Loughborough University Ethical Advisory Committee (R08-P102).

Before the experimental trials, the participants completed two familiarisations, which were identical to the experimental trials served to familiarise the participants with the experimental procedures and to measure their maximum oxygen uptake). The participants were asked to perform a discontinuous incremental exercise test protocol on an electronically braked cycle ergometer (Lode, Groningen, Netherlands. They cycled for 5 min during initial stage at 100 W and 3 min during subsequent stages until volitional exhaustion. Heart rate and rating of perceived exertion data collected from the initial stage was used to determine the workload for the subsequent stages. Expired air collected in a Douglas bag during the final 2 min in the initial stage and the final 1 min during subsequent stages was analysed for oxygen and carbon dioxide content (Servomex 1400, Crawley, East Sussex, United Kingdom), volume (Harvard Dry Gas Meter, Harvard Apparatus Ltd, Kent, United Kingdom) and temperature (Edale digital thermometer, Cambridge, UK). The participants completed four exercise sessions in randomised order separated by one week. The participants were asked to ingest a telemetry pill 10-12 h before the trial commenced, for the measurement of rectal temperature. On the day of the trial, two hours before coming to the laboratory, the participants were asked to consume at least 500 ml of fluid in an attempt to ensure euhydration. They were asked to consume the same amount of fluid prior to the subsequent trials to ensure that they were in similar hydration status for all trials. When the participants arrived at the laboratory, they were asked to empty their bladder in a container provided. Then, they had their nude body mass and height measured. They were asked to change into a short sleeve cotton shirt and a short pant. After that, they were asked to put on the heart rate monitor. The participants were asked to enter the climatic test chamber (Weiss Gallenkamp, Loughborough, UK) where the condition was set at ambient temperature 10.0°C and 60.0% RH for study A and at ambient temperature 30.0°C and 60% RH for study B . Immediately before exercise, they drank a bolus of the experimental drink (4 ml kg^{-1} body mass) with a further 1.5 ml kg^{-1} every 10 min during exercise. The drinks ingested during the experimental trials consisted of a sugar-free fruit drink (Tesco Ltd, Cheshunt, UK), prepared to the manufacturers guidelines: to this was added quantities of sucrose, glucose and fructose in a ratio of 50:25:25. The composition of the experimental drinks is presented in Table 7.1. All drinks were maintained at a temperature of 21°C prior to ingestion.

Table 7.1: Composition and energy content of experimental drinks.

Drink composition	0%	2%	4%	6%
Sucrose (g L^{-1})	0	10	20	30
Glucose (g L^{-1})	0	5	10	15
Fructose (g L^{-1})	0	5	10	15
Sodium (mmol L^{-1})	18	18	18	18
Potassium (mmol L^{-1})	2	2	2	2

Exercise continued until volitional exhaustion, whereby the participants were unable to maintain a pedal cadence of ≥60 revolutions/min despite verbal encouragement from the investigator.

During exercise, heart rate, rectal temperature and thermal sensation rating were recorded every 10 min and after exercise. Subjective feelings of thirst questionnaire was completed every 30 min and after exercise. Other measurements taken in this study were rating of perceived exertion every 10 min, expired gas samples every 15 min, cycling time to exhaustion and computer-based cognitive function every 30 min and after exercise. However, these data were not discussed in this thesis because they were part of the collaboration work with others (see Certificate of originality and Acknowledgements).

After the exercise, the participants exited the chamber. They were asked to remove the heart rate monitor. A final urine sample was collected and post trial nude body mass was measured. After that the participants were free to leave the laboratory. In all trials, the participants were asked to refrain from exercise and to consume their regular amount of meal and fluid the day before to ensure euhydration.

Statistical analysis

All data sets were checked for normative distribution using the Kolmogorov-Smirnov test. Parametric data are reported as mean ± standard deviation. A one way repeated measure ANOVA test was used to evaluate differences between trials for the physiological variables measured at a single time point. A two factors repeated measure ANOVA was used to evaluate differences of time and trial and an interaction effect of time and trial. A Student's paired t test followed by Bonferoni pair wise comparisons was used to locate the differences. A 2 way factorial ANOVA was used to compare pooled data of subjective feelings between cool and warm trials over time. The level of significant was set at $p<0.05$.

Statistical analysis was performed using SPSS version 16.0. for Windows.

Results

Pre-exercise body mass

Table 7.2 shows that pre-exercise body mass was similar ($P>0.05$) between each trial for both study A and study B. It suggests that the participants started the trial with similar hydration status.

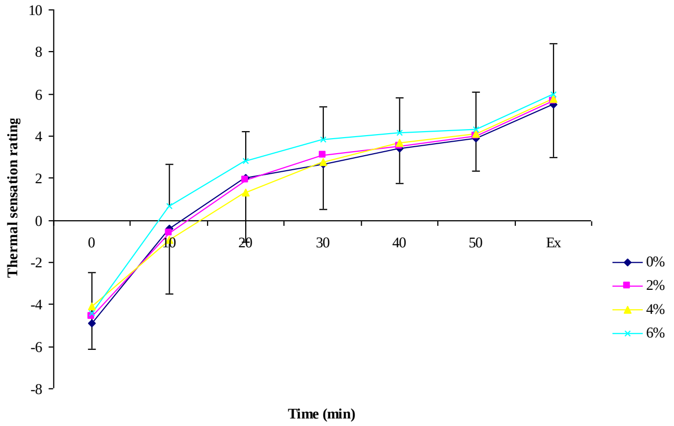
Table 7.2: Pre-exercise body mass (kg) for each trial in study A and study B. Values are mean ± SD.

Trial	Study A (cool)	Study B (warm)
0% CHO	73.48 ± 8.11	80.23 ± 6.78
2% CHO	73.43 ± 8.10	80.27 ± 6.95
4% CHO	73.47 ± 8.12	80.49 ± 6.94
6% CHO	73.45 ± 8.02	80.42 ± 6.86
P value	0.928	0.158

Thermal sensation rating

Statistical analysis on thermal sensation rating in study A showed a significant effect of trial ($P=0.045$, $\eta^2=0.214$) and time ($P<0.001$, $\eta^2=0.847$) but no interaction ($P=0.121$). In all trials, thermal sensation rating was not affected by the amount of carbohydrate in the drinks over the duration of exercise (Figure 7.1 (a)). In study B there was no effect of trial ($P=0.363$), a significant effect of time ($P<0.001$, $\eta^2=0.698$) and interaction ($P=0.003$, $\eta^2=0.173$). The thermal rating increased after 10 min exercise during 0% CHO trial and continued increasing until exhaustion. Similarly, the rating increased after 20 min exercise during 2, 4 and 6% CHO trial until exhaustion (Figure 7.1 (b)).

a) study A



b) study B

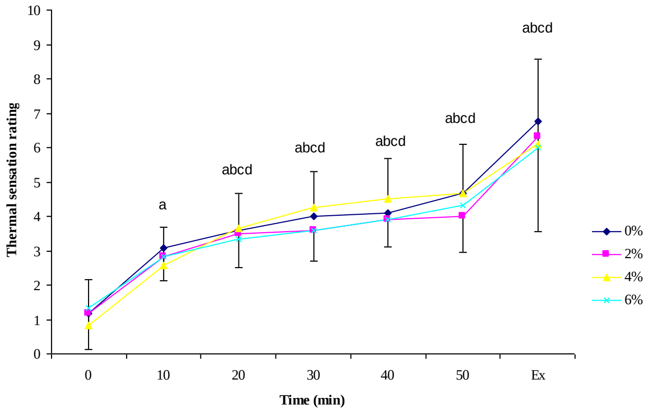
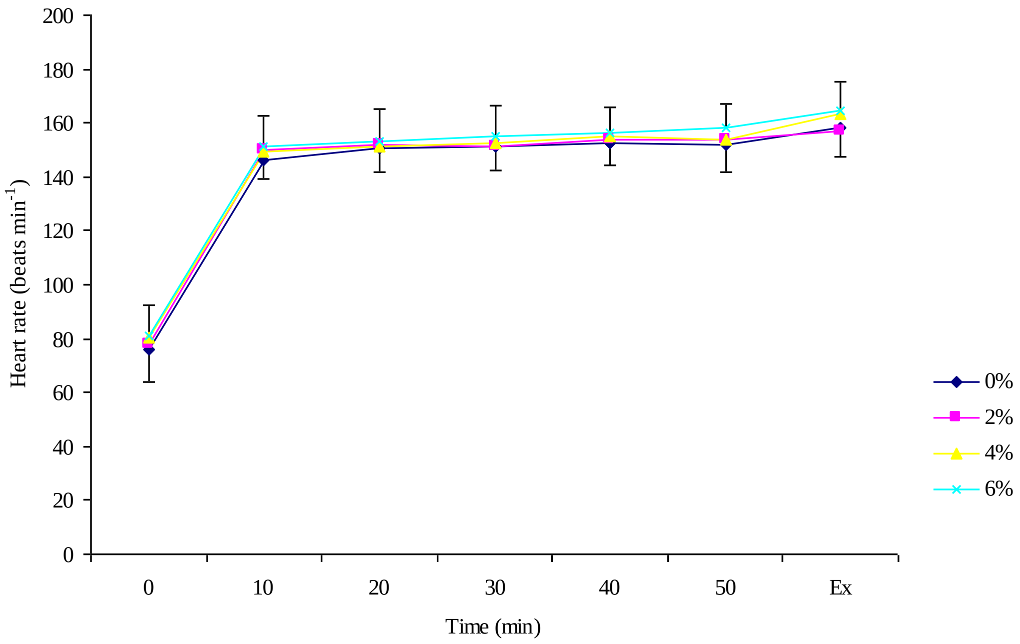


Figure 7.1: Thermal sensation ratings during exercise when ingesting 0, 2, 4 and 6% carbohydrate drinks in study A and B. Values are mean ± SD. abcd denotes 0, 2, 4 and 6% carbohydrate drink time point significantly different (P<0.05) from pre-exercise value, respectively.

Heart rate

Statistical analysis on heart rate in study A showed no effect of trial (P=0.256), a significant effect of time (P<0.001, η^2=0.986) and no interaction (P=0.898). In study B there was no effect of trial (P=0.481), a significant effect of time (P<0.001, η^2=0.964) and no interaction (P=0.053, η^2=0.130) (Figure 7.2).

a) study A



b) study B

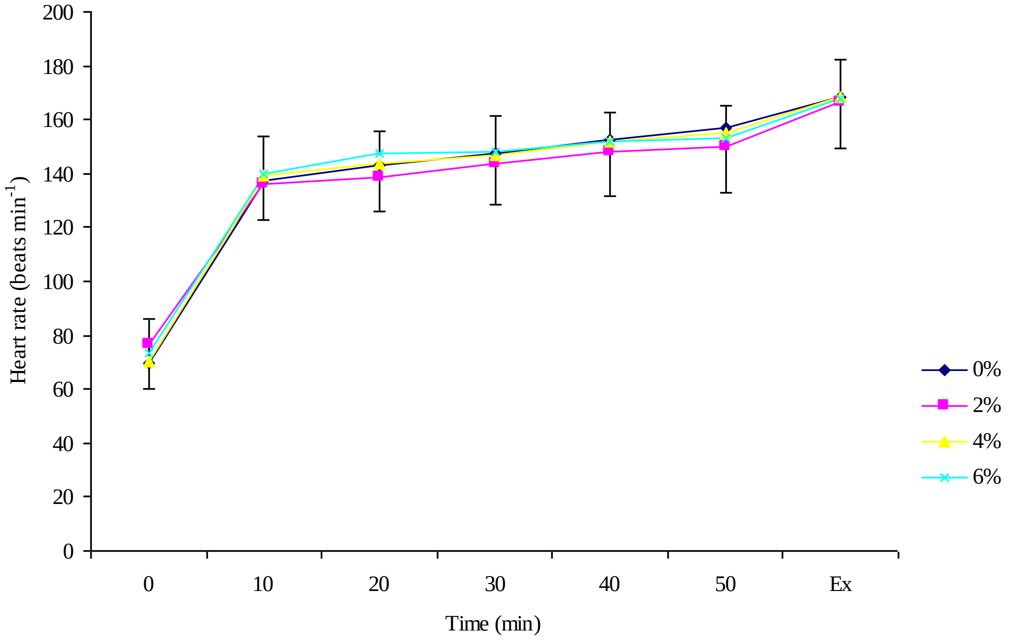
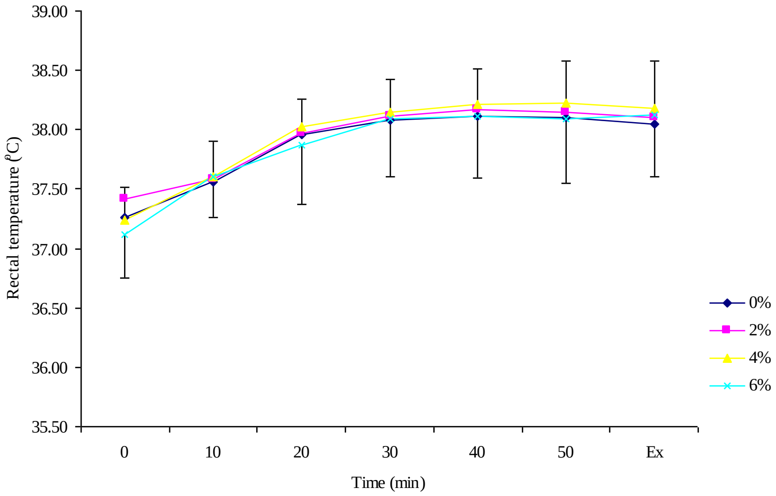


Figure 7.2: Heart rate during exercise when ingesting 0, 2, 4 and 6% carbohydrate drinks in study A and B. Values are mean ± SD.

Rectal temperature

Statistical analysis on rectal temperature in study A showed no effect of trial (P=0.806), a significant effect of time (P<0.001, η^2=0.849) and no interaction (P=0.891). In study B showed no effect of trial (P=0.782), a significant effect of time (P<0.001, η^2=0.931) and no interaction (P=0.103) (Figure 7.3).

a) study A



b) study B

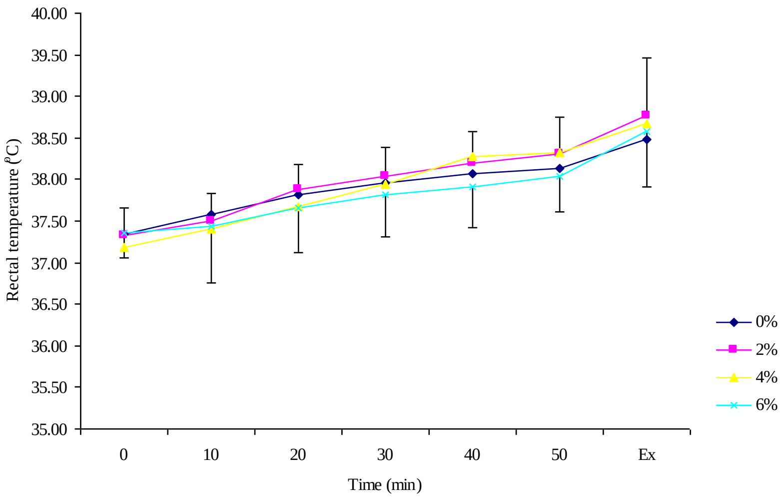


Figure 7.3: Rectal temperature during exercise when ingesting 0, 2, 4 and 6% carbohydrate drinks in study A and B. Values are mean ± SD.

Subjective feelings related to thirst

A summary of the statistical analysis for the subjective feelings related to thirst data for study A and B is given in Table 7.3. There were no significant differences between trials for all subjective feeling in study A. Similarly, no significant different between trials detected in study B except for bloatedness and nauseousness. Thirst, bloatedness, tiredness, throat dryness, throat scratchiness, concentration and energy ratings showed significant changes ($P<0.05$) over time in study A. In study B, the ratings for tiredness, alertness, head soreness, concentration, energy and nauseousness significantly changed ($P<0.05$) over the duration of exercise. Of the 14 subjective feelings, none of them showed a significant interaction between trial and time effect ($P>0.05$) in both studies.

Table 7.3: A summary of statistical analysis on subjective feelings related to thirst for study A and B

Subjective feeling	Study A (cool)					Study B (warm)					Figure
	Trial (P value)	Time (P value)	Effect size (η^2)	Interaction (P value)	Effect size (η^2)	Trial (P value)	Effect size (η^2)	Time (P value)	Effect size (η^2)	Interaction (P value)	
Thirst	0.385	0.004*	0.360	0.316		0.549		0.238		0.947	7.4 a,b
Stomach fullness	0.892	0.052	0.342	0.142		0.121		0.143		0.643	7.4 c,d
Mouth pleasantness	0.106	0.063	0.341	0.079	0.236	0.950		0.481		0.992	7.4 e,f
Mouth chalkiness	0.429	0.152		0.088	0.232	0.422		0.546		0.982	7.4 g,h
Bloatedness	0.480	0.005*	0.497	0.155		0.046*	0.309	0.171		0.380	7.4 i,j
Hunger	0.889	0.087	0.300	0.555		0.432		0.202		0.666	7.4 k,l
Tiredness	0.595	0.003*	0.658	0.574		0.663		<0.001*	0.718	0.366	7.4 m,n
Throat dryness	0.516	0.031*	0.382	0.327		0.101		0.350		0.207	7.4 o,p
Throat scratchiness	0.069	0.021*	0.521	0.805		0.249		0.075	0.258	0.348	7.4 q,r
Alertness	0.472	0.264		0.558		0.882		0.005*	0.612	0.913	7.4 s,t
Head soreness	0.467	0.559		0.684		0.143		0.010*	0.536	0.574	7.4 u,v
Concentration	0.498	0.036*	0.370	0.953		0.552		0.006*	0.632	0.806	7.4 w,x
Energy	0.140	0.010*	0.637	0.148		0.756		<0.001*	0.823	0.146	7.4 y,z
Nauseousness	0.464	0.207		0.083	0.234	0.002*	0.683	0.010*	0.583	0.955	7.4 ab,ac

* denotes significant different at P<0.05

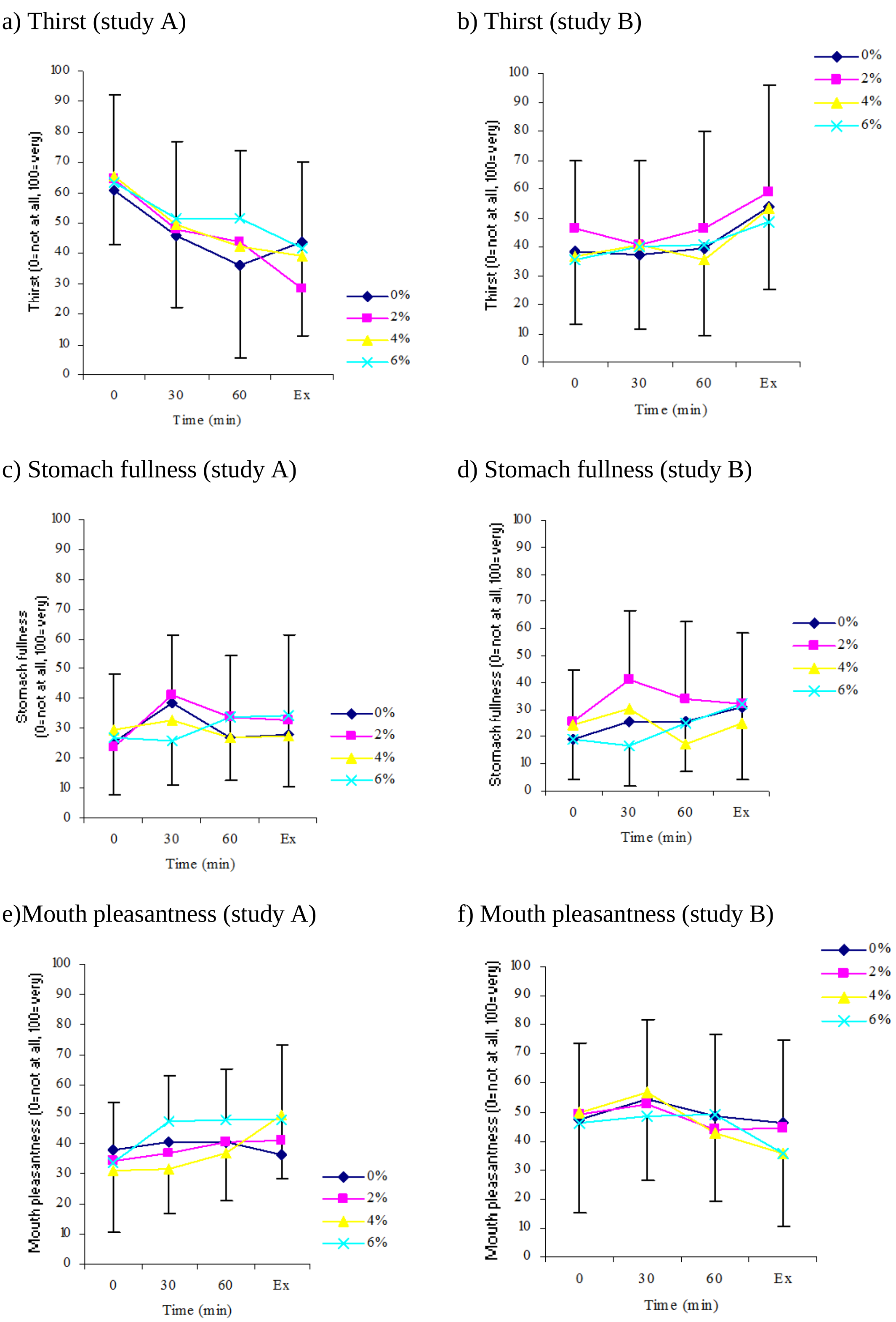


Figure 7.4: Ratings of subjective feelings related to thirst during exercise when ingesting 0, 2, 4 and 6% carbohydrate drink in study A and B. Values are mean ± SD.

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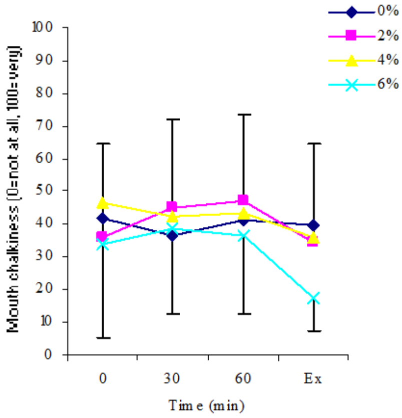
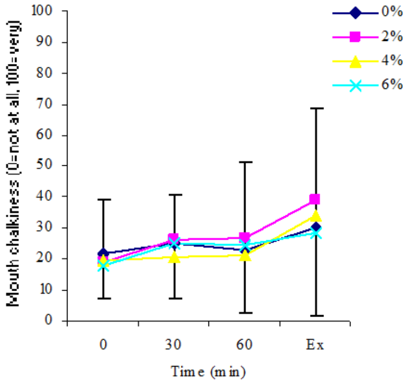
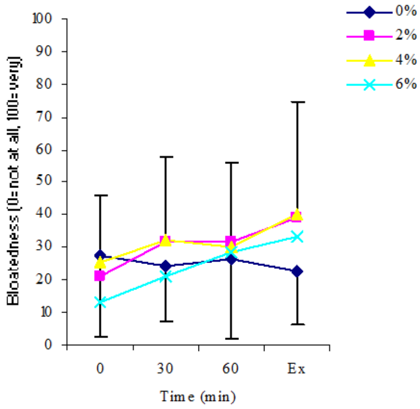
g) Mouth chalkiness (study A)



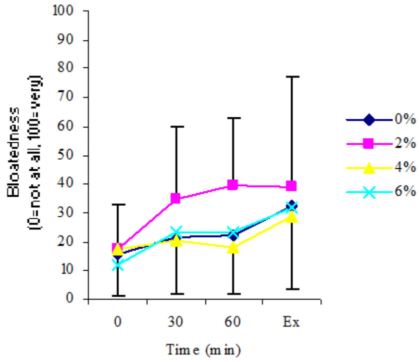
h) Mouth chalkiness (study B)



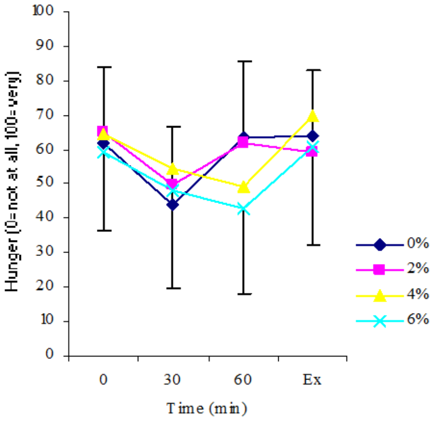
i) Bloatedness (study A)



j) Bloatedness (study B)



k) Hunger (study A)



l) Hunger (study B)

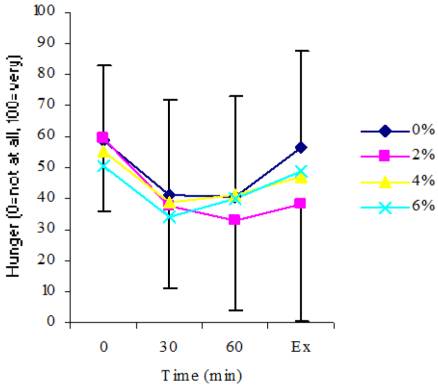


Figure 7.4: Ratings of subjective feelings related to thirst during exercise when ingesting 0, 2, 4 and 6% carbohydrate drink in study A and B. Values are mean ± SD.

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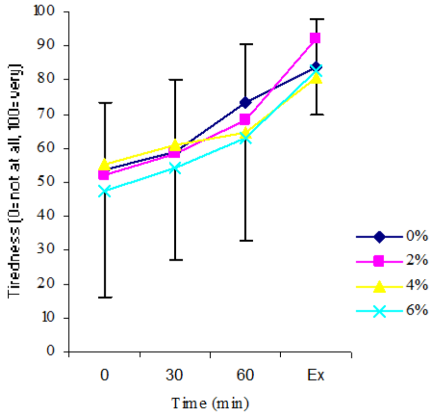
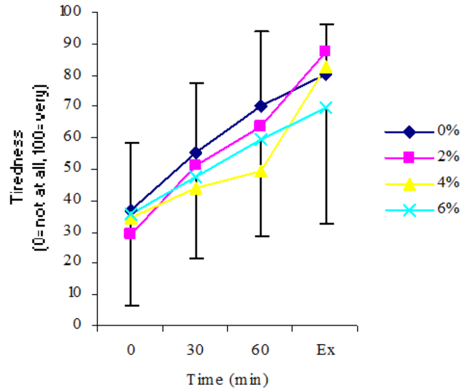
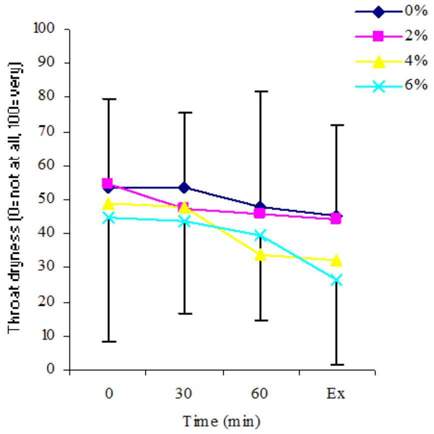
m) Tiredness (study A)



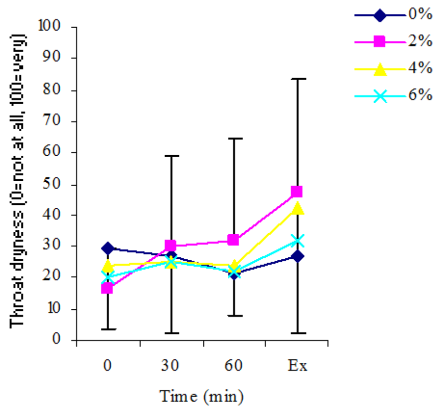
n) Tiredness (study B)



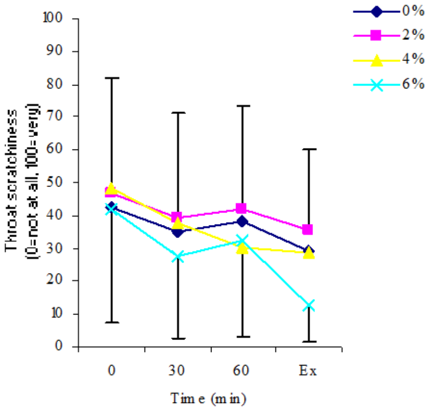
o) Throat dryness (study A)



p) Throat dryness (study B)



q) Throat scratchiness (study A)



r) Throat scratchiness (study B)

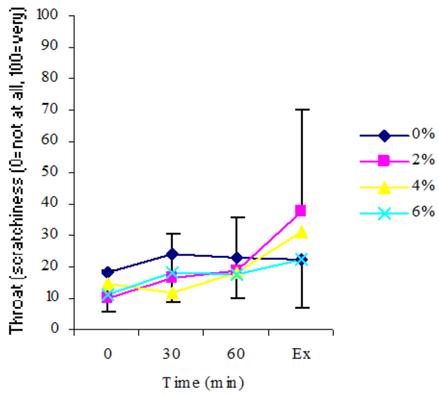


Figure 7.4: Ratings of subjective feelings related to thirst during exercise when ingesting 0, 2, 4 and 6% carbohydrate drink in study A and B. Values are mean ± SD.

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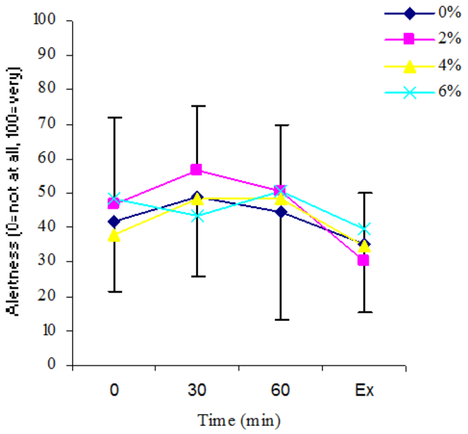
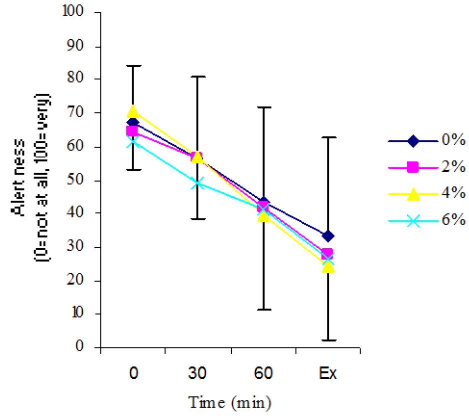
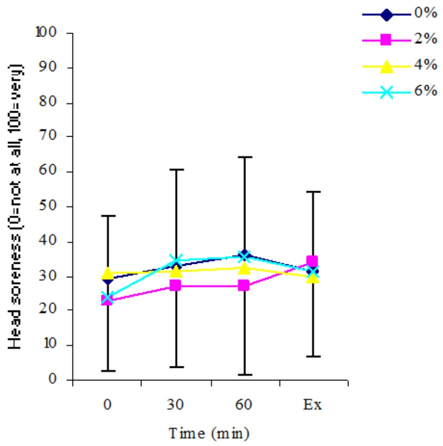
s) Alertness (study A)



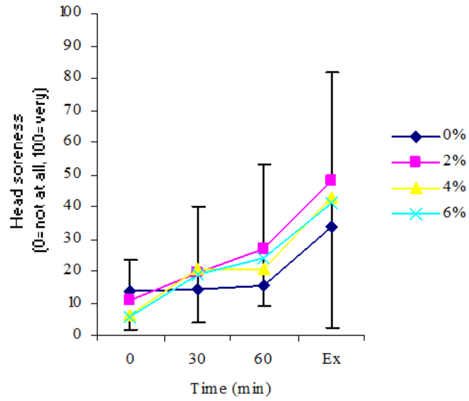
t) Alertness (study B)



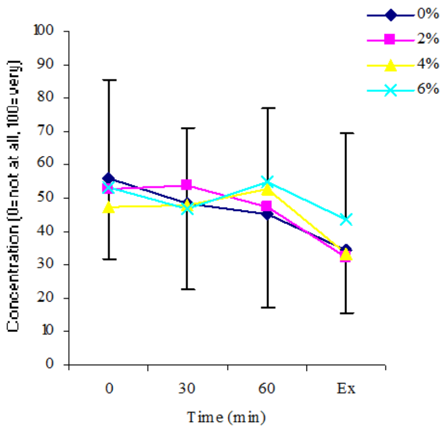
u) Head soreness (study A)



v) Head soreness (study B)



w) Concentration (study A)



x) Concentration (study B)

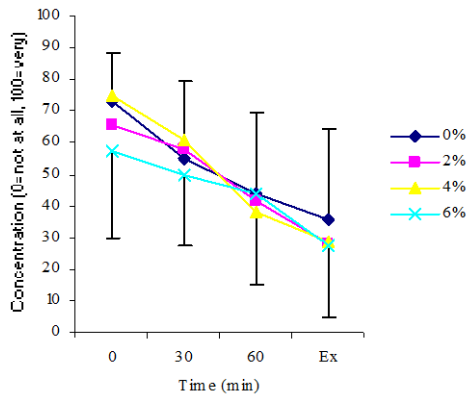


Figure 7.4: Ratings of subjective feelings related to thirst during exercise when ingesting 0, 2, 4 and 6% carbohydrate drink in study A and B. Values are mean ± SD.

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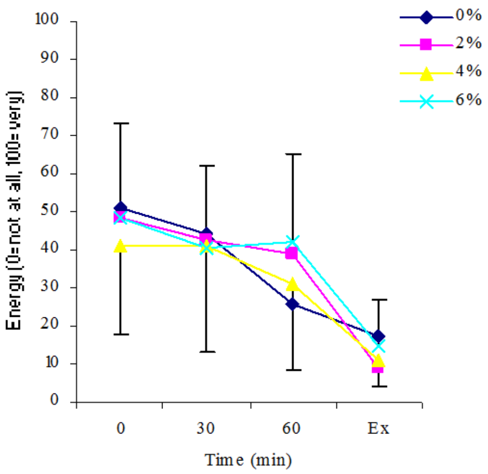
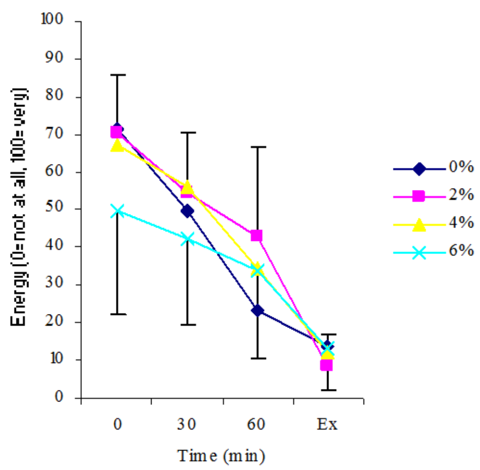
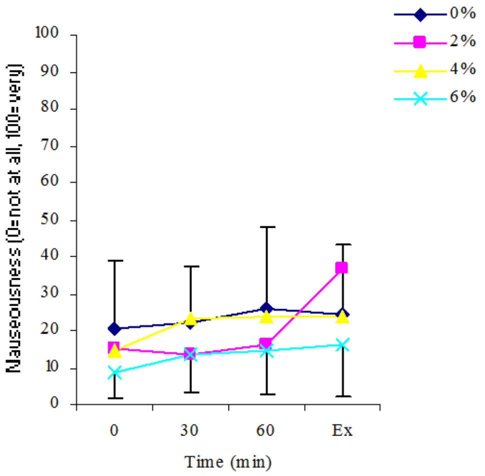
y) Energy (study A)



z) Energy (study B)



ab) Nauseousness (study A)



ac) Nauseousness (study B)

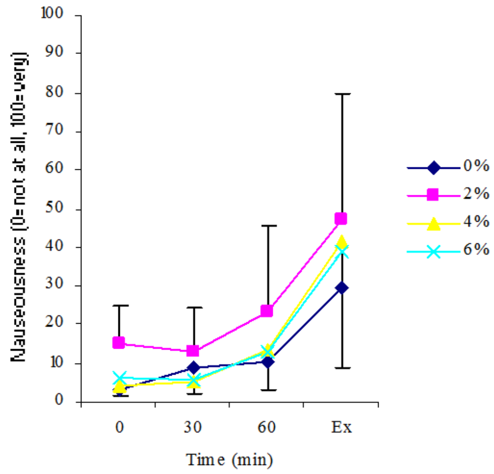


Figure 7.4: Ratings of subjective feelings related to thirst during exercise when ingesting 0, 2, 4 and 6% carbohydrate drink in study A and B. Values are mean ± SD.

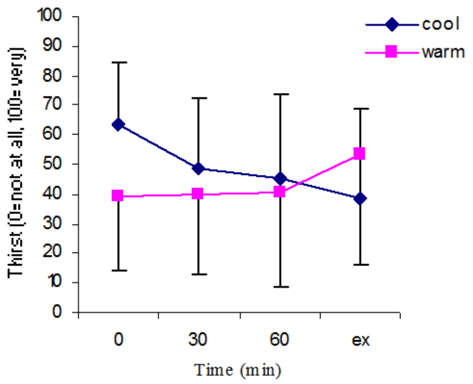
Since there were no significant interaction between time and trial for both study A and B, the data from all trials in both studies were pooled together and compared between cool and warm conditions. Table 7.4 shows the summary of results for the pooled data of 14 subjective feelings in cool and warm trials over time. The ratings for mouth pleasantness, mouth chalkiness, hunger, throat dryness, throat scratchiness and energy were significantly different ($P<0.05$) between trials. The ratings for bloatedness, hunger, tiredness, alertness, head soreness, energy and nauseous were significantly ($P<0.05$) changed over time. Only thirst and nauseous ratings showed the significant interaction of trial and time. In the cool trial, thirst rating decreased from 0 min to exhaustion, whereas in the warm trial, thirst perception was increased from 0 min to exhaustion (Figure 7.5a). The rating for nauseousness was increased from 0 min and continued increasing to 30, 60 min and at exhaustion on warm trial, but no changes for the nauseousness rating over the duration of exercise in the cool trial (Figure 7.5n).

Table 7.4: A summary of statistical analysis of pooled data (N=24) of subjective feeling ratings in cool and warm study. Values are mean ± SD.

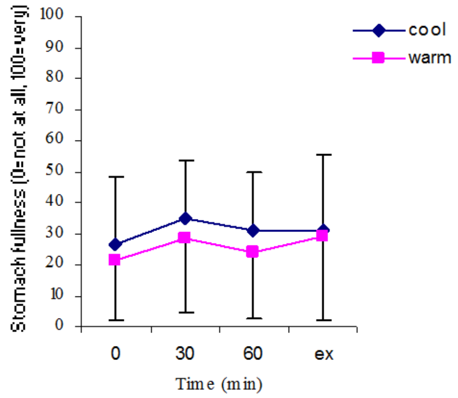
Subjective feeling	Trial (P value)	Effect size (η^2)	Time (P value)	Effect size (η^2)	Interaction (P value)	Effect size (η^2)	Figure
Thirst	0.336		0.714		0.037*	0.092	7.5a
Stomach fullness	0.533		0.449		0.902		7.5b
Mouth pleasantness	0.012*	0.069	0.339		0.294		7.5c
Mouth chalkiness	0.001*	0.113	0.928		0.298		7.5d
Bloatedness	0.762		0.047*	0.086	0.866		7.5e
Hunger	0.019*	0.061	0.026*	0.099	0.926		7.5f
Tiredness	0.843		<0.001*	0.108	0.181		7.5g
Throat dryness	0.002*	0.108	0.954		0.223		7.5h
Throat scratchiness	0.000*	0.134	0.964		0.070	0.077	7.5i
Alertness	0.085	0.033	0.020*	0.105	0.414		7.5j
Head soreness	0.233		0.033		0.114		7.5k
Concentration	0.204		0.861		0.644		7.5l
Energy	0.030*	0.053	<0.001*	0.543	0.117		7.5m
Nauseousness	0.623		<0.001*	0.206	0.047*	0.086	7.5n

* denotes significant different at P<0.05.

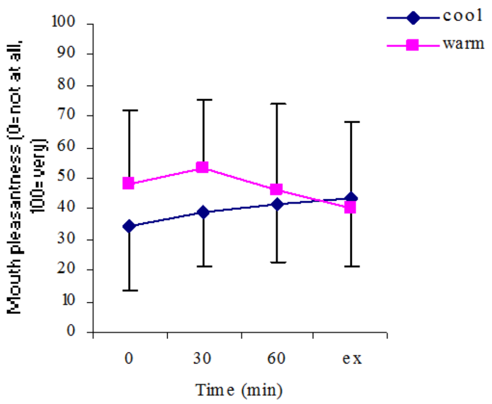
a) Thirst



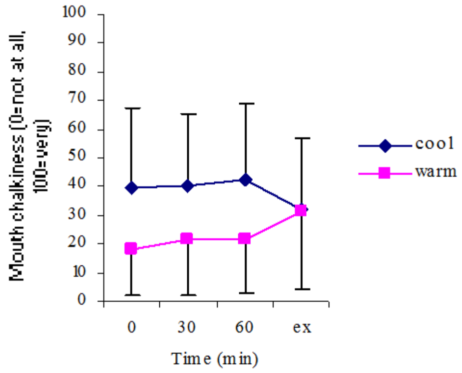
b) Stomach fullness



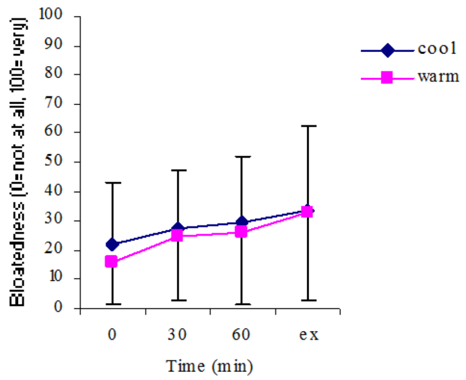
c) Mouth pleasantness



d) Mouth chalkiness



e)Bloatedness



f) Hunger

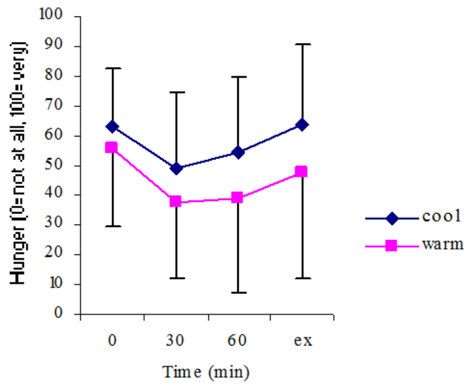


Figure 7.5: Ratings of subjective feelings related to thirst during exercise in cool and warm temperature. Values are mean ± SD.

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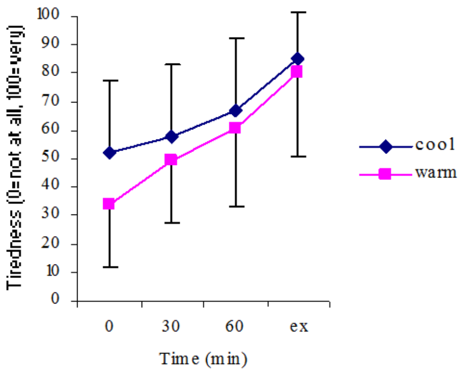
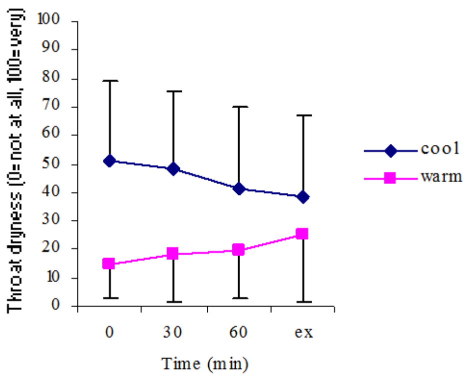
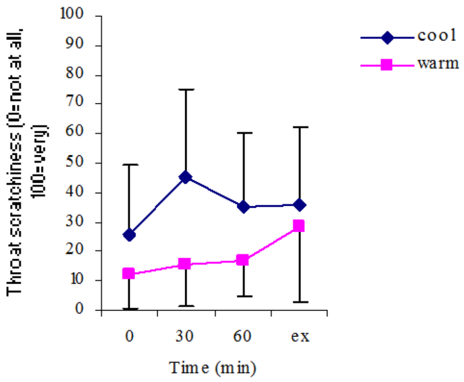
g)Tiredness



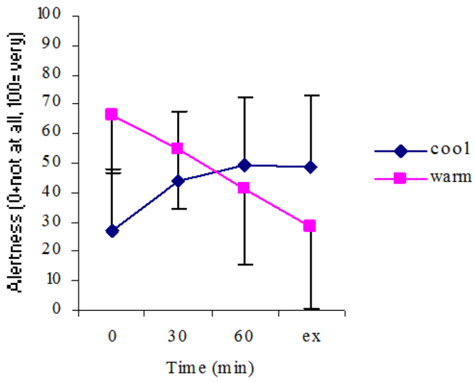
h) Throat dryness



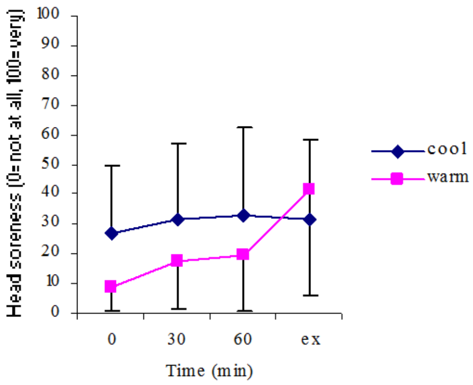
i) Throat scractchiness



j) Alertness



k) Head soreness



l) Concentration

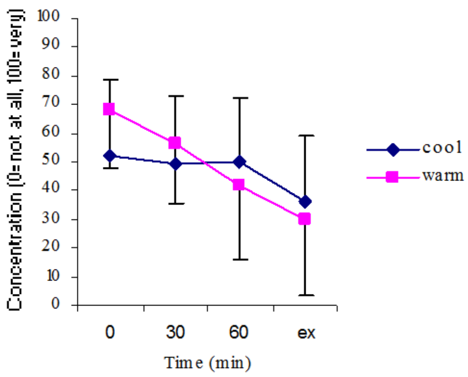


Figure 7.5: Ratings of subjective feelings related to thirst during exercise in cool and warm temperature. Values are mean ± SD.

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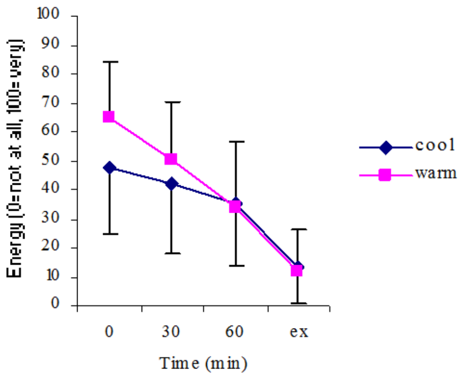
m) Energy



n) Nauseousness

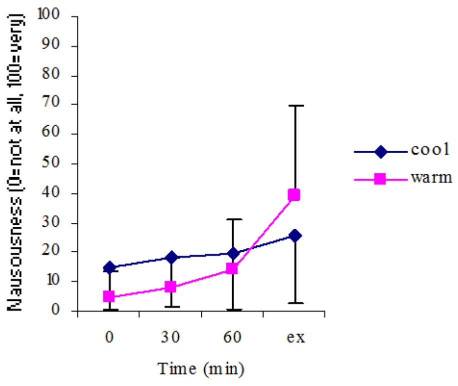


Figure 7.5: Ratings of subjective feelings related to thirst during exercise in cool and warm temperature. Values are mean ± SD.

Discussion

The main findings from this study show that ingestion of 0, 2, 4 and 6% CHO drink results in similar responses for subjective feelings of thirst when exercising both in cool and warm ambient temperature. However, subsequent pooled data analysis showed that thirst perception was different during exercise in the cool and warm temperature.

There was no significant (P>0.05) effect of trial and time interaction observed for all 14 subjective feelings measured during exercise in the cool (study A) and warm (study B) ambient temperature. Therefore, the varying carbohydrate content in the drinks did not affect the subjective feelings over the duration of exercise in the cool and warm environment.

Theoretically, no apparent differences between trial detected in terms of gastrointestinal discomfort indicators such as stomach fullness, bloatedness and nauseous may suggest that the rate of fluid intake equals the rate of gastric emptying, which caused no residual volume left in the stomach that make experimental beverages tolerable in both cool and warm conditions. Therefore, it is possible that the availability of fluid in the circulation could maintain the hydration status, and this may have not altered the subjective feelings such as thirst, tiredness, alertness, head soreness and concentration when ingesting carbohydrate drinks during exercise either in the cool or warm temperature. Even though this study did not have a direct measure for hydration status during exercise, previous work by Horswill et al. (2008) have shown that the maintenance of hydration status during exercise prevents the rise in core temperature. They conducted the study on the effect of carbohydrate intake on core temperature and metabolic responses during exercise at 65% VO_{2max} in 9 healthy men and found that the participants who were fed a fixed amount of carbohydrate and placebo during exercise only had a body mass change of 0.37 ± 0.63% and 0.56 ± 0.83%, respectively with no significant different (P>0.05) in core temperature between trials. In the present study, the rise in core temperature after 20 min of exercise in both study A (Figure 7.3 (a)) and B (Figure 7.3 (b)) were prevented in all trials and thus might suggest that the participants were not dehydrated to a level that stimulate the subjective feelings related to thirst.

In an attempt to generalise the findings, results from 4 beverage trials from both study A and B were pooled and subsequent comparison between cool and warm conditions were conducted. The findings showed that the thirst rating was increased over time during exercise in the warm, whereas the rating was decreased over time during exercise in the cool. This may suggest that thirst sensation is suppressed when the participants were exercising in the cool. Indeed, Kenefick et al. (2004) who investigated the effect of cold exposure on thirst sensation in healthy participants have shown that thirst was attenuated by 40% in resting and exercising individuals exposed to cold temperature.

In general, the results of this study are inconclusive with respect to the effect of fluid ingestion on subjective feelings of thirst during exercise both in cool and warm ambient conditions. The insignificant differences observed for most of the subjective feelings may due to the maintenance of hydration status during exercise both in cool and warm condition but further works are warranted to validate this assumption.

Chapter 8

General Discussion and Conclusions

The aim of this thesis was to examine the extent of knowledge on hydration and fluid balance in free-living individuals and to investigate the factors affecting thirst and fluid intake in resting and exercising people. This section presents a summary of the main findings of studies reported in this thesis and how they contributed to understanding the relationship between hydration, thirst and fluid balance as a whole.

Hydration and fluid intake in free-living people

Even though physiological thirst is adequate to stimulate drinking to ensure optimum hydration status, in some circumstances such as people who are performing strenuous exercise or hard labour with high rates of sweat losses, knowledge on hydration is important to ensure that people drink accordingly to maintain fluid balance. In chapter 3, it was found that the fitness professionals generally demonstrate substantial knowledge about the timing and benefits of fluid replacement before, during and after exercise, how to estimate their hydration status and the health consequences of lower or over consumption of water. Given the fact that the participants in this study were certified fitness professional, the substantial knowledge in this aspect of hydration is not surprising. Sport and exercise nutrition is one of the courses included in the modules required for the certification (National Register of Personal Trainers, 2010). On the other hands, most participants show some misconception about the type of beverages that hydrate the body. They misunderstood the fact that caffeinated beverages if consumed in moderation are actually not dehydrating. Misconception in some aspects of hydration may probably be due to widely dissemination of information which has not backed up by scientific evidence. Previous data have shown that athletes receive most of the information from sport magazines (Jacobson, Sobonya, & Ransone, 2001) while coaches from both magazines and professional conferences (Geijer, Pitney, & Brandenburg, 2009). While professional conferences may provide more sound knowledge about hydration, magazines may present some misinformation on hydration based upon questionable research. Therefore, investigation into the type of sources information obtained and the level of knowledge may be appropriate.

Furthermore, for the fitness professions (chapter 3), “feel thirsty” was one of the main responses given by the participants about how they predict their hydration status during exercise and in general during the day. The study reported in chapter 5, assessing the feasibility of subjective feelings of thirst as a marker of hydration status provides some supporting evidence for this concept. It is often suggested that thirst is not a good indicator of hydration status because it lags behind the body mass loss (Greenleaf & Harrison, 1986; Hubbard et al., 1990). However, the findings in chapter 5 show that subjective feelings related to thirst such as dry mouth, thirst perception, desire to drink and hunger ratings could potentially be used as a marker of hydration status to signify at least 1% body mass loss due to food and fluid restriction in free-living individuals. Taken together, the findings from chapter 3 and 5 in this thesis disputed the previous view that thirst is a poor indicator of hydration status (Armstrong, 2007) and water needs (Greenleaf, 1992).

Although the results of the present study in chapter 5 demonstrate the high possibility of using subjective feelings as markers of hydration status, it should be interpreted with caution. It was clear that most of the subjective feelings being measured in this study were significantly different between fasted and non-fasted trials. But the participants were not blinded to the trials because one trial involved taking breakfast 2 h before the measurement and on the other, nothing consumed since the previous night. It is perhaps not surprising that the subjects had greater perception of thirst and hunger on the fasted trial. In addition, the results of the present study should perhaps only be applied to healthy free-living young adults because the mean age of the participants in this study was 20 ± 1 years. Data have suggested that thirst perception declines with increasing age (Phillips et al., 1984b; Kenney & Chiu, 2001). Therefore, additional research into this matter would be appropriate to see whether the results would be reproducible in different populations under different settings.

Factors affecting thirst and fluid intake in resting and exercising individuals

One of the main findings of the study reported in chapter 3 was that "feel thirsty" was the main reason reported by participants as their stimulus for drinking during exercise and in general during the day. However, in chapter 4, it was found that thirst perception did not correlate with fluid intake, suggesting that thirst is not always the main reasons that stimulate drinking behaviour in free-living populations.

In chapter 4, the participants were free-living individuals going about their normal daily life. Availability of drinks, work pressure and environmental factors may also have influenced the fluid intake during the study period. Indeed, some evidence suggests that in everyday life, fluid intake could either be stimulated by other factors such as body fluid deficit (Maresh et al., 2004; Engell et al., 1987) or food intake (Saltmarsh, 2001). In addition, the participants were asked to weigh all their food and fluid intake and fill in an activity diary during 3 days of the study period. Getting them to do these tasks may interfere with their normal patterns of food intake and activity (Johansson et al., 1992; Goris & Westerterp, 1999) and may cause them hesitate to consume food and fluid and undertake activities as they would normally do.

Previous studies have shown that people increase their fluid intake to at least partially replace sweat losses when subjected to hot and temperate ambient temperatures (Engell et al., 1987; Maresh et al, 2004). On the other hand, limited existing literature suggests that thirst sensation is attenuated (Kenefick et al., 2004) and fluid intake reduced (Dann et al., 1997) when people were exposed to cold environments. In chapter 4, the data collection was conducted during a cool season (T = 8 ± 5°C, 86 ± 14% rh). The absence of relationship between thirst and fluid intake in that chapter may be due to the suppressed thirst sensation reported, hence affected the subsequent fluid intake in these participants. However, this finding was not supported in a study reported in chapter 6. The findings in chapter 6 demonstrate that exposure to cool or warm conditions resulted in similar subjective feelings of thirst despite the lower fluid intake in the cool compared to warm trial. It is speculated that the absence of a relationship between thirst and fluid intake in chapter 6 was probably because the participants were not dehydrated to a level that stimulated thirst and subsequent fluid intake. In this study, the body mass loss during 180 min of exposure was 0.25 ± 0.15% which was lower than the threshold value of 0.8% body mass loss as proposed by Wolf (1950b).

Another alternative explanation for the lower fluid intake during cool exposure in chapter 6 could be that the water was not palatable enough to stimulate drinking. In this study, the water was kept at temperature 15ºC for both the cool and warm trial. Although cool water may be more acceptable during (Szlyk et al. 1989; Sawka et al., 2007) and after (Sandick, Engell, & Maller, 1984) exercise in the heat, in cool environments, cool water perhaps may not be so pleasant. However, the study on the effect of drink temperature on fluid intake in individuals under cold stress is lacking, therefore further investigations are needed to validate this assumption. Besides, the search into mechanisms such as hormonal responses that could possibly affect the fluid intake during resting in cool environment is more difficult. Peripheral vasoconstriction may limit the amount of blood that could be withdrawn for the analysis of arginine vasopressin and other hormones that may play a role in the mechanisms of thirst and fluid intake. Thus, appropriate study design to enable blood withdrawal without compensating the subjective feelings response may be worthwhile for future research.

In chapter 7, thirst perception was found to be rated higher over time during exercise in the warm temperature, whereas the rating were decreased over time during exercise in the cool temperature. This finding therefore supports the previous assumption made in chapter 4 about the attenuation of thirst perception when the participants are exposed to cool temperature, therefore may be affected their fluid intake behaviour. Indeed, this finding agrees with the previous evidence that thirst is suppressed when individuals are resting or exercising in the cold (Kenefick et al., 2004).

Conclusions

The works described in this thesis have provided additional knowledge in the fields of hydration and fluid balance. The followings are the main conclusions that arise from this work:

1) The fitness professionals in this study demonstrate many acceptable perceptions that are in line with scientific evidence in the area of hydration, thirst and fluid intake. However, the present study also identifies the need for fitness professionals to accurately pick information based on sound evidence regarding their perspectives on hydration and fluid intake habits.

2) Thirst sensations do not correlate with fluid intake and water turnover in free-living participants during cool exposure, but mouth dryness and depression may explain the fluid intake behaviour in male and female participants of this study.

3) Subjective feelings related to thirst such as mouth dryness, thirst perception, desire to drink (water pleasantness) and hunger rating could be used as a marker of hydration status to indicate at least a 1% body mass loss due to food and fluid restriction in free living individuals.

4) When individuals are resting at different ambient temperatures, they consume more fluid in absolute and relative terms to replace fluid loss from sweating during warm compared to cool exposure. Besides, subjective feelings of thirst ratings and mood states do not affect the fluid intake during acute exposure in both warm and cool temperature. Other factors such as increases in central blood volume due to vasoconstriction or simply the absence of dehydration may explain the lower water consumption during resting in the cool environment.

5) Ingestions of fluid intake *per se*, either water or carbohydrate-containing beverages do not affect the subjective feelings during exercise in the cool and warm conditions. Other factors such as the hydration status might explain the inconsistencies in the ratings of subjective feelings related to thirst during exercise both in cool and warm ambient temperatures.

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Appendix A

The following subjective feelings questionnaire was completed as described in chapter 4 of this thesis (Engell et al., 1987).

Place a vertical mark on the lines below to indicate HOW YOU FELT, ON THE WHOLE, DURING THE DAY TODAY.

1) How weary did you feel during the day?

Not at all weary ______________________________ very weary

2) How dizzy did you feel during the day?

Not at all dizzy ______________________________ very dizzy

3) How did you generally feel during the day?

Not at all lightheaded ______________________________ very lightheaded

4) How sleepy did you feel during the day?

Not at all sleepy ______________________________ very sleepy

5) How tired did you feel during the day?

Not at all tired ______________________________ very tired

6) How irritable did you feel during the day?

Not at all irritable ______________________________ very irritable

7) How sore did your head feel during the day?

Not at all sore ______________________________ very sore

8) How well could you concentrate during the day?

Not at all well ______________________________ very well

9) How strong was your desire to eat and drink during the day?

Not at all strong ______________________________ very strong

10) Did you have trembling hands during the day?

Not at all trembling ______________________________ very trembling

11) How dry did your mouth feel during the day?

Not at all dry ____ very dry

12) How irritated did your mouth feel during the day?

Not at all irritated ____ very irritated

13) How pleasant did your mouth taste during the day?

Not at all pleasant ____ very pleasant

14) How bad did your mouth taste during the day?

Not at all bad ____ very bad

15) Did you have a chalk like taste in your mouth during the day?

Not at all severe ____ very severe

16) How dry did your throat feel during the day?

Not at all dry ____ very dry

17) How scratchy did your throat feel during the day?

Not at all scratchy ____ very scratchy

18) How warm did your throat feel during the day?

Not at all warm ____ very warm

19) Did you have chapped lips during the day?

Not at all severe ____ very severe

20) How thirsty did you feel during the day?

Not at all thirsty ____ very thirsty

21) How hungry did you feel during the day?

Not at all hungry ____ very hungry

22) How full did your stomach feel during the day?

Not at all full ____ very full

23) How alert did you feel during the day?

Not at all alert ____ very alert

24) How swollen did your tongue feel during the day?

Not at all swollen ____ very swollen

The following subjective feelings questionnaire was completed as described in chapters 5 and 6 of this thesis (Engell et al., 1987).

Subject: Trial:

Time:

Subjective feelings related to thirst

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

1) How dry does your mouth feel now?

Not at all dry ______________________________ very dry

2) How irritated does your mouth feel now?

Not at all irritated ______________________________ very irritated

3) How pleasant does your mouth taste now?

Not at all pleasant ______________________________ very pleasant

4) How bad does your mouth taste now?

Not at all bad ______________________________ very bad

5) Do you have a chalk like taste in your mouth now?

Not at all ______________________________ very severe

6) How dry does your throat feel now?

Not at all dry ______________________________ very dry

7) How scratchy does your throat feel now?

Not at all scratchy ______________________________ very scratchy

8) How warm does your throat feel now?

Not at all warm ______________________________ very warm

9) Do you have chapped lips now?

Not at all ______________________________ very severe

10) How thirsty do you feel now?

Not at all thirsty ______________________________ very thirsty

11) How pleasant would it be to drink some water now?

Not at all pleasant ______________________________ very pleasant

12) How hungry do you feel now?

Not at all hungry ______________________________ very hungry

13) How full does your stomach feel now?

Not at all full ______________________________ very full

14) How alert do you feel now?

Not at all alert ______________________________ very alert

15) How swollen does your tongue feel now?

Not at all swollen ______________________________ very swollen

16) How weary do you feel now?

Not at all weary ______________________________ very weary

17) How sleepy do you feel now?

Not at all sleepy ______________________________ very sleepy

18) How sore does your head feel now?

Not at all sore ______________________________ very sore

The following subjective feelings questionnaire was completed as described in chapter 7 of this thesis (Engell et al., 1987).

1) How thirsty do you feel now?

not at all thirsty ______________________________ very thirsty

2) How full does your stomach feel now?

not at all full ______________________________ very full

3) How pleasant does your mouth taste now?

not at all pleasant ______________________________ very pleasant

4) Do you have a chalk like taste in your mouth now?

not at all ______________________________ very severe

5) How bloated do you feel now?

not at all bloated ______________________________ very bloated

6) How hungry do you feel now?

not at all hungry ______________________________ very hungry

7) How tired do you feel now?

not at all tired ______________________________ very tired

8) How dry does your throat feel now?

not at all dry ______________________________ very dry

9) How scratchy does your throat feel now?

not at all scratchy ______________________________ very scratchy

10) How alert do you feel now?

not at all alert ______________________________ very alert

11) How does your head feel now?

not at all sore ______________________________ very sore

12) How well can you concentrate just now?

not at all well ______________________________ very well

13) How much energy do you feel you have now?

no energy ______________________________ full of energy

14) How nauseous do you feel now?

not at all nauseous ______________________________ very nauseous

Appendix B

The following TSS scale was completed as described in chapters 6 and 7 of this thesis (Parsons, 2003).

Thermal Sensation Scale

Subject: Trial: Time:

-10	cold impossible to bear
-9	
-8	very cold, shivering hard
-7	
-6	cold, light shivering
-5	
-4	most areas of the body feel cold
-3	
-2	some areas of the body feel cold
-1	
0	neutral
1	
2	some areas of the body feel warm
3	
4	most areas of the body feel hot
5	
6	very hot, uncomfortable
7	
8	extremely hot, close to limit
9	
10	heat impossible to bear

Appendix C

The following RPE scale was completed as described in chapter 7 of this thesis (Borg, 1998).

Rating of Perceived Exertion Scale

Subject: Trial: Time:

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	exhaustion

Appendix D

Loughborough University

School of Sport and Exercise Sciences

We are carrying out research looking at hydration in exercising people. As part of this research we are looking into the drinking behaviour of people who regularly exercise. Thank you for taking the time to answer our questionnaire.

The questionnaire is divided into two parts. Part A has questions about your demographic information, and Part B aims to assess your opinion and attitude towards your drinking behaviour. Your responses will remain confidential and will only be used for the purpose of this research. Thank you for your help and support with this questionnaire.

Please describe yourself.

Sex (male or female): ....female........

Height (in m, cm or ft and in): ....158 cm........

Weight (in kg or stone/lb): ....52 kg........

Age: ....26........

Occupation: ....fitness intructor........

Typical weekly exercise routine:
........walk - 30 min 3X........
........aquafit - 30 min 2X........
........teaching - 1h 2X........
....
....
....
....
....

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PART B

1. In your opinion, should you:

a) Drink before exercising? (please circle one) (ALWAYS) SOMETIMES NEVER

Why? to avoid dehydration

b) Drink when exercising or during short breaks in exercise? (please circle one)

(ALWAYS) SOMETIMES NEVER

Why? to hydrate the body

c) Drink after exercising? (please circle one) (ALWAYS) SOMETIMES NEVER

Why? to replace fluid loss

2. What, in you opinion, is the best drink for the human body:

a) For before exercise? water

Why? provide hydration

b) For during exercise? sport drink

Why? Contain energy and salt to stay hydrated

c) For after exercise? water

Why? easily absorb into the body

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d) Generally during the day? ....water..........

Why?..........same as (C)..........

....

....

3. What is your preferred/favourite drink

a) For before exercise ....water..........

b) For during exercise ....water..........

c) For after exercise ....~~Coffee~~ water..........

d) Generally during the day ....coffee..........

4. How do you decide when you need to drink

a) when exercising

..........feel thirsty..........

....

....

....

b) in general during the day?

..........feel thirsty..........

....

....

....

5. Rate the following drinks in terms of how good they are at providing water to the body. (1 = excellent, 5 = poor)

(please circle one number for each drink).

a)	Plain tap water	(1)	2	3	4	5
b)	Plain bottled mineral water	(1)	2	3	4	5
c)	Sparkling water	1	(2)	3	4	5
d)	Flavoured water	1	(2)	3	4	5
e)	Fruit juices	1	(2)	3	4	5
f)	Vegetable juices	1	(2)	3	4	5
g)	Milk	1	2	(3)	4	5
h)	Colas/lemonades etc	1	(2)	3	4	5
i)	Sports drinks	1	(2)	3	4	5
j)	Energy drinks	1	2	(3)	4	5
k)	Tea	1	2	3	(4)	5
l)	Coffee	1	2	(3)	4	5
m)	Chocolate	1	2	3	(4)	5
n)	Soup	1	2	3	(4)	5
o)	Any other drink (please name) ...... —	1	2	3	4	5

6. What problems, if any, can occur if we have too little water in our diet? List any or all that come to mind. Please write none if you think there are none.

tired, dehydration, lethorgy

7. What problems can occur if, if any, if we have too much water in out diet? List any or all that come to mind. Please write none if you think there are none.

always increase ~~toilet~~ urination

bloated

8a. Are you aware of how hydrated you are just now? (please circle as appropriate)

(YES) NO

8b. If yes, how do you know?

urine colour is yellowish

feel thirsty

9. How much do you typically drink in an average day? Give the amounts in pints, liters, glasses or any other way

a)	Plain tap water	1L
b)	Plain bottled mineral water	500 ml
c)	Sparkling water	–
d)	Flavoured water	–
e)	Fruit juices	–
f)	Vegetable juices	–
g)	Milk	–
h)	Colas/lemonades etc	–
i)	Sports drinks	–
j)	Energy drinks	–
k)	Tea	–
l)	Coffee	2 cups
m)	Chocolate	–
n)	Soup	–
o)	Any other drink or drinks (please name)	

....

10. From your observation of people attending your class or the same classes as you, how often do you think they drink:

a) before exercising ? (please circle one) ALWAYS SOMETIMES NEVER

b) during exercising ? (please circle one) ALWAYS SOMETIMES NEVER

c) after exercising? (please circle one) ALWAYS SOMETIMES NEVER

d) Do you think they drink the right amount? (please circle one) YES NO

Appendix E

The following table shows the results of Spearman correlation coefficient test for 18 subjective feelings related to thirst reported in Chapter 5 of this thesis.

	Dry mouth	Irritated mouth	Pleasant taste in mouth	Bad taste in mouth	Chalk like	Dry throat	Scratchy throat	Warm throat	Chapped lips	Thirsty	Water pleasantness	Hungry	Full stomach	Alert	Swollen tongue	Weary	Sleepy	Sorehead
Dry mouth	1.000	0.657**	-0.508**	0.635**	0.466**	0.617**	0.455**	0.014	0.432**	0.871**	0.870**	0.816**	-0.790**	-0.402**	0.345**	0.531**	0.371**	0.244
Irritated mouth	0.657**	1.000	-0.571**	0.760**	0.764**	0.664**	0.791**	-0.048	0.549**	0.763**	0.589**	0.638**	-0.558**	-0.255	0.645**	0.408**	0.393**	0.487**
Pleasant taste in mouth	-0.508**	-0.571**	1.000	-0.802**	-0.421**	-0.584**	-0.539**	0.029	-0.457**	-0.552**	-0.498**	-0.511**	0.447**	0.440**	-0.464**	-0.374**	-0.516**	-0.621**
Bad taste in mouth	0.635**	0.760**	-0.802**	1.000	0.657**	0.711**	0.740**	-0.062	0.581**	0.676**	0.560**	0.637**	-0.532**	-0.335*	0.556**	0.432**	0.528**	0.604**
Chalk like	0.466**	0.764**	-0.421**	0.657**	1.000	0.599**	0.750**	0.019	0.566**	0.543**	0.357**	0.546**	-0.417**	-0.199	0.587**	0.442**	0.379**	0.404**
Dry throat	0.617**	0.664**	-0.584**	0.711**	0.599**	1.000	0.792**	0.122	0.626**	0.711**	0.548**	0.575**	-0.434**	-0.293*	0.469**	0.454**	0.421**	0.368**
Scratchy throat	0.455**	0.791**	-0.539**	0.740**	0.750**	0.792**	1.000	-0.087	0.556**	0.519**	0.358**	0.518**	-0.339*	-0.271*	0.522**	0.477**	0.483**	0..429**
Warm throat	0.014	-0.048	0.029	-0.062	0.019	0.122	-0.087	1.000	0.029	0.048	0.177	-0.048	0.093	0.063	-0.008	-0.028	0.069	0.160
Chapped lips	0.432**	0..549**	-0.457**	0.581**	0.566**	0.626**	0.556**	0.029	1.000	0.495**	0.413**	0.522**	-0.333*	-0.190	0.565**	0.338*	0.388**	0.303*
Thirsty	0.871**	0.763**	-0.552**	0.676**	0.543**	0.711**	0.519**	0.048	0.495**	1.000	0.817**	0.789**	-0.746**	-0.321*	0.495**	0.507**	0.294*	0.354**
Water pleasantness	0.870**	0..589**	-0.498**	0.560**	0.357**	0.548**	0.358**	0.177	0.413**	0.817**	1.000	0.745**	-0.785**	-0.351**	0.310*	0.496**	0.306*	0.297*
Hungry	0.816**	0.638**	-0.511**	0.637**	0.546**	0.575**	0.518**	-0.048	0.522**	0.789**	0.745**	1.000	-0.815**	-0.355**	0.498**	0.507**	0.349**	0.362**
Full stomach	-0.790**	-0.558**	0.447**	-0.532**	-0.471**	-0.434**	-0.339*	0.093	-0.333*	-0.746**	-0.785**	-0.815**	1.000	0.251	-0.387**	-0.388**	-0.211	-0.213
Alert	-0.402**	-0.255	0.440**	-0.335*	-0.199	-0.293*	-0.271*	0.063	-0.190	-0.321*	-0.351**	-0.355**	0.251	1.000	-0.169	-0.564**	-0.676**	-0.211
Swollen tongue	0.345**	0.645**	-0.464**	0.556**	0.587**	0.469**	0.522**	-0.008	0.565**	0.495**	0.310*	0.498**	-0.387**	-0.169	1.000	0.249	0.263	0.459**

Weary	0.531**	0.408**	-0.374**	0.432**	0.442**	0.454**	0.477**	-0.028	0.338*	0.507**	0.496**	0.507**	-0.388**	-0.564**	0.249	1.000	0.494**	0.253
Sleepy	0.371**	0.393**	-0.516**	0.528**	0.379**	0.421**	0.483**	0.069	0.388**	0.294*	0.306*	0.349**	-0.211	-0.676**	0.263	0.494**	1.000	0.307*
Sorehead	0.244	0.487**	-0.621**	0.604**	0.404**	0.368**	0.429**	0.160	0.303*	0.354**	0.297*	0.362**	-0.213	-0.211	0.459**	0.253	0.307*	1.000

Correlational comparisons

** significant at p<0.01
* significant at p<0.05
2-tailed
N=56