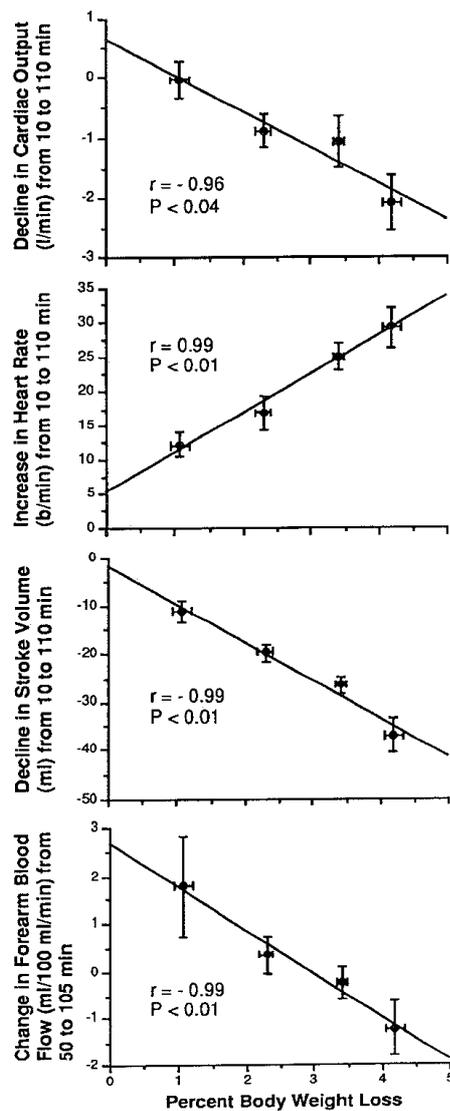


## **CHAPTER 1**

### **INTRODUCTION**

## 1.1 INTRODUCTION

Hypohydration (>2% body weight deficit) has been reported to increase cardiovascular and thermoregulatory strain and thus impair endurance exercise performance especially in hot conditions (Convertino *et al.*, 2000; Chevront *et al.*, 2003a; Murray, 2007; Sawka *et al.*, 2007). To avoid excessive dehydration (>2%), the American College of Sports Medicine (ACSM) *Position Stand on Exercise and Fluid Replacement* recommends endurance runners to drink *ad libitum* 0.4 to 0.8 L.hr<sup>-1</sup> during exercise (Sawka *et al.*, 2007). However, drinking fluid *ad libitum* is likely to lead to a body weight deficit of at least 2% (Fudge *et al.*, 2007). Fudge *et al.* (2007) further reported that “in conditions of modest dehydration (up to 3 L), fluid intake does not alter plasma volume, sweat rates or cardiovascular function”. This finding supports the earlier work of Chevront and Haymes (2001a) who reviewed the effect of *ad libitum* fluid ingestion on thermoregulatory responses and concluded that fluid replacement between 60-70% of sweat losses is effective in maintaining thermoregulation. However a systematic study of the effects of graded dehydration (1-5% BW loss) has shown increasing levels of thermal and circulatory strain with graduated fluid restriction (Figure 1.1) (Montain & Coyle, 1992b).



**Figure 1.1:** Influence of dehydration, as assessed by percent reduction in body weight after 2 hours of exercise, on change in cardiac output, heart rate, stroke volume, forearm blood flow during exercise (Montain & Coyle, 1992b)

Recent field studies have contradicted previous findings and suggest that maintaining fluid balance is not essential in athletic field settings such as marathon races (Noakes, 2007a; Noakes, 2007b). In reality, elite marathon runners only ingest approximately  $200 \text{ mL}\cdot\text{h}^{-1}$  of fluid during distance running races because they encounter difficulty in ingesting fluid whilst maintaining a fast speed of running ( $\sim 85\% \dot{V}O_{2\text{max}}$ ) (Noakes & Maharam, 2003). A number of studies have reported that greater body mass losses occurred in successful athletes who drank *ad libitum* during exercise (Cheuvront & Haymes, 2001a; Speedy *et al.*, 2001; Kao *et al.*, 2008) without affecting the

physiological markers of hydration status (Nolte *et al.*, 2010a; Nolte *et al.*, 2010b). It is thus not uncommon for elite marathon runners to experience large bodyweight changes and fluid losses as shown by the current marathon world record holder (now deceased) who lost 10% of body mass while establishing that record (Fudge & Pitsiladis, 2009). More recently, Zouhal *et al.* (2010) found that there was an inverse relationship between body weight change and finishing time in 643 marathon runners. These inconsistencies between laboratory and recent field studies have resulted in uncertainty among coaches, athletes and sport scientist regarding fluid replacement for athletes. Therefore, two questions arise (i) “*Does moderate hypohydration impair endurance performance?*” (ii) “*What is the critical threshold level of hypohydration to affect the circulatory and thermoregulatory responses during prolonged running performance?*”

Kenyan runners have dominated the middle and long distance running events at international level since the 1960s. Their outstanding success has captivated research’s seeking to discover the “secret” for the success of Kenyan elite distance running. There have been studies that have reported on the nutritional practices and daily lifestyle of Kenyan runners (Onywera *et al.*, 2004; Fudge *et al.*, 2006; Fudge *et al.*, 2008). These studies have shown that a negative energy balance with low fluid intake was practiced by the Kenyan runners prior to competition and during training. However, this practice did not impair the Kenyan runners’ performance as they have maintained their supremacy in endurance running for the past 40-50 years. Thus, Fudge *et al.* (2007) proposed that as hypohydration necessarily results in a decrease in body weight, there will be a reduced energy cost of running and consequently a reduced metabolic heat load and therefore delayed fatigue and enhanced running performance. However, whether the mechanism or mechanisms responsible for the Kenyan runners’ world class

performance is associated with body water loss and running economy is contentious and in need of further investigation.

As ambient temperature increases, sweat evaporation is a very effective means to provide a cooling effect for heat dissipation to achieve heat balance. Sweat evaporation limits the rise in core temperature and prevents the progressive development of hyperthermia. However, if fluid intake is less than sweat loss during exercise, it will lead to hypohydration which is associated with impaired endurance exercise performance (Sawka, 1992; Chevront *et al.*, 2003a; Murray, 2007; Maughan, 2010). The adverse effects of hypohydration associated with hyperthermia on prolonged exercise performance have been extensively studied, yet it is unclear whether the impairment to exercise performance is due to hyperthermia *per se*, or hypohydration *per se*, or a combination of hypohydration and hyperthermia. Concurrently, the perspective that hypohydration is the primary factor precipitating impaired endurance performance in hot conditions has been challenged with two alternative hypotheses: (i) Critical core temperature hypothesis and (ii) Circulatory strain hypothesis.

In a review of long distance running literature, the runners' core temperatures were in the range of 38 to 40.8°C as they completed the marathon / distance running races in varied environmental settings from 6 to 32°C (Chevront & Haymes, 2001b). It is unknown why some runners can tolerate such a high core temperature and completed the marathon races, but some runners tend to terminate exercise as core temperature nears 40°C (González-Alonso *et al.*, 1999b). Meanwhile, the increase in intracellular temperature will induce a heat shock protein 72 (HSP 72) response (Holtzhausen *et al.*, 1994). There are several reports documented that HSP 72 affords protection to heatstroke and also acts as a biomarker of multiple organ tissue damage (Huisse *et al.*,

2008; Ruell *et al.*, 2009; Dehbi *et al.*, 2010). Ravindran *et al.* (2005) found that resistance to dehydration damage in some cell lines correlates with the presence of a range of HSPs. The key question is whether these changes are causally linked; *does prolonged exercise in hot conditions coupled with hypohydration induce a heat shock protein response?* The ingestion of glutamine supplementation has been shown to induce HSP expression during hyperthermia (Ziegler *et al.*, 2005) and provide an ergogenic effect during dehydration (Hoffman *et al.*, 2010). Another question to be answered is *whether glutamine ingestion effectively enhances HSPs expression together with the restoration of fluid in dehydrated subjects during exercise in the heat.*

The Review of Literature in Chapter 2 surveys our current knowledge of the circulatory and thermoregulatory responses during prolonged exercise in the heat coupled with hypohydration and identifies avenues of enquiry that remain to be investigated. To further understand the effects of hypohydration and thermotolerance during prolonged exercise performance in hot climatic conditions, four studies were designed to investigate the underlying physiological mechanisms. Chapter 3 describes the research thesis design and the hypotheses and mechanisms tested as part of this thesis. Chapter 4 describes the common methodology which has been employed in this thesis. Chapter 5 investigates the hydration status of elite Kenyan distance runners competing in hot, humid conditions. Chapter 6 employs a specially constructed vest with pockets to insert sandbags of known weight, which were used to simulate the increase in bodyweight associated with simulated hyperhydration. In the same chapter hypohydration is induced via a prolonged walking protocol to attain a significant body weight deficit as a result of sweating. In Chapter 7 the effect of diuretic-induced dehydration on prolonged running performance in hot and cool climatic conditions is investigated. Chapter 8 investigates the effect of alanyl-glutamine ingestion on prolonged running performance

in hot and hypohydrated conditions. Finally, the key findings and recommendations are presented in Chapter 9.

## **CHAPTER 2**

### **LITERATURE REVIEW**

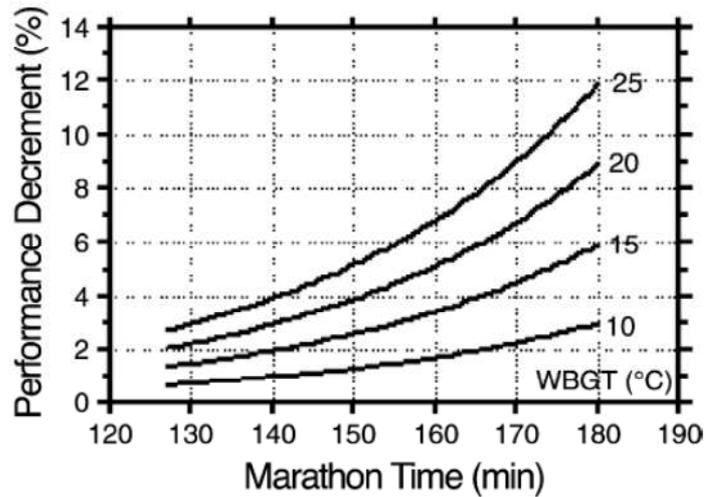
## **2.1 THE IMPACT OF WEATHER CONDITIONS ON PROLONGED EXERCISE PERFORMANCE**

Well trained marathon runners maintain an average running velocity that is equivalent to approximately 75 - 85%  $\dot{V}O_{2max}$  during marathon races (Davies & Thompson, 1979; Maughan & Leiper, 1983). The maintenance of such prolonged, high intensity exercise results in a considerable metabolic heat load that ultimately leads to a rise in body core temperature. This endogenous heat production is proportional to the velocity of running, so that there is a linear relationship between the intensity of running expressed in terms of %  $\dot{V}O_{2max}$  and rectal temperature (Davies, 1979). The magnitude of endogenous heat production in long distance races is approximately 10 times higher than at rest (Havenith, 2001). Associated with this high heat production is heat storage which must be regulated to avoid heat related illness and collapse. The so-called “prescriptive zone” describes a range of exercise intensities and ambient temperatures where body core temperature is well controlled with heat storage being limited by the rate of heat loss (Nielsen, 1938; Lind, 1963; Davies, 1979). As climatic conditions restrict the rate of heat loss, and / or the metabolic heat production increases, the athlete is in danger of moving beyond the “prescriptive zone”, with heat loss mechanisms unable to maintain thermal equilibrium. Thus marathon running performance is partly governed by climatic conditions (high ambient temperatures and high humidity) that restrict the rate of heat loss. High ambient temperatures reduce the temperature gradient from the body core to the surrounding environment, while high humidity impairs sweat evaporation. Under these conditions the only option for the athlete is to slow the rate of metabolic heat storage which requires a decrease in metabolic heat production by decreasing running velocity. This reduction in running velocity has been shown in a series of reports linking marathon race finishing times with climatic conditions (Trapasso & Cooper, 1989; Zhang *et al.*, 1992; Ely *et al.*, 2007; Ely *et al.*, 2008; Vihma, 2010).

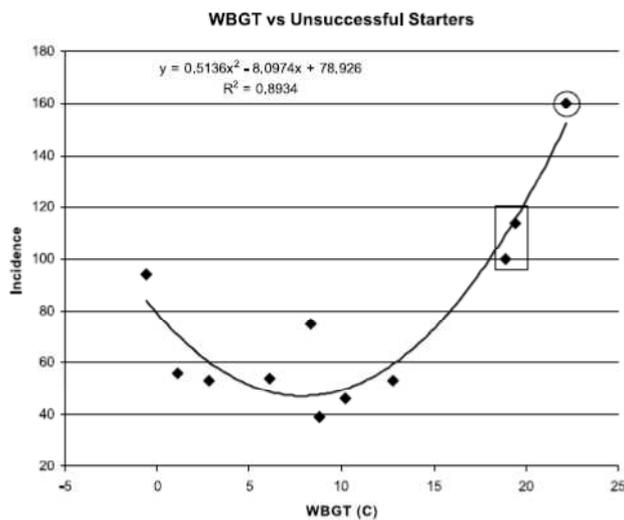
Although these reports tend to omit measures of sweating, hypohydration, convective airflow, changing terrain, radiant heat gain and more importantly relative humidity, they do suggest an ambient temperature of 8-15°C is an optimum 'air temperature' range for the conduct of marathon races (Zhang *et al.*, 1992).

Vihma (2010) analysed 28 years of meteorological reports linking finishing time results of runners completing the annual Stockholm Marathon (1980 to 2008). The meteorological data was comprehensive, including air and dew point temperature, ambient humidity, wind speed, occurrence of rain and solar radiation. The finishing time results for elite, intermediate and slower male and female runners showed that ambient /air temperature was significantly correlated with the finishing time in both fast and slower runners ( $r=0.66-0.73$ ). Similarly, Ely *et al.* (2007) constructed a nomogram to show the projected deficit in marathon finishing time relative to the Wet Bulb Globe Temperature index (WBGT) (Figure 2.1). The rise in the WBGT from 5°C to 25°C had an adverse impact on running performance in both men and women finishers across a wide-range of performances (fast and slow runners). These observations were in agreement with a controlled laboratory based study that concluded the optimal temperature for endurance exercise performance was 11°C compared with three other climatic conditions:  $3.6 \pm 0.3^\circ\text{C}$ ,  $20.6 \pm 0.2^\circ\text{C}$ , and  $30.5 \pm 0.2^\circ\text{C}$  with a relative humidity of  $70 \pm 2\%$  and an air velocity of approximately  $0.7 \text{ m}\cdot\text{s}^{-1}$  (Galloway & Maughan, 1997). Subjects rode a stationary exercise bicycle at 70% of their  $\dot{V}\text{O}_{2\text{max}}$  in 11°C and it was observed that their endurance time was ~30 min longer than when exercising in an ambient temperature of 31°C. Galloway and Maughan (1997) observed that prolonged cycle exercise was markedly reduced in the heat and was associated with a higher heart rate response and greater thermoregulatory strain (e.g., increased rectal

and weighted mean skin temperatures) when compared with other trials conducted in 3.6°C and 20°C.



**Figure 2.1** Nomogram showing the potential performance decrement (y-axis) based on projected marathon finishing time (x-axis) with increasing WBGT (Ely *et al.*, 2007)



**Figure 2.2** The 12 annual races of the Twin Cities Marathon from 1997 to 2008 showing unsuccessful runners per 1000 finishers plotted against start WBGT shows increasing risk with WBGT above 13°C. About 100-120 unsuccessful starters per 1000 finishers is borderline for a mass casualty incident (i.e. an event that produces more patients than available resources, such as ambulances and emergency room beds (Roberts, 2010)

More recently, Roberts (2010) conducted a retrospective review of the number of marathon race starters, finishers, and those with a documented medical problem on finishing the Twin Cities Marathon from 1997 to 2008. The study collated the WBGT with the incidence of medical problems and withdrawals from the race to estimate a “do not start” WBGT. The data indicates that when WBGT was  $>13^{\circ}\text{C}$ , the number and rate of finish line medical encounters and on-course marathon drop-outs begins to rise (Figure 2.2). The sum of the race dropouts and the race finish line medical encounters provides an indirect measure of heat stress encountered by the runners. Roberts (2010) concluded that endurance performance was impaired with increases in WBGT and that this effect was more pronounced in slower runners.

The aforementioned field studies on marathon runners and climatic conditions consistently reported that warm weather has a significant adverse impact on the endurance performance of these runners. However the now deceased Samuel Wanjiru from Kenya, the gold medallist of the Beijing Olympic Marathon 2008 ran 2 hours 6 minutes and 32 seconds in temperatures at least  $10^{\circ}\text{C}$  higher than typically observed in the Major Marathon Series which challenges the empirical evidence linking weather conditions with endurance performance. The average ambient temperatures during the Beijing Olympic Marathon ranged from  $22\text{-}27^{\circ}\text{C}$  with 65-80% rh, reported by the China Meteorological Administration (CMA). It seems that the warm weather had little or no effect on Wanjiru’s running performance, as he was able to maintain his performance by breaking the Olympic record by 2 minutes and 49 seconds. This performance was 7 seconds faster than his personal best time run in the Fukuoka Marathon 2007. Similarly, Naoko Takahashi, the winner of Sydney Olympic Woman’s Marathon in 2000 completed her race in  $35^{\circ}\text{C}$ , 55% rh climatic conditions in a time of 2 hours 23 minutes and 14 seconds which was 3 min ( $\sim 2.4\%$ ) slower than her personal best performance

time in much cooler conditions. Wanjiru and Takahashi's remarkable marathon victory in Beijing and Sydney respectively were not compatible with the nomogram (Figure 2.1) constructed by Ely *et al.* (2007). Therefore there is a need for more controlled laboratory studies that seek to understand the underlying mechanisms enabling the successful runners to perform well in marathon races despite the prevailing-hot climatic conditions.

## 2.2. RUNNING ECONOMY: EFFECTS OF ACUTE CHANGE IN BODY WEIGHT

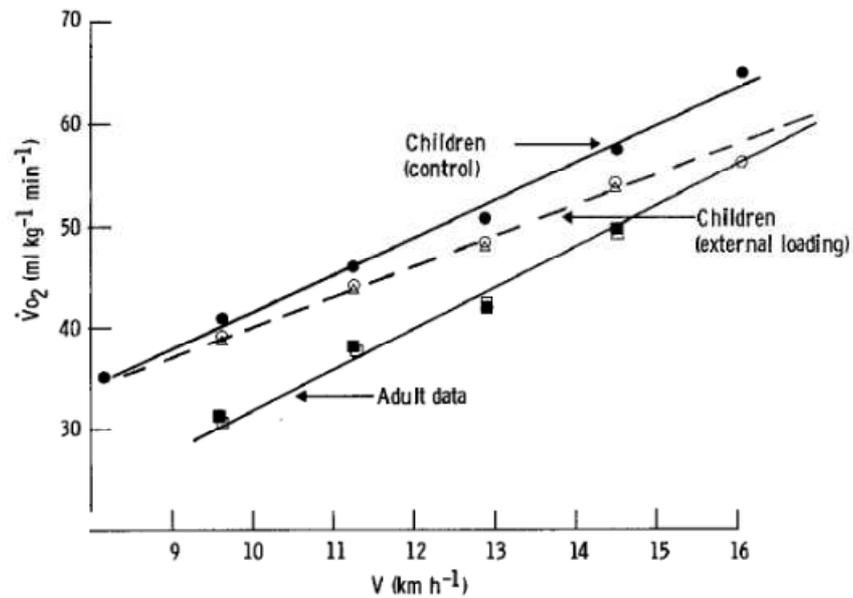
There are two primary goals in competitive distance running, (i) is to finish ahead of all other competitors and (ii) is to run the race as fast as possible. Seventy years ago Hill (1939) showed that for optimum conversion of chemical energy to mechanical work, the force applied and speed of movement in frog muscle must be matched so that force is one half and speed is one quarter of their respective maximal values. In humans, physical exercise is relatively inefficient with approximately 20 - 25% of the energy utilized producing mechanical work while the remaining energy produces heat within the active muscles (Gisolfi & Mora, 2000). If the energy utilization when running at a given velocity can be reduced, the time to fatigue will be delayed and the running velocity that can be sustained may potentially be increased. Thus minimizing the energy cost of running will enhance performance and as such is recognized as an important attribute for success as a distance runner.

Running economy (RE) is defined as the steady state oxygen uptake ( $\dot{V}O_2$ ) in terms of milli-litres per kilogram of bodyweight per minute ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) for a given submaximal running velocity. The metabolic energy demands for RE can be quantified by measuring the steady state  $\dot{V}O_2$ , which can be readily attained within 3 minutes of constant velocity running (Whipp & Wasserman, 1972). RE together with the athlete's maximum capacity to utilize oxygen ( $\dot{V}O_{2\text{max}}$ ) are two important measures that have been used to differentiate the more successful distance runners (Conley & Krahenbuhl, 1980; di Prampero *et al.*, 1986; Morgan *et al.*, 1989b; Saunders *et al.*, 2004; Sawyer *et al.*, 2010), with some researchers suggesting RE is a better predictor of endurance performance than  $\dot{V}O_{2\text{max}}$  (Daniels, 1985; Morgan *et al.*, 1989a; Daniels & Daniels, 1992; Morgan & Craib, 1992). The running velocity at  $\dot{V}O_{2\text{max}}$  combines both RE and

the athlete's  $\dot{V}O_{2\max}$  and as such is a common measure employed in making predictions about the endurance performance potential of a distance runner. Further factors e.g., physiology, biomechanics, training, environment and anthropometry appear to influence RE in well trained distance runners. Early research on distance running performance tested RE by measuring steady state oxygen uptake relative to body mass (oxygen cost per kilogram of body mass) at a given running velocity (Henry & DeMoor, 1950; Mayhew, 1977). Further research proposed that submaximal and maximal  $\dot{V}O_2$  attained during running should preferably be expressed as  $\text{mL.kg}^{-0.75}.\text{min}^{-1}$  rather than  $\text{mL.kg}^{-1}.\text{min}^{-1}$  in order to minimize the influence of body mass on  $\dot{V}O_2$  (Bergh *et al.*, 1991). Other studies have found that expressing RE in terms of the oxygen cost to cover a given distance ( $\text{mL.kg}^{-1}.\text{km}^{-1}$ ) rather than relative oxygen uptake ( $\text{mL.kg}^{-1}.\text{min}^{-1}$ ) is better in reflecting differences in oxygen cost during different velocities of running performance (Lucia *et al.*, 2006; Foster & Lucia, 2007). A further permutation is that of expressing RE in terms of caloric unit cost ( $\text{kcal.kg}^{-1}.\text{km}^{-1}$ ), which is sensitive to changes in running velocity when compared with  $O_2$  unit cost or  $\dot{V}O_2$  normalized per distance traveled ( $\text{mL.kg}^{-1}.\text{km}^{-1}$ ) (Fletcher *et al.*, 2009). This approach further considers the negative effect on RE when fat as an energy substrate is reflected in the increased oxygen utilization ( $\dot{V}O_2$ ,  $\text{L.min}^{-1}$ ). Body mass seems to be an important variable for these different approaches to determine RE, and as such a small change in body mass may significantly affect running economy.

A number of investigators have also documented the effect of external load on RE. When external weight is added to the torso of the body using a weighted jacket the oxygen uptake per kg of gross mass has been found to decrease both in children (Thorstensson, 1986; Davies, 1980) and in adults (Thorstensson, 1986). In the study by Davies (1980) a weighted jacket was used to increase gross weight by 5

and 10% of bodyweight in 23 children (12 - 13 years) who undertook 'steady state' treadmill running (8 – 16 km.h<sup>-1</sup>). The metabolic cost ( $\dot{V}O_2$  ml.kg<sup>-1</sup>.min<sup>-1</sup>) of running with added weight around the trunk expressed as a linear regression line showed a reduced slope when weight was added to the trunk. Thus when the  $\dot{V}O_2$  values were corrected for total mass (body mass plus the mass of the weight jacket) there was an apparent improvement in running economy which was independent of stride frequency and stride length (Figure 2.3). Thus, it was shown that the  $\dot{V}O_2$  (ml. kg<sup>-1</sup>. min<sup>-1</sup>) cost of running with the added weight at 14 to 16 km.hr<sup>-1</sup> was lower when compared with the unloaded condition. However, as these children were running at lower running speeds (e.g., 9 km.hr<sup>-1</sup>), no additional aerobic demand was required with the increasing external load from 5 to 10% of body weight. Davies (1980) concluded that in young athletic children with low bodyweight and a relatively high  $\dot{V}O_{2max}$  (ml. kg<sup>-1</sup>. min<sup>-1</sup>), the frequency of leg movement was not optimally matched to the force necessary to produce the most economic conversion of aerobic energy into mechanical work. Thorstensson (1986) similarly used vertical loading (weight jacket) and also showed a decrease in the  $\dot{V}O_2$  (ml. kg<sup>-1</sup>. min<sup>-1</sup>) cost of loaded running in children. However, in contrast to Davies (1980), Thorstensson (1986) also observed a decrease for the adult subjects, which was of a smaller magnitude than that observed in the children.



**Figure 2.3** The effect of added weight in improving the  $\dot{V}O_2$  cost of running in boys (aged 12-13 years). This illustrates that with added vertical load equivalent to 5% and 10% of bodyweight, there is not a proportional increase in  $\dot{V}O_2$  that might be expected (Davies, 1980)

Contrary to the findings of Davies (1980) and Thorstensson (1986), many studies have reported that an increase in body mass or external loading results in an increase in the energy cost of locomotion at submaximal velocities (Buskirk & Taylor, 1957; Cureton *et al.*, 1978; Cureton & Sparling, 1980; Jones *et al.*, 1986; Epstein *et al.*, 1987; Cooke *et al.*, 1991; Teunissen *et al.*, 2007). The discrepancy in the reports on the effect of external loading is likely to be related to the application of the external load with some studies using ankle weights and others using weight jackets. Cureton and Sparling (1980) reported excess body fat decreases performance in prolonged distance running by increasing the ratio of fat mass to fat free mass, thus requiring an increase in muscular activity to undertake running exercise with added fat mass. Women runners in the aforementioned study utilized more oxygen per unit of fat-free weight during submaximal running velocities compared with adult male runners. Jones *et al.* (1986) reported that the energy cost was increased 1% every 100 g increase in a pair of running shoes. The metabolic cost of running increased linearly when carrying an external

backpack weighing from 10 to 30 kg (10 kg increments) (Epstein *et al.*, 1987). Another study by Cooke *et al.*, (1991) investigated the effect of vertical and horizontal loading on oxygen uptake during treadmill running. They found horizontal loading with a weight jacket (5 and 10% body mass) significantly increased the  $\dot{V}O_2$  cost of the exercise. More recently Teunissen *et al.* (2007) reported that net metabolic rate increased by ~5% when subjects ran with an additional load equivalent to 10% of normal body mass compared with an unloaded trial.

Over the past four to five decades, Kenyan distance runners have dominated the Olympic Games, World Cross-Country Championships, IAAF World Track Championships, as well as major international road races and marathons. Several factors have been proposed to explain the extraordinary success of Kenyan distance runners, including diet and fluid intake before and during training. Kenyan runners were observed to be in negative energy balance with a low fluid intake regimen during intense training and prior to competition resulting in a short term reduction in body mass (Onywera *et al.*, 2004; Fudge *et al.*, 2006). Fudge *et al.* (2007) observed Kenyan distance runners and noted that they ingested ~1.1 L of plain water and ~1.2 L milky tea on a daily basis. Despite sweat loss associated with intense bouts of running the elite Kenyan distance runners do not appear to consume a significant volume of fluid before or during training to offset body water loss in the form of sweat. A review by Coyle (2004) suggested that a loss of body mass through sweat loss during marathon races may lower the oxygen cost of running. However, an extensive search of the research literature revealed only one study (Armstrong *et al.*, 2006) that investigated the question of hypohydration influencing running economy. Armstrong *et al.* (2006) found no effect of 5% hypohydration on the running economy of competitive distance runners exercising on a motor-driven treadmill at 70 and 85%  $\dot{V}O_{2max}$  in a euhydrated versus

hypohydrated state. While running economy did not change in the hypohydrated condition, the runners exhibited increased physiological strain despite the relatively moderate ambient temperature of 23°C. More recently, a study by Zouhal *et al.*, (2010) reported a significant inverse relationship between the degree of BW loss and race finishing time for a marathon race event (Zouhal *et al.*, 2010). Finishers who completed the race in <3 hours experienced a >3% BW deficit whereas finishers who required >4 hours to complete the race experienced <2% BW deficit. Therefore it could be speculated that the success of Kenyan runners may be related to a reduced body mass through sweat loss that would have the potential to reduce the energy cost of running. While there may be an intuitive link between a reduction in body mass (hypohydration) and energy cost of running, research to date has not established a clear relationship between improved RE as a consequence of hypohydration.

## **2.3 THERMOREGULATION DURING PROLONGED EXERCISE PERFORMANCE IN THE HEAT**

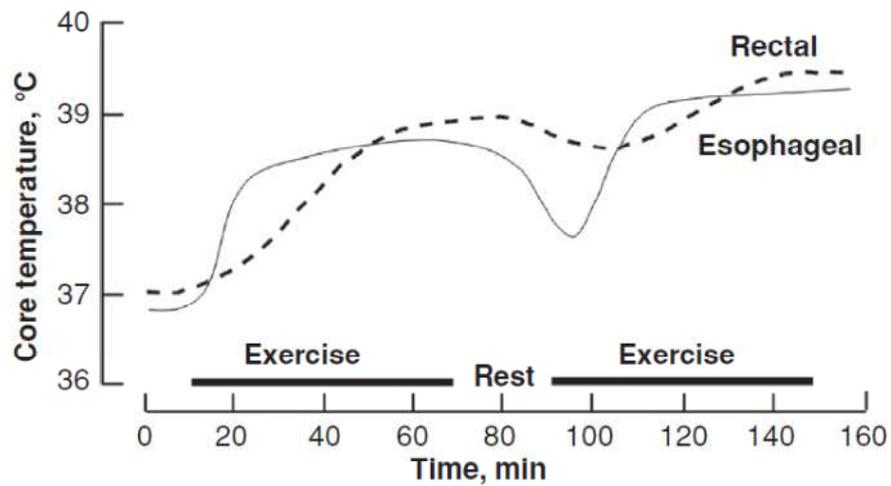
During exercise, skeletal muscle temperature increases and creates an increased temperature gradient between the active muscle, skin and prevailing ambient temperature. This conductance of metabolic heat may occur directly from deep within the active muscle to the skin and is sometimes referred to as “fixed conductance”. Increased muscle blood flow results in the transport of heat away from the active muscle and is sometimes referred to as “variable conductance” as it will change with the many regulatory systems governing blood flow (Hensel, 1973; Nadel, 1983). As the body core temperature ( $T_{\text{core}}$ ) increases, the heat produced is transported from the core to the skin surface through convection and from the surface of the skin to the environment mainly via sweat evaporation for heat dissipation (Nadel, 1983). Meanwhile skin blood flow (SkBF) is elevated 20-25-fold to facilitate heat dissipation and prevent progressive hyperthermia. Thus the exchange of heat from the skin to the environment is primarily via sweat evaporation and peripheral displacement of hot blood (Sawka *et al.*, 2011).

### **2.3.1 Core Temperature Measurements**

The measurement of internal body / core temperature ( $T_{\text{core}}$ ) is essential for the study of human thermoregulation. There are a number of sites used for measuring  $T_{\text{core}}$ : oral, tympanium, esophageal, gastrointestinal tract and rectum. Thermal physiologists should always consider the following requirements for deciding on the most appropriate site to measure  $T_{\text{core}}$ : (i) convenient and harmless; (ii) unbiased by environmental conditions; and (iii) the measured changes should quantitatively reflect small changes in arterial blood temperature (Shiraki *et al.*, 1986).

Esophageal temperature ( $T_{es}$ ) is measured by inserting a thermistor probe through the nose into the throat, and then swallowed so that the end of the thermocouple or thermistor probe is approximately level with the heart. The catheter length is approximately 25% of person's height. A topical analgesic gel is used on the catheter to reduce discomfort. The advantage of using  $T_{es}$  as a relatively non-invasive index of  $T_{core}$  for humans is the rapid response of  $T_{es}$  to a change in blood temperature adjacent to the heart (Shiraki *et al.*, 1986). The rapid response time for  $T_{es}$  is due to the low capacity of the esophagus and its proximity to the left atrium (Saltin & Hermansen, 1966).

Rectal temperature ( $T_{re}$ ) is widely employed by physiologists because it is a relatively non-invasive stable measurement site. A rectal thermistor or thermocouple is inserted within the rectum to approximately ~10 to 12 cm beyond the anal sphincter (ranging from 5-27 cm) (Nielsen & Nielsen, 1962). It provides a measure representative of the temperature of a large mass of deep body tissue. The insertion depth influences the  $T_{re}$  value measured by as much as 0.8°C (Mead & Bonmarito, 1949). The  $T_{re}$  sensor may slip to less than 5 cm beyond the anal sphincter during exercise; however, this problem can be solved by attaching a bulb / bead to the rectal thermistor at the desired depth that will hold the sensor in position. Figure 2.4 shows the  $T_{re}$  and  $T_{es}$  measurements during two exercise bouts separated by a 20 min rest period (Sawka *et al.*, 1988). During exercise and resting period, both  $T_{re}$  and  $T_{es}$  demonstrate similar patterns but  $T_{re}$  responds slower and slightly higher (~0.2 °C) than  $T_{es}$ . The measurement of  $T_{re}$  takes approximately 25-40 min to achieve steady state. (Nielsen & Nielsen, 1962; Saltin *et al.*, 1972) probably due to a lower rate of blood flow to the rectum compared to other measurement sites (Aulick *et al.*, 1981).



**Figure 2.4** Rectal and esophageal temperature responses to rest and exercise in the heat (Sawka *et al.*, 1988)

Tympanic temperature is measured by inserting a small sensor into the ear to touch to the tympanum, which reflects the temperature of blood in the internal carotid artery. The contact made between the thermocouple and the tympanic membrane can be very painful and uncomfortable.

An infrared thermometer is another alternative method to measure tympanic temperatures (Shinozaki *et al.*, 1988) although it is difficult to be confident that the alignment of the thermometer is measuring the tympanic temperature and not the skin surface within the ear. Since tympanic temperature values can be influenced by the ambient temperature, inner ear skin temperature and facial skin temperature (Greenleaf & Castle, 1972; McCaffrey *et al.*, 1975), caution is needed in accepting the temperature as a true reflection of core temperature.

Gastrointestinal (GI) temperature is measured using a swallowed “radio pill” to monitor continuous  $T_{core}$ . A sensor ingestion time of 6 hours before data collection is required to avoid both temperature fluctuations in the upper GI tract and sensor expulsion before

data collection (Lee *et al.*, 2000). Variation in sensor / pill mobility or location along the GI tract, the presence of water or food in the stomach and the temperature of ingested fluid will influence the GI temperature value (Kolka *et al.*, 1993; Byrne & Lim, 2007; Wilkinson *et al.*, 2008). Kolka *et al.* (1993) reported that GI temperature reached steady state faster than  $T_{re}$  while the response to changes in body temperature was slower than  $T_{es}$ . An advantage of the ingestible sensor is its wireless function compared with an indwelling rectal probe to measure  $T_{core}$ . Moreover,  $T_{core}$  measurements on a large group can be obtained simultaneously during the same event (Byrne *et al.*, 2006; Laursen *et al.*, 2006).

The reliability and validity of commonly used temperature devices to estimate core temperature during exercise in the heat was studied by Ganio *et al.* (2009) and Huggins *et al.* (2012). Ganio *et al.* (2009) found that a forehead sticker, oral temperature, temporal temperature, aural temperature and axillary temperature did not provide valid estimates of rectal temperature during indoor exercise in the heat. Intestinal temperature was the only measurement considered valid when compared with rectal temperature (the “gold standard”). Aural temperature underestimated core temperature when compared with rectal temperature (Huggins *et al.*, 2012). It is imperative to use an accurate and reliable method to assess  $T_{core}$  in hyperthermic exercising individuals. An improper assessment of  $T_{core}$  can lead to misdiagnosis of exertional heat stroke and possibly death.

### 2.3.2 Evaporative Heat Loss: Sweating

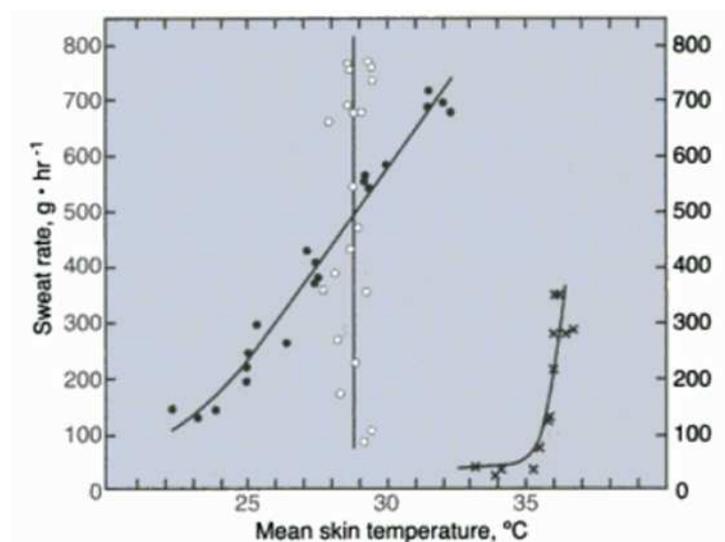
The ability to endure exercise in the heat depends on the effectiveness of muscle blood flow in delivering oxygen to contracting muscles and transporting heat from the exercising muscles to the skin, and ultimately the environment. Therefore, an optimal rate of sweating is crucial for heat dissipation via evaporative heat loss. Sweat glands are located within the dermis for sweat formation (Jenkins, 1986) and primarily serve this thermoregulatory function. Sweating appears to be under both sympathetic nervous control and humoral stimulation of  $\beta$ -adrenergic receptors on sweat glands. Sweating can be initiated by epinephrine (adrenalin) release in advance of any stimulus related to an increase in core temperature (sympathetic nervous control) (Pilardeau *et al.*, 1979). Sweat evaporation enhances heat dissipation by providing a cooling effect and thus contribute to heat balance / thermal equilibrium, especially when the ambient temperature is greater than 36°C. However, when the relative humidity and ambient temperature are both high, the evaporative cooling requirement ( $E_{\text{req}}$ ) will exceed the maximal evaporative cooling ( $E_{\text{max}}$ ) capacity. Therefore, sweat evaporation and heat loss under conditions of high ambient temperature and humidity will be relatively ineffective as sweat produced saturates clothing and drips to the ground.

#### **Threshold and Sensitivity of Sweating: Skin temperature vs. Core Temperature**

Body temperature is regulated by both central and peripheral thermal receptors which provide afferent input (e.g., core and skin temperatures) to the anterior hypothalamus, resulting in effector responses (e.g., sweating and SkBF) being initiated (Nadel *et al.*, 1971a; Hensel, 1973; Boulant, 2006). The effector response for heat dissipation is proportional to the displacement in both core and skin temperatures (Stolwijk & Hardy, 2010). Early studies observed that an elevation of  $T_{\text{sk}}$  initiated the sweating response

associated with a constant  $T_{\text{core}}$  (Nadel *et al.*, 1971a; Wenger *et al.*, 1975; Wenger & Roberts, 1980). Therefore it was thought that  $T_{\text{sk}}$  was more important in the control of sweating rather than internal temperature (rectal, oesophageal, tympanic) (Wyndham, 1973).

Stolwijk and Hardy (1966) and Nielsen *et al.* (1969) provided evidence that  $T_{\text{sk}}$  plays an important role in the regulation of sweating (Figure 2.5), and is not solely influenced by  $T_{\text{core}}$  (Benzinger & MD., 1967). Their data demonstrated that sweating was being evoked by alterations of  $T_{\text{sk}}$  while tympanic temperature remained unchanged, or declined, or increased (Stolwijk & Hardy, 1966). Nadel *et al.* (1971) also showed that  $T_{\text{sk}}$  can alter the degree of internal body temperature drive to the sweating mechanism. A low  $T_{\text{sk}}$  has an inhibitory effect on sweating whereas a high  $T_{\text{sk}}$  leads to a rise in  $T_{\text{core}}$  to maintain an effective temperature gradient between core and skin.



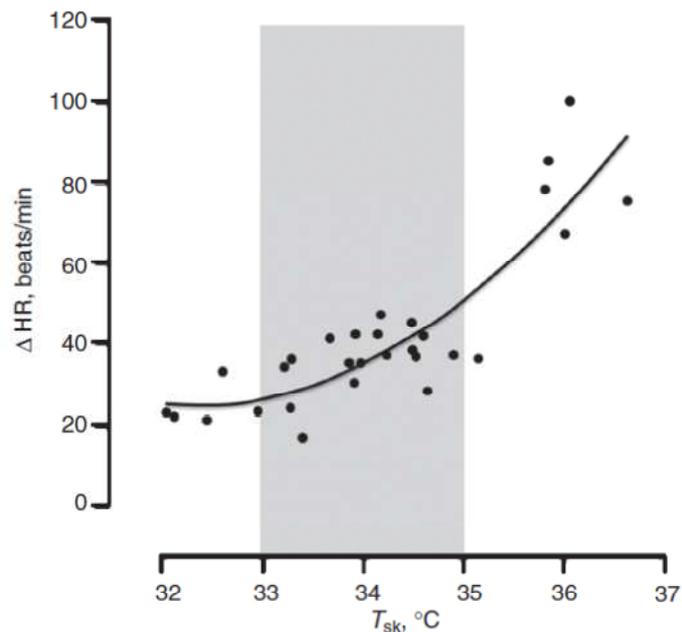
**Figure 2.5** Steady-state values of sweat rate plotted against the corresponding mean skin temperature values (Nielsen, 1969)

Open circle = exercise intensity from 90 to 235 W at constant environmental temperature of 20°C; Closed circle = constant exercise intensity (150W) at environmental temperatures from 5 to 30°C; x = experiments at rest at environmental temperatures from 25 to 44°C

It is generally agreed that  $T_{sk}$  is directly related to ambient temperature (Pedersen, 1969; Mairiaux *et al.*, 1987) and the changes in  $T_{sk}$  modify the central drive to sweat rate and peripheral blood flow (Davies, 1979). The level of  $T_{sk}$  influences the heat transfer by convection and radiation, while heat loss from sweat evaporation is determined by the saturated vapour pressure at the skin surface. With exercise in hot and humid climatic conditions, the water vapor pressure gradient between skin and air is lower as the skin-to-ambient temperature gradient is reduced. The  $T_{sk}$  increases due to the inability to evaporate sweat efficiently, however this will ultimately increase vapor pressure at the skin and facilitate the evaporation of sweat. The amount of heat transported to the skin surface by the SkBF must be exactly equal to the amount of heat which is given off by the evaporation of sweat to prevent an undesirable elevation in body heat storage. A high  $T_{sk}$  promotes pooling of blood in the peripheral vessels (Rowell *et al.*, 1971) and this vasoconstriction response might play a role in limiting the impairment of cardiac filling during exercise in the heat. If vasoconstriction occurs with an increasing demand for muscle blood flow during exercise, then  $T_{sk}$  will tend to fall, therefore inhibiting the increase in sweating while  $T_{core}$  will be elevated (Pedersen, 1969).

Pugh *et al.* (1971) and Davies (1979) found that intense prolonged exercise is limited when  $T_{sk}$  exceeds 28°C. Chevront *et al.* (2003b) further demonstrated the impact of warm-hot  $T_{sk}$  at constant  $T_{re}$  of ~ 37.5°C elevated HR during exercise (Figure 2.6). The  $T_{sk}$  was manipulated by wearing a chemical protective clothing ensemble and a liquid cooling garment which enabled stepwise increases in  $T_{sk}$  while maintaining a constant unchanged  $T_{re}$  across experimental trials. The results showed that HR rose exponentially with the increase of  $T_{sk}$  beyond ~ 35°C during 80-min of treadmill walking (4.9 km.hr<sup>-1</sup>) in a warm and dry climate (dry bulb temperature ~29.8°C; 30% rh). An equivocal observation of a constant  $T_{core}$  associated with  $T_{sk}$  elevations from 31

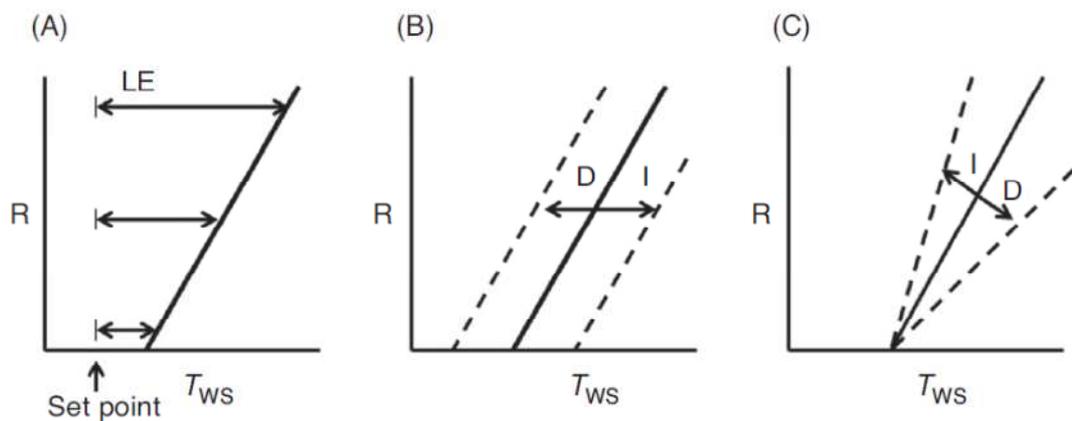
to 36°C was reported by Ely *et al.* (2010). Aerobic exercise performance in the heat was degraded by a high  $T_{sk}$  which was likely due to the redistribution of blood from the central to peripheral circulation (Rowell, 1986), as a consequence of an elevated SkBF (Sawka *et al.*, 2011).



**Figure 2.6** Influence of a skin cooling paradigm on heart rate with a constant core temperature during light-intensity treadmill walking exercise (Cheuvront *et al.*, 2003b)

On the contrary, Nadel and colleagues (1971) reported an increase in sweat rate was relative to the increase in internal temperatures at a fixed mean  $T_{sk}$ . This relationship was subsequently supported by other studies (Nadel *et al.*, 1974; Gisolfi & Wenger, 1984). Gisolfi and Wenger (1984) outlined the relationship between sweating rate and internal body temperature in a review paper. Figure 2.7 shows forcing function analyses with linear plots of individual mean body temperature and associated effector (e.g., sweating or SkBF) responses (A) to an increasing “load error” (B) and show a shift in threshold temperature or sensitivity (C) might appear (Gisolfi & Wenger, 1984). Threshold temperature shifts are often interpreted as reflecting central nervous system-

mediated changes in set point, while sensitivity shifts (slope of the sweating-body temperature relationship) are often interpreted as changes in peripheral input to the thermoregulatory controller (Bregelmann *et al.*, 1994; Cheuvront *et al.*, 2009). An elevation in the internal body temperature threshold during the onset of sweating and / or an attenuation of the increase in sweating relative to the increase in internal body temperature is known as impaired sweating responsiveness.



**Figure 2.7** Schematic diagram showing the idealized effector response, (e.g., sweating rate and SkBF) to increasing  $T_{ws}$  using forcing function analysis with linear plots: (A) Set point and the threshold  $T_{ws}$ , that elicits an effector responses, R. R Changes linearly with the load error (LE), or difference between the set point and  $T_{ws}$ ; (B) Idealized (parallel shift) increase (I) or decrease (D) in threshold temperature suggesting change in “set point” ; (C) Idealized increase or decrease in gain (slope or sensitivity) suggesting peripheral modification;  $T_{ws}$ , A weighted sum of tissue temperatures according to their relative contributions to control of thermoregulatory responses; R, response; D, decrease; I, increase; LE, load error (Gisolfi & Wenger, 1984)

### The Regulation of Sweating: Skin Temperature and Skin Blood Flow

Sweating and increased SkBF are the principal avenues of heat loss during prolonged exercise in the heat. It is well accepted that local warming of the skin increases SkBF and increases sweating rate (MacIntyre *et al.*, 1968; Nadel *et al.*, 1971a; Nadel *et al.*, 1971b), whereas local cooling decreases both SkBF and sweating rate (Van Beaumont & Bullard, 1965; Bullard *et al.*, 1967; Nadel *et al.*, 1971b; Crawshaw *et al.*, 1975;

Shibasaki *et al.*, 2006). However, it remains unclear whether the magnitude of the reduction in SkBF associated with local cooling and the underlying mechanism for attenuation of sweating by local skin cooling is a synergistic relationship. Wingo and colleagues (2010) evaluated the independent roles of SkBF and local  $T_{sk}$  on sweating rate by manipulating SkBF using vasoactive agents and measuring SkBF and sweating responses throughout whole body heating. The data demonstrated that local cooling attenuates sweating by independent effects of decreased SkBF and decreased local  $T_{sk}$  (Wingo *et al.*, 2010). Both low SkBF and local  $T_{sk}$  independently decrease sweating during whole body sweating. Wingo and colleagues (2010) suggest that the cooler areas of skin minimise excessive sweating and SkBF and might contribute to an increased central blood volume (BV) reserve. This may be a possible mechanism to explain why cooler environments have regularly been shown to be associated with superior marathon running performances.

## 2.4 HYPOHYDRATION DURING PROLONGED EXERCISE PERFORMANCE IN THE HEAT

### 2.4.1 Hydration

#### Hydration Terminology (Greenleaf, 1992)

<b>Euhydration:</b>	normal daily water variation
<b>Hyperhydration:</b>	new steady-state of increased water content
<b>Hypohydration:</b>	new steady-state of decreased water content
<b>Dehydration:</b>	process of losing water either from the hyperhydrated state to euhydration, or from euhydration downward to hypohydration
<b>Rehydration:</b>	process of gaining water from a hypohydrated state toward euhydration

Hydration is integral to the health and well being of humans. The human body needs water for anatomical and physiological functions, such as providing mass and form to the body, functioning as the medium and reagent for metabolic reactions, joint lubrication, transportation of substrate and also as the primary means for body heat dissipation (McArdle *et al.*, 2010). The total body water (TBW) in the human body can be compartmentalized into intracellular fluid (ICF) which represent approximately 60% of TBW and extracellular fluid (ECF), 40% of TBW, with ECF further divided into interstitial fluid and plasma volume (PV). ICF refers to fluid inside the cells, whereas ECF refers to fluids that flow within the microscopic spaces between cells (interstitial fluid). ECF provides most of the fluid lost through sweating, predominantly from blood plasma. Note that hemoconcentration occurs within the initial 10 min of upright exercise which causes a significant decrease in PV with minimal sweat production. Other factors such as a change in posture (Diaz *et al.*, 1979) or exercise intensity

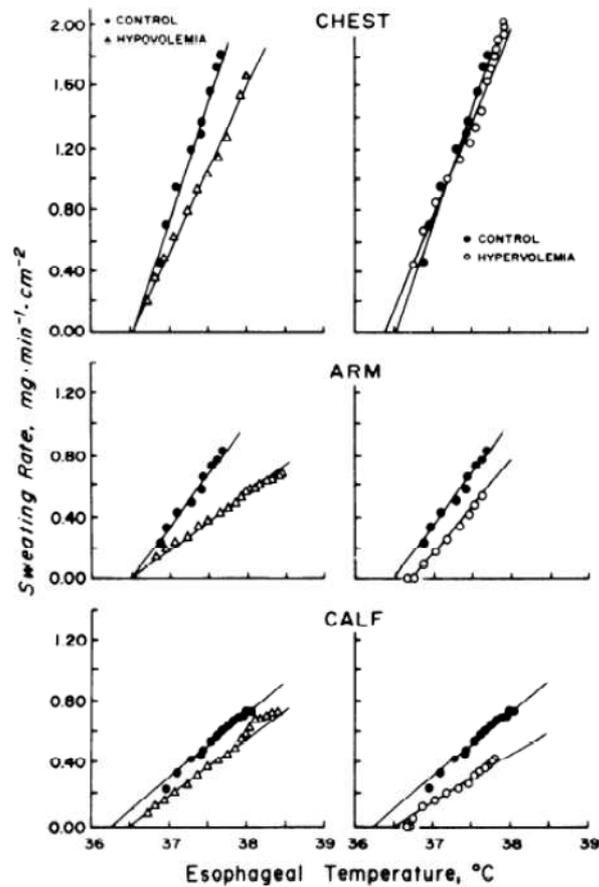
(Hagan *et al.*, 1980) and heat stress (Harrison *et al.*, 1975; Senay, 1975) are associated with a reduction in PV.

#### 2.4.2 Effects of Hypovolemia and Hyperosmolality on Sweat Rate

Prolonged exercise in the heat results in a body water deficit through sweating, which leads to a state of dehydration. This body water deficit lowers both intracellular and extracellular fluid volumes, resulting in hypovolemia and hyperosmolality which inhibits thermoregulatory responses (e.g., impairs sweat rate). Hypovolemia is defined as a less than “normal” blood volume. Blood volume (BV) represents the sum of erythrocyte volume and plasma volume (PV) (Sawka *et al.*, 2000). Erythrocytes, plasma proteins and PV can alter independent of each other to change BV.

Early studies concluded that exercise hyperthermia associated with dehydration was due to a fall in PV and subsequent reduction in SkBF (Adolph, 1947), which leads to excessive heat storage in the body. Thus, the maintenance of BV is an important factor for providing circulatory stability and thermoregulatory equilibrium in maximising endurance performance during exercise. As water and electrolytes flow from the plasma to the extracellular space, it results in a reduction in BV and reduced central venous pressure. Central venous pressure is further reduced by peripheral pooling of blood secondary to an increase in SkBF during exercise in the heat (Fortney *et al.*, 1983). This reduction is associated with decrements in SV, which may ultimately lead to a decline in  $\dot{Q}$ . When the average reduction of BV is greater than 7%, antidiuretic hormone (ADH) is released which may have an indirect effect on reducing sweating rate (Robertson *et al.*, 1976). Other studies have shown hypovolemia induced by a diuretic agent caused a decline in SV and  $\dot{Q}$  (Nadel *et al.*, 1980; Fortney *et al.*, 1981a) and thus reduced heat loss by raising temperature thresholds for cutaneous vasodilation and sweating.

Fortney and colleagues (1981b) had five men perform submaximal exercise on a cycle ergometer for 30 min at 30°C, 40% rh. Approximately 3.1% BW loss and an 8.7% reduction in BV were induced using a diuretic drug (50 mg triamterene and 25 mg dihydrochlorothiazide) without a change in plasma osmolality ( $P_{osm}$ ). The study clearly showed that the decrease in blood / plasma volume during the hypovolemia trial was related to the central integrative centres, competing with the thermoregulatory outflow by decreasing the sweat rate at any given body  $T_{core}$  (Figure 2.8). A combination of the reduced sweat rate and peripheral blood flow served to attenuate the rate of heat loss from the body core (Nadel *et al.*, 1980; Fortney *et al.*, 1981b). It was proposed that the reduction in BV, especially during dehydration, compromised the cutaneous circulation and offset the increase in sweat rate contributing to a higher  $T_{core}$  (Eklom *et al.*, 1970). Note that Fortney and colleagues (1981b) did not measure the heart rate (HR) responses during exercise in the heat (Fortney *et al.*, 1981b) while body temperatures were not extremely high (~38.5°C) towards the end of 30-min exercise. Therefore, whether a higher body temperature during the hypovolemia trial is solely related to a reduction in sweat rate, or the reduced BV increased cardiovascular strain together with a decline in cutaneous perfusion for sweating remains to be elucidated. Fortney *et al.* (1983) further reported on a similar study undertaken (Fortney *et al.* 1981b) which involved a hypovolemia trial where HR was 10 beats.min<sup>-1</sup> higher and SV was reduced by -14 mL.beat, while  $\dot{Q}$  declined by -2.2 mL.min<sup>-1</sup>. They proposed that the reduced cardiac filling pressure in the heat was mainly due to the peripheral pooling of blood in veins rather than a loss of PV which was supported by the pattern of SV over the course of exercise (Fortney *et al.*, 1983).



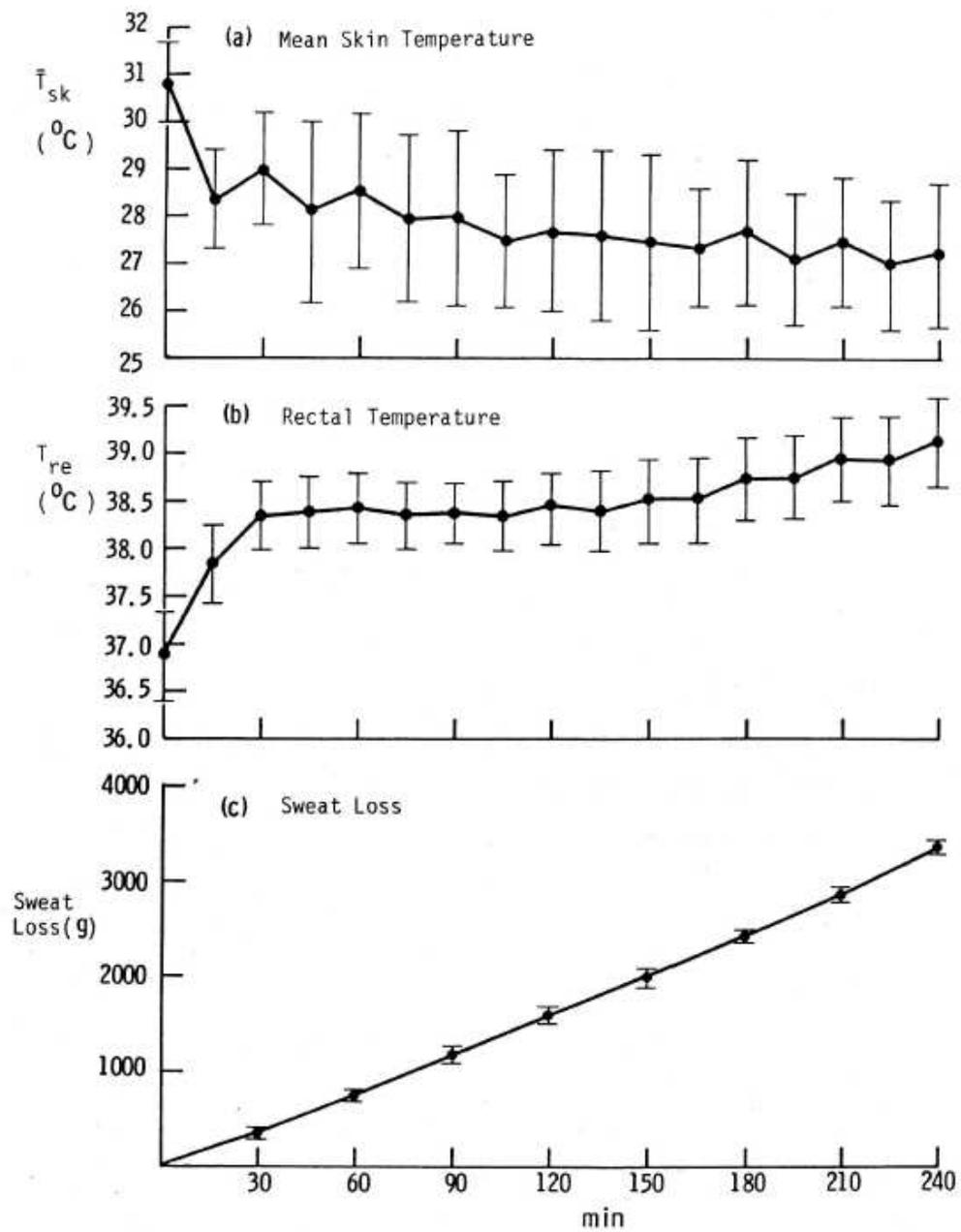
**Figure 2.8** The slope of the sweating rate-to- $T_{es}$  relationship was significantly reduced during hypovolemia for one typical subject (Fortney *et al.*, 1981b)

A decline in PV during exercise is accompanied by an increase in  $P_{osm}$  (Fortney *et al.*, 1981a).  $P_{osm}$  is controlled around a set-point of 280 – 290 mOsmol/kg in euhydrated subjects (Senay, 1979). Every 1-2% body mass loss incurred by dehydration increases  $P_{osm}$  by ~ 5 mOsmol/kg (Popowski *et al.*, 2001). An increase of  $P_{osm}$  by 1-2% will trigger osmoregulatory responses (e.g., the secretion of vasopressin by the kidney) to reduce fluid losses and stimulate the sensation of thirst to restore body fluid loss (Wolf, 1950). However, the thirst response in stimulating fluid ingestion seems to be relatively ineffective until an equivalent loss of 2-3% in body mass (Sawka & Montain, 2000).  $P_{osm}$  increases during exercise due to a greater loss of plasma water than plasma electrolytes through sweating; and an increase in osmotically active particles which are produced by the active muscles and diffuse into the vascular compartment. Another study of Fortney and colleagues (1984) reported that hyperosmolality modifies

thermoregulation by elevating thresholds for both cutaneous vasodilation and sweating even without decreases in PV. The onset of sweating was delayed, which imposed a limitation for heat dissipation (Fortney *et al.*, 1984).

The role of  $P_{\text{osm}}$  during exercise was examined by Takamata *et al.* (1998). These authors reported that the elevated body  $T_{\text{core}}$  threshold for cutaneous vasodilation during exercise could be the result of increased  $P_{\text{osm}}$  induced by exercise and it is not due to reduced PV or the intensity of the exercise itself. These findings were consistently reported by the same group of researchers who concluded that the elevated  $P_{\text{osm}}$  during hypohydration has an inhibitory influence on thermoregulatory cutaneous vasodilation and sweating by elevating the body  $T_{\text{core}}$  thresholds (Takamata *et al.*, 1997; Takamata *et al.*, 1998; Takamata *et al.*, 2001; Shibasaki *et al.*, 2009). However, the authors did not indicate the levels of dehydration during these experimental trials.

On the contrary, a linear relationship between sweat rate and time of exercise was observed ( $r=0.99$ ) during treadmill running in elite ultra-marathon athletes (Davies & Thompson, 1986). The authors reported that sweat rate was not affected by the level of dehydration. Their subjects had experienced a significant reduction in BW loss ( $5.5 \pm 0.8\%$ ) after 4 hours of exercise (Figure 2.9). There was no correlation between the volume of water consumed and  $T_{\text{re}}$  at 240-min of exercise.



**Figure 2.9** Mean skin temperature ( $\bar{T}_{sk}$ ,  $^{\circ}\text{C}$ ), rectal temperature ( $T_{re}$ ,  $^{\circ}\text{C}$ ) and sweat loss (g) of 10 well trained subjects during 4h treadmill exercise (Thompson, 1984)

### 2.4.3 Relationship between Hypohydration and Core Temperature

Exercise physiologists have long considered that a high core body temperature will accelerate fatigue and precipitate exhaustion (Craig & Cummings, 1966; Pugh *et al.*, 1967; Wyndham & Strydom, 1969; Costill *et al.*, 1970; Greenleaf & Castle, 1971; Gisolfi & Copping, 1974; Montain & Coyle, 1992a; Armstrong *et al.*, 1997). In 1969, Wyndham and Strydom postulated that there was a linear relationship between decreased body mass due to body water loss through sweating and the rise in  $T_{\text{core}}$  during and post-exercise. Thus they proposed that heat stroke was caused by dehydration (Wyndham & Strydom, 1969; Wyndham, 1977) and that dehydration was the primary cause of impaired prolonged exercise performance in hot conditions.

It has been suggested that an excessive rise in  $T_{\text{re}}$  with dehydration may be due to inadequate sweating (Greenleaf & Castle, 1971). A gradual decline in sweating has been noted in response to an elevated  $T_{\text{re}}$  during prolonged exercise in the heat (Wyndham *et al.*, 1966). Other researchers have concluded that sweating responds to body temperature regulation requirements and is not reduced appreciably by dehydration (Davies & Thompson, 1986). Pugh *et al.* (1967) reported an increased sweat rate in those runners experiencing a more pronounced level of dehydration during prolonged exercise. Of course a comparatively high sweat rate will in turn lead to a more rapid state of dehydration unless large fluid volumes are ingested. Whether there is a point when sweat rate diminishes during prolonged exercise is subject to debate. This debate centres on whether the condition(s) of hidromeiosis, skin wettedness or swelling of the keratinous layer is present.

Skin wettedness may ultimately reduce the sweat rate and impair heat loss with the result being a spiraling body  $T_{\text{core}}$ , impaired endurance performance and/or collapse

(Nadel *et al.*, 1971a; Candas *et al.*, 1979; Davies, 1979). In such conditions, the athlete may succumb to heat exhaustion or heat stroke with the latter being potentially fatal. Swelling of the keratinous layer of skin cells in response to changes in osmotic content of body fluid has been alluded to as a possible mechanism underlying hydromiosis (Brown & Sargent, 1965). With sweating providing one of the main avenues for heat loss, any compromise in this mechanism will severely stress the cardiovascular system with a greater demand for heat loss via peripheral blood flow whilst maintaining sufficient blood for the metabolic demands of the active muscles.

Craig and Cummings (1966) and Greenleaf and Castle (1971) found a greater increase in  $T_{re}$  during exercise in a dehydrated state than when body weight loss was completely replaced by water. However, dehydration during exercise in cool conditions leads to elevated  $T_{re}$  as reported by Pugh *et al.* (1967) and Greenleaf & Castle (1971) but it is not clear what thermoregulatory mechanisms are influenced by dehydration since  $T_{sk}$  was not measured in these studies. A number of exercise studies have also shown that there was no relationship between % body mass loss and body  $T_{core}$  (Noakes *et al.*, 1991; Sharwood *et al.*, 2004; Byrne *et al.*, 2006). A plausible explanation for these different findings might be due to the variable intensity of exercise in field studies. Therefore a dehydrated person may actually have a lower  $T_{core}$  because the intensity of exercise has decreased (Maughan *et al.* 1985). Recently, Lopez *et al.* (2011) and Casa *et al.* (2010) found a greater physiological strain (e.g., HR and  $T_{core}$  increased) together with performance decrements associated with dehydration exist in both laboratory (with the controlled relative intensity) and field settings (trail running speed). These studies revealed that constant controlled intensity running while may negatively impact on running performance with an elevated HR and body temperature. Therefore the dehydrated runners will either slow down their pace to prevent an increase in HR and

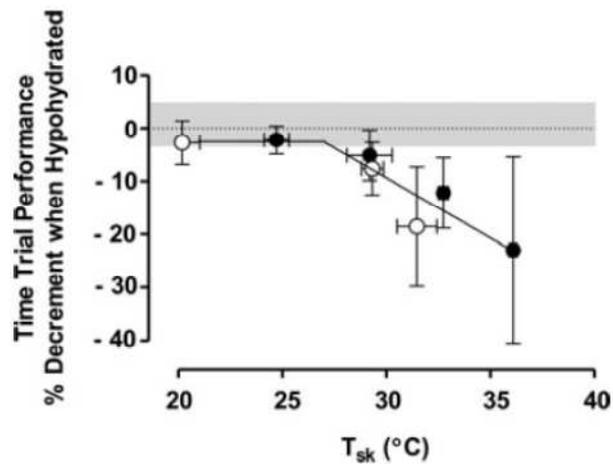
body temperature, or run at a higher intensity to keep up with euhydrated runners while experiencing a greater physiologic strain. These studies have been the catalyst for many subsequent studies that have focused on investigating the effects of dehydration on aerobic exercise performance.

#### 2.4.4 Relationship between Hypohydration and Skin Temperature

Another factor precipitating impaired endurance performance in hot conditions is an elevated  $T_{sk}$  associated with hypohydration. Recently, there is evidence that if  $T_{sk}$  can be maintained at a range of warm-hot (32-34°C), aerobic exercise performance can be preserved despite a high  $T_{re} \geq 40^\circ\text{C}$  (Ely *et al.*, 2009; Lee *et al.*, 2010). Kenefick *et al.* (2010) first evaluated the impact of hypohydration on aerobic exercise performance using incremental heat-stress conditions. Four groups of subjects ( $n=32$ ;  $n=8$  for each group) performed two experimental trials (euhydrated vs. hypohydrated) in four different ambient temperature conditions (10°C, 20°C, 30°C and 40°C) at 50%  $\dot{V}O_{2max}$  for 30 min and a self-paced time trial for 15 min. Exercise-heat exposure was employed to induce 4% BW loss. They reported that as  $T_{sk}$  exceeded 29°C, 4% BW loss degrades aerobic performance by ~ 1.6% for each additional 1°C increment in  $T_{sk}$  which was associated with a high SkBF. The authors concluded that increased  $T_{sk}$  is an important contributor to impaired aerobic performance when the subjects are hypohydrated (Kenefick *et al.*, 2010). The impaired prolonged exercise performance in the heat was likely caused by the redistribution of blood from the central to peripheral circulation (Rowell *et al.*, 1969) accompanied by an elevation in SkBF (Johnson, 1982). The high SkBF requirements consequently reduced cardiac filling, and elevated HR responses during exercise (Trinity *et al.*, 2010; Stöhr *et al.*, 2011).

A recent review reported that hot skin ( $>35^\circ\text{C}$ ) and body water deficits ( $<2\%$  body mass) impair aerobic performance (Sawka *et al.*, 2012). The authors employed segmented regression statistical analysis to approximate the  $T_{sk}$  threshold for performance impairment from three studies which employed similar experimental procedures and time trial performance tests (Cheuvront *et al.*, 2005; Castellani *et al.*, 2010; Kenefick *et al.*, 2010). They found that warmer skin (27.3°C) accentuated the decrement in

performance by ~ 1.3% for each additional 1°C rise in  $T_{sk}$  (Figure 2.10). This review concluded that hot skin accompanied by high SkBF, not high  $T_{core}$ , is the primary factor which impairs submaximal aerobic exercise performance when euhydrated and that hypohydration exacerbates this performance decrement.



**Figure 2.10** Percentage decrement in submaximal aerobic performance from euhydration as a function of skin temperature when hypohydrated by 3-4% of body mass (Sawka *et al.*, 2012)  
Filled circles represent 15min time trial (TT) tests; open circles represents 30 min TT tests.

#### **2.4.5 Fluid Replacement during Prolonged Exercise in the Heat**

When sweating becomes the primary means of heat dissipation, athletes are invariably advised to consume water with the volume being equivalent to the volume of sweat loss during exercise (Armstrong *et al.*, 1997). If fluids are not replaced at this rate, runners may experience dehydration (>2% of body mass loss) which is associated with an increase in cardiovascular and thermoregulatory strain (Montain & Coyle, 1992b; Sawka *et al.*, 1992; Sawka & Montain, 2000; Sawka & Noakes, 2007; Gonzalez *et al.*, 2009). The reality is that an elite marathon runner is likely to produce 2.0 liters of sweat for 2 to 2.5 hours, thus incurring a significant fluid deficit which cannot be replenished while running at ~ 80 - 85%  $\dot{V}O_{2max}$ . It is thus inevitable that well-trained marathon runners finish marathon races dehydrated. Aerobic exercise performance is likely to be adversely affected by hypohydration (Sawka, 1992; Cheuvront *et al.*, 2003a; Murray, 2007) although it is not clear what level of hypohydration challenges exercise performance. This is a controversial issue as commercial interests in fluid supplement drinks undertake their own research and invest considerable funds in marketing “sports drinks”. Hydration advice is therefore not always independent. Further research is warranted so that advice on fluid ingestion is based on a consensus of well designed and conducted research studies.

Fluid intake during prolonged exercise offsets the extent of dehydration and the magnitude of the associated increase in  $T_{re}$  (Wyndham & Strydom, 1969; Costill *et al.*, 1970; Nielsen *et al.*, 1971; Gisolfi & Copping, 1974; Wyndham, 1977). Wyndham and Strydom (1969) reported lower  $T_{re}$  in those runners who consumed fluid during a marathon race compared with those who chose not to drink. The amount of fluid lost in sweat and the rise in body temperature were closely correlated with BW. They attributed the lower  $T_{re}$  in this instance to the ingestion of water reducing the magnitude

of water deficit. Montain and Coyle (1992a) and Armstrong *et al.* (1997) further reported that the replenishment of fluid losses during exercise maintained cardiovascular function and lowered  $T_{re}$  compared with ingesting smaller volumes of fluid during exercise. Collectively, this evidence has been used by American College of Sports Medicine (ACSM) Position Stand: Exercise and Fluid Replacement (1996). This document concluded that athletes should be encouraged to drink “as much as possible/ tolerable” during prolonged exercise (Convertino *et al.*, 1996). With this advice it was presumed that endurance athletes, who ingested a volume of fluid equivalent to their fluid losses through sweating, would not succumb to heat stroke and would not find their performance to be impaired as consequence of dehydration. However, these guidelines are implicated in the increased prevalence of exercise-associated hyponatremia (EAH) (Noakes *et al.*, 2006) that has resulted in fatalities amongst less experienced endurance runners (Noakes, 2003). Moreover, no relationship between % body mass loss and body  $T_{core}$  has been reported during or following marathon and triathlon races (Noakes *et al.*, 1991; Sharwood *et al.*, 2002; Sharwood *et al.*, 2004; Byrne *et al.*, 2006; Laursen *et al.*, 2006). Noakes *et al.* (1991) measured the post-race  $T_{re}$ , together with levels of dehydration and running velocities to estimate absolute metabolic rates in marathon runners. They found that the post-race  $T_{re}$  in these runners was affected by the metabolic rate sustained during the latter section of the race, but not due to the level of dehydration. Sharwood *et al.* (2002, 2004) found the BW of triathletes who completed the 2000 and 2001 South African Ironman triathlon was significantly reduced (ranged from -2.6 to -10.7 %). This reduction was not associated with a higher  $T_{re}$  or greater prevalence of medical complications, but was associated with higher serum sodium concentrations. These studies have challenged the ACSM fluid replacement guidelines (Convertino *et al.*, 1996; Casa *et al.*, 2000) by documenting that dehydration does not necessarily lead to decrements in endurance

exercise performance and relatively low levels of dehydration do not cause an increase in  $T_{re}$  during endurance running and triathlon events (Sharwood *et al.*, 2002; Sharwood *et al.*, 2004)

## **2.5 CRITICAL CORE TEMPERATURE AND CIRCULATORY STRAIN HYPOTHESES**

More recently the view that dehydration is the primary factor precipitating impaired endurance performance in hot conditions has been challenged with researchers investigating two alternative hypotheses:

2.5.1 Critical core temperature hypothesis

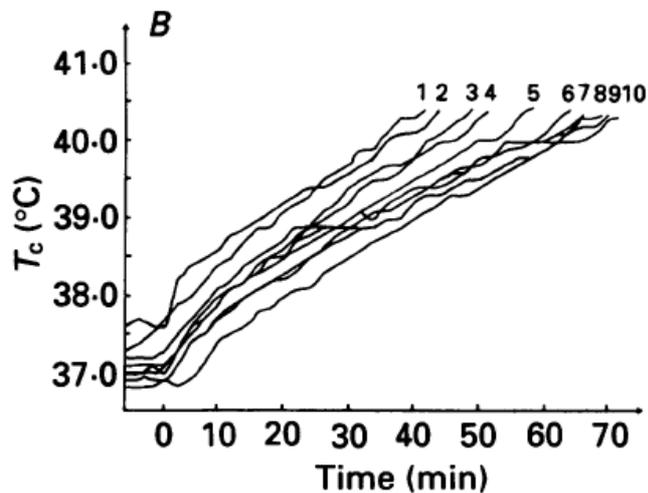
2.5.2 Circulatory strain hypothesis

### **2.5.1 Critical Core Temperature Hypothesis**

The hypothesis that a “critical core temperature” may limit prolonged exercise in hot conditions was introduced by Nielsen and colleagues (1993). Nielsen *et al.* (1993) proposed that an elevated body temperature provides peripheral feedback from thermally-sensitive sites to the central nervous system (CNS), resulting in reduced central neural drive to the exercising muscles in hot, dry environments. The reduced CNS activation of the exercising muscles may be an inhibitory protective mechanism against potentially lethal increases in body temperature.

Prior to Nielsen’s (1993) proposition, numerous animal studies in a wide range of mammalian species including rats (Fruth & Gisolfi, 1983), goats (Caputa *et al.*, 1986) and cheetah (Taylor & Rowntree, 1973) have shown evidence that the attainment of a critical core temperature limits endurance performance in the heat. In the study of Nielsen *et al.* (1993), eight well trained endurance cyclists undertook a heat acclimation training protocol which required them to perform a cycle bout at 50%  $\dot{V}O_{2max}$  for 90-min or until exhaustion each day over 9-12 days. The climatic conditions were 40-42°C and 10-15% rh. Thermoregulatory and circulatory physiological adaptations were

induced with undertaking this heat acclimation programme. As a result of the heat acclimation, they observed a lower rate of elevation in both HR and  $T_{\text{core}}$ ; while  $\dot{Q}$  and sweating responses were significantly increased and performance time was increased from 48 min to 80 min. Interestingly, subjects reached their point of fatigue at a similar level of  $T_{\text{core}}$  each day ( $\sim 39.7 \pm 0.15^\circ\text{C}$ ) even though the time exercising to fatigue increased in duration (Figure 2.11). Nielsen *et al.*, (1993) concluded that each cyclist discontinued the daily cycle bout because they had reached their own intrinsic critical  $T_{\text{core}}$ .



**Figure 2.11** Esophageal temperature plotted against time. One acclimating subject during ten consecutive days of exercise until exhaustion at  $40^\circ\text{C}$  (Nielsen *et al.*, 1993)

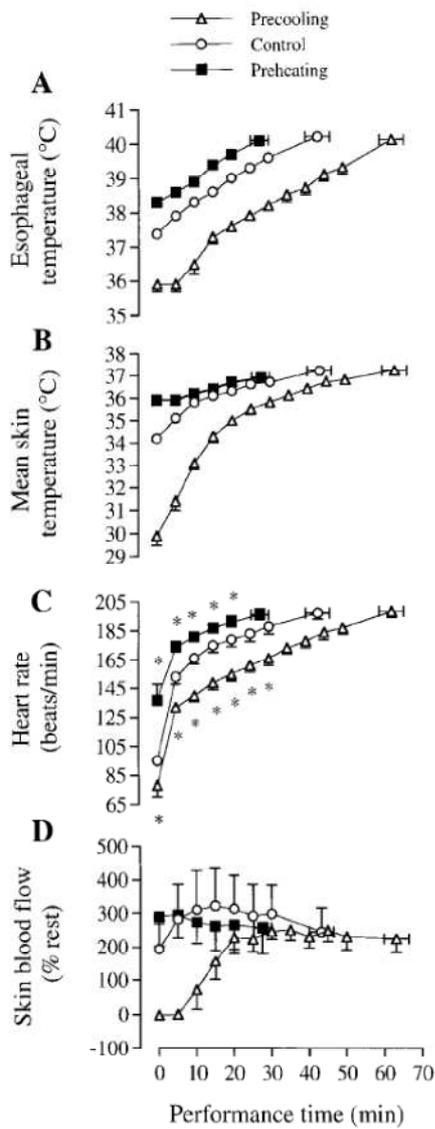
Nielsen *et al.*, (1997) subsequently had subjects perform a similar protocol in hot and humid conditions ( $35^\circ\text{C}$ , 87% rh) and the result was consistent with the previous findings. Mean  $T_{\text{core}}$  reached  $39.9 \pm 0.1^\circ\text{C}$  at the point of fatigue each day over 8-13 consecutive days of heat acclimation training with an improvement in exercise performance time from 45 to 52 min (Nielsen *et al.*, 1997). The high  $T_{\text{core}}$  had no significant effect on circulatory responses (e.g.,  $\dot{Q}$ , MAP, muscles and SkBF). Therefore,

Nielsen *et al.* (1993) suggested that the elevated  $T_{\text{core}}$  contributes to reducing motivation, which progressively reduces the drive to continue exercising during hyperthermia (Brück & Olschewski, 1987), but not circulatory failure.

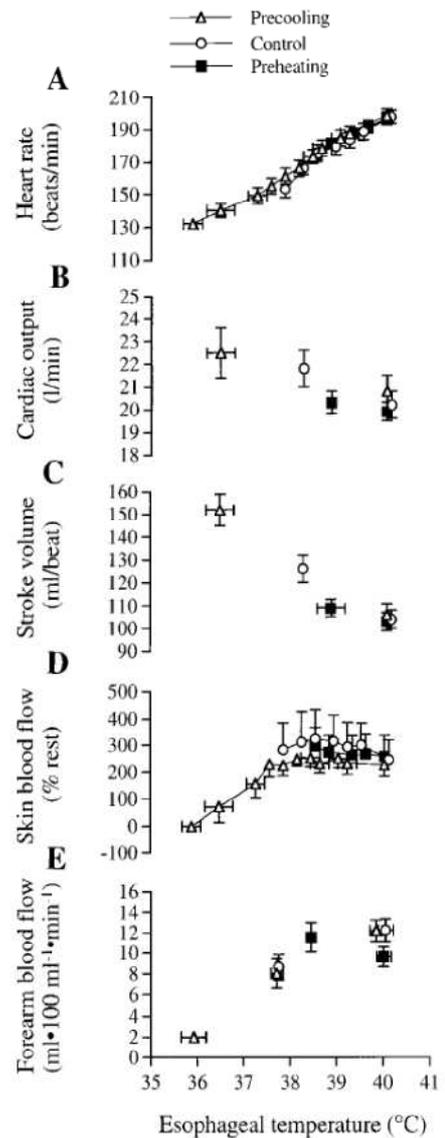
However, these findings were not entirely clear and were not consistent with the findings of a previous study by Jose *et al.* (1970). These researchers reported an increase in HR of 7 beats.min<sup>-1</sup> per 1.0°C increase in  $T_{\text{core}}$  during exercise after autonomic blockade by propranolol and atropine. It appears neither the increase in  $T_{\text{core}}$  nor incomplete blockade of sympathetic stimuli accounted for the rise of HR, but it is most likely related to the continual increase in  $T_{\text{core}}$  eliciting an increase in HR through direct effects on intrinsic HR at the sino-atrial node.

Later, Gonzalez-Alonso and colleagues (1999b) further concluded that prolonged exercise performance in the heat was associated with a high initial  $T_{\text{core}}$  and subsequent rate of heat storage. They conducted a study that required seven trained cyclists to be immersed in cool water (pre-cooled) condition, a control condition and warm water (pre-heated) condition prior to undertaking a prolonged bout of cycling in 40°C ambient temperature with different initial  $T_{\text{core}}$  (35.9, 37.4 or 38.2°C). Subjects fatigued at different time points: 28 min, 46 min and 63 min, respectively (Figure 2.12a and Figure 2.12b). Cessation of exercise was associated with a high  $T_{\text{es}}$  (40.1-40.2°C), elevated  $T_{\text{sk}}$  (37.0-37.2°C) and near maximal HR (98-99%). These thermoregulatory and circulatory responses were similar across all three conditions (including the control trial) at the point of fatigue regardless of differences in initial  $T_{\text{core}}$  (González-Alonso *et al.*, 1999b). Thus, the authors concluded that high body temperature *per se* causes fatigue in trained cyclists during prolonged exercise in an uncompensable hot environment. However, HR across the three conditions was conspicuously similar over most of the exercise

period (Figure 2.11b). González-Alonso and colleagues (1999b) seem to have ignored the fact that in all three conditions the cyclists were unable to maintain the exercise when their HR reached 98-99%  $HR_{max}$  which strongly indicates that circulatory strain may explain the aetiology of their fatigue.



**Figure 2.12a** Esophageal temperature (A), mean skin temperature (B), heart rate (C) and skin blood flow (D) during exercise in heat (40°C, 17% rh) during precooling, control, and preheating trials. Skin blood flow is referenced to resting baseline values obtained on arrival at the laboratory (0.2-1.2 V; n=4). Values are means  $\pm$  SE for 7 subjects (González-Alonso *et al.*, 1999b)  
\*Significantly different from control, *P*, 0.05.



**Figure 2.12b** Heart rate (A), cardiac output (B), stroke volume (C), skin blood flow (D), and forearm blood flow (E) plotted against core temperature during precooling, control, and preheating trials. Values are means  $\pm$  SE for 6 subjects (González-Alonso *et al.*, 1999b)

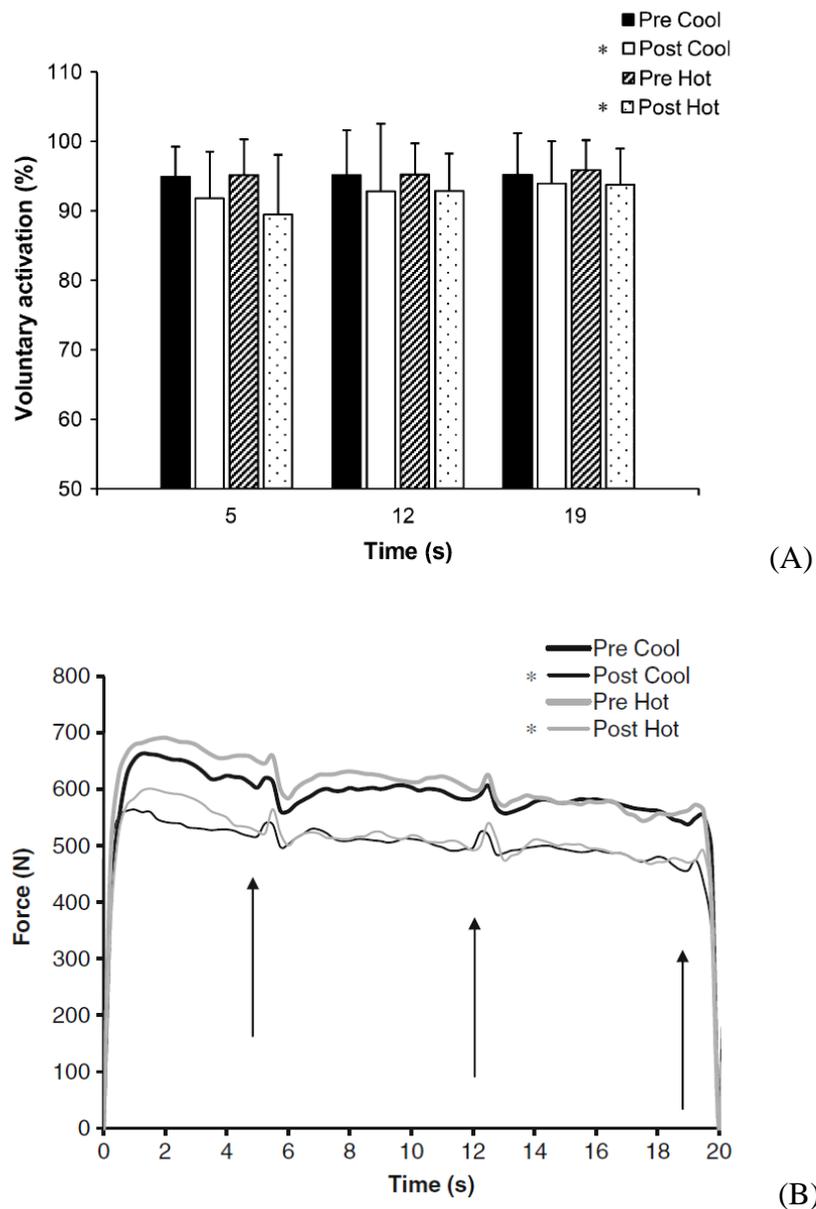
The study by González-Alonso and colleagues (1999b) has been further used as evidence to support the hypothesis that there may be a direct effect of high internal temperatures on the central nervous system (CNS) and muscle recruitment (Nybo & Nielsen, 2001). The force production during a maximal voluntary isometric (MVC) contraction of 120 s in exercised legs (knee extension) was progressively impaired in both cool (18°C) and hot (40°C) conditions but the reduction in muscle force development was much more pronounced in hyperthermic conditions. A superimposed electrical stimulus was applied to the femoral nerve (nervus femoralis) to assess the degree of voluntary activation. Although there was a hyperthermic-induced reduction in voluntary activation, the superimposed electrical stimulus evoked a force that was similar in normothermic and hyperthermic conditions. Conversely, a sustained MVC performed by the non-exercised wrist flexor muscles elicited similar reductions in force development at a  $T_{re}$  of ~39°C during both exercise-induced hyperthermia and passive heating trials. Collectively, these studies consistently reported that exercise terminates at a  $T_{re}$  of ~39 - 40°C (Nielsen *et al.*, 1993; Nielsen *et al.*, 1997; González-Alonso *et al.*, 1999b) and that this level of hyperthermia (~40°C) is associated with an inability of the central nervous system (CNS) to fully activate the skeletal muscles to produce or maintain the required force (i.e., central fatigue) (Nybo & Nielsen, 2001). However, Nybo & Nielsen (2001) seem to have overlooked the possibility that hypohydration might have been a limiting factor in their study. With the support of the earlier evidence of Coyle & Hamilton (1990) we speculate that fatigue during prolonged exercise is not confined to a reduction in CNS drive, and may involve both central and peripheral impairment of force generation. Coyle & Hamilton, (1990) suggested that the reduction in muscular strength following hypohydration is limited by the ability of the central nervous system to recruit motor units. While a more recent review article

(Judelson *et al.*, 2007) concluded that hypohydration reduced strength (by ~2%), power (~by 3%) and high intensity endurance (~by 10%). However, there is nevertheless considerable controversy in the research literature. Studies have demonstrated that hypohydration decreases strength (6-11%) (Bosco *et al.*, 1968; Schoffstall *et al.*, 2001) or have found no change in strength. These varied reports may reflect on the methods used to determine whether the maximal contractions are truly maximal. Only one of the aforementioned studies utilized electrically evoked contractions to differentiate whether a maximal effort produced a truly maximal force production (Greenleaf *et al.*, 1967; Montain *et al.*, 1998; Périard *et al.*, 2012). Thus it is unclear whether hypohydration impaired muscle strength or the voluntary effort made was insufficient to produce a truly maximal contraction during exercise.

The influence of hyperthermia-induced central fatigue on force production has been challenged by conflicting research findings. Recent studies have proposed that an elevated core temperature alone is not associated with the cessation of exercise in the heat (Saboisky *et al.*, 2003; Periard *et al.*, 2011; Périard *et al.*, 2011). Saboisky *et al.* (2003) reported that muscle force production is not limited by the attainment of a specific core temperature. Subjects performed a series of MVCs with the leg extensors (exercised muscles) and forearm flexors (non-exercised muscles) before and immediately after exercise under hot conditions (39.3°C; 60% rh). The capacity to produce force in non-exercised forearm flexor muscles was similar during a 5 s MVC, whereas muscle force production of the quadriceps declined in the exercised quadriceps. In addition, the reduction in force output of the exercised quadriceps was not accompanied by a significant change in the integrated electromyograph (iEMG), indicating the recruitment of motor units was similar but less force output was produced. Therefore, it was suggested that peripheral muscular fatigue may be present during

exercise-induced hyperthermia and this could be a consequence of hypohydration. Thus afferent feedback from the body core and muscle thermoreceptors inhibited voluntary activation to specific skeletal muscles in order to maintain cell integrity.

More recently, Périard *et al.*, (2011b) reported that a maximal voluntary contraction of the quadriceps was similarly attenuated during a 20 s MVC following a 40 km time trial in hot (35°C) and cool (20°C) conditions. The  $T_{re}$  reached 39.0°C and 39.8°C at time trial completion in the cool and hot condition trials, respectively. It was shown that the difference in core temperature (0.8°C) did not exacerbate the loss of force production in the heat. The reduction in voluntary activation was maintained throughout the contraction (Figure 2.13A) and the reduction in force production was similar between trials (Figure 2.13B). This provides evidence that fatigue is partly a consequence of the prolonged contractile activity of previously active muscles (attributed to peripheral fatigue), despite core temperatures  $\leq 39.9^\circ\text{C}$ . In a follow up study, Périard *et al.* (2011) investigated neuromuscular function by isolating the residual effects of exercise ( $T_{re}$ : 39.8°C) vs. passive hyperthermia ( $T_{re}$ : 39.5°C). Force production capacity declined at a faster rate following cycling to exhaustion in the heat, compared with passive hyperthermia during a 45 s MVC of quadriceps. Meanwhile mean voluntary activation throughout the sustained MVC was depressed following both active and passive hyperthermia. It was clearly shown that the loss of force production capacity during hyperthermia originated from both central and peripheral factors.



**Figure 2.13** Voluntary activation percent (A) and force production (B) during a 20 s maximal voluntary isometric contraction of the knee extensors with superimposed electrical stimulation at 5, 12 and 19 s prior to and following self-paced exercise in hot and cool conditions. \*indicate a significantly lower post-exercise mean voluntary activation / force production compared with pre-exercise ( $p < 0.05$ ) (Périard *et al.*, 2011).

### Is prolonged exercise in the heat limited by dehydration?

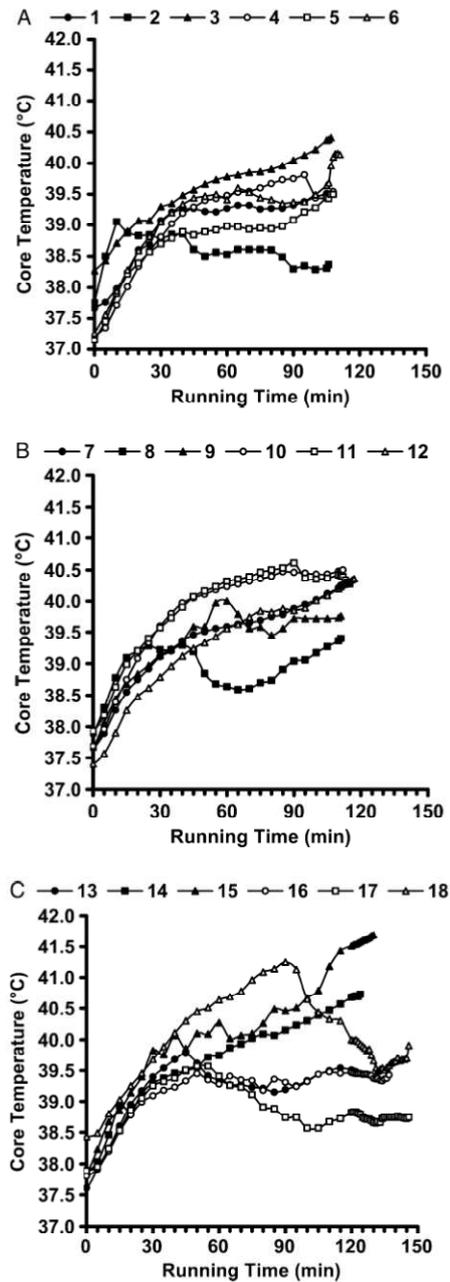
Dehydration might be another plausible factor involved in the early fatigue associated with a high  $T_{core}$ . Many studies have reported that fluid ingestion can attenuate the rise in  $T_{core}$  in hot conditions (Pitts *et al.*, 1944; Candas *et al.*, 1986; Montain & Coyle,

1992b; Sawka *et al.*, 1992; González-Alonso *et al.*, 1999a; Buono & Wall, 2000). Therefore, we speculate that there is a possibility that the subjects in these aforementioned heat acclimation studies (Nielsen *et al.*, 1993; Nielsen *et al.*, 1997) may have experienced a significant reduction in BW (and plasma volume) through sweating which might influence the circulatory responses to exercise and consequent fatigue. Nielsen *et al.* (1993, 1997) did not report whether there was any deleterious effects of dehydration during the two heat acclimation programmes employed in their research studies. Otherwise a comparative study could be undertaken between the subjects in the studies of Nielsen *et al.* (1993 and 1997) and those in the study of Gonzalez *et al.* (1999b), who experienced approximately 2.6% BW loss during the three conditions of the exercise trials.

In a parallel study by González-Alonso *et al.* (1999a), it was reported that progressive dehydration amounting to ~3.9% BW loss with hyperthermia (high  $T_{\text{core}}$  ~40°C) are primary factors underlying the early fatigue in hot environmental conditions compared with a euhydration trial. Gonzalez-Alonso *et al.* (1998) concluded that the decline in SkBF and muscle blood flow to the exercising muscles with dehydration was due to a reduction in  $\dot{Q}$  and systemic vascular conductance. However, when the subjects exercised in the euhydration trial, they experienced less thermoregulatory strain with a modest increase in  $T_{\text{core}}$  to ~38.2°C at the same duration of exercise (~135 min) as the dehydration trial (González-Alonso *et al.*, 1998). Similarly, Buono and Wall (2000) reported that subjects completed 60 min of exercise with 5% BW loss due to hypohydration resulting in a significant increase in both  $T_{\text{re}}$  and HR when in a hot environment (33°C) compared with temperate conditions (23°C). Hypohydration during exercise in the heat appears to reduce heat loss via decreases in whole body sweat rate

and forearm blood flow (Buono & Wall, 2000). Collectively, these studies suggest that a reduction in  $\dot{Q}$  due to hypohydration decreases skin and muscle blood flow, as well as whole body sweat rate, resulting in an increased  $T_{re}$  which impaired exercise performance in the heat.

Recently the concept of a “critical”  $T_{core}$  has been challenged by several observational “field” studies (Byrne *et al.*, 2006; Ely *et al.*, 2009; Lee *et al.*, 2010). Byrne *et al.* (2006) observed eighteen acclimatized soldiers who completed a half marathon race in warm environmental conditions (WBGT 23 - 28°C) in  $118 \pm 13$  min. All soldiers experienced a ~2.8% BW loss without any heat illness symptoms at the finish of the race and a mean post-race  $T_{re}$  of ~40°C (ranged from 38.3 - 41.7°C). Two very low  $T_{re}$  (Runner 2: 38.3°C and Runner 17: 38.7°C; Figure 2.14) measures were noted in 2 soldiers at the end of the race, whereas the other 16 soldiers’  $T_{re}$  was  $> 39.4^\circ\text{C}$ . These data indicate that exercise under these conditions is well tolerated despite the ~2.8% BW loss and elevated  $T_{re}$ . However, it should be noted that the reported average running pace in this study was significantly slower than elite distance runners who are able to race a half marathon in approximately 60-65 minutes.



**Figure 2.14** Individual core temperature response of 18 runners during the half marathon, presented in order of finishing time: (A) 105-111min, N=6; (B) 111-117 min, N=6; (C) 122-146 min, N=6 (Byrne *et al.*, 2006).

Similar observations under competitive marathon conditions have been reported with  $T_{re}$  exceeding 39°C associated with ~3% or greater BW loss without any sign of heat illness in cooler conditions at wet-bulb temperature of 17°C and 13.2°C (Pugh *et al.*, 1967; Maron *et al.*, 1977). Maron *et al.* (1977) suggested that the absence of clinical

signs of heat illness indicated that thermoregulation remained intact. Pugh *et al.* (1967) reported thermoregulatory response data on 77 marathon competitors who successfully or unsuccessfully finished the race. The first four placegetters lost ~5.8% BW with  $T_{re}$  reaching ~40.7°C at the conclusion of the race. More recently, Ely *et al.* (2009) reported that the velocity of running in warm (WBGT~ 27°C) and cool (WBGT~ 13°C) conditions was not affected by an elevated  $T_{re}$ . In fact, runners were able to accelerate at the end of the experimental trial regardless of an increase in  $T_{re}$  to >40°C (Ely *et al.*, 2009; Lee *et al.*, 2010).

The observations from these field studies do not support the critical  $T_{core}$  hypothesis and have provided strong evidence against the existence of a threshold  $T_{re}$  of ~ 40°C associated with fatigue. The difference in  $T_{core}$  between field studies and laboratory experimental trials may be attributed to fluctuations in running velocity in the field compared with constant velocity treadmill running in laboratory experiments. Furthermore, athletes may be more highly motivated when competing against other runners in field studies.

Dehydration >5% BW with marathon running exacerbates the rise in body temperature and associated upward drift in HR. This is well documented by Montain and Coyle (1992) who observed the magnitude of increase in esophageal temperature and heart rate to be linearly related to the level of dehydration during prolonged exercise in hot conditions (33°C dry bulb, 50% rh), but not in thermoneutral or cool conditions (Davies & Thompson, 1986). Davies and Thompson (1986) reported data on 10 ultramarathon athletes who ran on a motor driven treadmill for 4 hours at 65-70% of  $\dot{V}O_{2max}$  in thermoneutral conditions (dry-bulb temperature: 21°C, wet-bulb temperature: 17°C; 48%

rh). This represents the highest exercise intensity that can be maintained over this duration, yet neither thermoregulatory nor cardiovascular strain was found to be a limiting factor during this prolonged running experiment which was associated with 5.5% BW loss.

An increase in blood / plasma viscosity increases the work of the heart and may be a further factor which exacerbates both circulatory and thermoregulatory strain during exercise in the heat. Blood viscosity is determined by the haematocrit, erythrocyte aggregation, erythrocyte flexibility, fibrinogen concentration, platelet aggregation and plasma viscosity (Chien *et al.*, 1966; Gaudard & Varlet-Marie, 2003). Body water loss through sweating causes a decrease in plasma volume and an increase in total protein concentration, haematocrit haemoglobin concentration, consequently contributing to a rise in blood viscosity (Nose *et al.*, 1988). It has been reported that an increase in blood viscosity caused by thrombus formation in the coronary arteries and cerebral vessels has led to sudden death in hot conditions (Keatinge *et al.*, 1986). For cardiac function, the rise of blood viscosity is likely to increase heart pre-load and thus to decrease maximal stroke volume (Brun *et al.*, 2007). It seems reasonable to suppose that athletes' performance in the heat is disadvantaged by a higher blood viscosity. The increase in blood / plasma viscosity might cause a decline in venous flow velocity which might compromise venous return of blood to the central blood circulation. On the contrary, a lower whole blood viscosity represents a more fluid and freely flowing bloodstream, which facilitates better perfusion and supply to the muscles (Martin-e-Silva, 1988) during exercise (Brun, 2002).

## 2.5.2 Circulatory Strain Hypothesis

### Muscle blood flow during prolonged exercise in the heat

Exercising muscles require an approximate four-fold increase in blood flow to transport larger volumes of oxygen for greater fractional muscle recruitment (Andersen & Saltin, 1985). It has been widely accepted that O<sub>2</sub> delivery increases linearly with the intensity of exercise from rest to maximal oxygen uptake ( $\dot{V}O_{2\max}$ ), implying that O<sub>2</sub> delivery to locomotor limb muscle does not limit the attainment of  $\dot{V}O_2$  (Bevegard *et al.*, 1963; Rowell *et al.*, 1964b; Grimby *et al.*, 1966; Higginbotham *et al.*, 1986). However, the increase in  $\dot{V}O_2$  per unit work for high intensity exercise is attenuated due to the limitation of cardiac pumping capacity in humans (Astrand & Saltin, 1961). In addition, there is a restriction in systemic supply of O<sub>2</sub> at the skeletal muscles, but also limitations in diffusive O<sub>2</sub> transport from the muscle capillary to the mitochondria restrict the  $\dot{V}O_{2\max}$  (Roca *et al.*, 1989). To understand the contribution of the O<sub>2</sub> transport system to  $\dot{V}O_{2\max}$ , in large and small muscles, Mortensen *et al.* (2005) measured systemic haemodynamics, O<sub>2</sub> transport and  $\dot{V}O_2$  during incremental and constant cycling exercise to exhaustion and then measured systemic and leg haemodynamics and  $\dot{V}O_2$  during incremental cycling (whole body exercise) and one leg knee extensor exercise in male subjects. They found that the rate of rise in muscle blood flow and cardiac output were attenuated during incremental and constant load cycling. In contrast, the muscle blood flow and cardiac output during knee extensor exercise increased linearly to exhaustion (Mortensen *et al.*, 2005). There is a central limitation (e.g., an insufficient cardiac output) and peripheral limitations (e.g., a reduction in muscle blood flow) to aerobic power and capacity. If the central limitation exists during exercise in thermoneutral conditions, it is possible that the combination of both whole body exercise and heat

stress will exacerbate circulatory strain during prolonged exercise in hot environmental conditions.

### **Traditional vs. Contemporary Hypotheses**

The limiting factors during prolonged exercise performance in the heat have been investigated extensively during the recent decades and yet the existing field and laboratory evidence has not come to a conclusive underlying mechanism that accounts for the contribution of dehydration as a limitation to prolonged exercise. There have been two prevailing hypotheses on potential causes of cardiovascular drift: *Traditional* and *Contemporary Hypotheses*.

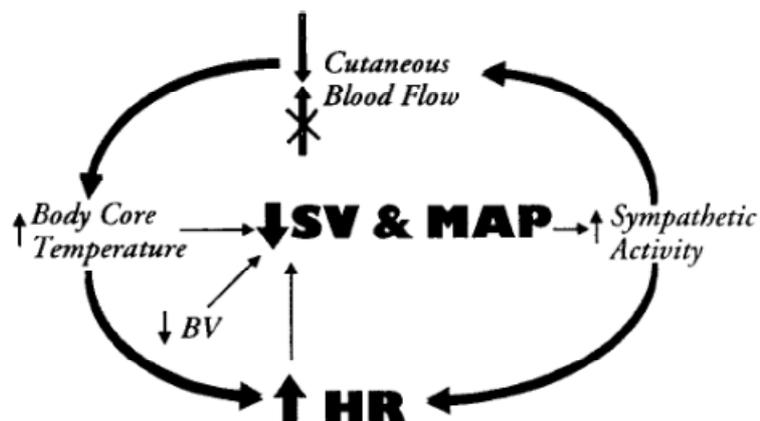
Rowell (1969) first introduced the classic concept of “cardiovascular drift” which is associated with a reduction in stroke volume due to a progressive increase in SkBF as  $T_{\text{core}}$  increases. Cardiovascular drift has been characterized as a “*downward drift in central venous pressure, stroke volume, pulmonary and systemic arterial pressure, and central blood volume, ...while at the same time a rise in heart rate maintains nearly constant cardiac output*” (Rowell, 1986). The rise in SkBF is primarily due to a partitioning of blood flow away from the viscera, renal and splanchnic regions to the skin to dissipate heat produced by exercising muscles (Rowell, 1974). At rest, the splanchnic region receives a blood flow of approximately 1200 to 1500 mL.min<sup>-1</sup>. Under warm conditions during submaximal exercise, splanchnic and renal vasoconstriction occurs to deliver 800 mL.min<sup>-1</sup> of blood to the skin (Rowell, 1974). As the exercise intensity increases to maximal ( $\dot{V}O_{2\text{max}}$ ), the blood flow to the splanchnic region reduces to 350 mL.min<sup>-1</sup> and is redistributed (1150 mL.min<sup>-1</sup> of blood flow) to active muscles (Rowell *et al.*, 1964a). This is necessary to meet the large increase in

muscle oxygen requirement during exercise. Meanwhile, the increase in heart rate offsets the decline in SV to maintain  $\dot{Q}$  (Rowell, 1974). However, if HR has drifted upward towards  $HR_{max}$  and SV continues to decline,  $\dot{Q}$  cannot be maintained. The decline in SV is thus suggested to be mediated by the Frank-Starling mechanism owing to a decrease in ventricular filling pressure.

The concept of a progressive increase in cutaneous blood flow (CBF) being the main factor contributing to the decline in SV during prolonged exercise has been supported for the past 25 years. However, the contemporary hypothesis suggests that the decrease in SV by increasing HR is due primarily to a reduction in ventricular filling time (Fritzsche *et al.*, 1999; Coyle & González-Alonso, 2001; Nassis, 2002). Increases in HR, by reducing filling time have the potential to decrease end-diastolic volume and SV (Turkevich *et al.*, 1988), provided that end-diastolic volume is not maximal (Poliner *et al.*, 1980). Fritzsche *et al.* (1999) reported that there is a strong inverse relationship between HR and SV during prolonged moderate-intensity cycling in thermoneutral conditions (27°C, <40% rh). The use of  $\beta$ -adrenergic blockade prevented the rise in HR, while SV declined between 15 – 55 minutes during prolonged exercise. Other possible factors that may cause the decline in SV (e.g., BV, CBF, forearm blood flow, forearm venous volume,  $T_{re}$  and  $T_{sk}$ ) were similar during both  $\beta$ -adrenergic blockade and control trials. CBF did not appear to be related to the decline in SV, suggesting that peripheral displacement of the blood volume was not responsible for the decline in SV. Similarly, a strong link between the decline in SV and a rise in HR during both cycling and treadmill running for 90 min at 60%  $\dot{V}O_{2max}$  ( $r=-0.65$  and  $r=-0.80$ , respectively) in thermoneutral environment (23.8 °C, 58.8% rh) was shown in a study by Nassis, 2002). It is worth noting that in Nassis *et al.*'s (2002) study, no fluid was provided throughout

the trials which significantly induced a ~ 3.3% and ~ 2.8% body mass loss associated with reductions in blood and PV during running and cycling trials, respectively. However, these results show that SV responses were not directly associated with the decline in BV. Indeed, a lower forearm SkBF was noted and it could be due to skin vasoconstriction and a further increase in  $T_{re}$  in the running trial. Therefore, both studies strongly proposed that the decline of SV is due to reduced ventricular filling time which is directly related to tachycardia.

If the rise of HR causes a decline in SV, what is the reason for the rise of HR? In a review on new perspectives in cardiovascular drift (Figure 2.15) it was suggested that increased  $T_{core}$  and sympathetic nervous activity are two likely possibilities (Coyle & González-Alonso, 2001). The rise in  $T_{core}$  is strongly correlated with HR during cardiovascular (CV) drift (Fritzsche *et al.*, 1999), whereas a lack of increase in  $T_{core}$  is accompanied by a lack of decline in SV without the effect of dehydration (González-Alonso *et al.*, 1995). Hyperthermia alone (i.e., increased in  $T_{re}$ ) during exercise in the heat has a significant effect on the HR response.



**Figure 2.15** New perspective regarding mechanisms for cardiovascular drift during prolonged exercise under conditions of maintained cardiac output and how it is exacerbated by dehydration, which acts primarily by causing hyperthermia (i.e., increased body core temperature) and hypovolemia (i.e., decreased blood volume) (Coyle & González-Alonso, 2001)

More recently, Trinity *et al.* (2010) confirmed that mild hyperthermia reduced SV during exercise which seemed to be due to the observed increase in HR *per se*. They used a similar experimental protocol as Fritzsche *et al.* (1999) but included two additional hyperthermic experimental conditions ( $\beta$  blocker *vs.* placebo) to examine the cardiovascular responses with hyperthermia. The hyperthermic conditions were achieved by having subjects wear a vinyl rain jacket and nylon / spandex leg coverings while two parabolic electronic heaters increased ambient temperature.  $T_{\text{core}}$  during both hyperthermic trials increased continually and was significantly greater than normothermic trials. The authors suggested that mild hyperthermia during exercise does not compromise heart function (Trinity *et al.*, 2010). The progressive increase in  $T_{\text{core}}$  elicited an increase in HR which was responsible for the decrease in SV.

There is a considerable volume of research that has reported a significant reduction of  $\dot{Q}$  with exercise-induced dehydration impairing aerobic performance as a consequence of circulatory and thermoregulatory strain (Sawka, 1979; Nadel *et al.*, 1980; Montain & Coyle, 1992a; Montain & Coyle, 1992b; González-Alonso *et al.*, 1995; González-Alonso *et al.*, 1997; González-Alonso *et al.*, 1998; González-Alonso *et al.*, 2000). Dehydrated subjects, ranging from 1.1 to 4.2% BW loss exhibited significant reductions in SV,  $\dot{Q}$  and SkBF with combined elevations in HR and esophageal temperature ( $T_{\text{es}}$ ) during exercise in the heat (Montain & Coyle, 1992b). In addition, total BV was reduced and was associated with a decline in SV when no fluid was ingested during 2 hours of exercise (Montain & Coyle, 1992a).

The factors related to the reduction in SV and  $\dot{Q}$  are not entirely clear. To resolve uncertainty arising from previous research, González-Alonso *et al.* (1995 and 1997)

conducted a series of studies to investigate the effects of dehydration on circulatory responses in an ambient temperature of 35°C (Table 2.1).

**Table 2.1:** Summary of González-Alonso *et al.* s' studies (1995 and 1997) investigating the effect of dehydration and hyperthermia during prolonged exercise

Variable	Study	
	González-Alonso <i>et al.</i> , 1995	González-Alonso <i>et al.</i> , 1997
T <sub>a</sub> , °C	35	35
Exercise duration , min	120	30
Exercise intensity , % $\dot{V}O_{2max}$	62 ± 2	70
% BW loss	4.9 ± 0.2	4.4 ± 0.2
HR at the end of exercise, beats.min <sup>-1</sup>	169 ± 2	178 ± 4
SV, % changes	↓28	↓20 ± 1
SV at the end of exercise, beats.min <sup>-1</sup>	108 ± 7	104 ± 6
$\dot{Q}$ , % changes	↓18 ± 3	↓13 ± 2
$\dot{Q}$ at the end of exercise, L.min <sup>-1</sup>	~18.5	18.4 ± 0.7
MAP, mmHg	↓ 5 ± 2	↓5 ± 2
T <sub>es</sub> at the end of exercise, °C	39.3 ± 0.2	39.3 ± 0.1
T <sub>sk</sub> at the end of exercise, °C	34.8 ± 0.2	34.6 ± 0.4
CBF, %	↓ 23 (% of 20 min value)	NM

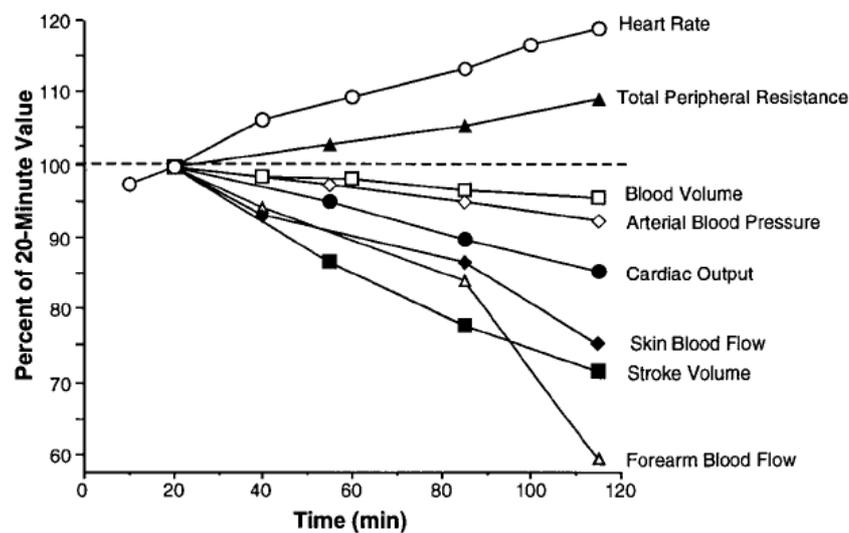
CBF, Cutaneous blood flow; NM, not measured / not mentioned; ↓, decrease; T<sub>a</sub>, ambient temperature; BW, body weight; HR, heart rate; SV, stroke volume;  $\dot{Q}$ , cardiac output; MAP, mean arterial pressure

González-Alonso *et al.* (1995) initially identified a relationship between the reduction in  $\dot{Q}$  and dehydration. Seven endurance-trained cyclists exercised at 62%  $\dot{V}O_{2max}$  for 120

min in the heat which resulted in a 4.9% BW loss with a progressive  $\sim 28\%$  and  $\sim 18\%$  decline in SV and  $\dot{Q}$  respectively which was associated with an increase in systemic and cutaneous vascular resistance (Table 2.1 & Figure 2.15). An approximate 17% increase in systemic vascular resistance coupled with the vasoconstrictor response of the cutaneous circulation limited the reductions in MAP. Therefore, heat dissipation was reduced, consequently promoting hyperthermia with the elevation in core temperature from 38.0 to 39.3°C in the heat. Conversely, the authors found the prevention of dehydration via fluid ingestion (replaced 95% of fluid losses) successfully maintained constant cardiovascular and thermoregulatory responses in both  $T_{\text{core}}$  ( $\sim 38.2^\circ\text{C}$ ) and SV (from 151-147 mL.beat<sup>-1</sup>) from 20- to 120-min of exercise (González-Alonso *et al.*, 1995). Similar observations were reported in a previous study by Hamilton *et al.* (1991). With the rise in  $T_{\text{core}}$  (stabilized at  $\sim 38.3^\circ\text{C}$ ), SV was attenuated ( $\sim 130$  mL.beat<sup>-1</sup>) during the fluid replacement trial when compared with no fluid replacement trial (2.9% BW loss) in thermoneutral conditions (22°C) at moderate intensity of exercise (70%  $\dot{V}O_{2\text{max}}$ ) (Hamilton *et al.*, 1991). However, the reductions in SV and  $\dot{Q}$  during the no fluid replacement trial were not as large as that observed in González-Alonso *et al.* (1995) study (i.e.,  $\downarrow 15\%$  compared with  $\downarrow 28\%$  in SV;  $\downarrow 7\%$  compared with  $\downarrow 18\%$  in  $\dot{Q}$ ) possibly due to a lower level of dehydration while subjects were exercising in a cooler environmental conditions.

However, it is unclear whether the inability to maintain  $\dot{Q}$  is due to hyperthermia *per se*, or dehydration *per se*, or the combination of dehydration and hyperthermia. González-Alonso *et al.* (1997) carried out another study with a higher relative exercise intensity (72%  $\dot{V}O_{2\text{max}}$ ) and noted that  $\dot{Q}$  and systemic vascular resistance were significantly reduced at the end of exercise in both dehydrated and hyperthermic conditions (Table

2.1), but these reductions were not found in hyperthermia or dehydration alone. To investigate the specific effect of dehydration, another group of subjects were tested in a cold environment (2°C) with ~4.1% BW loss. Thereafter, intravenous infusion of a BV expander (dextran solution) was used during an additional bout of exercise (30 min at 70%  $\dot{V}O_{2max}$ ) in the dehydration trial with the purpose of restoring BV. González-Alonso *et al.* (1997) found that cardiovascular responses were similar when compared with a control (euhydrated) trial. Remarkably, when hyperthermia was prevented, the decline in SV with dehydration was due to reduced BV. Meanwhile cutaneous vasoconstriction can be prevented if the subjects exercise in a cold environment that lowers  $T_{sk}$ , and increases the  $T_{core}$ -to- $T_{sk}$  gradient. Based on these studies, it appears that dehydration itself reduces SV by 7%, but when it is combined with hyperthermia, the decline in SV is 3-4 times greater (20-28%) (Figure 2.16).



**Figure 2.16** A redrawn summary of the effects of dehydration and concomitant hyperthermia from González-Alonso *et al.* (1995) (Coyle & González-Alonso, 2001)

## 2.6 THERMOTOLERANCE, HEAT SHOCK PROTEINS AND GLUTAMINE INGESTION

During environmental heat exposure and exercise, appropriate physiological heat dissipation prevents life-threatening hyperthermia. Current research has demonstrated that mammalian species have developed different ways to deal with heat stress. Heat shock proteins (HSPs) in polytene chromosomes were first discovered in 1962 (Ritossa, 1962) and subsequent studies reported that most HSPs have strong cytoprotective effects (Lindquist & Craig, 1988; Hightower, 1991; Moseley, 1997; Welch, 1992; Kregel, 2002; Sonna *et al.*, 2002; Campisi *et al.*, 2003). HSPs are categorized into families on the basis of their molecular weight (Table 2.2).

**Table 2.2** Cellular locations and proposed functions of mammalian heat shock protein families (Kregel, 2002)

HSP Family	Cellular Location	Proposed Function
HSP 27 (sHSP)	Cytosol, nucleus	Microfilament stabilization, antiapoptotic
HSP 60	Mitochondria	Refolds proteins and prevents aggregation of denatured proteins, proapoptotic
<b>HSP Family:</b>		
HSP 72 (Hsp70)	Cytosol, nucleus	Antiapoptotic
HSP 73 (Hsc 70)	Cytosol, nucleus	Protein folding, cytoprotection
HSP 75 (mHSP70)	Mitochondria	Molecular chaperones
HSP 78 (GRP78)	ER	Molecular chaperones
HSP 90	Cytosol, ER, nucleus	Cytoprotection, molecular chaperones
HSP 110/104	Cytosol	Regulation of steroid hormone receptors, protein translocation
		Protein folding

HSP, heat shock protein; sHSP; small HSP; ER, endoplasmic reticulum

Most research has studied HSP 72, because it is recognised as the most heat sensitive and highly inducible heat shock protein (Sonna *et al.*, 2007; Ruell *et al.*, 2009). The induction of HSP 72 was associated with the development of thermotolerance, which is

defined as the ability of a cell or organism to become resistant to heat stress after a prior sublethal heat exposure (Landry *et al.*, 1982; Yamada *et al.*, 2007). The expression of HSP 72 varies in different cells, but, on the average, HSP 72 in the intact body seem to operate for several hours following the heat stress and remain elevated for 3-5 days (Kregel, 2002). It was shown that the greater the initial heat dose, the greater the magnitude and duration of thermotolerance (Blake *et al.*, 1990; Kiang *et al.*, 1994). However, the response of HSP 72 is not heat-specific and can be induced by a variety of stressors including ischemia (Mestril & Dillmann, 1991; Marber *et al.*, 1995), energy depletion (Sciandra & Subject, 1983; Lee, 1987; Febbraio *et al.*, 2004), hypoxia (Hahn & Li, 1982; Wen *et al.*, 2002) and exercise training (Hung *et al.*, 2005; Chen *et al.*, 2007) etc.

At the molecular level, it has been postulated that the HSP 70 family is responsible for preventing protein denaturation, processing denatured protein and protein fragments that are caused by hyperthermia (Kregel, 2002). At the organ level, accumulation of HSPs reverses the increase in intestinal permeability to prevent the pathological changes associated with endotoxin release (Moseley & Gisolfi, 1993) and endotoxin tolerance (Hotchkiss *et al.*, 1993).

The presence of HSP 72 in plasma or serum can act as a danger signal (Asea *et al.*, 2002) and an aid in the diagnosis of heat stroke (Huisse *et al.*, 2008; Ruell *et al.*, 2009; Dehbi *et al.*, 2010). In an animal study, Dehbi *et al.* (2010) investigated the expression of HSP 60 and HSP 72 in baboons subjected to environmental heat stress to induce moderate ( $T_{\text{core}}: 42.5^{\circ}\text{C}$ ) and severe heatstroke ( $T_{\text{core}}: \geq 43.5^{\circ}\text{C}$ ). The authors reported that severe heat stroke triggered a marked release of HSP 72 but not HSP 60 into the circulation and in multiple organs (i.e., liver) of heat stressed baboons. Non-survivors displayed

significantly higher HSP 72 than survivors. Similarly, it has been reported that plasma HSP 70 levels were significantly increased in a heatstroke patient compared with healthy controls (Huisse *et al.*, 2008). Therefore, it was suggested that HSP 72 may serve as a prognostic indicator of heatstroke and also a biomarker of multiple organ tissue damage.

During a competitive running event, a positive correlation was found between plasma HSP 72 concentration and final  $T_{re}$  immediately after a 14-km run (Ruell *et al.*, 2006). Plasma HSP 72 concentration was significantly higher in hyperthermic runners with more severe symptoms of heat illness than control runners (asymptomatic). The data indicates that, the final  $T_{re}$  accounts for 42% of the variance in plasma HSP 72. Another study by Ruell *et al.*, (2007) further reported that no difference was found in lymphocyte HSP 72 levels between hyperthermic patients and control subjects (without symptoms or signs of heat illness) immediately after a 14 km running race. Similarly, seven “heat intolerant” subjects exhibited higher physiological strain but no difference was found in HSP 72 levels immediately after a 2 hours heat tolerance test in a climate chamber (40°C, 40% rh) (Moran *et al.*, 2006). A unique feature was found by Ruell *et al.* (2007) that lymphocyte HSP 72 was elevated in hyperthermic athletes 2 days after the 14 km run and the level of elevation was associated with the degree (post race rectal temperatures) and duration (race time plus cooling time) of hyperthermia. They also found that a  $T_{re}$  of at least 39°C is necessary for elevated lymphocyte HSP 72 two days later. These findings were in agreement with other studies that lymphocyte HSP 72 did not increase immediately following exercise even though  $T_{re}$  was increased (Niess *et al.*, 2002; Simar *et al.*, 2004).

The precise mechanisms for the improvement in cellular thermotolerance in association with the increase in HSP levels have not been determined. Ravindran *et al.* (2005) reported that resistance to dehydration damage in some cell lines correlates with the presence of a range of HSPs. Therefore, it has raised the question of whether the successful runners are able to adapt to dehydration and the associated high metabolic heat loads, or whether they are able to acquire a tolerance to dehydration due to the induction of HSPs.

During competitive distance running, elite runners will run at their fastest speed to win the race. The relatively high intensity of the race will most likely lead to an increase in  $T_{re}$  and the level of dehydration. A recent field study (Zouhal *et al.*, 2010) observed that the fastest runners (less than 3 hours finishers) completing a marathon race were the most dehydrated when compared with slower runners with a  $> 3$  hours finishing time. With sweat rates during marathon races exceeding 2.0-3.0 L.hr<sup>-1</sup> (Armstrong, 1986), it is not uncommon for these athletes to experience significant levels of dehydration over the course of the race. It thus appears that the significant level of dehydration and success in marathon races were irrespective of ACSM guidelines (Sawka *et al.*, 2007).

A number of studies have found that glutamine can be a potent enhancer for stress induced HSP expression *in vitro* and *in vivo* and improve cell survival against a variety of stressors in humans as well as animals (Nissim *et al.*, 1993; Ehrenfried *et al.*, 1995; Wischmeyer *et al.*, 1997; Chow & Zhang, 1998; Wischmeyer *et al.*, 2003; Ziegler *et al.*, 2005; Chang *et al.*, 2006; Singleton & Wischmeyer, 2006; Morrison *et al.*, 2006). Singleton and Wischmeyer (2006) demonstrated that oral glutamine administration in rats significantly enhanced the expression of HSP and improved survival following hyperthermia. An increase in HSP 72 expression and a marked protection of intestinal

epithelial cells against lethal heat and oxidant injury were shown in unstressed cells after 2 hours of glutamine administration (Wischmeyer *et al.*, 1997). Morrison *et al.* (2006) showed that glutamine-mediated cellular protection after heat stress injury is related to heat shock factor-1 expression and cellular capacity to activate an HSP response. In a human study, Ziegler *et al.* (2005) reported that parenteral alanyl-glutamine dipeptide administration 0.5 g/kg per day for 7 days upregulates serum HSP 70 concentration and reduces the “critically ill patients” length of stay in an Intensive Care Unit (ICU) and days on a ventilator.

From a hydration or rehydration perspective, Lima *et al.* (2002) reported that an alanyl-glutamine-based oral rehydration fluid increased water and electrolyte intestinal absorption in a rat model of secretory diarrhoea induced by cholera toxin. It was also shown that glutamine can stimulate sodium intestinal absorption in rabbit (Nath *et al.*, 1992). More recently, Hoffman *et al.* (2010) demonstrated that an oral rehydration beverage containing L-alanyl-L-glutamine (0.05 and 0.2 g.kg<sup>-1</sup> body mass per liter) provided an ergogenic effect by increasing fluid and electrolyte (Na<sup>+</sup>) uptake and increased performance time to exhaustion during mild dehydration in ten physically active males. Based on the aforementioned studies, glutamine supplementation is likely to be an enhancer for HSPs expression together with the restoration of fluid in dehydrated subjects during exercise in the heat.

## 2.7 SUMMARY

Presently, it is a matter for debate whether dehydration is the primary factor precipitating impaired endurance performance in hot conditions and how distinct levels of dehydration impact upon the circulatory and thermoregulatory responses to exercise and the environment. There may be a threshold level of dehydration at which the effects of dehydration and hyperthermia become additive

Previous investigations have proposed that prolonged exercise performance is partly governed by climatic conditions especially at high ambient temperatures and high humidity, which restricts sweat evaporation and hence the rate of heat loss from the body (Galloway & Maughan, 1997; Vihma, 2010). Therefore, under these conditions the only option for the athlete is to decrease the metabolic heat production by decreasing running velocity (Ely *et al.*, 2007; Ely *et al.*, 2008). Moreover, the number and rate of finish line medical encounters and on-course marathon drop-outs begins to rise when WBGT is  $>13^{\circ}\text{C}$  (Roberts, 2010). It is likely that the increase in WBGT has a significant impact on prolonged exercise performance. However, Samuel Wanjiru (now deceased) and Naoko Takahashi, both gold medallists of the Beijing Olympic Men's Marathon 2008 and Sydney Olympic Woman's Marathon 2000 respectively, demonstrated that elite performance in hot weather conditions appears to be well maintained by highly motivated elite athletes. Meanwhile, a number of studies have been published about the negative impact of hypohydration on endurance performance (Montain & Coyle, 1992b; Sawka, 1992; Chevront *et al.*, 2003a). These studies are now subject to intense scrutiny, along with assertions that fluid ingestion should match the volume of sweat lost or the athlete should simply let their sensation of thirst dictate the ingestion of fluid. There is a need for more controlled laboratory studies that seek to understand the underlying mechanisms enabling the successful runners to perform well

in marathon races despite the apparent effect of prevailing hot climatic conditions contributing to significant hypohydration and hyperthermia.

Over the past four to five decades, Kenyan distance runners have dominated the Olympic Games, World Cross-Country Championships, IAAF World Track Championships, as well as major international road races and marathons. Kenyan distance runners' negative energy balance with a low fluid intake regimen during intense training and prior to competition has been proposed to explain their extraordinary success (Onywera *et al.*, 2004; Fudge *et al.*, 2006). Coyle (2004) suggested that a loss of body mass through sweat loss during marathon races may lower the oxygen cost of running. If the energy utilization when running at a given velocity can be reduced, the time to fatigue will be delayed and the running velocity that can be sustained may potentially be increased. Therefore it could be speculated that the success of Kenyan runners is related to a reduced body mass through sweat loss, low energy diet and minimal fluid intake that would have the potential to reduce the energy cost of running.

The view that dehydration is the primary factor precipitating impaired endurance performance in hot conditions has been challenged with multiple hypotheses. These hypotheses have been proposed to explain the underlying mechanisms of hyperthermia during prolonged exercise performance in the heat. According to the critical core temperature hypothesis, the aetiology of fatigue during exercise in the heat is likely due to a high internal body temperature (Nielsen *et al.*, 1993; Nielsen *et al.*, 1997; González-Alonso *et al.*, 1999b). It is also suggested that exercise-induced hyperthermia leads to a reduction in central neural drive to the exercising muscles in hot and dry environments (Nybo & Nielsen, 2001). The reduced CNS activation of the exercising

muscles may be an inhibitory protective mechanism and represent a “safety brake” against hyperthermic catastrophe during prolonged exercise. However, recent studies have proposed that an elevated core temperature alone is not associated with the cessation of exercise in the heat (Saboisky *et al.*, 2003; Périard *et al.*, 2011). Therefore, we speculate that dehydration might be another plausible factor involved in the early fatigue associated with a high core temperature.

During exercise in hot environments, the distribution of blood flow to the cutaneous circulation and exercising muscles creates competition for the maintenance of  $\dot{Q}$  (Rowell, 1974; Fritzsche *et al.*, 1999; Trinity *et al.*, 2010). As exercise continues, if heart rate has drifted upward towards  $HR_{max}$  and SV continues to decline,  $\dot{Q}$  cannot be maintained. Ultimately, a decline in  $\dot{Q}$  will limit  $O_2$  delivery and uptake to exercising muscle causing fatigue. However, the factors related to the reduction in SV and  $\dot{Q}$  are not entirely clear. González-Alonso *et al.* (1995 and 1997) investigated the effects of dehydration (~4% BW loss) on circulatory responses in different environmental conditions. They found that when hyperthermia was prevented, the decline in SV with dehydration was due to reduced blood volume. Conversely, evidence from Hamilton *et al.* (1991) demonstrated that the reductions in SV and  $\dot{Q}$  during the no fluid replacement trial were not as large as that observed by González-Alonso *et al.* (1995). This could be explained by a lower degree of dehydration (~3% vs. ~4% BW loss) and subjects were exercising in cooler environmental conditions (22°C vs. 35°C). Research is thus required to explain part of the discrepancy in circulatory responses to different levels of dehydration and manipulation of environmental stress (WBGT) during prolonged exercise.

It is speculated that the expression of HSPs may play an important role in the development of thermotolerance and protection from cellular damage (Yamada *et al.*, 2008) associated with dehydration (Ravindran *et al.*, 2005). However, the precise mechanisms for the improvement in cellular thermotolerance in association with the increase in HSP levels have not been delineated. Oral glutamine administration in rats has been shown to enhance the expression of HSPs and improved survival following hyperthermia (Singleton & Wischmeyer, 2006). Moreover, when subjects ingested an oral rehydration beverage containing L-alanyl-L-glutamine, it provided an ergogenic effect by increasing fluid and electrolyte (Na<sup>+</sup>) uptake (Hoffman *et al.*, 2010). Based on the aforementioned studies, glutamine supplementation is likely to be an ergogenic aid, increasing fluid restoration and HSPs expression during mild dehydration in the heat.

## **CHAPTER 3**

### **RESEARCH THESIS DESIGN**

### **3.1 RESEARCH THESIS DESIGN**

To investigate the effects of hypohydration and thermotolerance during prolonged exercise performance in the heat, one observational field study and three laboratory experiments were undertaken. This thesis tested the primary hypothesis that hypohydration together with a progressive increase in circulatory and thermoregulatory strain impairs prolonged exercise performance in the heat.

#### **STUDY ONE:**

This study investigated the hydration status of elite Kenyan distance runners competing in hot, humid conditions. This study tested the hypothesis that the elite runners would finish the long distance races with more than 3% BW loss in warm humid climatic conditions. It also tested the hypothesis that elite runners would compensate well for the increase in ambient temperature by an increased sweat rate regardless of the volume of fluid ingested or body weight loss.

#### **STUDY TWO:**

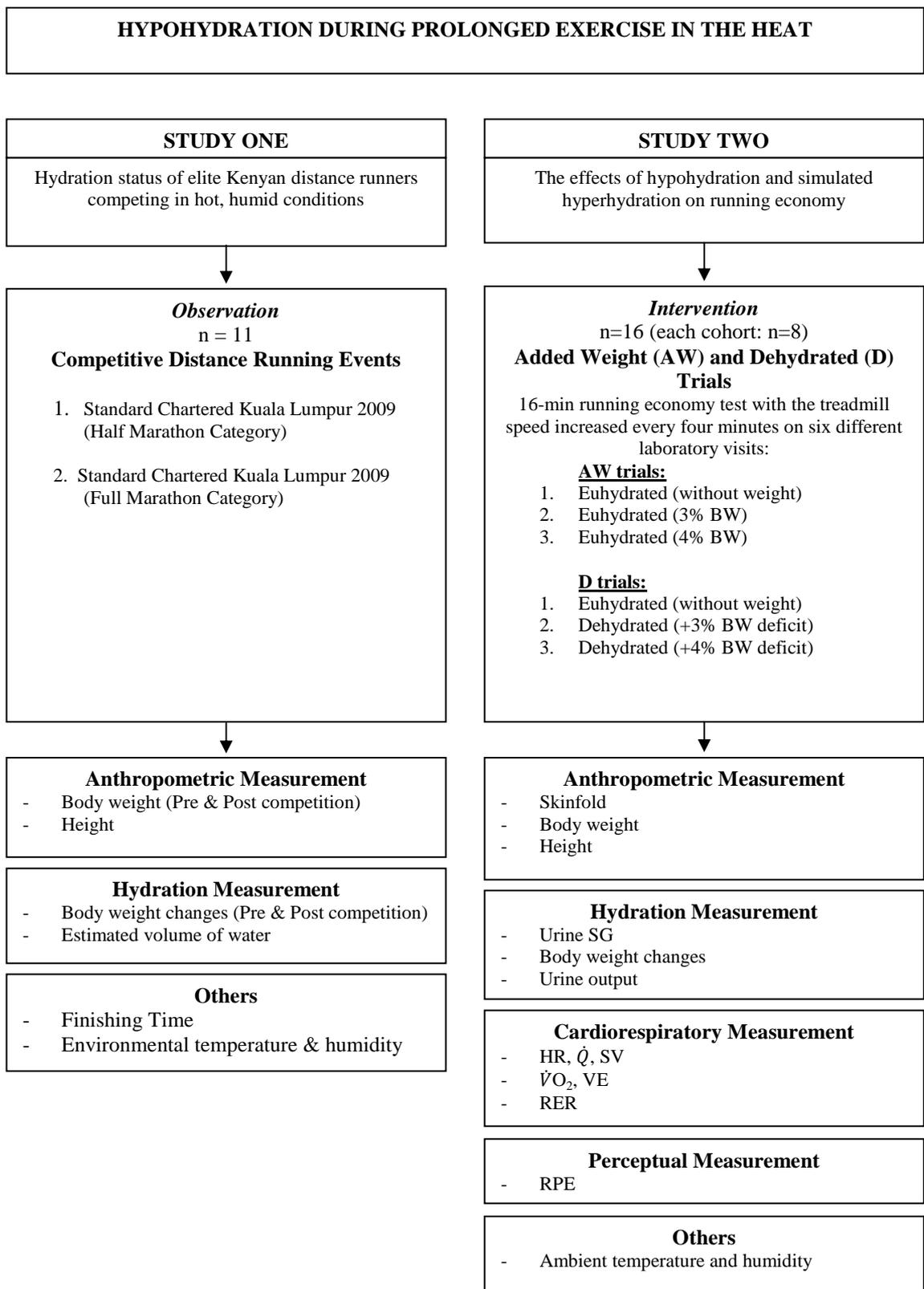
This study tested the hypotheses that (i) hypohydration would reduce the oxygen cost of running proportionally with the degree of hypohydration (-3% and -4% BW); and (ii) simulated hyperhydration would increase the oxygen cost of running proportionally with the degree of added weight (+3% and +4% BW).

**STUDY THREE:**

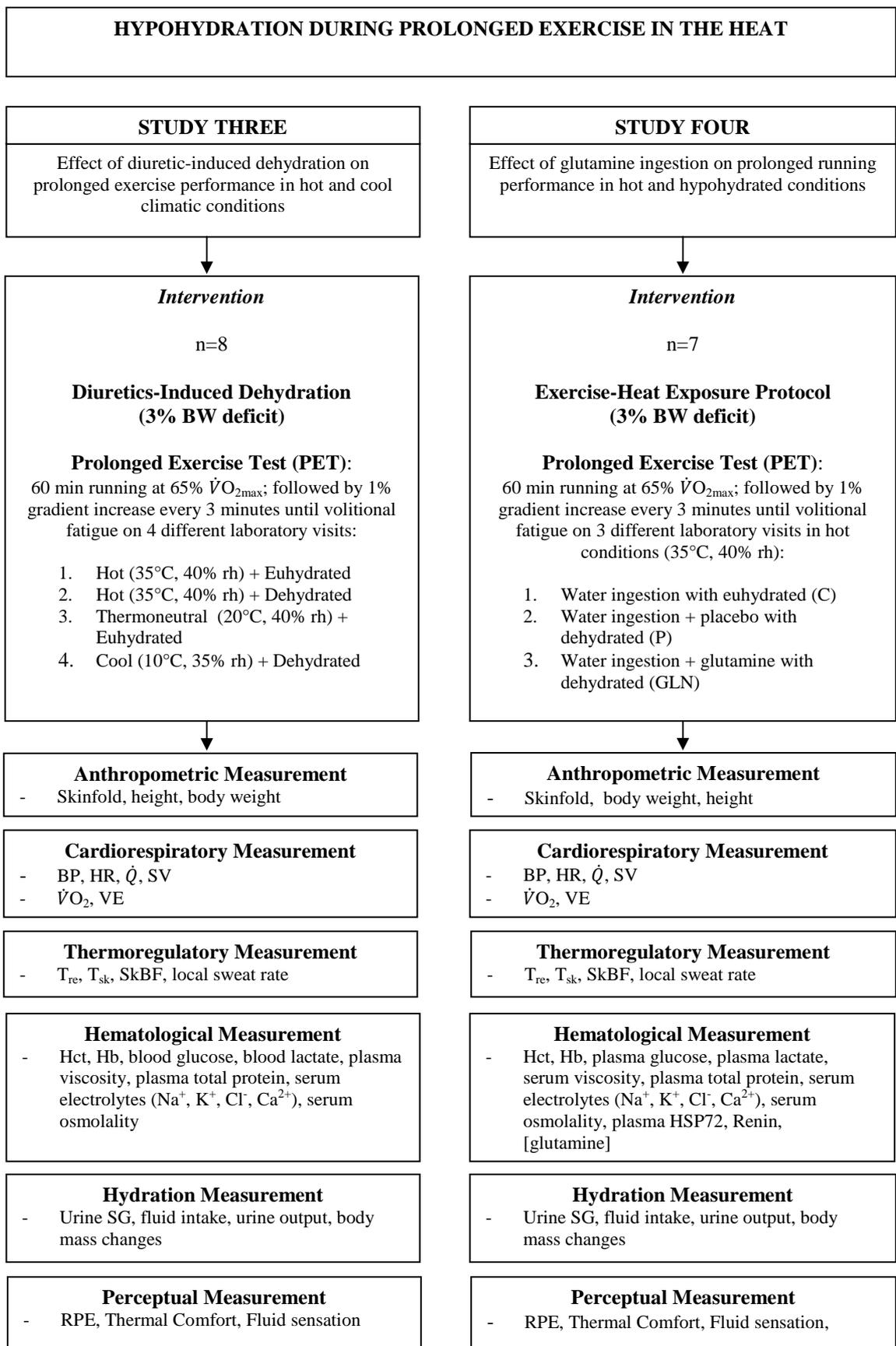
This study tested the hypothesis that mild dehydration would impair prolonged running performance in hot (35°C, 40% rh) but not in cool (10°C; 35% rh) conditions. It also tested the hypothesis that mild dehydration would not cause either circulatory strain or thermoregulatory strain in cool conditions.

**STUDY FOUR:**

This study tested the hypothesis that the administration of alanyl glutamine would attenuate exercise-induced decrease in plasma glutamine concentration. It also tested the hypothesis that the ingestion of alanyl-glutamine would induce plasma HSP 72 expression during prolonged exercise in hot conditions and in a hypohydrated state.



**Figure 3.1** Thesis Research Design



**Figure 3.1** (continued)

## **CHAPTER 4**

### **METHODOLOGY**

## **4.1 ANTHROPOMETRIC MEASUREMENTS**

The following anthropometric characteristics were measured and recorded for each subject.

### **4.1.1 Height and Weight: Computed Body Mass Index (BMI) and Body Surface Area ( $A_D$ )**

The standing height without shoes of each subject was measured using a wall mounted height stadiometer (Harpenden Stadiometer, Holtain Limited, UK) to the nearest half centimetre. Subjects were instructed to position their heads in the Frankfort Horizontal Plane during the standing height measurement.

Body mass was determined to the nearest 0.001 kg using an electronic scale (Mettler Toledo ID1 Multi Range Scale, Columbus, OH, USA) with shoes removed and with exercise shorts. The electronic scale was calibrated prior to the experimental trials using known weights. Body mass index (BMI) was calculated from height and weight:  $BMI (kg.m^{-2}) = Weight / (Height)^2$ . Body surface area ( $A_D$ ) was calculated using the equation described by DuBois and DuBois (1916):  $A_D (m^2) = 0.20247 \times Height (m)^{0.725} \times Weight (kg)^{0.42}$

### **4.1.2 Skinfold Determination of Thickness: Computed Percentage of Body Fat**

Body density was calculated using seven skinfolds thickness measurements according to the method of Jackson & Pollock (1978). The seven skinfold thickness sites were: triceps, chest, midaxillary, subscapular, suprailiac, abdomen, and anterior mid-thigh were measured twice to the nearest 0.5 mm using skinfold calliper (Holtain, Crymych, UK) (Jackson & Pollock, 1978) on the right side of the body. A third measurement of

skinfold thickness was taken when inconsistent values ( $>2\text{mm}$ ) were obtained. Percentage of body fat was estimated using Siri's equation:  $(495 / \text{Body Density}) - 450$  (Siri & Lukaski, 1993).

The abovementioned procedures for height, weight and skinfold measurements were used in Study Two, Study Three and Study Four.

## **4.2 PRELIMINARY MEASUREMENTS**

### **4.2.1 Submaximal Exercise Test**

The submaximal exercise test was undertaken in thermoneutral conditions (20°C, 40% rh) and involved 4 x 4 min of continuous steady state running. The four treadmill velocity stages were 10, 12, 14, 16 km.h<sup>-1</sup> with 0% gradient. Heart rate (HR) was measured with a ProTrainer™ heart rate monitor (Polar Electro Oy, Kemele, Finland) and the Borg rating scale of perceived exertion (RPE) values (Borg, 1982) were recorded during the last 15 sec of each stage. Oxygen uptake ( $\dot{V}O_2$ ) was measured for 60 s using the Douglas Bag technique. The respiratory gas analyzers were calibrated immediately prior to the analysis of the expired respiratory gas samples. Expired gas fractions were measured using O<sub>2</sub> and CO<sub>2</sub> analysers (Model 2-3A and Model CD-3A respectively, Ametek, Thermo Instruments, Pittsburg, PA) calibrated with a known beta gas (O<sub>2</sub>:15.9%; CO<sub>2</sub>: 4.03%) and outside air (O<sub>2</sub>:20.93%; CO<sub>2</sub>: 0.03%). Expired gas volume was measured using a dry gas flow meter (Parkinson-Cowan, England). Standard temperature pressure dry values were calculated according to corrected barometric pressure and temperature.

### **4.2.2 Maximum Oxygen Uptake Test ( $\dot{V}O_{2max}$ Test)**

An incremental gradient run on a treadmill to volitional fatigue was undertaken to determine  $\dot{V}O_{2max}$  followed by 5 min of recovery walk. The treadmill velocity was set at 12 km.hr<sup>-1</sup> and the gradient was increased by 2% every 2 min. HR and RPE values were recorded during the last 15s of each stage of the treadmill run. Maximal heart rate, maximal oxygen consumption, time to exhaustion and perceived exertion were obtained at the point of volitional fatigue, usually within the final ~ 10s of exercise. Subjects

were assisted in fitting the nose clip and inserting the mouthpiece of Hans Rudolph respiratory gas collection valve in the final minutes of the run to volitional fatigue. A hand signal from the subjects indicated that they were close to their exercise limit and at this point expired respiratory gas collection began.

The  $\dot{V}O_{2\max}$  value at exhaustion was accepted when two of the following three criteria were met:

1. A plateau in  $\dot{V}O_2$  despite further increases in exercise intensity / gradient.
2. A respiratory exchange ratio (RER) of 1.10 or above.
3. A plateau in HR despite further increases in exercise intensity / gradient.

(Howley *et al.*, 1995)

The submaximal and  $\dot{V}O_{2\max}$  tests data were used to determine the treadmill velocity that elicits 65, 70, 75 and 80%  $\dot{V}O_{2\max}$  for Study Two and 65%  $\dot{V}O_{2\max}$  for the subsequent prolonged exercise tests (PETs) in Study Three and Study Four.

### **4.3 CARDIORESPIRATORY MEASUREMENTS**

#### **4.3.1 Heart Rate (HR)**

Heart rate (HR) was monitored and recorded at 5 min interval during experimental trials using a ProTrainer™ heart rate monitor (Polar Electro Oy, Kemele, Finland).

#### **4.3.2 Mean Arterial Pressure (MAP)**

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a sphygmomanometer (Accoson, Harlow, UK) at rest, 30 min and within the final minutes prior to when the treadmill was stopped. Mean arterial pressure (MAP) was calculated as follows:  $MAP = (0.33 \times SBP) + (0.67 \times DBP)$ .

#### **4.3.3 Oxygen Uptake ( $\dot{V}O_2$ )**

Expired respiratory gas was collected over a 60 s period using the Douglas Bag technique. Gas analyzers were calibrated immediately before the analysis of the gas samples. Expired gas fractions were measured using O<sub>2</sub> and CO<sub>2</sub> analysers (Model 2-3A and Model CD-3A respectively, Ametek, Thermox Instruments, Pittsburg, PA) which were calibrated immediately prior to the respiratory gas collections with a known beta gas (O<sub>2</sub>:15.9%; CO<sub>2</sub>: 4.03%) and outside air (O<sub>2</sub>:20.93%; CO<sub>2</sub>: 0.03%). Expired gas volume was measured using a flow meter (Parkinson-Cowan, England). Standard temperature pressure dry values were calculated according to corrected barometric pressure and temperature.

#### 4.3.4 Cardiac output ( $\dot{Q}$ )

Cardiac output ( $\dot{Q}$ ) was measured using the CO<sub>2</sub>-rebreathing equilibrium technique (Collier, 1956) towards the end of each stage of the preliminary submaximal exercise test. Subjects were required to breathe via a respiratory valve (model 2700, Hans Rudolph, Kansas City, MO) with a mouthpiece and nose clip. They were required to rebreathe with a gas mixture of approximately 15% CO<sub>2</sub> and 85% O<sub>2</sub> for approximately 12 seconds for the determination of  $\dot{Q}$ . The fraction of expired CO<sub>2</sub> was measured on breath by breath basis by continuously sampling at the mouthpiece with a CO<sub>2</sub> analyser (Beckman LB-2, Beckman Instrument, IL, USA) calibrated with outside air (CO<sub>2</sub>: 0.03%) and a beta gas of known concentration (CO<sub>2</sub>:15%) before the testing. Mixed venous PCO<sub>2</sub> equilibrium was invariably attained during the rebreathing procedure. The criteria for PCO<sub>2</sub> equilibrium were as follows:

- (i) equilibrium was attained within 12s after initiation of the rebreathing procedure and
- (ii) PCO<sub>2</sub> varied <1 Torr for a 5-s period. Estimates of cardiac output were adjusted for haemoglobin concentration using the equations of McHardy, (1967).

Stroke volume (SV) was calculated from the  $\dot{Q}$  and HR determinations:  $SV = \dot{Q} / HR$ . Arterio-venous differences in  $\dot{V}O_2$  content (a-vO<sub>2</sub> diff) was calculated using the Fick equation:  $\dot{V}O_2 = Q \times a-vO_2 \text{ diff}$

#### 4.3.5 Respiratory Exchange Ratio (RER)

Respiratory exchanged ratio (RER) was determined using the following equation:

$$\text{RER} = \dot{V}\text{CO}_2 / \dot{V}\text{O}_2$$

Where  $\dot{V}\text{CO}_2$ : Expired carbon dioxide

$\dot{V}\text{O}_2$ : Oxygen uptake

The abovementioned procedures for cardiorespiratory measurements were used in Study Two, Three and Four.

## 4.4 THERMOREGULATORY MEASUREMENTS

### 4.4.1 Skin Temperature ( $T_{sk}$ ) and Rectal Temperature ( $T_{re}$ ): Computed Mean Skin Temperature ( $\bar{T}_{sk}$ )

Skin ( $T_{sk}$ ) and rectal ( $T_{re}$ ) temperatures were monitored continuously and recorded at 1-min and 5-min intervals respectively.  $T_{sk}$  was measured at four sites using iButton™ temperature sensors: positioned according to anatomical landmarks identifying the skin sites required for calculating mean skin temperature ( $\bar{T}_{sk}$ ) using the equation of Ramanathan (1964). iButton™ have an inbuilt data logger (Maxim Integrated Products, Sunnyvale, CA, USA) for subsequent data downloading (van Marken Lichtenbelt et al., 2006). These were attached to the skin using narrow strips of Opsite™ surgical bandage.  $\bar{T}_{sk}$  was calculated using the equation:

$$\bar{T}_{sk} = 0.3 (T_{chest} + T_{arm}) + 0.2 (T_{thigh} + T_{leg}) \text{ (Ramanathan, 1964).}$$

A thermistor probe (YSI 400 Series; Mallinckrodt Medical, Kansas City, MO) was inserted to a depth 12 cm past the anal sphincter.  $T_{re}$  data was logged on a portable temperature data logger (Digi-Sense® Thermistor Thermometer, Chemopharm, US), which was calibrated before the study in a water bath with a platinum resistance probe (T1091).

### 4.4.2 Skin Blood Flow (SkBF)

A laser Doppler skin probe (MP1-V2, moorLAB™, Moor Instruments Ltd, Devon, UK) with a probe holder and a double-sided adhesive disc was attached onto a clean skin surface at the level of the right medio-ventral upper-arm, below the deltoid (near to the iButton™). Skin blood flow (SkBF) was recorded via a Laser-Doppler perfusion monitor (moorLAB™, Moor Instruments Ltd., Devon, UK). The data was later

analyzed using the Moor Instruments associated software (moorLAB v2.01, Mec Instruments, Wilmington, DE, USA). Values are expressed as arbitrary units (AU). All data was normalised to resting skin blood flow during 5 min resting period (baseline) as a percentage change.

The abovementioned procedures for thermoregulatory measurements were used in Study Three and Study Four.

## **4.5 HYDRATION MEASUREMENTS**

### **4.5.1 Urine Specific Gravity (USG)**

A urine sample was collected in a disposable container for urine specific gravity analysis using a digital specific gravity refractometer (Atago<sup>®</sup> UG- $\alpha$ , Japan) with measurement range from 1.0000 to 1.0600, with a measurement accuracy of  $\pm 0.0010$ . Several drops of distilled water were dripped onto the prism surface for calibration and approximately 0.5 mL of urine sample was used to measure specific gravity. Duplicate measurements were made.

### **4.5.2 Sweat Loss**

Sweat loss was calculated as the difference in pre- and post-exercise nude body mass, corrected for fluid intake, urine output and respiratory water loss (Mitchell *et al.*, 1972).

The abovementioned procedures for hydration measurements were used in Study Two, Study Three and Study Four.

## **4.6 HAEMATOLOGICAL MEASUREMENTS**

### **4.6.1 Haemoglobin & Haematocrit: Computed Plasma Volume Changes**

Venous blood was collected into 1 mL tubes containing EDTA as anticoagulant (Vacutainer, Greiner Bio-one, Kremsmunster, Austria). Haemoglobin (Hb) concentration was analysed (Sysmex KX-21N Hematology Analyser, Kobe, Japan) and haematocrit (Hct) percentage was estimated using a microcentrifuge (Hawksley) and micro haematocrit reader (Hawksley CE/15006, UK). Triplicate measurements were made of haematocrit and haemoglobin. Hb and Hct values were used to calculate the percentage changes in plasma volume (PV) as follows:

$$\% \Delta PV = \left\{ \left( \frac{[Hb]_C}{Hb_T} \times \frac{(100-Hct_C)}{(100-Hct_T)} \right) / (100-Hct_C) - 1 \right\} \times 100$$

(Dill & Costill, 1974)

Where C refers to resting and T refers to exercise values for Hct and Hb

#### 4.6.2 Glucose and Lactate

There are two methods for measuring glucose and lactate:

- Glucose assay: (i) Glucose oxidase colorimetric analysis (Study Four)
- (ii) Automated glucose oxidase method (Study Three)
- Lactate assay: (i) Lactate dehydrogenase (LDH) method (Study Four)
- (ii) Automated lactate oxidase method (Study Three)

##### Glucose Assay

4 mL of venous blood was collected using a lithium heparin tube as anticoagulant and centrifuged at 2,200 g for 10 min at 4°C (Super T 21, Kendro Laboratory Products, NC, USA). One aliquot of plasma was frozen and stored at -20°C for later analysis of glucose. Plasma glucose was determined using a *glucose oxidase colorimetric analysis kit (TR-1511-200 Thermo Electron Noble Park, Victoria, Australia)*. Samples were prepared by mixing 450 µL of glucose reagent with 4 µL of plasma. After vortex mixing, the plasma samples were kept on ice, followed by incubation in a heating block at 37°C for 10 min. After 10 min, 200 µL of standards and plasma samples were pipetted into a 96-well plate. All the standards and plasma samples were assayed in duplicate. Colour absorption was measured at a wavelength of 600 nm in a microplate reader (Benchmark Plus, Bio-Rad, CA, USA). The unknown concentration was calculated using the absorbance of the glucose standards.

##### Lactate Assay

250 µL of blood was deproteinized in 500 µL of 0.6 M perchloric acid (PCA). This solution was mixed well and stored frozen at -20°C. On the day of analysis, the frozen sample was thawed, centrifuged and the supernatant was retained. Blood lactate was determined using the lactate dehydrogenase (LDH) method (Annan, 1975).

The following solutions were used for this assay:

**1 L of Hydrazine Buffer ingredients:**

Hydrazinium Sulphate	13.0g
Hydrazine Hydrate	50 mL
EDTA	2.0 g

**Reagent cocktail ingredients:**

Hydrazine Buffer	1 mL per sample
Nicotinamide Adenine Dinucleotide (NAD)	1 mg per sample
Lactate Dehydrogenase (LDH) [Sigma-Aldrich <sup>®</sup> catno L2500]	5.0 $\mu$ L per sample

The reagent cocktail was prepared fresh on the day of analysis. 1 mL of reagent cocktail was pipetted into a cuvette and the initial absorbance reading was obtained using a spectrophotometer (UV-1601; Shimadzu, Tokyo, Japan) at 340 nm. 50  $\mu$ L of sample (supernatant) was later pipetted into the cuvette, mixed well and incubated at room temperature for 30 min. The final absorbance reading was taken at the same wavelength. The unknown concentration was calculated using the absorbance of a series of lactate standards.

**Plasma Glucose and Lactate**

Duplicate measurements were made of plasma glucose and lactate using the automated glucose oxidase and lactate oxidase methods, respectively (EML 105; Radiometer, Copenhagen, Denmark).

#### **4.6.3 Serum Osmolality**

Venous blood was collected using a clot activator tube for serum separation and allowed to clot for at least 30 min. After centrifugation at 2,200 g for 10 min at 4°C (Super T 21, Kendro Laboratory Products, NC, USA), serum samples were stored at -20°C for later analysis.

The osmolality of serum was measured using a cryoscopic osmometer (OSMOMAT 030, Gonotec GmbH) to determine the freezing point depression. The OSMOMAT 030 was calibrated with 50µL distilled water and 50µL of calibration solution prior to the osmolality measurement.

Serum samples were thawed and inverted for several times prior to the centrifugation at 5000 rpm for 5 min at 15°C (Hermle Z360K Centrifuge, Germany). A serum sample volume of 50 µL was pipetted into a clean dry vessel and measurements were made in duplicate.

#### **4.6.4 Serum Electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>)**

105 µL serum samples were analysed for electrolyte (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>) concentrations using the ABL80 Flex (Radiometer, Copenhagen, Denmark).

#### **4.6.5 Plasma Total Protein**

Plasma total protein was analysed using a total protein kit (Stanbio Total Protein LiquiColor<sup>®</sup>, Texas). 4  $\mu\text{L}$  of plasma sample was added to 400  $\mu\text{L}$  of reagent (Stanbio Total Protein LiquiColor<sup>®</sup> Reagent). The solution was mixed well using a vortex mixer and allowed to stand in the heating block at 37°C for approximately 10 min. 200  $\mu\text{L}$  of solution was transferred into a 96-well microplate. The absorbance of sample ( $A_{\text{sample}}$ ) and standard ( $A_{\text{standard}}$ ) were measured against the blank on a microplate reader at 550 nm within an hour and the unknown concentration calculated using the absorbance of the protein standards.

#### **4.6.6 Plasma Viscosity**

Plasma viscosity was measured using viscometer (Cone and plate Viscometer, LVT with CP-40, Wells-Brookfield). Prior to the viscosity measurement, a water bath which was connected to the viscometer was set at 37°C and 0.5 mL of calibration oil (2.5CS) was used for calibration. 0.5 mL of plasma was pipetted into the sample cup and the viscometer reading (% of torque) was recorded after 15 min of temperature equilibrium. Two different shear rates (90 and 225  $\text{sec}^{-1}$ ) were used to determine the plasma viscosity; any dial reading below 1% was not considered and the measure was repeated at a lower shear rate.

#### **4.6.7 Plasma Heat Shock Protein (HSP) 72**

Plasma HSP72 was analysed using R and D Systems kits (DYC1663, Minneapolis, MN, USA) according to manufacturer instructions and previous research (Molvarec *et al.* 2009). All measurements were made in duplicate. Plasma HSP72 samples were diluted 1/5 in a solution of 1 mM EDTA, 0.5% Triton X-100 in PBS pH 7.4. 100  $\mu\text{L}$  of capture antibody diluted with PBS to a concentration of 360  $\mu\text{g}\cdot\text{mL}^{-1}$  was added to each well and left at room temperature overnight. The wells were washed 3 times using wash buffer (400  $\mu\text{L}$ ). 300  $\mu\text{L}$  of block buffer was added to each well, left at room temperatures for 90 min, and washed (3 times). 100  $\mu\text{L}$  of standards (2000, 1000, 500, 250, 125, 62.5, 31.25  $\text{pg}\cdot\text{mL}^{-1}$ ) and samples were added to wells, incubated at room temperature for 2 hours and washed (3 times). 100  $\mu\text{L}$  of diluted detection antibody was added in each well, incubated for 2 hours and washed (3 times). 100  $\mu\text{L}$  of diluted streptavidin was added to each well, incubated for 20 min in the dark and washed (3 times). 100  $\mu\text{L}$  of substrate was added in each well and incubated for 20 min. 50  $\mu\text{L}$  of stop solution was added and the absorbance of each well was measured using a microplate reader (Benchmark Plus, Bio-Rad, CA, USA) at 450 with 570 nm used for wavelength correction. A logistic 5PL (Rodbard) curve fit was used and unknown concentrations calculated from the standard curve.

#### **4.6.8 Plasma Renin**

Plasma renin was analysed using an Elisa Renin kit (Renin Active Elisa, IBL International, GMBH). 50  $\mu\text{L}$  of Standard (0; 4; 16; 32; 64; 128  $\text{pg}\cdot\text{mL}^{-1}$ ), Control and samples were added to 150  $\mu\text{L}$  of Assay Buffer in all wells and incubated for 90 min at room temperature on a plate shaker at  $\sim 700$  rpm. The wells were rinsed 4 times with 300 $\mu\text{L}$  wash solution. 100 $\mu\text{L}$  of Enzyme Conjugate was dispensed in all wells,

followed by another 90 min of incubation and 4 times of the wash procedure. 100  $\mu\text{L}$  of Substrate Solution was added to each well and incubated for 15 min at room temperature. The enzymatic reaction was stopped by adding 100  $\mu\text{L}$  of Stop Solution to each well. The absorbance of each well was measured using a microplate reader (Benchmark Plus, Bio-Rad, CA, USA) at 450 nm. Unknown concentrations were calculated from the standard curve.

#### **4.6.9 Plasma Glutamine**

Plasma Glutamine was assayed using a fluorometric method in two parts (Grossie *et al.*, 1993). Glutamine was converted to glutamate by glutaminase. The resulting glutaminase was then assayed by measuring NADH formed by the conversion of glutamate to  $\alpha$ -ketoglutarate and ammonia by glutamate dehydrogenase.

##### ***Part One:***

12.5  $\mu\text{L}$  of sample, standard or water was added to 15  $\mu\text{L}$  of glutaminase (Sigma Chemical CO.) and incubated for 60 min at 37°C in a heating block. For the blank tube, 27.5  $\mu\text{L}$  of acetate buffer (0.5M, pH 4.9) and 12.5  $\mu\text{L}$  of sample were mixed and incubated for 60 min at 37°C. 90  $\mu\text{L}$  of cold perchloric acid (0.4M) was mixed with the solution and centrifuged for 10 min at 5000 rpm (Hermle Z360K Centrifuge, Germany).

##### ***Part Two:***

70  $\mu\text{L}$  of sample from *Part One* and 20  $\mu\text{L}$  of NAD were added to 1 mL of pyrophosphate buffer in fluorometric cuvettes and fluorescence measured using an Aminco Bowman Series 2 fluorometer (Thermo Electron, WI, USA). The fluorescence

was set to an excitation wavelength of 342 nm (4 nm slit width), an emission wavelength of 459 nm (16 nm slit width) and a sensitivity of 715 V. 5 µL of glutamate dehydrogenase was added into the cuvettes, incubated at room temperature for 45 min and the fluorescence read again.

### *Calculations*

The calculations of glutamine and glutamate in plasma were as below:

$$\text{Glutamine} = \left\{ \frac{(R_2GLN - R_1GLN) - (R_2B - R_1B)}{(R_2STD - R_1STD)} \times \text{STD concentration} \right\} - \text{glutamate}$$

$$\text{Glutamate} = \left[ \frac{(R_2GLU - R_1GLU) - (R_2B - R_1B)}{(R_2STD - R_1STD)} \right] \times \text{STD concentration}$$

Where:

R<sub>1</sub> and R<sub>2</sub> represent the different stages of the assay;

GLN, GLU, STD and B represent the results for glutamine, glutamate, standard and the blank, respectively.

The abovementioned procedures for haematological measurements were used in Study Three and Study Four.

## **4.7 PERCEPTUAL MEASUREMENTS**

Thirst sensations, ratings of perceived exertion and thermal comfort were recorded at 10-min intervals throughout the testing and at exhaustion point for Study Three and Study Four. These measurements were obtained in a random order to eliminate an ordering effect.

### **4.7.1 Perceived Thirst Sensation**

Perceived thirst sensation was identified using a nine-point thirst scale with verbal anchors ranging from 1 ("not thirsty at all") to 9 ("very, very thirsty") (Engell *et al.*, 1987).

### **4.7.2 Ratings of Perceived Exertion (RPE)**

Ratings of perceived exertion (RPE) were obtained using the 15-point Borg scale ranging from 6 ("very, very light") to 20 ("very, very hard") (Borg, 1982).

### **4.7.3 Thermal Comfort Scale**

Thermal comfort was recorded from a seven point scale (Bedford, 1936) ranging from 1 ("much too cold") to 7 ("much too hot").

The abovementioned procedures for perceptual measurements were used in Study Three and Study Four.

## **CHAPTER 5**

### **STUDY ONE: HYDRATION STATUS OF ELITE KENYAN DISTANCE RUNNERS COMPETING IN HOT, HUMID CONDITIONS**

## **5.0 HYDRATION STATUS OF ELITE KENYAN DISTANCE RUNNERS COMPETING IN HOT, HUMID CONDITIONS**

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## 5.1 ABSTRACT

**Objective:** The purpose of this study was to determine the hydration status and practices of elite runners during competitive distance running events in a tropical climatic environment. As such this is a descriptive field study that is necessarily limited by the practicalities of conducting studies at several mass participation endurance running competitions. Nevertheless the compliance of a group of elite Kenyan distance runners who volunteered to participate in this study was excellent, thus providing a rare opportunity to obtain data on truly elite athletes. **Methods:** The study was undertaken in conjunction with the Standard Chartered Kuala Lumpur Marathon 2009 (Half marathon and Full Marathon categories). A total of 11 complete data sets were collected from elite Kenyan male runners. The change in BW following each race was calculated from measurements of the runner's BW immediately before and after the race. **Results:** The elite Kenyan runners ran at a fast speed  $\sim 18 \text{ km}\cdot\text{hr}^{-1}$  and drank a small volume of water ( $\sim 120 \text{ mL}$ ) which resulted in a significant body weight deficit (3.0 %). An inverse relationship between  $A_D$  and the percentage change of BW loss ( $r=-0.65$ ;  $p=0.03$ ) and between sweat rate and race time ( $r=-0.82$ ;  $p=0.002$ ) was found. **Conclusion:** This present study demonstrates that the elite Kenyan runners completed their races in warm, very humid climatic conditions ( $\sim 26^\circ\text{C}$ ;  $\sim 90\% \text{ rh}$ ) with  $\sim 3\%$  BW loss. They completed the races as the fast finishers in this present study but ran slower than they were capable because of the prevailing heat and humidity. The greater reduction in BW of the elite runners may simply be a consequence of their faster running velocity, increased rate of heat storage and increased sweat loss. Interestingly, these elite runners were able to adapt well by increasing their sweat rate regardless of the volume of fluid ingested or percentage of BW loss in the warm humid conditions. We speculate that elite Kenyan runners are likely to be adapted to regular intense training and racing in warm to hot environmental conditions. As such they would be

heat acclimatised and able to tolerate high body temperatures. They may also possibly be able to tolerate dehydration through the protective effect of heat shock proteins. Other factors such as  $\dot{V}O_{2\max}$ , percentage of  $\dot{V}O_{2\max}$  that can be sustained, running economy, morphological characteristics and genetics factor are known to be important for success in marathon running.

## 5.2 INTRODUCTION

### *Race Information*

The Standard Chartered Kuala Lumpur (SCKL) Marathon 2009 is an annual-mass participation running event held in Dataran Merdeka, Kuala Lumpur, Malaysia. This event was open to runners from the general public to enter and it was sanctioned by the Malaysia Amateur Athletic Union (MAAU), International Association of Athletics Federations (IAAF), and the Association of International Marathons and Distance Races (AIMS). It is one of the biggest running events in Southeast Asia. The race was divided into five categories: (i) Full Marathon, (ii) Half Marathon, (iii) 10km, (iv) 5km Family Fun Run and (v) the “kid’s” dash. A total of 12,500 participants ran in this event: 1,900 runners in the full marathon category and 2,800 runners in the half marathon category.

Hypohydration (fluid loss equivalent to >2% body weight deficit) has been reported to increase cardiovascular and thermoregulatory strain, ultimately impairing endurance exercise performance especially in hot conditions (Convertino *et al.*, 2000; Chevront *et al.*, 2003; Murray, 2007; Sawka *et al.*, 2007). The danger of inadequate fluid intake during marathon running was reported by Wyndham and Strydom in the late 1960s. Their study showed that >3% of body weight (BW) deficit during two 32 km races elicited an elevation of body core temperature that predisposed the runners to hyperthermia. Subsequently, these researchers encouraged athletes to consume 300 mL of fluid at 20-min intervals ( $0.9 \text{ L}\cdot\text{h}^{-1}$ ) during strenuous exercise (Wyndham, 1977). Consequently, ingesting fluid during prolonged exercise has become a common practice among runners because maintaining a euhydrated state is thought to result in better endurance performance and attenuates the risk of dehydration and hyperthermia when

exercising in warm-hot and humid climatic conditions. The maintenance of a euhydrated state is problematic when considering the high volume of sweat loss in well-trained marathon runners coupled with the intensity of running a marathon (80 – 85 %  $\dot{V}O_{2max}$ ) and the relatively low rate of gastric emptying and absorption.

Caution in interpreting the findings of laboratory based research is needed as there can be vast differences when compared with field based observations that are subject to the vagaries of the prevailing weather conditions. Pugh and colleagues (1967) reported their observations made on 77 competitors in the 1966 Witney Marathon (Air temperature: 22 - 23.5°C; relative humidity: 52 - 58% rh) where they found that the first four runners to finish the race at an average speed of 15.5 km.hr<sup>-1</sup> (range 15.19 to 15.95 km. hr<sup>-1</sup>) exhibited the highest rectal temperatures (>40.0°C), together with a significant volume of sweat production contributing to a high percentage of BW loss (5.8%). The study implied that tolerance to a high body core temperature and body fluid loss are necessary for success in marathon running. Meanwhile, the International Amateur Athletic Association (IAAF) rules governing the running of marathons at that time prohibited any fluid being ingested during the first 7 miles (11.2km) of the race.

In 1975 the American College of Sports Medicine (ACSM) released a *Position Statement on Prevention of Heat Injuries during Distance Running* which recommended that distance runners should frequently ingest 400-500 mL of fluid immediately prior to, and during long distance running races. A decade later, the ACSM (1987) *Position Stand on the Prevention of Thermal Injuries during Distance Running* further suggested runners should consume 100-200 mL of fluid every 2-3 km during races to prevent thermal injuries. To some extent the ACSM Position Stand was

misguided on that regardless of the number of participants in a marathon 25 years ago; fluid refreshment stations were limited to every 5 kilometres. Clearly, the ingestion of arbitrary volumes of fluid does not take account of differences in the velocity of running (percentage of  $\dot{V} O_{2\max}$  sustained), climatic conditions, heat acclimatisation or the physique of runners which will differentially affect the heat load incurred. The possible influence of physique on heat load was recognised by Pugh *et al.*, (1967) who proposed that a greater surface area / body-weight ratio is advantageous for heat dissipation, which means runners of smaller physical stature could maintain better heat balance compared with runners of larger physical stature (Marino *et al.*, 2000).

Previous laboratory based research has consistently reported that dehydrated athletes will experience an elevation in body core temperature and a reduction in sweat rate which was thought to increase the risk of developing heat illness (Sawka & Coyle, 1999; Sawka *et al.*, 2001). The ACSM (2007) *Position Stand on Exercise and Fluid Replacement* advocates athletes to drink (*ad libitum*) according to their dictates of thirst, while body weight should be maintained to no more than 2% body weight (BW) loss during exercise (Sawka *et al.*, 2007). It is suggested that if fluids are not replaced at this rate, athletes may experience a performance limiting level of dehydration (>2% of BW loss) which is associated with an increase in cardiovascular and thermoregulatory strain (Montain & Coyle, 1992; Sawka *et al.*, 1992; Sawka & Montain, 2000; Sawka & Noakes, 2007; Gonzalez *et al.*, 2009). Aerobic exercise performance is likely to be adversely affected by hypohydration (Sawka, 1992; Cheuvront *et al.*, 2003; Murray, 2007; Maughan, 2010) although, no relationship between the degree of dehydration and post-race rectal temperature was found during marathon and triathlon races (Noakes *et al.*, 1988; Noakes *et al.*, 1991; Sharwood *et al.*, 2002; Sharwood *et al.*, 2004; Byrne *et al.*, 2006; Laursen *et al.*, 2006). Noakes *et al.* (1991) measured the post-race rectal

temperature, levels of dehydration, running velocities and estimated absolute metabolic rates in marathon runners. They found that the post-race rectal temperature in these runners was affected by the metabolic rate sustained during the latter section of the race, but not due to the level of dehydration. Sharwood *et al.* (2002, 2004) found body weight in triathletes who completed the 2000 and 2001 South African Ironman Triathlon to be significantly reduced (ranged from -2.6% to -10.7%). This reduction was not associated with a higher rectal temperatures or greater prevalence of medical complications, but was associated with higher serum sodium concentrations. These studies concluded that dehydration does not necessarily lead to a decrement in aerobic exercise performance. Numerous studies have also shown that greater body mass losses are observed in successful athletes who drank *ad libitum* during exercise (Cheuvront & Haymes, 2001; Speedy *et al.*, 2001; Kao *et al.*, 2008). More recently, a significant inverse relationship between the degree of body weight loss and race finishing time for a marathon race was reported by Zouhal *et al.* (2010). They found that the finishers who completed the race in <3 hours experienced a >3% BW deficit whereas finishers who required >4 hours to complete the race experienced <2% BW deficit. Unfortunately, the aforementioned field studies focused on slower runners and did not obtain measurements from elite runners. Moreover, Zouhal *et al.*, (2010) collected their data from six major “city marathons” in cool but not in warm / hot conditions.

The purpose of the present study was to determine the hydration status in elite runners during competitive distance running event in a tropical climate. We hypothesized that (i) the elite runners would complete the long distance races with more than 3% BW loss in warm humid climatic conditions (high WBGT); and (ii) the elite runners would cope with the warm humid climatic conditions (high WBGT) an increased sweat rate regardless of the volume of fluid ingested or body weight loss.

## **5.3 METHODS**

### **5.3.1 Subjects**

All runners from the Standard Chartered Kuala Lumpur Marathon 2009 (which combined the Half marathon and Full Marathon categories) were invited to participate in the study on the day of the race. Before participation, the nature of study was explained to the race competitors and verbal consent was obtained. Prior approval to conduct the study was obtained from Sports Centre Research Committee, University of Malaya. The hypothesis of this study was not mentioned to prospective subjects as this information could influence the runners in altering their usual fluid replacement behaviour. At the end of the races, a complete data set was collected from 11 elite Kenyan male runners.

### **5.3.2 Experimental Procedures**

All measurements were collected on the day of the race: Standard Chartered KL Marathon 42.2 km and 21.1 km 2009. Prior to the start of each race, subjects' age and race numbers were recorded. The body mass of each subject was measured to the nearest 0.1 kg using a digital weighing scale (SECA 803, Germany) while in their race attire (running singlet, shorts and socks) without shoes on. Body height was measured to the nearest 0.5 cm using a portable wall-mounted roll-up measuring tape (SECA 205, Germany). To estimate fluid intake during races, subjects were required to remember the volume of fluid intake or total number of drinks provided at the water stations during the races. They were advised that the cups at each water station contained 200 mL of water and sports drinks. The water stations were located approximately 5 km apart. Immediately following the race, subjects were towelled dry and body mass was

measured without shoes on. Subjects were required to avoid consuming fluid after crossing the finishing line until the body mass measurements were collected. Body mass was measured within 5 minutes of the athlete completing the race. Finishing time and ranking / position were provided by the event organisers.

Sweat loss was calculated as the change in body mass, with correction for fluid intake and urine loss immediate post race finish. Metabolic fuel oxidation and respiratory water losses were not accounted for in calculating sweat loss. Average rate of metabolic heat production for the whole race was estimated based on the assumption that heat liberation during running approximates  $4 \text{ kJ.kg}^{-1} \text{ body mass.km}^{-1}$  (Dennis & Noakes, 1999; Byrne *et al.*, 2006). Therefore the heat production (W) during the race was calculated using: Heat Production (W) = pre body mass (kg) \* running speed ( $\text{m.s}^{-1}$ ) \* 4 J (Nielsen, 1996). Body surface area ( $A_D$ ) was calculated using:  $A_D (\text{m}^2) = (0.202 \times W^{0.425} \times H^{0.725})$  (Du Bois & Du Bois, 1916), where W = Weight of body (kg); H = Height of body (m)

### ***Reliability of Digital Weighing Scale Measurement***

Ten numbered digital weighing scales, all the same model (SECA 803, Germany) were used throughout the study. The digital weighing scales were calibrated using two known weight of 10 kg on a flat, firm surface. The reliability of each of these weighing scales was determined by three repeated measurements. The intra and inter-weighing scales difference were less than 1%.

### ***Environmental Measurement***

The wet bulb globe temperature and humidity (WBGT) were measured every minute before, during and at the end of races by using Microtherm Heat Stress WBGT meter (Cassella Measurement Ltd, United Kingdom) (Table 5.1). The device was positioned at the start and finish area.

#### **5.3.4 Statistical Analysis**

Descriptive statistics was used to determine the mean and standard deviation of the environmental measurements (ambient temperature and relative humidity), as well as the subjects' age, morphological characteristics (body mass, height, BMI,  $A_D$ ,  $A_D$ /Weight Ratio), running performance (finishing time, running speed), fluid balance variables (volumes of drinks, body mass deficit,  $\Delta$  % BW, sweat rate), metabolic rate and heat production. Paired *t*-test was used to examine the difference between pre and post body mass. Pearson's product moment correlation coefficient (*r*) was used to examine the strength of the relationships between the morphological characteristics, fluid balance variables, heat production and running performance. All values are reported as mean  $\pm$  SD. Statistical significance was accepted as  $p < 0.05$ .

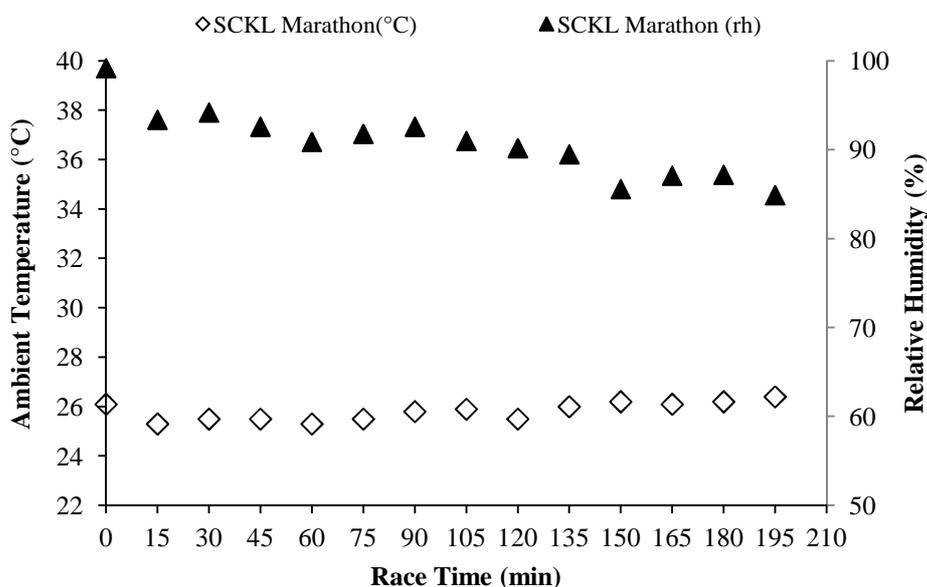
## 5.4 RESULTS

### 5.4.1 Environmental Conditions

The environmental conditions during the Standard Chartered Kuala Lumpur (SCKL) Marathon 2009 were hot and humid (Table 5.1 and Figure 5.1). Subjects who completed the races did not experience any symptoms of heat illness.

**Table 5.1** Environmental conditions during the Standard Chartered Kuala Lumpur (SCKL) Marathon 2009

Race	Start Time	Finish Time	Ambient Temperature (°C)	WBGT (°C)	Relative humidity (%)
SCKL Marathon (42.2 km)	5.00 am	8.00 am	25.8 ± 0.3	25.0 ± 0.4	91.8 ± 3.1
SCKL Half-Marathon (21.1 km)	6.15 am	8.15 am	26.0 ± 0.3	25.1 ± 0.2	89.2 ± 2.5



**Figure 5.1** Ambient temperature and relative humidity measurements during the Standard Chartered Kuala Lumpur (SCKL) marathon 2009

### **5.4.2 Subjects**

The average age and anthropometric characteristics (body mass, height, BMI,  $A_D$  and  $A_D$ / Weight ratio) of the elite runners are shown in Table 5.2. Table 5.3 lists the descriptive data on running performance and hydration level on each individual of the elite runners that completed 21.1 km and 42.2 km distance races.

### **5.4.3 Hydration Level**

Body mass significantly decreased during the SCKL Marathon 2009 by ~1.8 kg in Kenyan runners. During the race, it was clearly shown that these runners who ran at a fast speed ~18 km.hr<sup>-1</sup> and drank a small volume of water (Table 5.2) experienced a significant body weight deficit (3.0 %).

**Table 5.2** General characteristics of the elite Kenyan distance runners in Standard Chartered Kuala Lumpur (SCKL) Marathon 2009

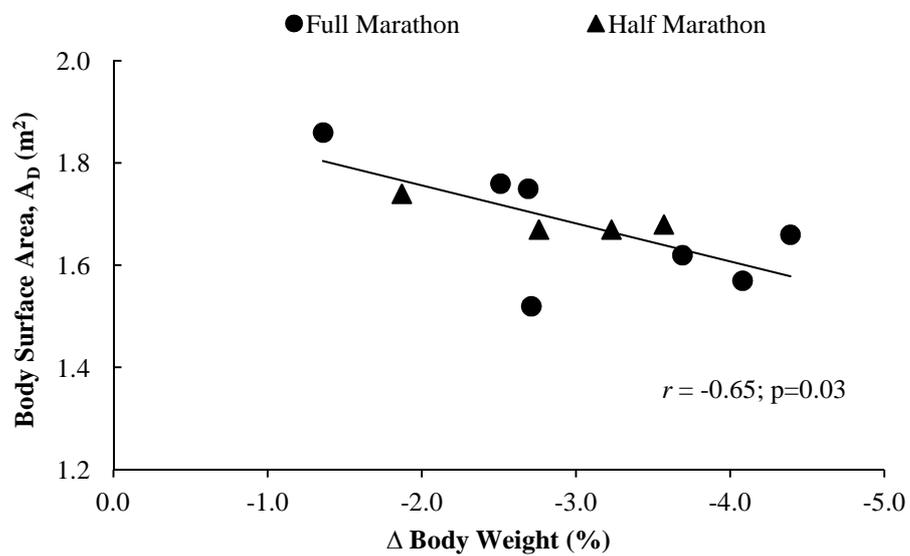
	Elite		
	SCKL Marathon 2009		
	42.2 km	21.1 km	Both
<b>Sample Size (n)</b>	7	4	11
<b>Age (years)</b>	27.1 ± 2.9	23.8 ± 1.0	25.9 ± 2.9
<b>Height (cm)</b>	169.2 ± 10.1	169.4 ± 1.3	169.3 ± 7.8
<b>Body Mass (kg)</b>	59.0 ± 4.7	59.9 ± 2.8	59.4 ± 4.0
<b>BMI (kg.m<sup>-2</sup>)</b>	20.7 ± 1.9	20.9 ± 0.9	20.8 ± 1.6
<b>A<sub>D</sub> (m<sup>2</sup>)</b>	1.68 ± 0.12	1.69 ± 0.03	1.68 ± 0.09
<b>A<sub>D</sub>/ Weight ratio</b>	0.0284 ± 0.0010	0.0283 ± 0.0010	0.0283 ± 0.0008
<b>Finishing time (min)</b>	148.2 ± 10.7	68.9 ± 2.2	
<b>Running Speed (km.h<sup>-1</sup>)</b>	17.1 ± 1.2	18.6 ± 0.2	17.7 ± 1.2
<b>Ranking (range)</b>	12 ± 10 (1-28)	3 ± 1 (1-4)	
<b>Volume of Drinks (mL)</b>	110.7 ± 173.1	125.0 ± 119.0	115.9 ± 149.3
<b>BW deficit (kg)</b>	1.79 ± 0.58	1.70 ± 0.40	1.75 ± 0.50
<b>Δ% BW</b>	3.1 ± 1.1	2.9 ± 0.7	3.0 ± 0.9
<b>Sweat Rate (L.hr<sup>-1</sup>)</b>	0.77 ± 0.21	1.59 ± 0.44	1.07 ± 0.46
<b>Heat Production (W)</b>	1122.6 ± 121.9	1239.3 ± 58.0	1165 .1 ± 115.7

**Table 5.3** Descriptive data on running performance and hydration level on each individual elite Kenyan runner (n=11) that completed 42.2 km and 21.1 km in SCKL Marathon 2009

Subject	Distance (km)	Finishing time (hr: min : sec)	Running Speed (km.h <sup>-1</sup> )	Position	Estimated Volume of Drinks (mL)	BW deficit (kg)	Δ% BW
1	42.2	2:17:03	18.48	1	75	2.5	4.39
2		2:22:10	17.81	5	75	1.7	2.69
3		2:23:41	17.62	7	500	0.9	1.36
4		2:24:03	17.58	8	50	2.1	3.69
5		2:26:23	17.30	12	25	1.4	2.71
6		2:39:23	15.89	22	25	1.5	2.51
7		2:47:40	15.10	28	25	2.4	4.08
8	21.1	1:07:12	18.84	1	100	1.9	3.23
9		1:08:10	18.57	2	300	2.1	3.57
10		1:08:19	18.53	3	50	1.2	1.87
11		1:08:37	18.45	4	50	1.6	2.76

#### 5.4.4 Effect of $A_D$ , Heat Production, Running Speed and Race Time on % BW loss

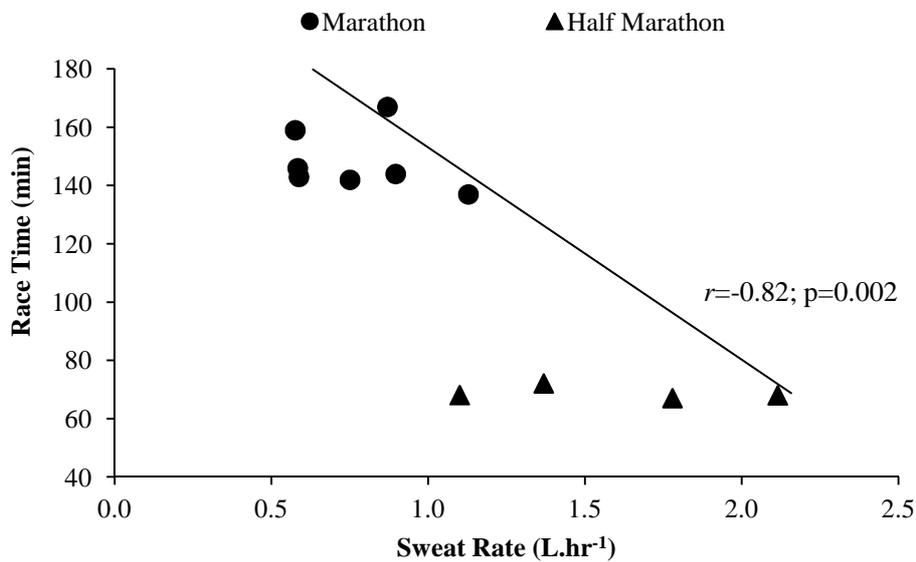
An inverse relationship between  $A_D$  and the percentage change of BW loss for the elite Kenyan runners was found (Figure 5.2;  $r=-0.65$ ;  $p=0.03$ ). No significant relationship was detected between the heat production and the % BW loss ( $r=-0.47$ ;  $p=0.141$ ), the running speed and the % BW loss ( $r=-0.108$ ;  $p=0.752$ ), and the race time and the % BW loss ( $r=0.13$ ;  $p=0.707$ ).



**Figure 5.2** Relationship between the body surface area ( $A_D$ ,  $m^2$ ) and the percentage change of body weight loss in elite Kenyan runners ( $n=11$ ) during different competitive distance running events (Full Marathon and Half Marathon)

#### 5.4.5 Effect of $A_D$ , Heat Production, Running Speed and Race Time on Sweat Rate Responses

A significant inverse relationship between sweat rate and race time was found (Figure 5.3;  $r=-0.82$ ;  $p=0.002$ ). The regression analyses did not detect any significant relationship between sweat rate and running speed ( $r=0.599$ ;  $p=0.052$ ) or  $A_D$  ( $r=-0.10$ ;  $p=0.774$ ). It was shown that sweat rate failed to demonstrate any significant relationship with heat production during the race ( $r=0.349$ ;  $p=0.293$ ).



**Figure 5.3** Relationship between the race time (min) and the sweat rate (L.hr<sup>-1</sup>) in elite Kenyan runners (n=11) during different competitive distance running events (Full Marathon and Half Marathon)

## 5.5 DISCUSSION

To our knowledge, this is the first study to report the hydration status of elite Kenyan distance runners during competitive distance running events in a tropical climatic environment. We have collected 11 complete data sets from Kenyan runners during Standard Chartered Kuala Lumpur Marathon 2009 in 42.2 and 21.1 km categories. According to the final results (race time and finishing position) provided by the event organiser, the top positions from 1<sup>st</sup> - 10<sup>th</sup> in full marathon and 1<sup>st</sup> - 6<sup>th</sup> in half marathon were dominated by Kenyan runners. These results were not surprising as the International Association of Athletics Federation (IAAF) Statistics Office (updated 19 July 2012) reported that 6 of the 10 current world ranked athletes in half and full marathon events are Kenyan runners. To compare the Kenyan runners' current performance with their best performance time, we have searched this information by using their name through the IAAF website and an athletics database ([www.all-athletics.com](http://www.all-athletics.com)) (Table 5.4).

The average ambient temperature and relative humidity for each race date was obtained from an online weather services site that maintains historical weather data: Weather Underground ("Weather History and Data Archive"). For example, if the marathon event took place on 25 October 2009 in Chuncheon, then the weather data for Chuncheon on the same day would match that race. We found that the Kenyan runners' performance in the tropical climate of Kuala Lumpur was slower than their previous best performance except for Subject 5 who ran 12 sec faster than his previous best marathon performance during the Istanbul Eurasia Marathon, Turkey (2009) with an ambient temperature of ~19°C. Note that the ambient temperature during their best performance events was 15 - 22°C lower than the SCKL Marathon 2009 (Table 5.4). Therefore, it is reasonable to speculate that the warm and humid environmental

conditions are likely to have had an adverse impact on running performance in these Kenyan runners during the SCKL Marathon 2009.

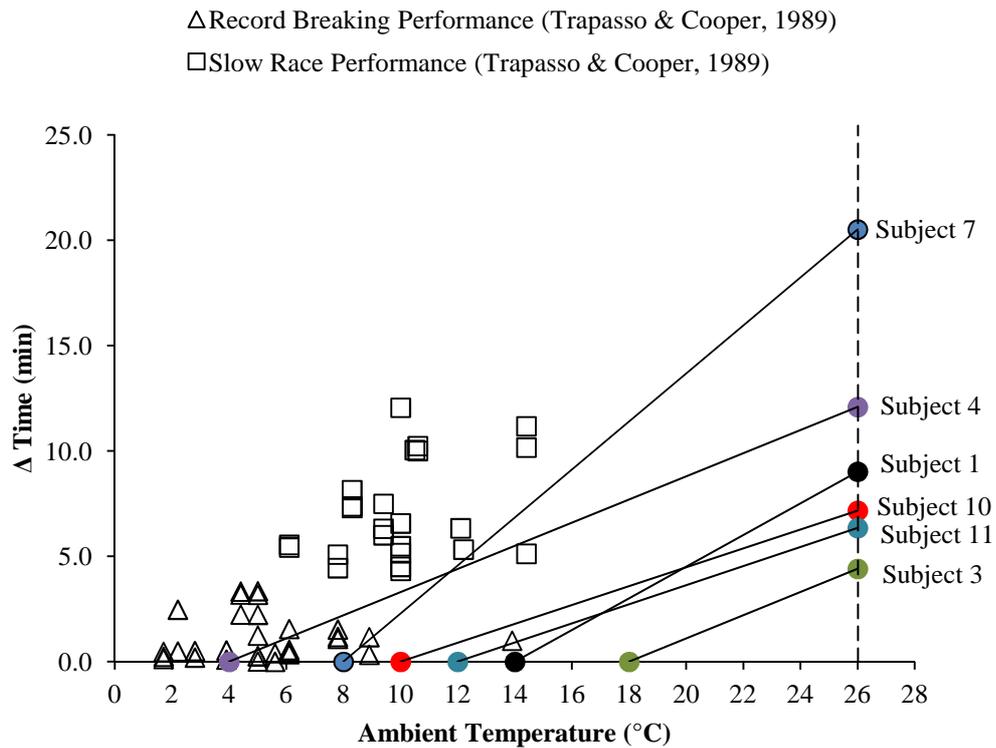
**Table 5.4** Comparison between current performance time and the previous best performance time in 7 elite Kenyan runners

Subject	Current Performance (hr:min:sec)	Best Performance (hr:min:sec)	Year	Venue	Weather	$\Delta$ Performance (min:sec)
1	2:17:03	2:08:01	2009	Chuncheon, South Korea	~14°C; 81% rh	9:02 (7% ↓)
3	2:23:41	2:19:00	2009	Nairobi, Kenya	~18°C	4:41 (3.2% ↓)
4	2:24:03	2:11:54	2009	Koeln, Germany	~4°C; 88% rh	12:09 (9.2% ↓)
5	2:26:23	2:26:35	2009	Istanbul, Turkey	~19°C; 77% rh	00:12 (0.8% ↑)
7	2:47:40	2:26:49	2007	Zurich, Switzerland	~8°C; 80% rh	20:51 (14% ↓)
<i>Mean</i>	<i>2:27:46</i>	<i>2:18:28</i>				<i>9:23 (6.8% ↓)</i>
10	1:08:19	1:01:02	2000	Palma de Mallorca, Spain	~10°C	7:17 (11.8% ↓)
11	1:08:37	1:02:02	2008	Venlo, Netherlands	~12°C; 71% rh	6:35 (10.23% ↓)
<i>Mean</i>	<i>1:08:28</i>	<i>1:01:32</i>				<i>6:56 (11% ↓)</i>

↓ Performance decrement  
 ↑ Performance improvement

The reduction in running velocity with increased climatic stress has been previously observed in a series of reports linking marathon race finishing times with climatic conditions (Trapasso & Cooper, 1989; Zhang *et al.*, 1992; Ely *et al.*, 2007; Ely *et al.*,

2008; Vihma, 2010). To determine the impact of weather on running performance and to confirm our result with previous findings, we combined our data with the meteorological data and performances of the top 3 finishers in the past 30 Boston Marathons (1958-1987) (Trapasso & Cooper, 1989). Trapasso & Cooper (1989) found that in 25 of the 31 record breaking performances during previous Boston Marathons the wet bulb temperature was below 7.8°C, whereas in 22 out of 24 cases of unusually slow race performances the wet bulb temperature was 7.8°C or above ( $r=0.74$ ). Figure 5.4 clearly demonstrates that our data shows a similar trend in the relationship between increases in ambient temperature and the resultant adverse effect on running performance. The Kenyan runners in the present study (Subject 1, 3, 4, 7, 10 and 11) ran slower (from 4 min 41 sec to 20 min 51 sec over the course of the marathon) than their previous best performance when the ambient temperature increased. Subject 7 experienced the greatest performance decrement (14%), ~20 min slower than his previous best performance time. This may reflect a misjudgement in the pacing strategy adopted in the early stages of the marathon which hastened the rate of heat storage and ultimately caused a slowing of pace in the final stages of the race (see the plot on Figure 5.4 for Subject 7).



**Figure 5.4** Relationship between the change in performance time and ambient temperature during Boston Marathon (1958-1987) (Trapasso & Cooper, 1989) and the present study (Subject 1, 3, 4, 7, 10, 11).  
 Dashed line: Ambient temperature during SCKL Marathon 2009  
 At 0-min: Ambient temperature during previous best performance time in 6 elite Kenyan runners

Furthermore, the high humidity (~90% rh) together with the warm conditions (~26°C) during the SCKL Marathon 2009 is not conducive to sustained fast running over an extended duration. It has been suggested that when the humidity is high, the rate at which sweat evaporates from the skin is lower than that observed in dry environmental conditions (Maughan *et al.*, 2012). Maughan *et al.* (2012) demonstrated that a faster rise in heat storage was associated with a higher humidity (80 vs. 24 % rh trials) at the same power output of cycling exercise (70%  $\dot{V}O_{2max}$ ) in hot climatic conditions (30°C). Therefore, a faster rate of rise of core temperature during exercise was reported despite ongoing sweat losses. It has also been shown that skin wettedness may cause a swelling in the superficial keratinous cell layers of the skin causing a physical impairment in sweating (Brown & Sargent, 1965; Candas *et al.*, 1979).

The first finding of this study showed that the elite runners completed their race with a ~3% BW loss. Note that subject 1 and subject 8 (Table 5.3) who placed 1<sup>st</sup> in full marathon and half marathon categories respectively, experienced >4% and >3% BW loss, respectively in each race. The mean BW loss (in percentage terms) observed in this study was comparable with a recent observational field study (Zouhal *et al.*, 2010), in which “fast runners” who completed the marathon race <3 hours experienced >3% BW loss. This is consistent with other studies showing the fastest finishers in endurance races are often those who are the most dehydrated (Sharwood *et al.*, 2004; Kao *et al.*, 2008). In the present study, the elite runners consumed an average of 120 mL of fluid throughout the long distance races. These elite runners seem to prefer to consume a small volume of fluid without following the drinking guidelines of the ACSM (Sawka *et al.*, 2007), and yet this behaviour did not appear to adversely affect their running performance during the race. This observation was supported by a recent meta-analysis on the effects of exercise-induced dehydration on exercise performance (Goulet, 2011). Five studies which used time-trial cycling protocols simulating real-world conditions were analysed. Goulet (2011) concluded that none of these studies showed a significant deleterious effect (e.g., percentages change in power output) on exercise performance with ~ 4% BW loss. Robinson *et al.* (1995) reported that 2.3% BW loss improved exercise performance compared with a euhydration trial in 20°C ambient temperature. Conversely, approximately 1.5 L of fluid replacement based on total sweat loss did not induce any physiological benefits for the subjects. In fact, it produced an uncomfortable feeling of gastric distension and significantly reduced the mean distance covered in 60 min from 43.1 to 42.3 km ( $p<0.05$ ) (Robinson *et al.*, 1995). The ideal fluid intake regimen may vary according to exercise duration and intensity, climatic conditions, physique and extent of heat acclimation. Moreover, fluid ingestion during exercise does not necessarily ensure an adequate replacement of body water

stores. Both gastric emptying and intestinal absorption can be adversely affected by variable exercise intensity (Neufer *et al.*, 1989). It has been suggested that strenuous exercise at 70%  $\dot{V}O_{2\max}$  or greater reduces the availability of fluid ingested during exercise (Costill & Saltin, 1974; Maughan *et al.*, 1990). The shock, or increased vibration, resulting from running may induce gastrointestinal (GI) disturbance. At exercise intensities above 70%  $\dot{V}O_{2\max}$ , a delay in gastric emptying has been observed (Neufer *et al.*, 1987). The relatively small volume of fluid the elite runners ingested may reflect the high running velocity under warm conditions with excessive humidity levels. In order to prevent GI disturbance and discomfort, fast runners may well deliberately limit their fluid ingestion. Running at  $\sim 18 \text{ km}\cdot\text{hr}^{-1}$  while ingesting a large volume of fluid (equivalent to sweat loss) is likely to lead to gastric distention and a slowing in running velocity. As we did not measure any hydration markers on Kenyan runners prior to the races (e.g., plasma volume, urine specific gravity, serum osmolality), therefore there is a possibility that 2-3% BW deficit has occurred before the starting of the races.

The second important finding was that the elite runners were able to compensate well by increasing the sweating rate regardless of the volume of fluid ingested or percentage of BW loss in the warm conditions. It has been proposed that the reduction in sweat rate was significantly associated with plasma hypovolemia ( $r=0.53$ ) (Sawka *et al.*, 1985). The sweat rate response declines in subjects who are progressively dehydrated from 3, 5 to 7% of body weight loss despite elevations in core temperature. Note that a loss of BW through sweating may not change the plasma volume levels. This might be due to the plasma volume being defended in a hypovolemic state via a reduction in capillary hydrostatic pressure (Zappe *et al.*, 1993) with an increase in plasma protein concentration (higher plasma oncotic pressure), and thus enhanced vascular

absorption (Fortney *et al.*, 1981; Convertino, 1987). We found sweating rate is mainly affected by duration of exercise ( $r=-0.83$ ;  $p=0.002$ ) whereas running velocity,  $A_D$  and heat production have minimal impact on sweating rate.

A study by Laursen *et al.* (2006) investigated the relationship between core temperature and hydration during an Ironman triathlon and found that a decrease in body mass of 3% occurred while urine specific gravity and plasma  $[Na^+]$  measures remained within the normal range. Laursen *et al.* (2006) proposed that the 3% BW loss was likely to have occurred from the loss of intracellular body water during the process of glycogen and triglyceride oxidation (Pastene *et al.*, 1996), and it was not due to the loss of extracellular body water. Similarly, several studies have suggested that the water released on oxidation of glycogen is an obligatory source of body water replenishing body fluids during endurance exercise (Astrand & Saltin, 1964; Olsson & Saltin, 1970; MacLaren & Lanaghan, 1983). Fink *et al.* (1975) found that glycogen bound water is more available in hot conditions (40°C) than in cool conditions (9°C) as exercising in the heat requires greater demands on muscle glycogen metabolism. The availability of this endogenous source of water will offset water loss from intracellular space and ultimately mask the true level of dehydration. Moreover, core body temperature during the triathlon was reported to be only ~1°C above normal (Laursen *et al.*, 2006). It is most probable that ~3% BW loss is not a critical threshold level to affect the circulatory and thermoregulatory responses during prolonged running performance.

Zouhal *et al.* (2010) suggested that levels of dehydration in excess of 2% BW may be ergogenic for fast runners. The view of reducing BW to lower the oxygen cost of running may potentially improve endurance running in some athletes has previously been reported (Myers & Steudel, 1985; Jones *et al.*, 1986; Noakes, 2000; Coyle,

2004). Jones *et al.* (1986) found that the energy cost was increased 1% every 100 g increase in a pair of running shoes. Likewise, a greater energy cost was observed when an additional weight was added to the ankle (Myers & Steudel, 1985). However, an alternative interpretation by Armstrong *et al.* (2011) on Zouhal *et al.* (2010) field study proposed that the faster runners drink less during the races and the observation that they run faster may not be due to the reduced volume of water they ingested. The runners may have run faster if they were better hydrated. Therefore the published conclusion by Zouhal *et al.* (2010), that “body weight loss during a marathon race may be ergogenic” cannot be substantiated.

It is of interest to note that Saltin *et al.*, (1995) compared Kenyan runners who exhibited better running economy compared with Scandinavian athletes while their  $\dot{V}O_{2\max}$  was similar. Kenyan athletes have also been shown to have a low BMI and an ectomorphic somatotype (characterized by long, slender legs) which could be a contributing factors for their superior running economy (Larsen, 2003; Larsen *et al.*, 2004) and metabolic efficiency (Saltin *et al.*, 1995).

The environmental conditions during the races in the current study were warm and extremely humid (~26°C, ~90% rh) (Table 5.1). There is a possibility that runners competing in hot environments might be either advantaged or disadvantaged by their physique. Our data demonstrated that the greater percentage changes of body weight loss in elite runners were affected by a smaller  $A_D$  (Figure 5.2). Dennis and Noakes (1999) stated that heat production in running depends on body mass and heat loss depends on body surface area. It also has been suggested that  $A_D$ /Weight ratio is another important determinant for heat gain and heat loss during exercise in hot climatic conditions (Epstein *et al.*, 1983). A large  $A_D$ /Weight ratio indicates that a high

efficiency in thermoregulatory system for heat loss while a small  $A_D/Weight$  ratio facilitates heat gain from the environment. Marino *et al.* (2000) showed that light runners produce less heat compared with heavier runners at similar running speeds, and thus they can run faster before becoming hyperthermic and succumbing to heat exhaustion. Heavier runners might be more disadvantaged than lighter runners during a marathon in hot and humid conditions (Dennis & Noakes, 1999). These observations suggest that a greater heat load is incurred by heavier runners when compared with lighter runners during exercise in hot environments (Marino *et al.*, 2000; Marino *et al.*, 2004). It is also notable that the loss of body mass reduces absolute heat production or absolute aerobic power (Drinkwater & Horvath, 1979). The higher the intensity of exercise, the greater the increase in metabolic rate and heat production (Nadel *et al.*, 1977).

In summary, this present study demonstrates that the elite runners completed their races in warm humid climatic conditions with ~3% BW loss (ranged from 1.4 to 4.4%). The Kenyan runners completed the races as the fast finishers in this study but ran slower than their previous best performance. This may have been a deliberate strategy to avoid the adverse effects of the heat and very high humidity during each race. Our data on seven of the Kenyans demonstrated that they were unable to come near to their personal best time for the marathon or half marathon. Therefore the Kenyans experienced ~3% BW loss and their performance was adversely affected. The reduction of BW may be simply an association or it could be a cause-effect relationship for the deterioration of running performance. Interestingly, we found that Kenyans are able to compensate well by increasing their sweat rate regardless of the volume of fluid ingested or percentage of BW loss in warm conditions. We speculate that elite runners might be well adapted to dehydration, with regular training in hot conditions increasing their tolerance to high

body temperatures and body water deficits. They may also possibly be able to tolerate dehydration through the protective effect of heat shock proteins. Other factors such as  $\dot{V}O_2\text{max}$ , capacity to sustain a high percentage of  $\dot{V}O_2\text{max}$  for an extended period of time, running economy, genetics factor and anthropometric characteristics may play an important role for success in marathon running.

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## **CHAPTER VI**

### **STUDY TWO: THE EFFECTS OF HYPOHYDRATION AND SIMULATED HYPERHYDRATION ON RUNNING ECONOMY**

## **6.0 THE EFFECTS OF HYPOHYDRATION AND SIMULATED HYPERHYDRATION ON RUNNING ECONOMY**

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**Keywords:** Running economy, hypohydration, simulated hyperhydration

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## 6.1 ABSTRACT

**Purpose:** This study investigated the effects of hypohydration and simulated hyperhydration on running economy (RE). **Methods:** Sixteen well trained male distance runners performed a control trial in a euhydrated state (**AW0** / **D0**) and undertook either 2 added weight (**AW**) trials or 2 dehydration (**D**) trials. Each trial consisted of four incremental submaximal running velocities (65, 70, 75, 80% of  $\dot{V}O_{2\max}$  with 4 min stages for each velocity) on a motorised treadmill in thermoneutral conditions (20°C; 40% rh). Hyperhydration was simulated with added weight to the torso which was equivalent to 3% (**AW3**) and 4% (**AW4**) body weight (BW) while 3% (**D3**) and 4% (**D4**) BW deficit was induced via an exercise-heat exposure protocol. **Results:** Subjects were euhydrated prior to all **AW** and **D** trials as indicated by urine specific gravity <1.010. RE at each velocity was expressed as oxygen uptake ( $\dot{V}O_2$ , mL.kg<sup>-1</sup>.min<sup>-1</sup> and mL.kg<sup>-0.75</sup>.min<sup>-1</sup>), caloric unit cost ( $C_R$ , kcal.kg<sup>-1</sup>.km<sup>-1</sup>) and gross oxygen cost of running (mL.kg<sup>-1</sup>.km<sup>-1</sup>).  $\dot{V}O_2$  increased significantly with running velocity ( $r=0.999$ ;  $p<0.001$ ). Subjects in **D** group required a significantly greater oxygen uptake during the **D3** and **D4** trials over the range of treadmill velocities compared with **D0** trial (ANCOVA;  $p<0.01$ ). For the **AW** group, no significant differences in oxygen uptake were found between **AW0**, **AW3** and **AW4** trials (ANCOVA;  $p>0.05$ ). HR increased linearly with each increment in velocity for all trials. A ~4% body mass loss in **D4** trial significantly increased the heart rate response when compared with **D0** (ANCOVA,  $p=0.012$ ) while there was no significant difference in oxygen uptake between the two conditions. **Conclusion:** The study shows that (1) hypohydration did not reduce the oxygen cost of running proportionally with the bodyweight deficit (**D3** and **D4**) and (2) simulated hyperhydration did not increase the oxygen cost of running proportionally with the added gross weight (**AW3** and **AW4**).

## 6.2 INTRODUCTION

Kenyan runners have been the most successful group in international long distance races over the past two decades. The International Association of Athletics Federation (IAAF) Statistics Office (updated 19 July 2012) reported that 6 of the 10 current world ranked athletes in half and full marathon events are Kenyan runners. Kenyan dominance in long distance races has increased the athletes, coaches and sports scientists' interest in gaining insight into the factors contributing to such outstanding world class performances.

One interesting observation has shown that Kenyan distance runners ingest daily fluid volumes below the prevailing recommendation of the American College of Sports Medicine (ACSM) (Sawka *et al.*, 2007). Despite sweat loss associated with intense bouts of running the elite Kenyan distance runners do not appear to consume fluid before or during training. Fudge *et al.* (2007) observed Kenyan distance runners and noted that they ingested ~1.1 L of plain water and ~1.2 L milky tea on a daily basis. Our unpublished data (Chapter 5) shows elite Kenyan runners in half and full marathon races consumed a small amount of water (<100 mL) and experienced >3% body weight (BW) deficit during such events, yet were the fastest runners, invariably winning these events or finishing amongst the top ten placegetters. A recent published study similarly supports our observations and has further shown a significant inverse relationship between the degree of body weight loss and race finishing time for a marathon race event (Zouhal *et al.*, 2010). Finishers who completed the race in <3 hours experienced a >3% BW deficit whereas finishers who required >4 hours to complete the race experienced <2% BW deficit. It could be speculated that part of the success of Kenyan runners may be related to a reduced body mass through sweat loss that would have the potential to reduce the energy cost of running. This is not a new proposition as Coyle

(2004) suggested in a review on fluid and fuel intake during exercise that a loss of body mass during marathon races may lower the oxygen cost of running. However, such speculative statements lack definitive experimental evidence to support such a conclusion.

Running economy (RE) has been identified as one of the main physiological factors contributing to the endurance performance of Kenyan distance runners (Bosch *et al.*, 1990; Saltin *et al.*, 1995; Larsen, 2003; Lucia *et al.*, 2006). Kenyan distance runners have been shown to exhibit superior RE when compared with Scandinavian runners with a similar  $\dot{V}O_{2\max}$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) (Saltin *et al.*, 1995). RE is determined as the oxygen uptake or energy cost relative to body weight when running at a sub-maximal steady state velocity. Therefore, a small change in body weight may affect oxygen uptake during running. This has led to the speculative comment that endurance runners who experience an acute reduction (~3-4%) in body weight through sweating may possibly improve their RE in terms of a lower oxygen utilisation for a given submaximal running velocity (Coyle, 2004).

The theoretical concept of an increased body mass or external load leading to an increase in the energy cost of locomotion at a submaximal velocity is not novel (Jones *et al.*, 1986; Epstein *et al.*, 1987; Cooke *et al.*, 1991; Teunissen *et al.*, 2007). Jones *et al.* (1986) reported that energy cost was increased 1% every 100 g increase in a pair of running shoes. The metabolic cost of carrying an external backpack weighing from 10 to 30 kg was observed to increase linearly with 10 kg increments (Epstein *et al.*, 1987). Another study investigated the effect of vertical and horizontal loading on oxygen uptake during treadmill running (Cooke *et al.*, 1991). The authors found horizontal loading with a weight jacket (5 and 10% body mass) significantly increased the  $\dot{V}O_2$

cost of the exercise. Hyperhydration could theoretically (i) produce performance advantages by delaying dehydration and reducing cardiovascular strain (Greenleaf *et al.*, 1997), or (ii) have no effect (Cooke *et al.*, 1991; Latzka *et al.*, 1997; Latzka *et al.*, 1998), or (iii) impair exercise performance as a result of the body weight gain associated with hyperhydration (van Rosendal *et al.*, 2009).

There are few studies in the research literature which have investigated the effect of hypohydration and hyperhydration on RE. One such study by Armstrong *et al.* (2006) tested ten highly trained collegiate distance runners who completed four 10 min treadmill running bouts at either 70%  $\dot{V}O_{2\max}$  or 85%  $\dot{V}O_{2\max}$  with either euhydrated or hypohydrated conditions on four separate days (23°C, 50% rh). The authors reported that a 5% BW deficit had no effect on running economy at 70 and 85%  $\dot{V}O_{2\max}$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ), although increased circulatory strain was evident with an increase in heart rate (HR), together with a decrease in both stroke volume (SV) and cardiac output ( $\dot{Q}$ ) in a thermoneutral environment.

In a study of hyperhydration using creatine and glycerol supplementation, Beis *et al.*, (2011) found that an increase in body mass ( $\sim+1.3\%$ ) did not negatively impact on RE at 60%  $\dot{V}O_{2\max}$  in both cool (10°C) and hot (35°C) conditions. However, the elevated body mass was associated with reduced circulatory strain and an improved thermoregulatory response to an ambient temperature of 35°C. Beis *et al.* (2011) proposed that future studies should investigate the relationship between hypohydration and RE with a faster running speed to confirm the possible beneficial effect of hyperhydration during endurance running competition in hot conditions.

Following a search of the research literature, we found that there is a lack of well-controlled investigations on the effect of dehydration and hyperhydration on RE in thermoneutral conditions. Thus, the present study investigates the effects of hypohydration (-3 and -4% BW) and simulated hyperhydration (+3 and +4% BW) on RE. We hypothesized that (1) hypohydration would reduce the oxygen cost of running proportionally with the degree of bodyweight loss and (2) simulated hyperhydration would increase the oxygen cost of running proportionally with the increase in bodyweight associated with hyperhydration.

## **6.3 METHODS**

### **6.3.1 Subjects**

Sixteen endurance trained male runners aged 41 to 54 volunteered to participate in this study. They were physically fit and maintained their running training for an average of 70 km/week, with a minimum of 5 training sessions per week. They were non-smokers with no medical history of chronic disease or orthopedic problems affecting their mobility or gait. Subjects were ranked on the basis of a high or low  $\dot{V}O_2$  (ml. kg<sup>-1</sup>. min<sup>-1</sup>) when running on the treadmill at 10 km.hr<sup>-1</sup> and were randomly assigned (pair matched on the basis of their RE at 10 km.hr<sup>-1</sup>) to one of two groups: Added Weight (**AW**) or Dehydration (**D**). The experimental protocols and potential risks were explained verbally and subjects gave their written informed consent prior to their participation in this study. The study was approved by the Human Research Ethics Committee of The University of Sydney (Ref No. 11766).

### **6.3.2 Preliminary Testing**

All subjects completed a Medical History & Screening Questionnaire and the Canadian Physical Activity Readiness Questionnaire (PAR-Q). Resting blood pressure and heart rate were measured while the subjects rested in a seated position. Standing height and weight were subsequently measured to the 0.1 cm and 0.001 kg respectively. A familiarization of treadmill running was undertaken prior to the submaximal test so that all subjects were well practiced in this mode of exercise.

### **6.3.2.1 Submaximal Exercise Test**

A submaximal exercise test was undertaken on a motor driven treadmill at 10 km.h<sup>-1</sup> (0% grade), followed by 12, 14, 16 km.h<sup>-1</sup> of steady state running (4 min for each stage) in thermoneutral conditions (20°C, 40% rh). Submaximal heart rate (HR) and  $\dot{V}O_2$  (mL.kg<sup>-1</sup>.min<sup>-1</sup>) over the final minute of each 4 min stage was used to construct a linear relationship between HR and  $\dot{V}O_2$  (mL.kg<sup>-1</sup>.min<sup>-1</sup>). Maximum HR was measured with a Polar heart rate monitor (Polar Electro Oy, Kemele, Finland) recording the highest HR attained over the “all out” effort of the final two laps of a 5 km race conducted within the previous 3 months. The submaximal treadmill test data and maximal heart rate (HR<sub>max</sub>) measured towards the conclusion of a 5 km race were used to construct a linear regression equation to predict  $\dot{V}O_{2max}$  and the treadmill velocity that elicits 65%, 70%, 75%, 80% of  $\dot{V}O_{2max}$  for the subsequent running economy tests. To lower the risks of an adverse events on our subjects, an actual  $\dot{V}O_{2max}$  test was not conducted as the subjects were aged >40 years old (ACSM, 2000).

### **6.3.3 Anthropometric Measurements**

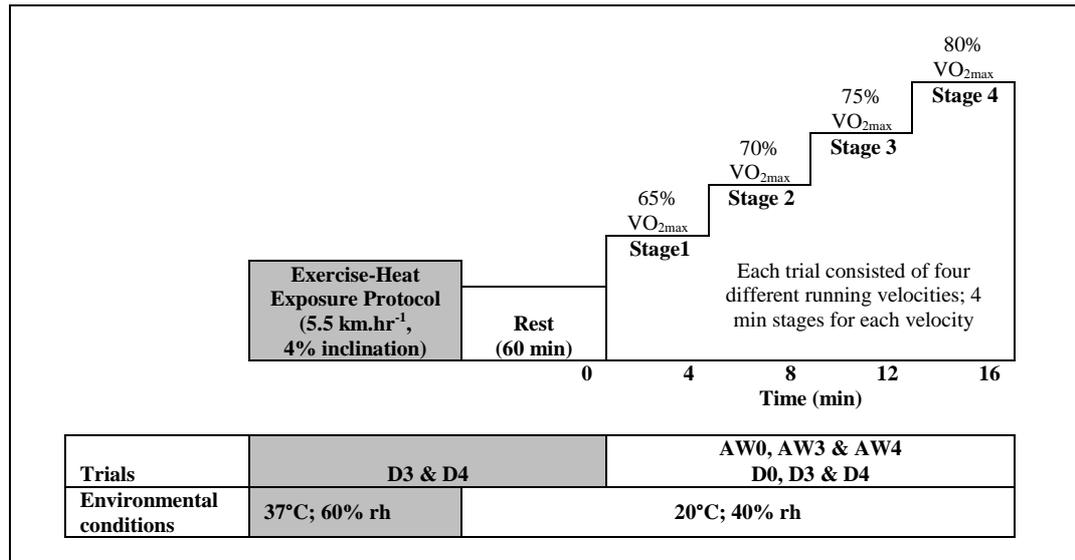
Subjects were required to empty their bladder of urine before the body mass measurement. Body mass and height (with running shorts and socks only) were determined using a digital weighing scale (Mettler Toledo ID1 Multi Range Scale, Columbus, OH, USA) and a height stadiometer (Harpenden Stadiometer, Holtain Limited, UK), respectively.

### 6.3.4 Experimental Design

Subjects were familiarised with the experimental procedures by running at a range of velocities on a motorized treadmill before the first experimental trial. Subjects from the added weight (**AW**) and the dehydration (**D**) groups performed three randomised experimental trials separated by 3-7 days. They performed a control trial (**AW0** or **D0**) in a euhydrated state and undertook either 2 added weight (**AW3** & **AW4**) or 2 dehydration (**D3** & **D4**) trials. Hyperhydration was simulated with added weight in the form of carefully measured sandbags fitted closely to the torso in a purpose made sleeveless vest. The added weight was equivalent to 3% (**AW3**) and 4% (**AW4**) body weight while 3% (**D3**) and 4% (**D4**) body weight deficit was induced via an exercise-heat exposure protocol. Each trial consisted of four different submaximal running velocities as follows: 65, 70, 75 and 80%  $\dot{V}O_{2\max}$ ; with 4 min stages for each velocity; (see below Table 6.1) on the treadmill in thermoneutral conditions (20°C; 40% rh) (Figure 6.1).

**Table 6.1** Four relative running intensities (%  $\dot{V}O_{2\max}$  – ml. kg<sup>-1</sup>. min<sup>-1</sup>) which performed by the added weight (**AW**) and the dehydration (**D**) groups during the running economy tests

<b>Trials</b>	<b>Treadmill Velocity (km.hr<sup>-1</sup>)</b>			
	65% $\dot{V}O_{2\max}$	70% $\dot{V}O_{2\max}$	75% $\dot{V}O_{2\max}$	80% $\dot{V}O_{2\max}$
<b>AW0, AW3, AW4</b>	10.1 ± 0.6	11.4 ± 0.7	12.8 ± 1.2	14.1 ± 1.5
<b>D0, D3, D4</b>	10.9 ± 1.4	12.0 ± 1.3	13.3 ± 1.5	14.5 ± 1.5



**Figure 6.1** Schematic representation of experimental design for **AW** trials and **D** trials

#### 6.3.4.1 Experimental Protocol

All subjects abstained from alcohol, caffeine, medications and vigorous exercise for 24 hours prior to the experimental testing. They were instructed to drink a prescribed volume 6 ml. kg BW<sup>-1</sup> of water every 2 - 3 hours throughout the day before and on the day of testing to maintain a euhydrated state. Upon arrival at the laboratory, a urine sample was collected for determination of urine specific gravity (USG) using a digital refractometer (Atago<sup>®</sup> UG- $\alpha$ , Japan) and body mass was measured with the pre-weighed sports attire (shorts, socks and shoes). A USG of  $\leq 1.020$  (Armstrong *et al.*, 1994) was required as part of the inclusion criteria.

#### *Exercise-Heat Exposure Protocol*

For **D** group, subjects undertook an exercise-heat exposure protocol to induce 3% and 4% body weight deficit during **D3** and **D4** trials in randomized order prior to the

commencement of the running economy test. Prior to undertaking the exercise-heat exposure protocol, subjects inserted a sterilised disposable thermistor probe (YSI 400 series, Mallinckrodt Medical, USA) 12 cm past the anal sphincter and a Polar Trainer™ heart rate monitor (Polar Electro Oy, Kemele, Finland) was secured on the chest. Rectal temperature ( $T_{re}$ ) data was logged on a portable temperature data logger (Digi-Sense® Thermistor Thermometer, Chemopharm, US). Subjects performed a walking protocol in an environmental chamber (37°C, 60% rh) at 5.5 km.hr<sup>-1</sup> with 4% inclination. Air flow of 2.44 m.s<sup>-1</sup> was generated by a fan which was placed 1 m in front of the subject to increase evaporative sweat loss after 30 min of the walking protocol. Subjects walked at an exercise: rest ratio of 25 min walking and 5 min rest.  $T_{re}$  and heart rate (HR) were monitored continuously and the 15 point Borg scale rating of perceived exertion (RPE) (Borg, 1982) was recorded every 25 min. Body mass was measured during each rest interval. The subjects stopped walking when their target body weight deficit was achieved. The mean exercise time to induce 3% and 4% of BW loss were  $91.9 \pm 14.4$  min and  $129.1 \pm 19.8$  min, respectively. None of the subjects'  $T_{re}$  exceeded 39.0°C, or displayed signs or symptoms of exercise-induced heat illness (E.g., dizziness, headache, nausea, etc), or indicated they had reached volitional fatigue.

After completion of the dehydration protocol, subjects were positioned supine and four ice-packs were placed on the chest (left and right side) and groin areas to enhance body cooling.  $T_{re}$  decreased to a 37.5°C after 20 min of cooling. Thereafter, subjects took a shower and rested for 40 min. They continued with the RE test on the same day with either 3% or 4% BW deficit.

### ***Running Economy (RE) Tests***

A urine sample was collected and body mass together with the pre-weighed sports attire (shorts, socks and shoes) was measured prior to the RE tests. Water was ingested when there was a need to titrate added weight to meet the target body mass. A Polar Trainer™ heart rate monitor (Polar Electro Oy, Kemele, Finland) including the transmitter was strapped to the chest for the HR to be monitored during exercise. For the **AW** group, subjects wore a weight jacket containing sand bags equivalent to 3% or 4% BW placed in pockets of the weight jacket. The weight of the sand bags was evenly distributed around the torso and adjusted tightly to the torso, which was intended to simulate the hyperhydration added weight effect. For **D** group, subjects performed the RE tests with either 3% or 4% BW deficit. All RE tests were undertaken in thermoneutral conditions (20°C, 40% rh) without convective air flow.

The revolutions of the treadmill belt were manually counted for 60 s to confirm the treadmill speed on a LED display while the subject ran at each steady state velocity. Expired respiratory gas ( $\dot{V}O_2$ ) was collected over the final minute of each stage of the four submaximal speeds using the Douglas bag technique. Gas samples were analyzed for oxygen ( $O_2$ ) and carbon dioxide ( $CO_2$ ) concentration using Ametek  $O_2$  and  $CO_2$  analyzers (Thermox Instruments, Pittsburg, PA). Total volumes of expired air were measured using a dry gas flow meter (Parkinson-Cowan, England). HR and relative perceived exertion (RPE) values were recorded within the final 15 s of each speed of running. The subjects were instructed to wear the same sports attire and running shoes during each experimental trial.

### **6.3.5 Statistical Analysis**

Power and Sample Size Calculation (PS) software by Dupont and Plummer (1997) was used to determine the sample size. A three-way (Group x Trial x Time) repeated ANOVA was performed using Statistical Package for Social Sciences (SPSS 18.0, Chicago, IL) to determine the interaction between and within the independent and dependent variables. When significant interaction effects were established, pair wise differences were identified using Tukey's HSD post hoc analysis procedure. In addition, a one-way between-groups analysis of covariance (ANCOVA) to compare the differences between added weight or dehydration trials based on their respective baseline measurements. Significant differences in subjects' physical and physiological characteristics, and hydration measurements were identified using independent and paired *t*-tests. Pearson Correlation analysis was used to determine the relationship between running economy and running velocity. Data are presented as mean  $\pm$  SD. In all cases, significance was accepted at significant level  $p < 0.05$ .

## 6.4 RESULTS

### 6.4.1 Subjects

Physical and physiological characteristics of the subjects are presented in Table 6.2.

There were no significant differences in these variables between groups.

**Table 6.2** Mean  $\pm$ SD for physical and physiological characteristics of added weight (AW) and dehydration (D) participant groups

	Participant Group	
	AW (n=8)	D (n=8)
Age (years)	44.3 $\pm$ 4.2	45.3 $\pm$ 2.9
Body mass (kg)	71.1 $\pm$ 6.0	74.8 $\pm$ 8.4
Height (cm)	174.3 $\pm$ 6.9	175.3 $\pm$ 7.2
$\dot{V}O_2$ at 10 km.hr <sup>-1</sup>	33.1 $\pm$ 2.1	32.6 $\pm$ 1.8
Predicted $\dot{V}O_{2max}$ (mL.kg <sup>-1</sup> .min <sup>-1</sup> )	58.6 $\pm$ 6.3	58.2 $\pm$ 6.6
Predicted HR <sub>max</sub> (beats.min <sup>-1</sup> )	178 $\pm$ 2	176 $\pm$ 3
10 km Performance Time (min)	40.3 $\pm$ 5.1	40.2 $\pm$ 3.6
21 km Performance Time (min)	91.1 $\pm$ 12.0	90.6 $\pm$ 9.0
Training volume (km.wk <sup>-1</sup> )	67.3 $\pm$ 26.9	73.1 $\pm$ 21.6

Values are mean  $\pm$  SD

$\dot{V}O_2$ , oxygen uptake; Predicted HR<sub>max</sub>, predicted maximal heart rate,  $\dot{V}O_{2max}$ , maximal oxygen uptake

### 6.4.2 Hydration Measurements

Table 6.3 presents the hydration measurements of two groups (Added Weight Group and Dehydration Group) of subjects before RE tests. Subjects were well hydrated on presentation to the laboratory with urine specific gravity <1.020 and baseline body mass similar within trials. The two methods employed to increase and decrease body mass were most effective in changing gross body mass by ~3% and ~4% from baseline

measures in the added weight and dehydration groups, respectively ( $p < 0.05$ ). USG values were found to be greater in **D3** and **D4** during pre-exercise measurements but this was not significant when compared with respective baseline values and **D0**.

**Table 6.3** Mean  $\pm$ SD for hydration measurements prior to each RE test in both Added Weight (**AW**) and Dehydration (**D**) trials

	Variables		
	USG	Body Mass (kg)	$\Delta$ Body mass (%)
<i><b>AW Trials</b></i>			
<b>AW0</b>			
Pre-exercise	1.0046 $\pm$ 0.0041	71.43 $\pm$ 6.22	
<b>AW3</b>			
Baseline		71.03 $\pm$ 6.04	
Pre-exercise	1.0046 $\pm$ 0.0022	73.17 $\pm$ 6.22*	+3.00*
<b>AW4</b>			
Baseline		71.40 $\pm$ 6.20	
Pre-exercise	1.0047 $\pm$ 0.0041	74.00 $\pm$ 6.11*^	+4.00*^
<i><b>D Trials</b></i>			
<b>D0</b>			
Pre-exercise	1.0082 $\pm$ 0.0033	74.31 $\pm$ 8.58	
<b>D3</b>			
Baseline	1.0084 $\pm$ 0.0036	75.14 $\pm$ 8.10	
Pre-exercise	1.0101 $\pm$ 0.0060	72.79 $\pm$ 7.69*	-3.11 $\pm$ 0.42*
<b>D4</b>			
Baseline	1.0086 $\pm$ 0.0060	75.19 $\pm$ 8.09	
Pre-exercise	1.0093 $\pm$ 0.0065	72.22 $\pm$ 7.99*#	-3.91 $\pm$ 0.65*#

\*, ^, # significantly different from baseline, **AW3** and **D3**, respectively ( $p < 0.05$ )

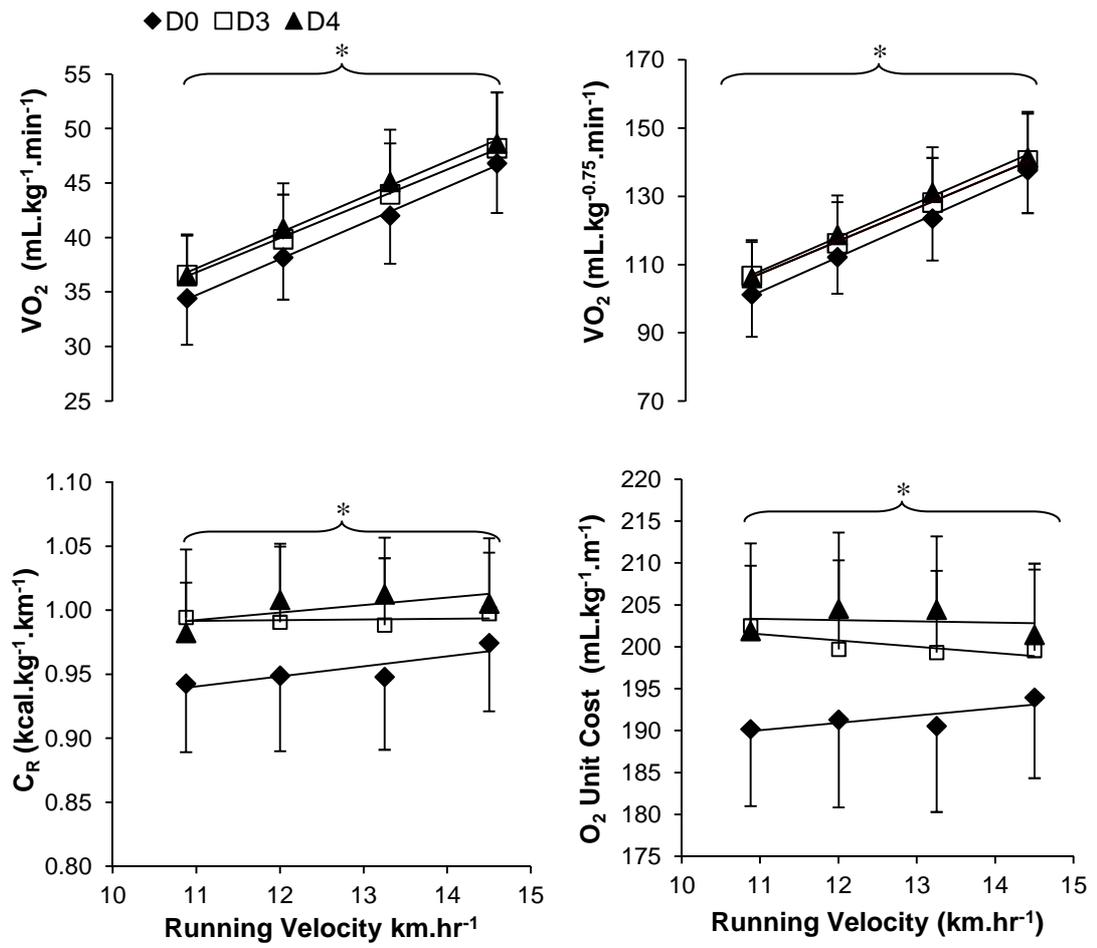
### 6.4.3 Running Economy

Figure 6.2 and Figure 6.3 depict RE during four different running velocities in three different conditions in both **D** and **AW** groups, respectively. RE at each running velocity was expressed as oxygen uptake ( $\dot{V}O_2$ , mL.kg<sup>-1</sup>.min<sup>-1</sup> and mL.kg<sup>-0.75</sup>.min<sup>-1</sup>), caloric unit cost ( $C_R$ , kcal.kg<sup>-1</sup>.km<sup>-1</sup>) and gross oxygen cost of running (mL.kg<sup>-1</sup>.km<sup>-1</sup>). As expected,  $\dot{V}O_2$  (mL.kg<sup>-1</sup>.min<sup>-1</sup> and mL.kg<sup>-0.75</sup>.min<sup>-1</sup>) increased linearly with running velocity in all conditions (Table 6.4; ANOVA,  $p < 0.001$ ).

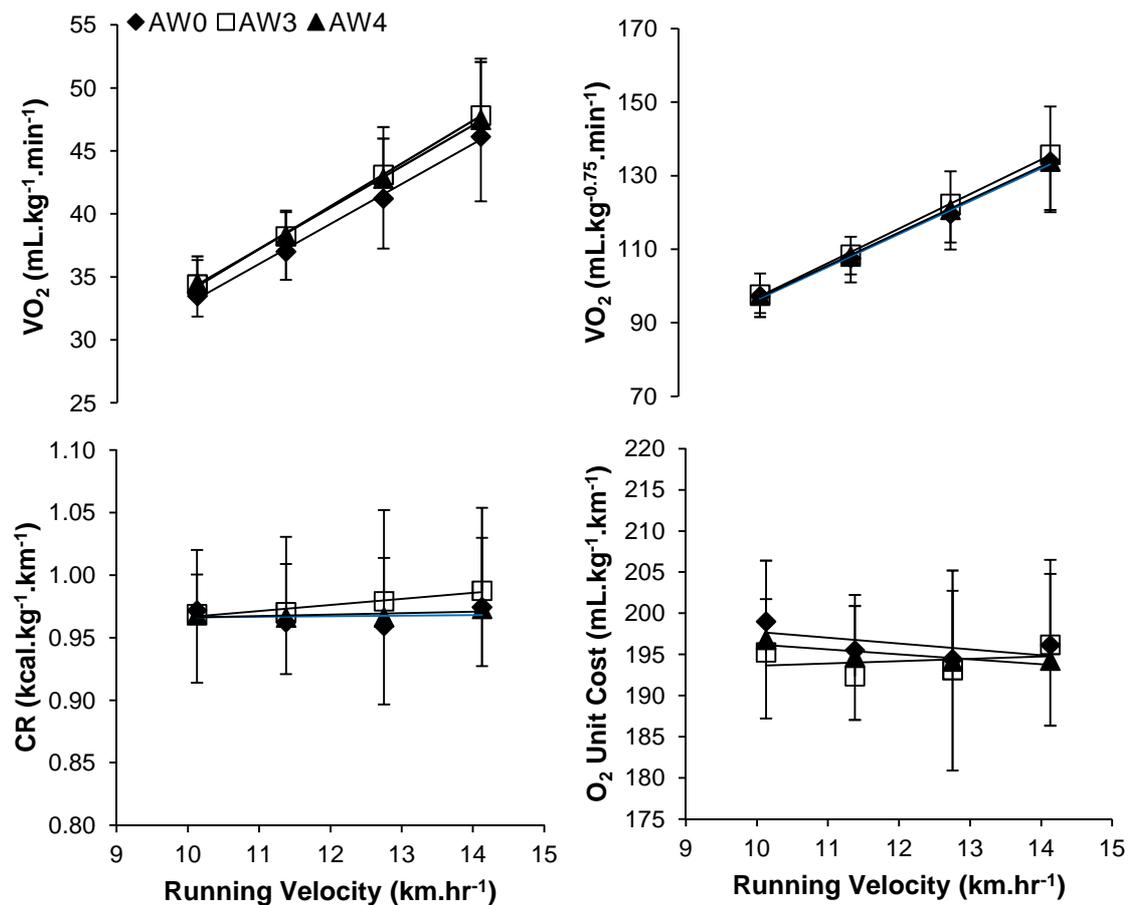
**Table 6.4** Relationship between running economy and running velocity during **AW** (n=8) and **D** (n=8) trials

Trials	Running Economy	
	$\dot{V}O_2$ , mL.kg <sup>-1</sup> .min <sup>-1</sup>	$\dot{V}O_2$ , mL.kg <sup>-0.75</sup> .min <sup>-1</sup>
<b>AW0</b>	$r=0.93$	$r=0.95$
<b>AW3</b>	$r=0.92$	$r=0.92$
<b>AW4</b>	$r=0.96$	$r=0.96$
<b>D0</b>	$r=0.70$	$r=0.77$
<b>D3</b>	$r=0.72$	$r=0.80$
<b>D4</b>	$r=0.77$	$r=0.85$

Subjects in **D** group required a significantly greater  $\dot{V}O_2$  (mL.kg<sup>-1</sup>.min<sup>-1</sup> and mL.kg<sup>-0.75</sup>.min<sup>-1</sup>), caloric unit cost,  $C_R$  (kcal.kg<sup>-1</sup>.km<sup>-1</sup>) and gross oxygen cost of running (mL.kg<sup>-1</sup>.km<sup>-1</sup>) during the **D3** and **D4** trials over the range of treadmill velocities when compared with **D0** trial (ANCOVA;  $p < 0.01$ ; Figure 6.3). For the **AW** group, no significant differences in  $\dot{V}O_2$  (mL.kg<sup>-1</sup>.min<sup>-1</sup> and mL.kg<sup>-0.75</sup>.min<sup>-1</sup>), caloric unit cost,  $C_R$  (kcal.kg<sup>-1</sup>.km<sup>-1</sup>) and gross oxygen cost of running (mL.kg<sup>-1</sup>.km<sup>-1</sup>) were found between **AW0**, **AW3** and **AW4** trials (ANCOVA;  $p > 0.05$ ; Figure 6.4).



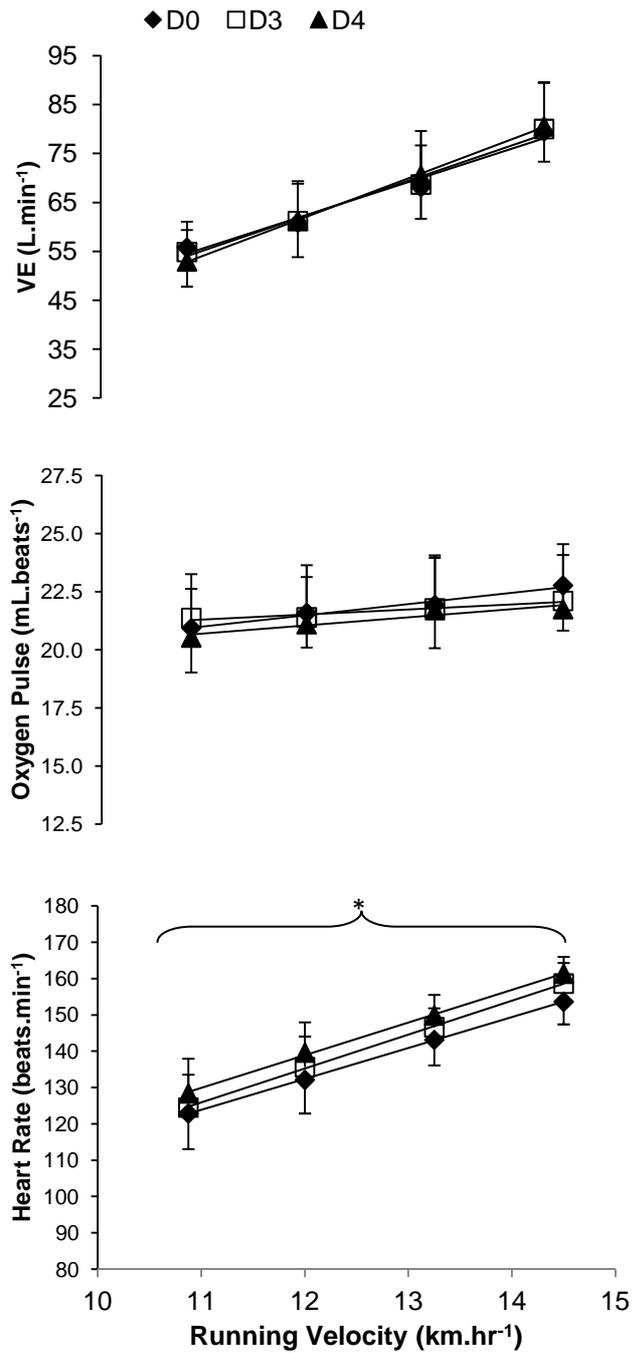
**Figure 6.2**  $\dot{V}O_2$  (mL.kg<sup>-1</sup>.min<sup>-1</sup>, mL.kg<sup>-0.75</sup>.min<sup>-1</sup>), caloric unit cost,  $C_R$  (kcal.kg<sup>-1</sup>.km<sup>-1</sup>) and gross oxygen cost of running (mL.kg<sup>-1</sup>.km<sup>-1</sup>) at running velocities that elicit 65, 70, 75 and 80%  $\dot{V}O_{2max}$  during **D** trials (n=8)  
 \* Significantly different from **D0** (ANCOVA, p<0.05)



**Figure 6.3**  $\dot{V}O_2$  (mL.kg<sup>-1</sup>.min<sup>-1</sup>, mL.kg<sup>-0.75</sup>.min<sup>-1</sup>), caloric unit cost,  $C_R$  (kcal.kg<sup>-1</sup>.km<sup>-1</sup>) and gross oxygen cost of running (mL.kg<sup>-1</sup>.km<sup>-1</sup>) at running velocities that elicit 65, 70, 75 and 80%  $VO_{2max}$  during AW trials (n=8)

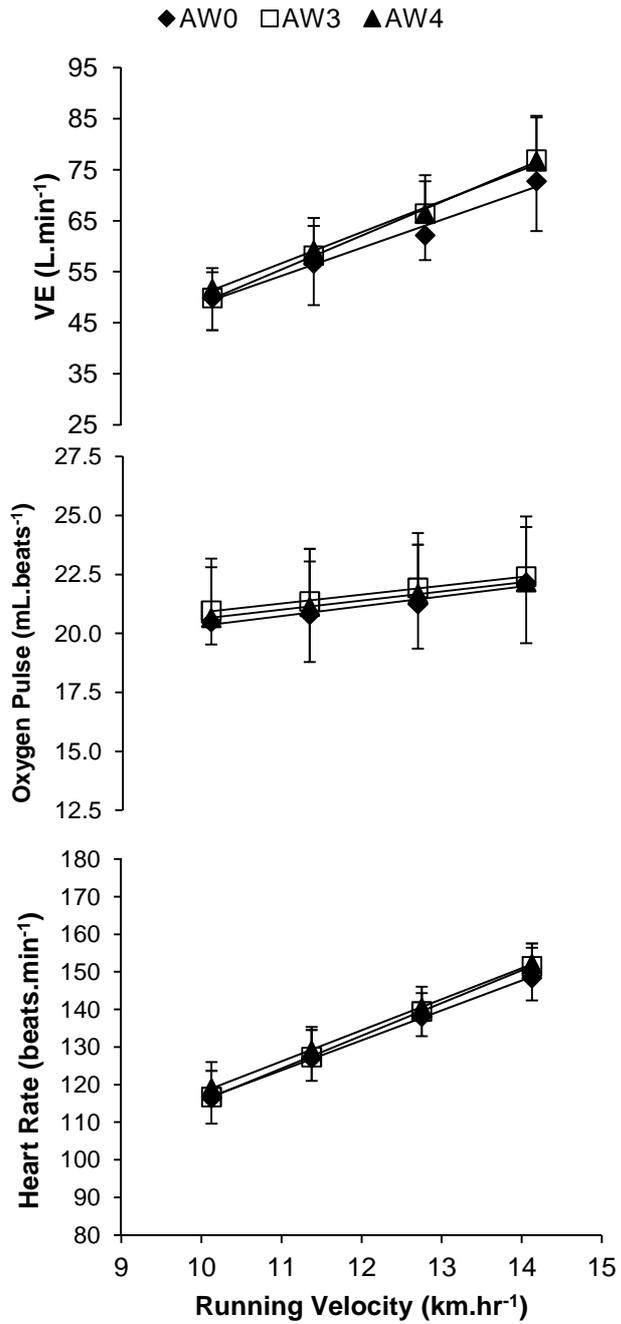
#### 6.4.5 Cardiorespiratory Responses

Heart rate (HR) increased linearly  $\sim 10$  beats.min<sup>-1</sup> with each increment in running velocity for all trials in both AW and D groups (ANOVA,  $p < 0.001$ ; Figure 6.4 & Figure 6.5). A  $\sim 4\%$  body mass loss in D4 trial significantly increased the circulatory strain when compared with D0 (ANCOVA,  $p = 0.012$ ; Figure 6.4). However, an additional weight load (AW3 and AW4 trials) had no significant effect on HR response when compared with AW0 trial (ANCOVA,  $p > 0.05$ ; Figure 6.5). No significant differences in oxygen pulse and VE were found in both groups (ANCOVA;  $p > 0.05$ ; Figure 6.4 & Figure 6.5).



**Figure 6.4** Heart rate (beats.min<sup>-1</sup>), oxygen pulse (mL.beats<sup>-1</sup>) and pulmonary ventilation (L.min<sup>-1</sup>) at running velocities that elicit 65, 70, 75 and 80%  $\dot{V}O_{2max}$  during **D** trials (n=8)

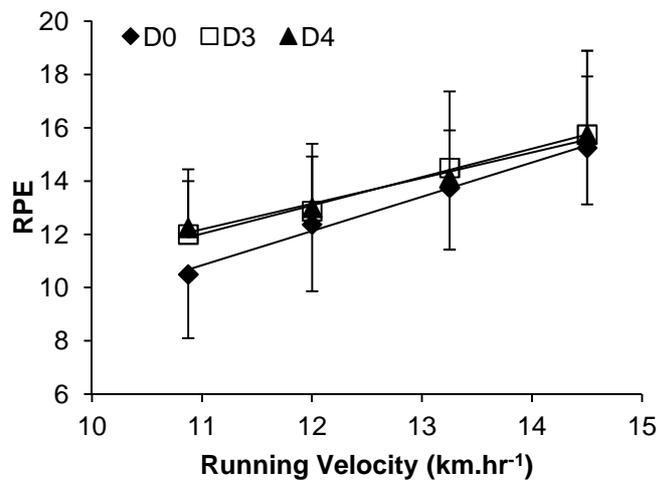
\* significantly different from **D0** (ANCOVA; p<0.05)



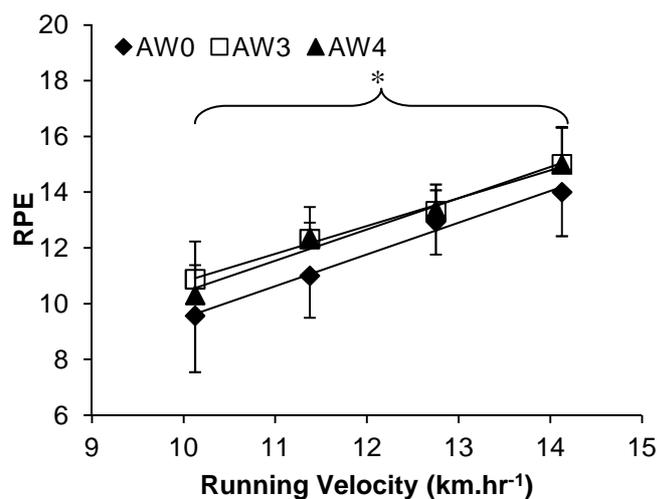
**Figure 6.5** Heart rate (beats.min<sup>-1</sup>), oxygen pulse (mL.beats<sup>-1</sup>) and pulmonary ventilation (L.min<sup>-1</sup>) at running velocities that elicit 65, 70, 75 and 80%  $\dot{V}O_{2max}$  during **AW** trials (n=8)

### 6.4.6 Perceptual Response

Subjects in both groups reported the RPE as significantly more difficult with each increase in running velocity in all trials (ANOVA,  $p < 0.001$ ; Figure 6.6 and Figure 6.7). Interestingly the RPE was significantly higher across the four running velocities in the **AW3** and **AW4** trials when compared with the **AW0** (ANCOVA;  $p < 0.05$ ; Figure 6.7) but no significant difference between trials was noted in the **D** group (ANCOVA;  $p > 0.05$ ; Figure 6.6).



**Figure 6.6** RPE at running velocities that elicit 65, 70, 75 and 80%  $\dot{V}O_{2max}$  during **D** trials (n=8)



**Figure 6.7** RPE at running velocities that elicit 65, 70, 75 and 80%  $\dot{V}O_{2max}$  during **AW** trials (n=8)

\* significantly different from **AW0** (ANCOVA;  $p < 0.05$ )

## 6.5 DISCUSSION

This study investigated the effect of simulating the bodyweight gain associated with a hyperhydrated state (+3 and +4% BW) and when dehydrated (-3% and -4% BW) on RE when exercising at 65 to 80%  $\dot{V}O_{2\max}$  in thermoneutral conditions. The results of this present study show that (1) hypohydration did not reduce the oxygen cost of running proportionally with the decrease in body mass (Figure 6.1) and (2) hyperhydration did not increase the oxygen cost of running proportionally with the added load (Figure 6.3).

The oxygen cost of running often referred to as running economy (RE), when coupled with  $\dot{V}O_{2\max}$ , is recognized as an important determinant of distance running performance (Daniels & Daniels, 1992). A number of investigators have expressed different ideas on how running economy should be determined and expressed (Daniels, 1985; Bergh *et al.*, 1991; Pate *et al.*, 1992; Bourdin *et al.*, 1993; Fletcher *et al.*, 2009). The method of expressing RE may add to the inconsistency and variability of RE data and yet there is no clear standard used by researchers e.g., compare the single measure of  $\dot{V}O_2$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) at 16  $\text{km}\cdot\text{hr}^{-1}$  (Costill *et al.*, 1971) with the multiple measures from below average racing pace to racing pace (Maughan & Leiper, 1983). Bergh *et al.* (1991) proposed that submaximal and maximal  $\dot{V}O_2$  attained during running should preferentially be expressed as  $\text{mL}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$  rather than  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  in order to minimize the influence of body mass on  $\dot{V}O_2$ . This approach is supported by studies which have consistently shown an inverse relationship between body mass and  $\dot{V}O_{2\max}$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) during running (Pate *et al.*, 1992; Bourdin *et al.*, 1993).

Other studies have found that expressing RE in terms of the oxygen cost to cover a given distance ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{km}^{-1}$ ) rather than relative oxygen uptake ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) is

better in reflecting differences in oxygen cost during different velocities of running performance (Lucia *et al.*, 2006; Foster & Lucia, 2007). A further permutation is that of expressing RE in terms of caloric unit cost ( $\text{kcal.kg}^{-1}.\text{km}^{-1}$ ), which is sensitive to changes in running velocity when compared with  $\text{O}_2$  unit cost or oxygen uptake normalized per distance traveled ( $\text{mL.kg}^{-1}.\text{km}^{-1}$ ) (Fletcher *et al.*, 2009). This approach further considers the decrease in RE when fat as an energy substrate is reflected in the increased oxygen utilization ( $\dot{V}\text{O}_2$ ,  $\text{L.min}^{-1}$ ). To corroborate the sensitivity of dehydration and hyperhydration (-4% to +4% BW) on RE at four different running velocities, our data is presented in four different expressions: (i)  $\dot{V}\text{O}_2$  ( $\text{mL.kg}^{-1}.\text{min}^{-1}$ ); (ii)  $\dot{V}\text{O}_2$  ( $\text{mL.kg}^{-0.75}.\text{min}^{-1}$ ); (iii) caloric unit cost,  $C_R$  ( $\text{kcal.kg}^{-1}.\text{km}^{-1}$ ) and (iv) gross oxygen cost of running ( $\text{mL.kg}^{-1}.\text{km}^{-1}$ ) in both hypohydrated and hyperhydrated trials (Figure 6.2 & Figure 6.3).

### ***Hypohydration effects on RE***

Contrary to our primary hypothesis, our data does not show a significant improvement in RE with a reduction in body weight (Figure 6.2) to the extent of 3% and 4% BW deficit as a consequence of being hypohydrated. The ratio standard expression ( $\text{mL.kg}^{-1}.\text{min}^{-1}$ ), as a power function with exponents for body mass ( $\text{mL.kg}^{-0.75}.\text{min}^{-1}$ ) and  $\text{O}_2$  unit cost ( $\text{mL.kg}^{-1}.\text{km}^{-1}$ ) were statistically ~5-6% greater when the subjects experienced 3-4% BW deficit compared with the euhydrated state (**D0**) equivalent to the running velocities that elicited 65 to 75%  $\dot{V}\text{O}_{2\text{max}}$ . These observations are similar to Greenleaf *et al.* (1971) who observed  $\dot{V}\text{O}_2$  ( $\text{L.min}^{-1}$ ) to be higher during their hypohydration trial (-5.2% BW) when compared with *ad libitum* condition (-1.63% BW) and their hyperhydration trials (+1.2% BW). These researchers (Greenleaf *et al.* 1971) suggested

that the small increase in  $\dot{V}O_2$  (~8.4%) observed in their study would not represent an important physiological change during exercise.

Care must be taken when examining RE in prolonged exercise where there is a marked level of dehydration which is associated with many other disturbances to homeostasis. Davies & Thompson (1986) observed that progressive dehydration has limited influence on RE when running duration is less than 50 min. However, an increase in  $\dot{V}O_2$  (+9.1%  $\dot{V}O_{2max}$ ) associated with ~5.5% BW loss became pronounced after 50 min in the course of constant velocity running at 65-70%  $\dot{V}O_{2max}$  for 4 hours duration (Davies & Thompson, 1986). These researchers further proposed that this pronounced metabolic drift could be partly attributed to increased free fatty acid (FFA) oxidation, increased pulmonary ventilation (VE), increased work of the cardiac muscle (HR), increased metabolic rate of the liver, increased circulating catecholamines and possibly an increased motor unit recruitment with a reduced contribution from the parallel and series elastic components. Furthermore, observations made on RE in prolonged running may include an increased  $\dot{V}O_2$  consequent to the adoption of grossly altered gait patterns with extreme muscle fatigue. The increase in  $\dot{V}O_2$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) suggests an increase in the relative intensity of exercise when expressed in terms of %  $\dot{V}O_{2max}$ . Armstrong *et al.* (2006) reported that RE at 75% or 85%  $\dot{V}O_{2max}$  with 5% BW loss did not differ from when in a euhydrated state, which indicates dehydration at the level of the current study has neither a beneficial or adverse effect on RE. Note that the subjects in Armstrong *et al.*'s (2006) study were instructed to run for 10-min during the RE tests. Therefore, it is likely that a short duration of running is sufficient to establish a “steady state” with ~5% BW loss has limited influence on RE (Davies & Thompson, 1986). In fact, the authors further explained that highly trained runners were able to cope with

added physical stress imposed by dehydration without a change in RE (Armstrong *et al.*, 2006).

Previous studies have found Kenyan runners to be in negative energy balance with a low fluid intake regimen prior to intense training, and prior to competition resulting in a significant reduction in body mass (Onywera *et al.*, 2004; Fudge *et al.*, 2006). An individual learns to produce specific patterns of muscle recruitment and has the capability to adapt to training (Bonacci *et al.*, 2009). Therefore, training in a hypohydrated state might be one of the factors responsible for the improvement in RE, as a result of adaptations in motor unit recruitment. Collectively, we speculate that Kenyan runners have trained and adapted to these conditions. However there is no published data indicating that there is an adaptive metabolic response to hypohydration. A reduction in body weight should have a beneficial effect on running performance by reducing the energy cost of running.

### ***Hyperhydration effects on RE***

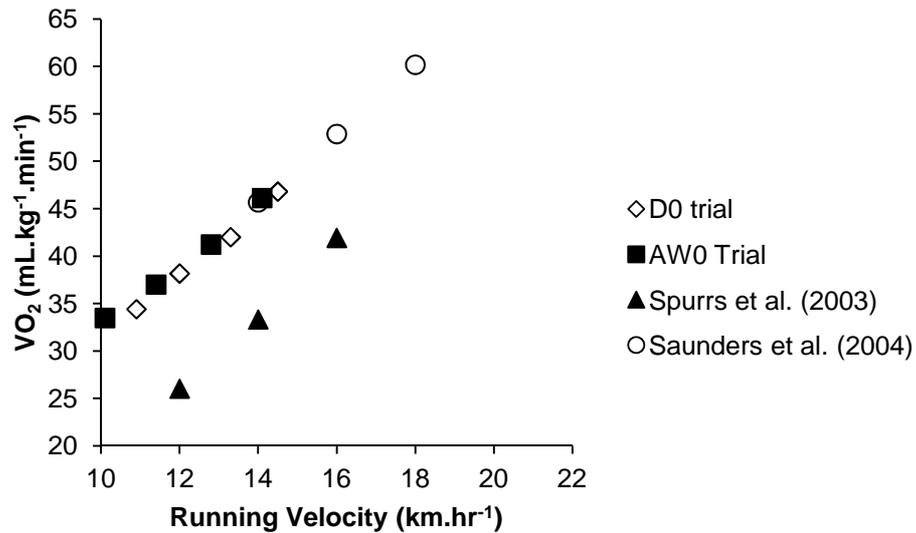
There are many studies that have shown that additional external loading of weight in the form of a weight jacket or weight belt around the torso of the body during running and walking results in an increased energy cost i.e. an increase in  $\dot{V}O_2$  (Cureton & Sparling, 1980; Davies, 1980; Cooke *et al.*, 1991; Teunissen *et al.*, 2007). Davies (1980) reported that  $\dot{V}O_2$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) during exercise increased in proportion to external loading up to 5% and 10% of body weight when wearing a weight jacket. Teunissen and colleagues (2007) attached flexible lead strips symmetrically to a padded belt that wrapped tightly around the subjects' waist to determine the effects of added weight (10, 20 and 30% increments) while a harness system was used to simulate reduced gravity (-

25, -50 and -75% of 100% BW) on RE. Runners loaded with additional weight incurred a significant increment in net metabolic rate whereas when the runners ran with reduced weight that simulated reduced gravity, net metabolic rate decreased proportionally. Interestingly, Cooke *et al.* (1991) documented that vertical loading with 5 and 10% of body mass using a weight jacket did not produce a significant increase in  $\dot{V}O_2$  due to the economy in energy expenditure with vertical loading. The authors also explained that the experimental design of their study (e.g., combination of vertical and horizontal loading) might be one of the reasons which influenced the final results. Similarly, addition of external weight to male runners from 2.6 to 11.6% (2.2 to 10.5 kg) using a harness had no significant effect on  $\dot{V}O_2$  ( $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) during running indicates that additional loading up to 11.6% bodyweight does not alter running form and economy (Cureton & Sparling, 1980). This seems difficult to reconcile with the external added weight in this study being as much as 10.5 kg; which would intuitively elicit an increase in the  $\dot{V}O_2$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ).

One possible explanation for these results is that when added weight is evenly distributed around the torso the additional oxygen cost is minimised and may be offset by an added contribution from the series and parallel elastic component of muscles and tendons at no additional metabolic cost. It is well known that when running the muscles begin to be activated milli-seconds prior to foot contact with the ground. As the weight of the body loads the muscles (quadriceps and triceps surae) on landing the muscles are stretched and lengthened thus performing repeated eccentric contractions (Cavagna, 1977). The muscles subsequently perform a concentric contraction in propelling the person forward and upward (Cavagna *et al.*, 1964; Cavagna *et al.*, 1968; Asmussen & Bonde-Petersen, 1974). It would seem that the greater the pre-activation in setting an “active stiffness in the muscle, the greater the subsequent contribution there is to a

forceful concentric contraction (Wilson *et al.*, 1994). This sequence is referred to as the “stretch-shortening cycle” (SSC) and not only involves stretching of the myofilaments, but also stretching of tendons and ligaments. These structures behave like springs, stretching and re-coiling (Komi & Bosco, 1978). The faster a person runs the greater the contribution from the SSC to upward and forward motion. Conversely, when runners maintain a bent legged style of running e.g., groucho running there is a very limited contribution from the SSC resulting in poor RE (McMahon *et al.*, 1987). It has been postulated that performing plyometric training e.g., depth jumping with an added load in the form of holding dumbbells increases the level of pre-activation more than without this loading. If this is correct then some of the increased  $\dot{V}O_2$  with added external weight when running at faster speeds may be offset by an increased contribution from the SSC (Figure 6.3).

Plyometric training has been shown to improve running economy (Paavolainen *et al.*, 1999; Turner *et al.*, 2003; Saunders *et al.*, 2006). Such training involves depth jumping, bounding and hopping movements which are thought to increase the number of motor units recruited in the pre-activation stage or contributing to an earlier onset of pre-activation. The outcome is a reduced  $\dot{V}O_2$  cost for running particularly at high velocities. Spurrs *et al.* (2003) reported that the increase in lower leg musculotendinous stiffness through a 6-weeks of plyometric training resulted in an improvement in RE. However, their finding is not convincing because the reported  $\dot{V}O_2$  measurements at 12, 14 and 16 km.hr<sup>-1</sup> are exceptionally low to the extent that we have been unable to locate any other studies reporting similar  $\dot{V}O_2$  RE data. Figure 6.8 compares our data with that reported by Spurrs *et al.* (2003) and other studies (Abe *et al.*, 1998; Saunders *et al.*, 2004).



**Figure 6.8** Comparison of the running economy data ( $\dot{V}O_2$ , mL.kg<sup>-1</sup>.min<sup>-1</sup>) in our subjects (**AW0** and **D0** trials) and with previous research (Spurr's *et al.*, 2003; Saunders *et al.*, 2004).

It is acknowledged that carrying an additional load closer to the centre of the body mass has minimal influence on the oxygen cost during running. However when additional load is added distally to the limbs, there is a pronounced increase in the oxygen cost of running (Myers & Steudel, 1985). Myers and Steudel (1985) reported that aerobic demand was increased by 1% for every extra kilogram carried on the trunk. This data was identical to our data depicted in Figure 6.3, where an additional ~3% of  $\dot{V}O_2$  (mL.kg<sup>-1</sup>.min<sup>-1</sup>) was needed to carry 3% and 4% additional weight across four different running velocities when compared with the control trial (**AW0**).

The sensitivity of oxygen cost of running during **AW** and **D** trials might be offset by the day to day variance in measurement of RE. It has been reported that the range extends to  $\pm 3\%$  to 5% in a group of well trained distance runners, whereas the largest within subject variation was 9% (Daniels *et al.*, 1984). Cooke *et al.* (1991) repeated three measures of  $\dot{V}O_2$  performed on one subject and produced an average  $\pm 2$  mL.kg<sup>-1</sup>.min<sup>-1</sup>

$\dot{V}O_2$  for each speed and load condition. When circadian variation, training activity, footwear, running experience and the length of treadmill ‘accommodation’ are strictly controlled the intra-individual variation is reported to be 1.6% (Morgan *et al.*, 1991). Further studies suggest that 90% of this variance in day to day RE is biological and 10% is technical error (Armstrong & Costill, 1985). In the present study we have ensured that each subject was wearing the same footwear and had a similar training activity during the days before testing. To minimise circadian variation, all tests were conducted at the same time of day for each individual throughout the experimental trials. We have calculated the variance between trials in  $\dot{V}O_2$  ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) for both groups. We found that the variance ranged from ~1.4% to ~5.6% and ~0.4% to ~3.4% in **D** and **AW** groups respectively, which is identical to the study by Daniels *et al.* (1984).

### ***Heart Rate and Perceptual Responses***

In agreement with the results of Armstrong *et al.* (2006), our data also shows that dehydration induced by an exercise-heat exposure protocol imposed a circulatory strain on the subjects during exercise. There is a possibility that there was a residual effect of the dehydration protocol on subsequent measurement of RE despite the cooling procedures that were initiated at the end of the prolonged exercise-heat exposure protocol. A greater HR response was found when the subjects were running with 3 and 4% BW deficit (Figure 6.4). The raised HR response observed may be associated with sweat losses causing a state of hypovolemia (Cheuvront *et al.*, 2010). Hypovolemia has the potential to lower venous return and central filling pressure, although cardiac output is maintained throughout exercise due to an increase in HR which offsets a progressive reduction in stroke volume (Rowell *et al.*, 1966). Conversely, simulated hyperhydration did not alter plasma volume and as such the HR response remained similar across the

**AW** trials (Figure 6.5), indicating that added weight equivalent to 3-4% body weight did not have a significant impact on the cardiovascular response.

Interestingly, subjects' perceptual ratings (RPE) between **D** trials were similar (Figure 6.6) despite the HR responses being significantly higher in **D3** and **D4** trials across increasing running velocities (Figure 6.4). Conversely, the increase in RPE in **AW3** and **AW4** trials when compared with **A0** trial (Figure 6.7) were not associated with the increase HR in **AW3** and **AW4** trials when compared with **A0** trial (Figure 6.5). The subjects may have assumed that running with a heavier body weight would deteriorate their running performance whereas running with a lighter body weight would enable them to run faster.

### ***Conclusion***

In conclusion, this study has shown that (1) hypohydration (-3 and -4% BW) did not reduce the oxygen cost of running proportionally with the degree of hypohydration and (2) hyperhydration (+3 and +4% BW) did not increase the oxygen cost of running proportionally with the degree of hyperhydration. None of the runners in the present study gained any beneficial effect in running economy with hypohydration. The additional oxygen cost is minimised during hyperhydration trials as the added weight is evenly distributed around the torso and may be offset by an added contribution from the series and parallel elastic component of muscles and tendons at no additional metabolic cost.

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## **CHAPTER 7**

### **STUDY THREE: EFFECT OF DIURETIC-INDUCED DEHYDRATION ON PROLONGED RUNNING PERFORMANCE IN HOT AND COOL CLIMATIC CONDITIONS**

## **7.0 EFFECT OF DIURETIC-INDUCED DEHYDRATION ON PROLONGED RUNNING PERFORMANCE IN HOT AND COOL CLIMATIC CONDITIONS**

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## 7.1 ABSTRACT

To investigate the effects of hypohydration on prolonged running performance in hot and cool conditions, eight male runners (age:  $32.8 \pm 6.1$  yr; body mass, BM:  $72.2 \pm 6.5$  kg; height:  $177.9 \pm 9.3$  cm;  $\dot{V}O_{2\max}$ :  $65.5 \pm 6.4$  mL/kg/min) undertook four 60 min trials of treadmill running at 65%  $\dot{V}O_{2\max}$  followed by 1% gradient increase every 3 min until volitional fatigue. The four 60 min trials are follows: 1) euhydrated in 20°C (**E20**); 2) dehydrated in 10°C (**D10**); 3) euhydrated in 35°C (**E35**) and 4) dehydrated in 35°C (**D35**). A diuretic (Lasix<sup>®</sup> 1 mg /kg BM) was used in **D10** and **D35** to induce ~3% BM deficit. Subjects reached their point of fatigue at ~95%, of  $HR_{\max}$  ( $177 \pm 10$  beats/min) and rating of perceived exertion (RPE) was equally high in all trials ( $18.4 \pm 1.1$ ). Time to fatigue in the **E20** ( $75.3 \pm 3.5$  min) and **D10** ( $73.7 \pm 4.2$  min) was significantly longer ( $p < 0.05$ ) than the **E35** ( $64.7 \pm 6.8$  min) and **D35** ( $40.6 \pm 13.6$  min). At 25-min of running, cardiac output in all trials was similar  $\sim 22.2 \pm 4.1$  L.min<sup>-1</sup>, while heart rate (HR) was ~23 and ~14 beats.min<sup>-1</sup> higher in **D35** ( $p < 0.001$ ) and **E35** ( $p < 0.05$ ) respectively, when compared with **E20** and **D10** ( $\sim 143$  beats.min<sup>-1</sup>). Mean skin temperatures ( $T_{sk}$ ) were ~6°C lower in **E20** and **D10**, compared with **E35** and **D35** ( $p < 0.001$ ) at 25-min and at fatigue. Similarly, body core temperature ( $T_{re}$ ) was ~0.5°C lower in **E20** and **D10** compared with **E35** and **D35** ( $p < 0.05$ ) at 25-min but there was no significant difference between **D35** ( $39.2 \pm 0.5^\circ\text{C}$ ) and **D10** ( $38.8 \pm 0.4^\circ\text{C}$ ) at fatigue. Thirst sensation increased over time and was significantly higher in **E35** and **D35**, compared with **E20** and **D10** ( $p < 0.05$ ). Running performance in **E35** and **D35** was impaired, in association with elevated ambient temperature, but not hypohydration. This performance decrement was approximately 10-35 minutes less endurance time in **E35** and **D35** respectively and was further associated with a greater upward HR drift, elevated  $T_{sk}$ , and  $T_{re}$ . Furthermore, mild dehydration did not have an adverse effect on the running performance in cool conditions. RPE was similar across all conditions

despite thirst sensation being greater in the hot conditions. In conclusion mild dehydration (~4.5% BW loss) was shown to have a significant effect on endurance performance in hot conditions. However, this level of dehydration did not adversely affect endurance performance in cool conditions.

## 7.2 INTRODUCTION

Hypohydration has been reported to impair exercise performance when body water loss amounts to 1-2% of pre-exercise body mass (Armstrong *et al.*, 1985). This is a relatively low level of hypohydration that endurance athletes experience during most of their training sessions in hot climatic conditions. Well trained endurance athletes have sweat rates greater than 2.0 L.hr<sup>-1</sup> in such conditions, so that they may incur a body weight deficit of ~5% in completing a marathon. The current Position Stand of the American College of Sports Medicine (ACSM) on Exercise and Fluid Replacement states that dehydration (>2% body weight loss) will compromise exercise performance especially during warm / hot environments (Sawka *et al.*, 2007). The ACSM Position Stand further suggests athletes should replace fluid according to the dictates of thirst to maintain <2% body weight loss. In fact, it is not physically possible to replace body water loss by ingesting fluid at a volume of ~2% body weight (A well-trained 70 kg runner will need to ingest approximately 1.4 L.hr<sup>-1</sup>) while running a marathon at an average pace equivalent to 80 to 90%  $\dot{V}O_{2max}$ .

A plethora of laboratory-based studies have consistently reported that dehydration increases circulatory and thermoregulatory strain during exercise with the consequence for athletes being impaired exercise performance especially in hot conditions (Sawka *et al.*, 1985; Montain & Coyle, 1992; González-Alonso *et al.*, 1995; Galloway & Maughan, 1997; Roy *et al.*, 2000; Chevront *et al.*, 2005). Montain and Coyle (1992) undertook a systematic study of the effects of graded dehydration (1 - 5%) which showed increasing levels of thermal and circulatory strain with graduated fluid restriction. In support of this finding, González-Alonso *et al.* (1995) investigated cyclists in a euhydrated and dehydrated state and found that when fluid was ingested cardiovascular function and thermal homeostasis were maintained. In the dehydrated state the cyclists were

hyperthermic, cardiac output and stroke volume were reduced together with an increase in plasma catecholamines and cutaneous vascular resistance. In this situation cutaneous vasoconstriction maintains mean arterial pressure but limits heat loss.

In contrast, Goulet (2011) recently undertook a meta-analysis to determine the effect of exercise-induced dehydration on time-trial cycling performance at average ambient temperature and relative humidity of  $26.0 \pm 6.7^{\circ}\text{C}$  and  $61 \pm 9\%$ . The results demonstrated that 4% body weight loss did not affect outdoor cycling performance and the author concluded with the suggestion that fluid replacement during exercise according to the thirst sensation could maximise endurance performance. Furthermore, Zouhal and colleagues (2010) recently reported that there was a significant inverse relationship between percentage body weight change and marathon finishing time. Their data shows the fastest runners who completed the marathon in less than 3 hour had lost  $>3\%$  body weight in cool conditions ( $9-16^{\circ}\text{C}$ ) with 60 - 82% relative humidity (rh).

With these conflicting studies, we propose that prolonged exercise in the heat with mild dehydration ( $\sim 3\%$  body weight loss) imposes an increasing level of strain on the cardiovascular and thermoregulatory systems; however, this mild level of dehydration remains tolerable in cool environments. Therefore we hypothesized that (i) mild dehydration will impair prolonged running performance in hot ( $35^{\circ}\text{C}$ , 40% rh) but not in cool ( $10^{\circ}\text{C}$ ; 35% rh) conditions; (ii) mild dehydration will not cause significant circulatory strain in cool conditions.

## 7.3 METHODS

### 7.3.1 Subjects

Eight male endurance runners participated in this study (Table 7.1). Before providing written informed consent, subjects were fully informed of the experimental procedures including the risks and benefits associated with participation in the study. They completed a Physical Activity Readiness Questionnaire (PAR-Q) and Medical History & Health Screening Questionnaire. This study conforms with the current Declaration of Helsinki Guidelines and was approved by the Human Research Ethics Committee of The University of Sydney (Ref No.12450).

**Table 7.1** Mean  $\pm$ SD for physical and physiological characteristics of the subjects

	n=8
Age (years)	32.8 $\pm$ 6.1
Height (cm)	177.9 $\pm$ 9.3
Weight (kg)	73.2 $\pm$ 6.5
% Body Fat	8.0 $\pm$ 2.5
Body surface area, $A_D$ (m <sup>2</sup> )	1.91 $\pm$ 0.14
Maximal oxygen uptake (mL. kg <sup>-1</sup> .min <sup>-1</sup> )	65.5 $\pm$ 6.4
Training volume (km.week <sup>-1</sup> )	71.8 $\pm$ 24.7
10 km Performance Time (min)	36.9 $\pm$ 2.2
21.1 km Performance Time (min)	85.2 $\pm$ 4.8

### 7.3.2 Anthropometric Measurements

Height and body weight with running shorts and socks were determined using a height stadiometer (Harpenden Stadiometer, Holtain Limited, UK) and an electronic scale (Mettler Toledo ID1 Multi Range Scale, Columbus, OH, USA), respectively. Seven sites of skinfolds thickness (triceps, chest, midaxillary, subscapular, suprailiac, abdomen, and anterior thigh) were measured twice to the nearest 0.5 mm using skinfold callipers (Holtain, Crymych, UK) to estimate percent body fat (Jackson & Pollock, 1978). A third measurement of skinfold thickness was taken when inconsistent values (>2.0 mm) were obtained. Percentage of body fat was estimated using Siri's equation:  $(495 / \text{Body Density}) - 450$  (Siri & Lukaski, 1993).  $A_D$  was calculated using the equation described by DuBois and DuBois (1916):  $A_D \text{ (m}^2\text{)} = 0.20247 \times \text{Height (m)}^{0.725} \times \text{Weight (kg)}^{0.42}$

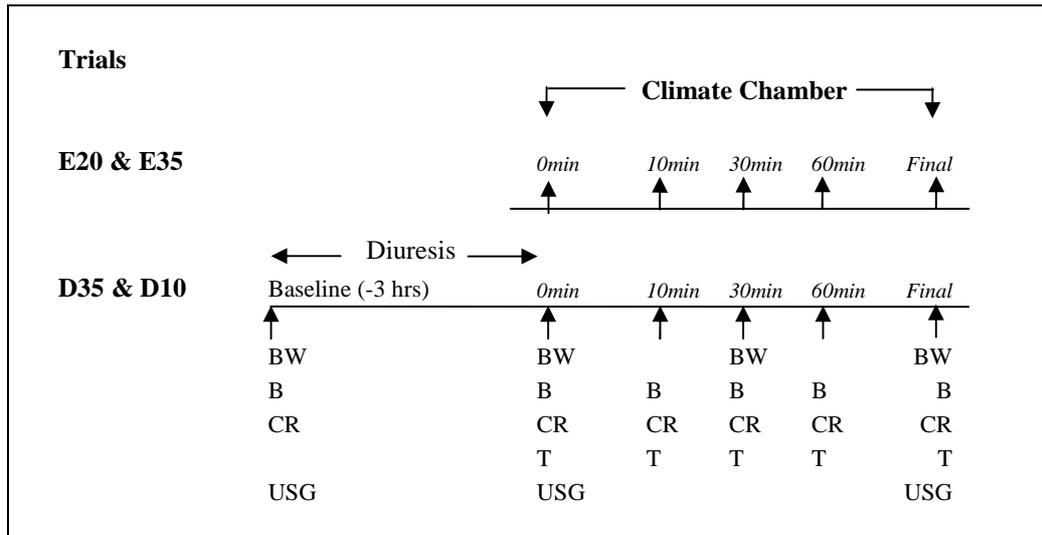
### 7.3.3 Preliminary Testing

A period of treadmill running familiarization was undertaken prior to the submaximal test. A submaximal exercise test was undertaken in thermoneutral conditions (20°C, 40% RH) and involved 4 x 4 min of continuous steady state running. The treadmill velocity ranged from 10, 12, 14 to 16 km.hr<sup>-1</sup> with 0% gradient. Subjects then completed an incremental gradient run (12 km.hr<sup>-1</sup>; 2% gradient increased every 2 min) to volitional fatigue to determine maximal oxygen uptake ( $\dot{V}O_{2\text{max}}$ ) and maximal heart rate ( $HR_{\text{max}}$ ). The linear regression equation correlating treadmill velocity with the corresponding oxygen uptake and heart rate was extrapolated to the measured  $HR_{\text{max}}$ , to verify the  $\dot{V}O_{2\text{max}}$  and to determine the treadmill velocity corresponding to 65%  $\dot{V}O_{2\text{max}}$  for each subject's subsequent series of prolonged exercise tests (PETs).

### 7.3.4 Experimental Design

To investigate the effect of hypohydration during hot and cool conditions, subjects performed four PETs in four different climatic conditions as follows: **E20**: euhydrated in thermoneutral conditions [20°C, 40% relative humidity (rh)]; **E35**: euhydrated in hot conditions (35°C, 40% rh); **D35**: dehydrated in hot conditions (35°C, 40% rh); **D10**: dehydrated in cool conditions (10°C, 35% rh). These PETs were undertaken in randomised order, with at least 7 days between each trial.

To ensure subjects were euhydrated prior to all experimental trials, they were instructed to drink a prescribed volume of approximately 6 mL.kg BW<sup>-1</sup> every 2-3 hours, 4-5 sessions per day of water on the day before and during the day of testing. For the dehydration trials (**D35** and **D10**), a diuretic was administered by orally ingesting 40-80 mg (1 mg.kg BW<sup>-1</sup>) of furosemide (Lasix<sup>®</sup>), to elicit the desired ~3% of body mass loss. The diuretic tablet was ingested 3 hours prior to the dehydration trials, after emptying the urinary bladder. No fluid or food was ingested prior to the commencement of each PETs during the diuresis period. Subjects were rested in thermoneutral conditions. Figure 7.1 shows a schematic of the experimental design and timeline for data collection.



**Figure 7.1** Schematic representation of the experimental design and protocols for four experimental trials (**E20**, **E35**, **D35** and **D10**)

Abbreviations: BW (Body weight, blood pressure); B (Blood sample); CR (Heart rate,  $\dot{V}O_2$ , cardiac output at 10, 30, 60 and final); T ( $T_{re}$ ,  $T_{sk}$  and  $SkBF$  at every minute); USG (Urine specific gravity). The order of experimental trials was randomised.

#### 7.3.4.1 Experimental Protocol

Subjects were required to refrain from any strenuous physical activity, maintain a similar food intake and avoid the consumption of caffeine and alcohol within 24 hours before arrival at the laboratory for the experimental trials. To assist subjects in maintaining the same food intake prior to each PET, they maintained a 24 hour record of food and fluid ingestion prior to the first PET, and thereafter repeated this food and fluid intake in the 24 hours prior to each subsequent PET.

#### *Diuretics-induced Dehydration Protocol*

Upon arrival at the laboratory for the dehydration trials (**D35** and **D10**), subjects were required to void their urinary bladder and collect a mid-stream sample of urine in a disposable urine container. Subjects' baseline body mass with pre-weighed sports attire

including shorts, socks and shoes were weighed. Thereafter, subjects consumed the diuretic (Lasix<sup>®</sup>, 1 mg.kg BW<sup>-1</sup>) together with 100 mL of plain water. Urine was collected throughout the diuresis process using a 3 L urine container. Urine volume and body mass were weighed to determine 3% body mass deficit prior to the commencement of both the **D35** and **D10** PETs. The mean time taken for the dehydration protocol was approximately 2.0 to 2.5 hr. The subjects did not report any adverse experience with the administration of diuretics, despite the discomfort of frequent urination and a prolonged period of waiting to achieve the desired level of diuresis.

### ***Prolonged Exercise Tests (PETs)***

#### *Preparation*

Prior to the commencement of all PETs, subjects were asked to void their bladder of urine and a mid-stream sample of urine was collected. A sterilised disposable thermistor probe (YSI 400 series, Mallinckrodt Medical, USA) secured by a sterilised bead positioned 12 cm from the tip of the probe was then inserted beyond the anal sphincter to a depth of 12 cm. A Polar Trainer<sup>™</sup> heart rate monitor (Polar Electro Oy, Kemele, Finland) was secured on the chest and four iButton<sup>™</sup> temperature sensors (Maxim Integrated Products, Sunnyvale, CA, USA) were attached to the skin at four sites. An indwelling cannula was inserted into the cephalic vein in the forearm and an extension tube of 20 cm was attached to it to allow for repeated blood sampling and was kept patent by flushing the extension tube with heparinised saline. A resting blood sample was collected after maintaining a seated position for 15 min in a thermoneutral condition to allow equilibration.

Body mass with pre-weighed sports attire and all instrumentation were weighed in the

climate chamber. Subjects maintained a seated position for 5-10 min while resting blood pressure (BP) was measured. A laser Doppler skin blood flow probe (MP1-V2, MoorLAB™, Moor Instruments Ltd, Devon, UK) was placed on the right medio-ventral upper arm. The baseline values of skin blood flow (SkBF) were recorded while subjects stood 5 min on the treadmill with their arms in a relaxed dependent position hanging by their sides. Resting HR and  $\dot{V} O_2$  were measured immediately prior to the commencement of each PET.

During PETs, subjects were required to run at 65%  $\dot{V} O_{2max}$  for an hour followed by an incremented gradient run (+1% every 3 min) until volitional fatigue. A fan was positioned at approximately 1 m distance in front of the treadmill which created an artificial wind resistance of  $\sim 12 \text{ km}\cdot\text{hr}^{-1}$ . All subjects were instructed and encouraged to run for as long as possible. In compliance with ethical approval, exercise was terminated if a  $T_{re}$  of  $39.9^\circ\text{C}$  was attained. Time to exhaustion was recorded as a measure of exercise performance. Water from pre-measured water bottles was available for the subjects to drink *ad libitum* throughout each PET.

Following completion of each PET, clothed body mass, and subsequently sports attire and water bottles were weighed to determine sweat loss. Subjects were instructed to wear the same sports attire during each PET. All experimental trials were conducted at approximately the same time of day for each subject.

#### 7.3.4.2 Hydration Measurements

The same electronic scale ( $\pm 0.001$  kg; Mettler Toledo ID1 Multi Range Scale, Columbus, OH, USA) was used to measure the subjects' body mass and weight of their sports attire and water bottles throughout the study. The electronic scale was calibrated prior to each of the experimental trials.

Urine samples were collected prior to the administration of a diuretics tablet (Lasix<sup>®</sup>), pre-PET and post-PET. Urine specific gravity (USG) was analysed using a digital urine specific gravity refractometer (Atago<sup>®</sup> UG- $\alpha$ , Japan) to determine the hydration status: USG <1.020 was considered as euhydrated.

Sweat loss was calculated as the difference in pre- and post-exercise nude body mass, corrected for fluid intake, sweat contained in sports clothing and running shoes, urine output and respiratory water loss (Pugh *et al.*, 1967; Mitchell *et al.*, 1972). Whole body sweat rate was estimated as:  $\text{Sweat Rate (L/min)} = \text{Sweat loss (L)} / \text{Performance time (min)}$

### 7.3.4.3 Cardiorespiratory Measurements

Oxygen uptake ( $\dot{V}O_2$ ) and cardiac output ( $Q$ ) were measured at 0, 10, 30, 60 min and in the final minutes prior to the cessation of exercise.  $\dot{V}O_2$  was measured for 60 s using the Douglas Bag technique. Gas analyzers were calibrated immediately before the analysis of the expired respiratory gas samples. Expired gas fractions were measured using  $O_2$  and  $CO_2$  analysers (Model 2-3A and Model CD-3A respectively, Ametek, Thermox Instruments, Pittsburg, PA) calibrated with a known gas ( $O_2$ :15.9%;  $CO_2$ : 4.03%) and ambient air ( $O_2$ :20.93%;  $CO_2$ : 0.03%). Expired gas volume was measured using a dry gas flow meter (Parkinson-Cowan, England).

Cardiac output ( $\dot{Q}$ ) was determined using the  $CO_2$ -rebreathing equilibrium technique of Collier et al (1956), controlled and monitored with software written in LabVIEW Program (National Instruments, Austin, Texas, USA). Subjects breathe via a respiratory valve (model 2700, Hans Rudolph, Kansas City, MO) with a mouthpiece and nose clip. They were required to rebreathe with a gas mixture of approximately 15%  $CO_2$  and 85%  $O_2$  for approximately 12 seconds for the determination of  $\dot{Q}$ . The fraction of expired  $CO_2$  was measured with a  $CO_2$  analyser (Beckman LB-2, Beckman Instrument, IL, USA) calibrated with ambient air ( $CO_2$ : 0.03%) and a gas of known concentration ( $CO_2$ :15%) before the testing. Stroke volume (SV) was calculated from the  $\dot{Q}$  and HR determinations:  $SV = \dot{Q} / HR$ . Arterio-venous differences in  $\dot{V}O_2$  content (a-v $O_2$  diff) was calculated using the Fick equation:  $\dot{V}O_2 = Q \times a-vO_2 \text{ diff}$

Heart rate (HR) was monitored at 5 min interval during each PET. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a sphygmomanometer (Accoson, Harlow, UK) at rest, 30 min and during the final

minutes when the subjects signalled their inability to maintain the exercise. Mean arterial pressure (MAP) was calculated as:  $(0.33 \times \text{SBP}) + (0.67 \times \text{DBP})$ .

#### 7.3.4.4 Thermoregulatory Measurements

Rectal ( $T_{\text{re}}$ ) and skin ( $T_{\text{sk}}$ ) temperatures were monitored continuously and recorded at every 5 min and 1 min respectively.  $T_{\text{re}}$  data was logged on a portable temperature data logger (Digi-Sense<sup>®</sup> Thermistor Thermometer, Chemopharm, US), which was calibrated before the study in a water bath with a platinum resistance probe (T1091).  $\bar{T}_{\text{sk}}$  was measured at four sites using iButton<sup>™</sup> temperature sensors, which have an inbuilt data logger for subsequent data downloading (van Marken Lichtenbelt *et al.*, 2006). The skin sites used for measuring  $T_{\text{sk}}$  were: (i) halfway between the acromium process and nipple (chest), (ii) deltoid, (iii) anterior mid-thigh and (iv) right lateral calf. The iButtons<sup>™</sup> were attached to the skin using narrow strips of Opsite<sup>™</sup> surgical bandage.  $\bar{T}_{\text{sk}}$  was calculated using the equation:  $\bar{T}_{\text{sk}} = 0.3 (T_{\text{chest}} + T_{\text{arm}}) + 0.2 (T_{\text{thigh}} + T_{\text{leg}})$  (Ramanathan, 1964).

SkBF was measured at a site on the right upper arm (below deltoid) using a Laser-Doppler perfusion monitor (moorLAB<sup>™</sup>, Moor Instruments Ltd., Devon, UK). At 10, 30, 60 and during the final minutes of exercise, subjects were instructed to place their right arm on the treadmill side guiderail for 30-60 s to minimise the vibration and movement artifacts from a swinging arm. The data was later analysed using the Moor Instruments associated software (moorLAB v2.01, Mec Instruments, Wilmington, DE, USA). Values are expressed as arbitrary units (AU). All data was normalised to resting SkBF (baseline) as a percentage change.

#### **7.3.4.5 Haematological Measurements**

Venous blood samples (7 ml) were drawn at 0, 10, 30, 60 and during the final minutes of exercise. Haemoglobin concentration was analysed (Sysmex KX-21N Hematology Analyser, Kobe, Japan) and haematocrit percentage was estimated using a microcentrifuge (Hawksley) and micro haematocrit reader (Hawksley CE/15006, UK). Haemoglobin and haematocrit values were used to calculate the percentage change in plasma volume (Dill & Costill, 1974). Triplicate measurements were made of haematocrit and haemoglobin. Serum osmolality and plasma viscosity were measured using an osmometer (OSMOMAT 030, Gonotec GmbH) and viscometer (Cone and plate Viscometer, LVT with CP-40, Wells-Brookfield), respectively. Duplicate measurements were made of blood glucose and lactate using the automated glucose oxidase and lactate oxidase methods, respectively (EML 105; Radiometer Pacific, Copenhagen, Denmark).

#### **7.3.4.6 Subjective Reporting**

Perceived thirst sensation was identified using a nine-point thirst scale with verbal anchors ranging from 1 ("not thirsty at all") to 9 ("very, very thirsty") (Engell *et al.*, 1987). Ratings of perceived exertion (RPE) were obtained using the 15-point Borg scale ranging from 6 ("very, very light") to 20 ("very, very hard") (Borg, 1982) and thermal comfort was recorded from a seven point scale (Bedford, 1936) ranging from 1 ("much too cold") to 7 ("much too hot). Thirst sensations, RPE and thermal comfort were recorded at 10-min intervals throughout the testing and at the point of exhaustion. These measurements were obtained in a random order to eliminate any ordering effect.

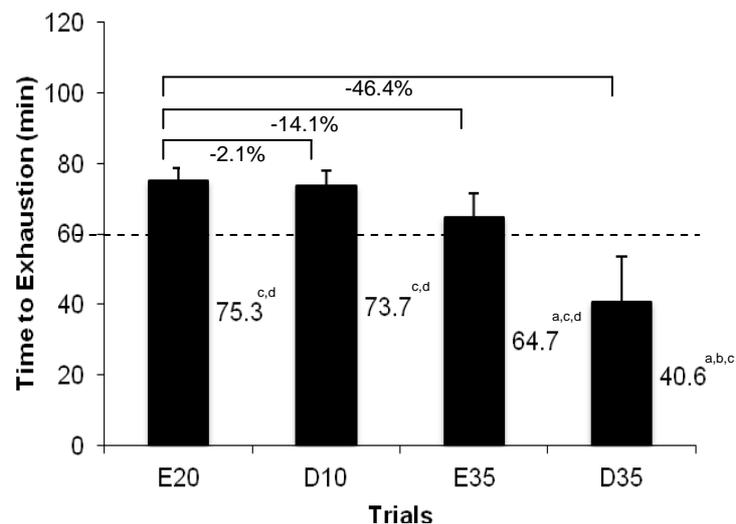
#### **7.3.5 Statistical Analysis**

Power and Sample Size Calculation (PS) software by Dupont and Plummer (1997) was used to determine the sample size. A two-way (time x trial) repeated ANOVA was performed using Statistical Package for Social Sciences (SPSS 18.0, Chicago, IL) to compare significance difference within and between treatments. Where significant interaction effects were established, pairwise differences were identified using Tukey's HSD post hoc analysis procedure. Where appropriate, differences between trials were also identified using one-way analysis of variance with repeated measures. All values are expressed as mean  $\pm$  SD and the significance level was accepted at  $p < 0.05$ .

## 7.4 RESULTS

### 7.4.1 Time to Exhaustion

Figure 7.2 shows time to exhaustion in four different conditions (**E20**, **E35**, **D10** and **D35**) and % decrement in performance from **E20**. Subjects managed to complete 60-min of running in all trials and proceed to incremental runs until exhaustion except in **D35**. Running performance time was significantly longer in **E20** and **D10** compared with **E35** and **D35** ( $p < 0.05$ ). A significant difference was found between **E35** and **D35** ( $p = 0.001$ ) while the **D35** trial exhibited the greatest decrement of performance from **E20** when compared with the performance time in **D10** and **E35**.



**Figure 7.2** Time to exhaustion during four experimental trials (**E20**, euhydrated in 20°C; **D10**, dehydrated in 10°C; **E35**, euhydrated in 35°C; **D35**, dehydrated in 35°C)

a, b, c, d indicate a significant difference from **E20**, **D10**, **E35** and **D35**, respectively ( $p < 0.05$ )

#### 7.4.2 Hydration Status

Hydration status was similar prior to the commencement of **E20** and **E35**, as well as before Lasix<sup>®</sup> administration in **D35** and **D10**, as indicated by a similar body mass and urine specific gravity measurements (Table 7.2;  $p>0.05$ ). Two and half hours after administering Lasix<sup>®</sup>, subjects in the **D10** and **D35** experienced a body mass deficit of  $3.04 \pm 0.39 \%$  and  $3.13 \pm 0.27 \%$ , respectively. The differences in hydration status and ambient temperature resulted in a greater magnitude of percentage change in body mass during the post exercise period in **E35** compared with the other trials ( $p<0.01$ ). Sweat rate was significantly higher in **E35** and **D35** compared with **E20** and **D10** (Table 7.2;  $p<0.001$ ), which was associated with a greater fluid intake in the hot conditions.

**Table 7.2** Hydration status determined by percentage change of body mass, urine specific gravity, sweat rate, and fluid intake during PETS

Variable and Time	Trials			
	E20	D10	E35	D35
<b>Body Mass (kg)</b>				
Baseline		74.31 ± 6.61		74.10 ± 7.12
0 min	73.90 ± 6.81	72.08 ± 6.41	73.89 ± 7.17	71.80 ± 6.79
Final	72.63 ± 6.67	71.22 ± 6.39	72.24 ± 7.01	71.04 ± 7.11
<b>% Δ Body Mass</b>				
Baseline – 0 min		3.04 ± 0.39		3.13 ± 0.27
Baseline – 30 min	0.57 ± 0.16	3.33 ± 0.47	0.87 ± 0.22	4.06 ± 0.36
Baseline – 60 min	1.21 ± 0.22	3.95 ± 0.47	1.36 ± 0.35	4.04 ± 0.62
Baseline – Final	1.73 ± 0.35	4.14 ± 0.59	2.22 ± 0.52	4.49 ± 0.74
0 min – Final	1.73 ± 0.35	1.10 ± 0.47	2.22 ± 0.52 <sup>b,d</sup>	1.35 ± 0.65
<b>Urine Specific Gravity</b>				
Baseline		1.006 ± 0.004		1.006 ± 0.006
0 min	1.009 ± 0.004	1.006 ± 0.001	1.006 ± 0.006	1.006 ± 0.001
Final	1.009 ± 0.007	1.008 ± 0.002	1.008 ± 0.005	1.007 ± 0.003
<b>Sweat Rate (L.min<sup>-1</sup>)</b>				
0 min – 30 min	0.34 ± 0.15	0.21 ± 0.07	0.69 ± 0.13	0.73 ± 0.13
0 min - 60 min	0.76 ± 0.33	0.65 ± 0.18	1.04 ± 0.23	0.70 ± 0.36
0 min - Final	1.34 ± 0.21	0.98 ± 0.29	2.08 ± 0.27 <sup>a,b</sup>	2.08 ± 0.47 <sup>a,b</sup>
<b>Fluid Intake (mL)</b>				
0 min– 30 min	73 ± 51	114 ± 82	147 ± 77	177 ± 77
0 min – 60 min	79 ± 69	118 ± 78	150 ± 77	101 ± 69
0 min – Final	153 ± 106	232 ± 153	297 ± 149 <sup>a</sup>	244 ± 81

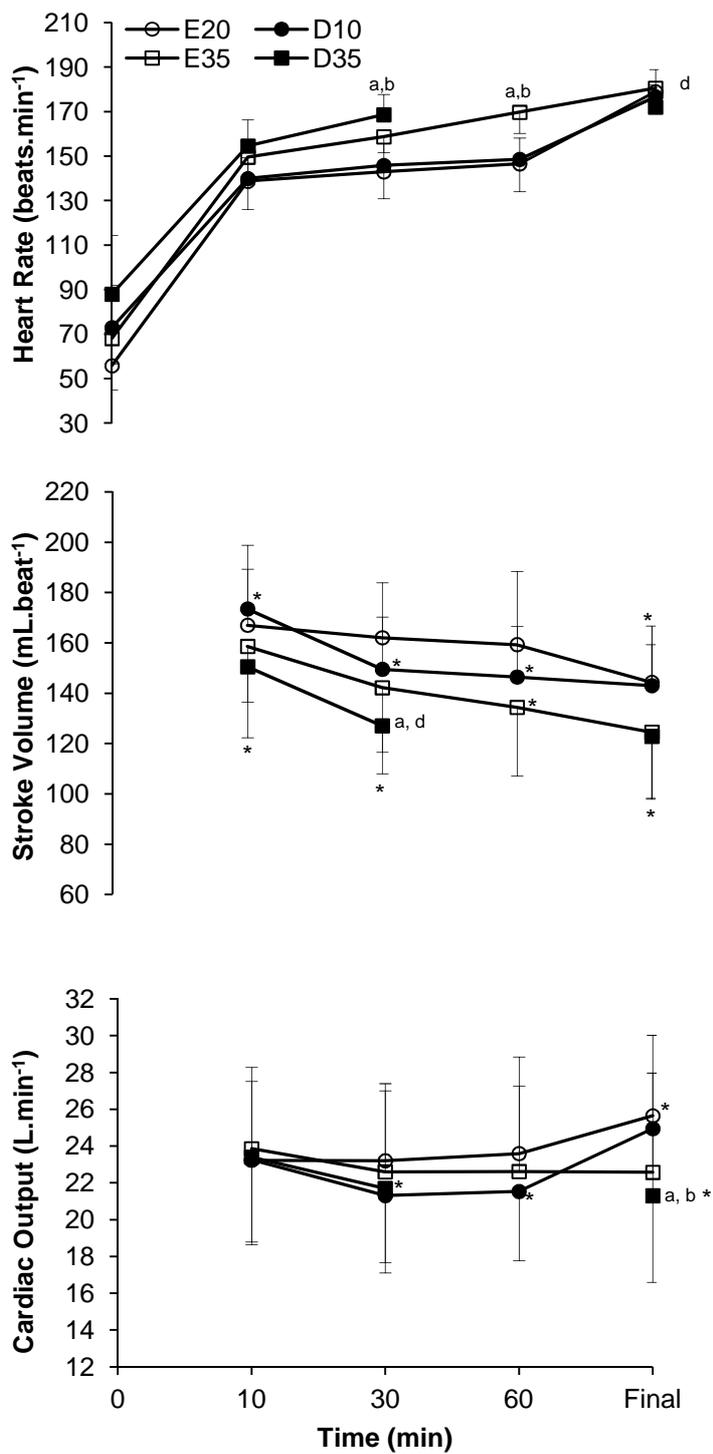
Baseline: Prior to the diuretics administration

<sup>a, b, c, d</sup> Significantly different from **E20, D10, E35** and **D35** respectively (p<0.05)

### 7.4.3 Cardiorespiratory Responses

After 25-min running, HR was significantly higher ( $p < 0.05$ ) in the hot conditions (**E35**:  $157 \pm 9$  beats.min<sup>-1</sup>; **D35**:  $166 \pm 9$  beats.min<sup>-1</sup>) compared with **E20**:  $142 \pm 12$  beats.min<sup>-1</sup> and **D10**:  $144 \pm 10$  beats.min<sup>-1</sup> (Figure 7.3). Concurrently, SV was similar  $162 \pm 22$ ,  $150 \pm 21$ ,  $142 \pm 26$  mL.beat<sup>-1</sup> in **E20**, **D10** and **E35**, respectively, except for a significant reduction in **D35** ( $127 \pm 19$  mL.beat<sup>-1</sup>) compared with **E20** ( $p = 0.023$ ).  $\dot{Q}$  (**E20**:  $23.2 \pm 4.2$ , **D10**:  $21.3 \pm 4.2$ , **E35**:  $22.6 \pm 4.4$ , **D35**:  $21.7 \pm 4.0$  L.min<sup>-1</sup>) and MAP ( $\sim 101 \pm 11$  mmHg) during steady state exercise in all conditions were similar (Figure 7.4), except for a significant decrease of MAP in **E35** trial compared with **D10** trial ( $p = 0.22$ ). Subjects were fatigued at  $96 \pm 3$ ,  $95 \pm 5$ ,  $97 \pm 3$ ,  $93 \pm 5\%$  of HR<sub>max</sub> in **E20**, **D10**, **E35** and **D35** respectively (Figure 7.3). HR<sub>max</sub> was significantly higher ( $\sim 4\%$ ) in **E35**:  $180 \pm 10$  beats/min compared with **D35**:  $172 \pm 10$  beats.min<sup>-1</sup>. SV at exhaustion was slightly lower in **E35** and **D35** compared with **E20** and **D10** but no statistical difference was found between trials (Figure 7.3;  $p > 0.05$ ). Concurrently,  $\dot{Q}$  at exhaustion was significantly reduced in **D35** compared with **E20** and **D10** (Figure 6.3;  $p = 0.006$  and  $p = 0.003$ , respectively). MAP was significantly lower in **E35** and **D35** than **E20** (Figure 7.4;  $p < 0.05$ ).

Mean oxygen uptake ( $\dot{V}O_2$ ) was similar ( $\sim 66.1 \pm 3.4\% \dot{V}O_{2max}$ ) between all conditions during the initial 60 min of level running (Figure 7.5). However, as exercise progressed to the final incremental stage,  $\dot{V}O_2$  reached  $75.9 \pm 10.4\% \dot{V}O_{2max}$  and  $69.5 \pm 1.3\% \dot{V}O_{2max}$  at the point of exhaustion in the **E35** and **D35** trials respectively, and was significantly lower than  $91.0 \pm 8.5\% \dot{V}O_{2max}$  and  $89.9 \pm 8.5\% \dot{V}O_{2max}$  in the **E20** and **D10** trials respectively ( $p < 0.01$ ).

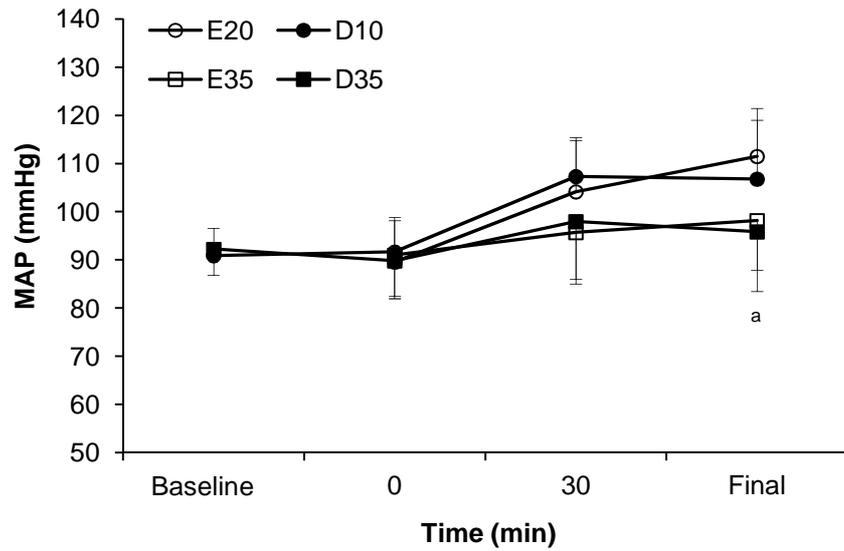


**Figure 7.3** Heart rate, stroke volume and cardiac output responses during PETs

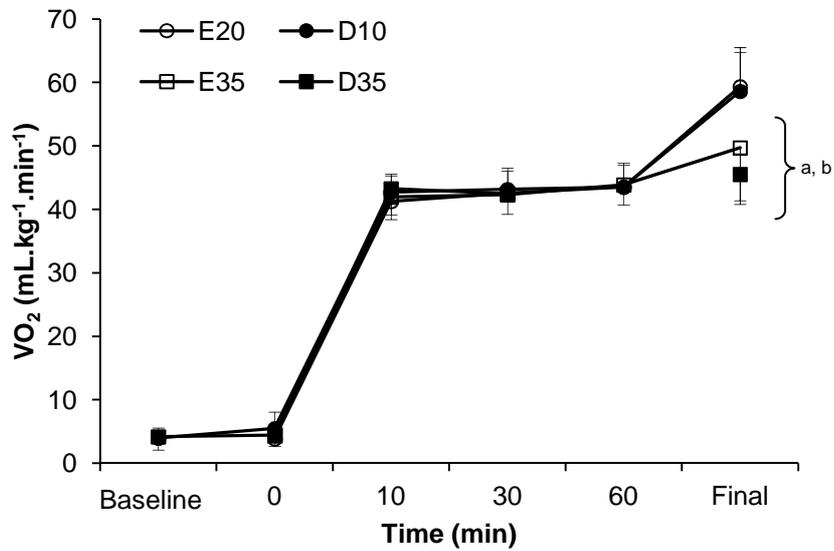
a, b, d Significantly different from **E20**, **D10** and **D35** respectively ( $p < 0.05$ )

\* Significantly different from 10 min ( $p < 0.05$ )

Note: Only one subject managed to complete the 60-min run in **D35** trial. Therefore, the time point at 60-min was excluded from the graph.



**Figure 7.4** Mean arterial pressure (MAP) prior to the diuretic administration (baseline), at 0, 30 and the final point of exhaustion during PETs  
<sup>a</sup> Significantly different from **E20**( $p < 0.05$ )



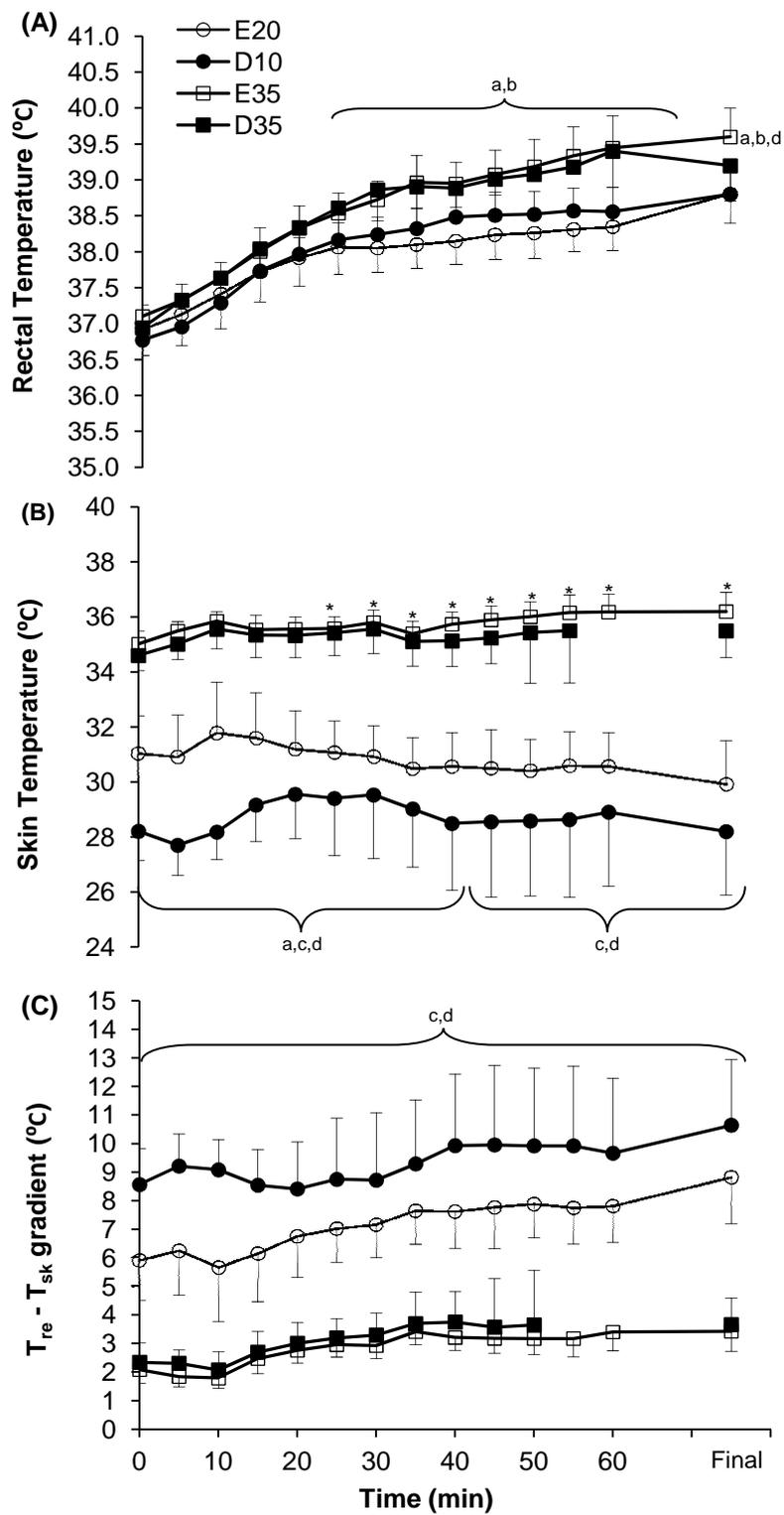
**Figure 7.5** Mean oxygen uptake ( $\dot{V}O_2$ ) prior to the diuretic administration (baseline), at 0, 10, 30, 60-min and the final point of exhaustion during PETs.  
<sup>a, b</sup> Significantly lower than **E20** and **D10** trials ( $p < 0.01$ )

#### 7.4.4 Thermoregulatory Responses

The initial rectal temperature ( $T_{re}$ ) in all trials was similar  $\sim 36.9 \pm 0.3^\circ\text{C}$  (Figure 7.6A) and from 5 min onwards,  $T_{re}$  increased significantly in all trials ( $p < 0.001$ ). At 25-min of running,  $T_{re}$  was  $\sim 0.5^\circ\text{C}$  lower in **E20** and **D10** compared with **E35** and **D35** ( $p < 0.05$ ). Subjects stopped exercise with  $T_{re}$ :  $38.7 \pm 0.4^\circ\text{C}$ ,  $38.8 \pm 0.4^\circ\text{C}$ ,  $39.6 \pm 0.4^\circ\text{C}$  and  $39.2 \pm 0.5^\circ\text{C}$  in **E20**, **D10**, **E35** and **D35**, respectively.  $T_{re}$  in **E35** was significantly greater compared to other trials ( $p = 0.001$ ). No significant difference was found between **D35** ( $39.2 \pm 0.5^\circ\text{C}$ ) and **D10** ( $38.8 \pm 0.4^\circ\text{C}$ ) at fatigue.

Resting mean skin temperatures ( $\bar{T}_{sk}$ ) was significantly lower in **D10** trial compared with other trials (Figure 6.6B;  $p < 0.001$ ). At 25-min running,  $\bar{T}_{sk}$  was maintained at  $31.1 \pm 1.2^\circ\text{C}$ ,  $29.4 \pm 2.1^\circ\text{C}$  and  $35.4 \pm 0.8^\circ\text{C}$  during **E20**, **D10** and **D35**, respectively, but not in **E35** ( $35.6 \pm 0.4^\circ\text{C}$ ), which was significantly elevated compared with resting  $\bar{T}_{sk}$  ( $p < 0.05$ ). Mean  $T_{sk}$  remained  $\sim 6^\circ\text{C}$  higher in **E35** and **D35**, compared with **E20** and **D10** trials throughout the PETs ( $p < 0.05$ ). No difference in  $\bar{T}_{sk}$  was found between **E20** and **D10** after 45 min and when nearing the cessation of exercise.

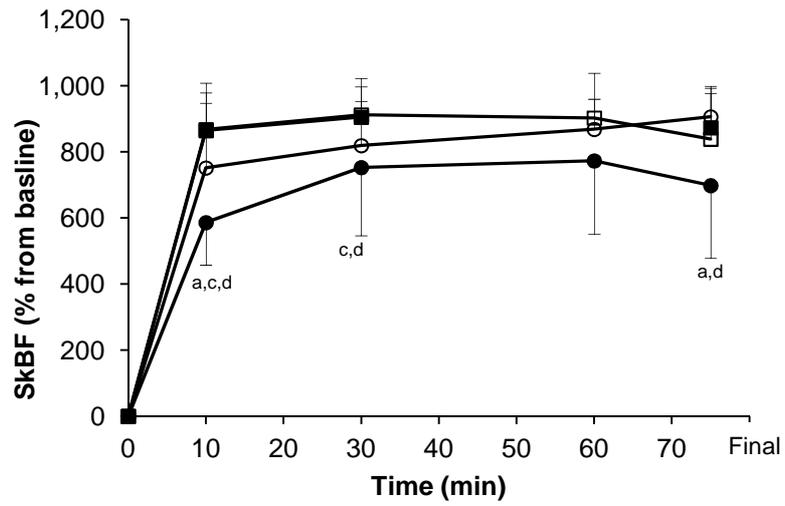
The  $T_{re}$ - $T_{sk}$  gradient was significantly greater in **E20** and **D10** from the start of exercise towards the point of exhaustion compared with **E35** and **D35** (Figure 7.6C;  $p < 0.05$ ). SkBF was significantly lower in **D10** compared with other trials from resting towards the exhaustion point (Figure 7.7;  $p < 0.05$ ).



**Figure 7.6** Rectal temperature (A), mean skin temperature (B) and core-to-skin temperature ( $T_{re} - T_{sk}$ ) gradient (C) during PETs

a, b, c, d Significantly different from E20, D10, E35 and D35, respectively ( $p < 0.05$ )

\* Significantly different from resting value ( $p < 0.05$ )

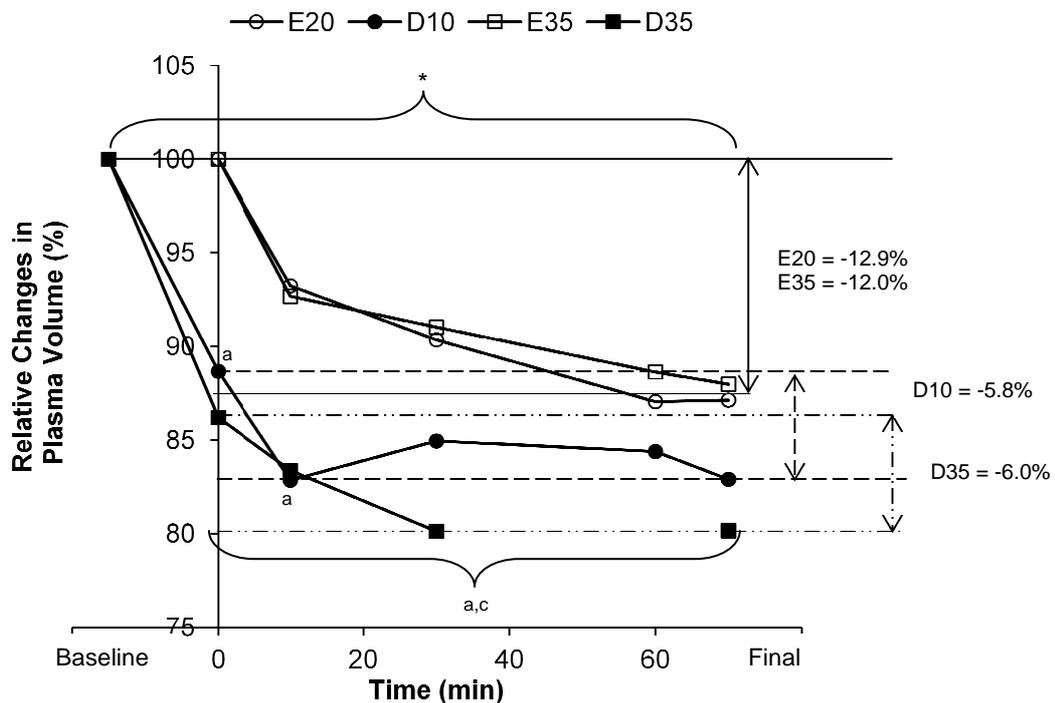


**Figure 7.7** Skin blood flow (SkBF) at 0, 10, 30, 60-min and the final point of exhaustion during PETs

<sup>a, c, d</sup> Significantly different from **E20**, **E35** and **D35** respectively ( $p < 0.05$ )

### 7.4.5 Haematological Responses

Plasma volume was significantly lower in the dehydration trials (**D10**:  $-11.3 \pm 6.7\%$  and **D35**:  $-13.8 \pm 9.8\%$ ) than in the euhydration trials (**E20** and **E35**) before the commencement of exercise due to the diuresis effect following administration of furosemide (Lasix<sup>®</sup>) (Figure 7.8;  $p < 0.001$ ). Plasma volume was further reduced over time in the **E20**:  $-12.9 \pm 6.4\%$ , **D10**:  $-17.1 \pm 3.4\%$ , **E35**:  $-12.0 \pm 5.0\%$  and **D35**:  $-19.8 \pm 6.6\%$  ( $p < 0.05$ ). Plasma volume in **D10** was lower than **E20** during the first 10 min of running; thereafter it remained similar until exhaustion. No significant difference in plasma volume was found between the two euhydration trials (**E20** vs. **E35**) as well as between the two dehydration trials (**D10** vs. **D35**). However, plasma volume in **D35** was significantly lower than **E20** and **E35** throughout the experimental trials ( $p < 0.05$ ).



**Figure 7.8** Plasma volume changes prior to the diuretic administration, at 0, 10, 30, 60-min and final point of exhaustion during PETs

<sup>a, c</sup> Significantly different from **E20** and **E35**, respectively ( $p < 0.05$ )

\* Significantly different from resting value ( $p < 0.05$ )

The baseline values of plasma viscosity in the dehydration trials (**D10** and **D35**) were similar to the euhydration trials (**E20** and **E35**) immediately before commencement of the trials. Plasma viscosity was significantly higher in the dehydration trials due to the diuretic effect of Lasix<sup>®</sup> compared with the euhydration trials during PETs (Table 7.3;  $p < 0.01$ ). Plasma viscosity significantly increased over time in all trials ( $p < 0.01$ ). Similarly, serum osmolality rose significantly from resting pre-exercise to the point of exhaustion in all trials (Table 7.3;  $p < 0.05$ ), however, the elevated serum osmolality measures were similar between trials.

No significant difference was observed in blood glucose or lactate levels at baseline in all condition trials (Table 7.3;  $p > 0.05$ ). At exhaustion, blood glucose and lactate levels were significantly increased ( $p < 0.05$ ) and no significant difference was found between trials ( $p > 0.05$ ) except that a greater blood glucose level was found in **D35** when compared with other trials ( $p < 0.05$ ).

**Table 7.3** Haematological Responses during PETS

Variable and Time	Trials			
	E20	D10	E35	D35
<b>Plasma Viscosity (mPa.s)</b>				
Baseline		1.34 ± 0.11		1.33 ± 0.09
0 min	1.29 ± 0.05 <sup>b,d</sup>	1.55 ± 0.13 <sup>*,a,c</sup>	1.29 ± 0.09 <sup>b,d</sup>	1.52 ± 0.13 <sup>*,a,c</sup>
Final	1.58 ± 0.13 <sup>#</sup>	1.89 ± 0.17 <sup>#,a</sup>	1.63 ± 0.07 <sup>#</sup>	1.93 ± 0.13 <sup>#,a</sup>
<b>Serum Osmolality (mosmol.kg<sup>-1</sup>)</b>				
Baseline		288 ± 6		287 ± 8
0 min	286 ± 9	294 ± 5 <sup>*</sup>	287 ± 7	296 ± 12
Final	299 ± 8 <sup>#</sup>	304 ± 13 <sup>#</sup>	300 ± 5 <sup>#</sup>	307 ± 13 <sup>#</sup>
<b>Glucose (mmol.L<sup>-1</sup>)</b>				
Baseline		5.64 ± 1.23		5.54 ± 0.84
0 min	5.47 ± 0.65	5.38 ± 0.94	5.39 ± 0.70	5.23 ± 0.53
Final	6.89 ± 1.33 <sup>#</sup>	7.10 ± 1.72 <sup>#</sup>	6.73 ± 1.36 <sup>#</sup>	7.99 ± 1.64 <sup>#,a,b,c</sup>
<b>Lactate (mmol.L<sup>-1</sup>)</b>				
Baseline		1.44 ± 0.14		1.56 ± 0.23
0 min	1.35 ± 0.43	1.49 ± 0.45	1.49 ± 0.43	1.99 ± 0.49 <sup>a</sup>
Final	5.66 ± 1.96 <sup>#</sup>	5.84 ± 2.50 <sup>#,c</sup>	3.95 ± 1.85 <sup>#</sup>	4.83 ± 2.06 <sup>#</sup>

Baseline: Prior to the diuretics administration

<sup>a, b, c, d</sup> Significantly different from E20, D10, E35 and D35 respectively (p<0.05)

<sup>\*</sup> Significantly different from baseline value (p<0.05)

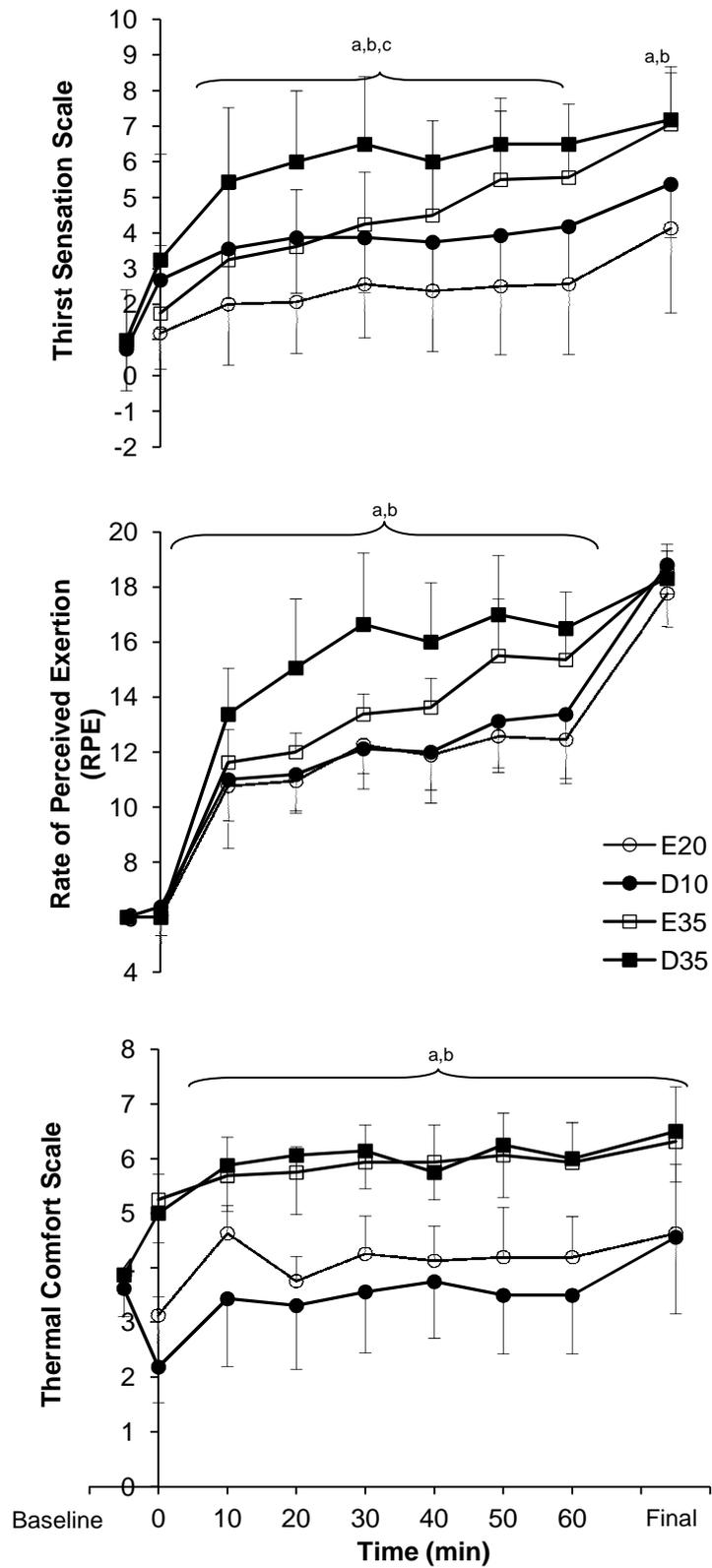
<sup>#</sup> Significantly different from 0-min value (p<0.05)

#### 7.4.6 Subjective Responses

The sensation of thirst was similar: **E20**:  $1.2 \pm 1.0$ , **D10**:  $2.7 \pm 1.4$ , **E35**:  $1.8 \pm 1.9$  and **D35**:  $3.3 \pm 3.0$  in all trials prior to the commencement of exercise. From 10 min onwards, the thirst sensation was greater in **D35** ( $6.0 \pm 2.0$ ) compared with the other trials (Figure 7.9;  $p < 0.05$ ). The sensation of thirst being greater in the hot (**E35** and **D35**) conditions compared with thermoneutral (**E20**) and cool (**D10**) conditions at the exhaustion point ( $p < 0.05$ ).

Ratings of perceived exertion (RPE) increased significantly across all conditions (Figure 7.9;  $p < 0.01$ ). From 10 min onwards, subjects reported their RPE to be significantly harder in **D35** trial compared to **E20** and **D10** trials (Figure 7.9;  $p < 0.05$ ). At exhaustion, RPE was similar across all conditions (**E20**:  $17.8 \pm 1.2$ , **D10**:  $18.8 \pm 1.1$ , **E35**:  $18.6 \pm 0.8$  and **D35**:  $18.3 \pm 1.2$ ).

Thermal comfort scores were similar during both hot conditions (**E35** and **D35**) throughout the trials (Figure 7.9;  $p > 0.05$ ). A significant difference was noted in **E20** and **D10**, which was lower than **E35** and **D35** ( $p < 0.01$ ) indicating that subjects perceived the conditions to be significantly cooler in both the cold and thermoneutral conditions.



**Figure 7.9** Rating of perceived exertion and thermal comfort scale prior to the diuretic administration (baseline), at 10 min intervals during PETs  
 a, b, c Significantly different from E20, D10 and E35 respectively ( $p < 0.05$ )

## 7.5 DISCUSSION

The main finding of this study is that dehydration had a marked effect on prolonged exercise in hot climatic conditions whereas there was a limited effect of dehydration on exercise performance in cool conditions. Our data exhibited a significant difference in performance time between **D35** and **E35** (Figure 7.2), which shows there was a negative effect of dehydration on endurance performance time in the heat. It is interesting to note that dehydration had a minimal effect on endurance performance in cool conditions despite subjects experiencing a ~4% BW deficit towards the end of exercise in the **D10** trial. A cool environment with the air temperature range from 8 to 15°C is possibly most suitable for running a marathon race (Zhang *et al.*, 1992). This was supported by both genuine competitive races and laboratory testing (Galloway & Maughan, 1997; Ely *et al.*, 2007) on running and cycling performance, respectively. Based on a nomogram developed by Ely *et al.* (2007) the running time for 120 min at 10°C resulted in a performance decrement of ~1.5% whereas when running for 120 min with a higher ambient temperature (25°C) the performance decrement was ~4.5%. Similarly, Galloway & Maughan (1997) found that subjects' cycling endurance time during a trial in 10.5°C was ~40 min longer than when cycling in an ambient temperature of 30.5°C.

Urine specific gravity (USG) measurements confirmed that subjects were euhydrated prior to the commencement of exercise in the euhydration trials and before the administration of the diuretics drug in the dehydration trials to attain ~3% BW prior to the experimental trials. Unexpectedly USG in all trials remained similar regardless of a 3-4% BW loss using diuretics as a method to induce dehydration. The diuretic drug was administered prior to the commencement of exercise while progressive dehydration occurred through sweat loss during exercise in the heat. USG measurement might not be accurate to determine acute dehydration. To our knowledge there are limited

studies which have examined the sensitivity of USG during acute dehydration induced by the diuretics method. It has been reported that USG in dehydration trials using diuretics is significantly lower than a control trial, but no explanation was made by the authors (Watson *et al.*, 2005). It remains unclear why the measures of USG are lacking in sensitivity.

In the present study, the reduction in % PV values (11-14%) associated with ~3% BW loss prior to the experimental trials is similar to that reported in the research literature (Caldwell *et al.*, 1984; Roy *et al.*, 2000). Plasma volume decreases were mainly due to a differential fluid loss across the fluid compartments of the body via urine formation during diuresis in the dehydration trials (**D10** and **D35**) and sweat excretion during prolonged exercise in all trials. In general, the reduction in plasma volume and elevation in plasma / serum osmolality are associated with the severity of dehydration incurred. However, we found that serum osmolality was similar across all trials (Table 7.3) regardless of a significant difference in plasma volume (Figure 7.8) combined with a greater percentage of body weight loss in the dehydration trials (Table 7.3). Our data shows serum osmolality remained unchanged between trials. We speculate that a significant difference in serum osmolality can only be detected if >4.5% BW loss is achieved. These results are supported by previous studies in which the dehydration level from 1.5 to 4.2% did not elevate serum osmolality despite a reduction in plasma volume in either hot or cold conditions (González Alonso *et al.*, 2000). A large increment in plasma osmolality was observed with no further reduction in plasma volume when a 5% BW loss was elicited (Sawka *et al.*, 1985). In the present study, a slight increase of serum osmolality in all trials would have potentially mobilized fluid from the intracellular and extracellular fluid space, so that plasma volume loss can be replenished or maintained throughout the exercise (Nose *et al.*, 1988b).

It is clear from our data that *ad libitum* fluid intake did not replace sweat loss, resulting in subjects becoming further dehydrated during the prolonged exercise tests in all trials (Table 7.3). However, this further reduction in body weight did not affect the change in plasma volume in **D10** after 10 min of running. This observation appears directly related to the cool environment, which resulted in a lower sweat rate (~53%), lower SkBF (~176%), together with a 45% greater time to exhaustion compared with **D35** (Figure 7.2). It has been shown that both reduced SkBF and local  $T_{sk}$  independently decrease sweating during whole body sweating (Wingo *et al.*, 2010). This may be a possible mechanism to explain why cooler environments have regularly been shown to be associated with better performance in marathons. Similarly endurance time to exhaustion in the present study was much improved in the cool climatic condition.

In the present study, we also found that dehydration trials (**D10** and **D35**) compared with the euhydration trials (**E20** and **E35**) resulted in a higher plasma viscosity (Table 7.3), reflecting a reduction of plasma volume (Tatsuya *et al.*, 2004). We propose that the increase in plasma viscosity causes a decline in venous flow velocity which might compromise venous return of blood to the central blood circulation. Maintenance of blood volume is crucial to optimize cardiovascular ( $\dot{Q}$ , MAP) and thermoregulatory systems (Fortney *et al.*, 1981; Nose *et al.*, 1988a), otherwise exercise performance in hot conditions will be adversely affected.

With increased heat stress, we found that the HR drifted approximately  $14 \text{ beats}\cdot\text{min}^{-1}$  from 10 to 45-min in **E35** and **D35** trials. We did compare the trials at 60-min because only one subject managed to complete the 60-min steady state run in **D35** trial. Therefore comparisons between trials were made at 45-min. We also found that the increased HR (approaching ~97 and ~93%  $\text{HR}_{\text{max}}$  in **E35** and **D35**, respectively)

reduced SV (**E35**: ~21%, **D35**: ~18% from 10-min), ultimately compromised  $\dot{Q}$  (**E35**: -7.8%, **D35**: -10.5% from 10-min onwards; to exhaustion (see Figure 7.3). MAP remained constant throughout the initial 60-min of constant velocity level running, while a slight increase was observed at the point of exhaustion (~7% from 0-min). However, this slight increment in MAP was not sufficient to compensate for a peripheral displacement of blood volume in the hot condition trials (**E35** and **D35**), resulting in a decline in  $\dot{Q}$ . Both hypovolemia and peripheral vascular dilation in the hot condition trials are consistent with the research and findings of Rowell *et al.* (1966). During **D10** trial, SV (~148 mL.beat<sup>-1</sup>), HR (~147 beats.min<sup>-1</sup>) and  $\dot{Q}$  (~21.4 L.min<sup>-1</sup>) were maintained and accompanied with an increase in MAP after 30 min of steady state exercise despite incurring a pronounced level of dehydration. This indicates that dehydration (3-4% body weight loss) has minimal to no effect on prolonged exercise performance in cool conditions.

During all PETs,  $\dot{V}O_2$  was essentially constant over the initial 60 min of running at a constant velocity and zero gradient even though the dehydration level and ambient temperature were varied between trials (Figure 7.5). Interestingly a 5% BW loss did not change submaximal  $\dot{V}O_2$  during exercise compared with a euhydrated state (Armstrong *et al.*, 2006). However, running in hot conditions either with or without dehydration at the point of exhaustion resulted in a similar reduction in  $\dot{V}O_{2max}$  (Figure 7.5) and a lower HR<sub>max</sub> compared with **E20** and **D10** trials (Figure 7.3). These results suggesting that the impairment of  $\dot{V}O_{2max}$  with heat stress alone or with the combination of dehydration is tightly coupled with the decline in SV (Wingo *et al.*, 2012) and a consequent 8-11% reduction in  $\dot{Q}$ . We suggest that a compromised  $\dot{Q}$  reduced oxygen delivery to the exercising muscles which caused the onset of fatigue during exercise in

the heat (Mortensen *et al.*, 2005).

It has been proposed that hypohydration-mediated increases in  $T_{re}$  during exercise in the heat results in a decline in heat loss from the body (Sawka *et al.*, 1985; Montain & Coyle, 1992; Buono & Wall, 2000). Roy and colleagues (2000) reported that hypohydration (~3.8% BW loss) exaggerated thermal and cardiovascular strain by increasing  $T_{re}$  and HR during 60 min of submaximal cycling exercise in thermoneutral conditions (22-24°C; 35-45% rh). The authors proposed that heat storage in their hypohydration trial was greater than their euhydration trial, due to a 14.6% decrease in plasma volume coupled with an increase in  $T_{re}$  ( $1.6 \pm 0.1^\circ\text{C}$ ). González-Alonso *et al.* (1995) have also reported a significant increase in  $T_{re}$  with 4.9% BW loss during prolonged cycling for 120 min at 62%  $\dot{V}O_{2\text{max}}$  in a temperate environment (20°C). However, our study did not show similar results when compared with previous findings.  $T_{re}$  was identical in both hot condition trials (**E35** vs. **D35**) regardless of a 2-3% BW difference throughout the 60 min of PETs (Figure 7.6). At the point of exhaustion,  $T_{re}$  in **E35** was significantly higher than in **D35** due to an increased running duration of 25 min. The average  $T_{re}$  was equally high in both hot condition trials (~39.1°C) compared with thermoneutral and cool condition trials (~38.4°C). This may possibly be due to our subjects' training status, as athletes are adapted to heat transfer from the exercising muscles and body core through increased skin blood flow and via evaporative sweat loss. Surprisingly we did not observe any significant variations in sweat rate during the dehydrated state and euhydrated state trials (**D35**:  $2.08 \pm 0.47 \text{ L}\cdot\text{min}^{-1}$  vs. **E35**:  $2.08 \pm 0.27 \text{ L}\cdot\text{min}^{-1}$ ) (Table 7.2). Armstrong *et al.* (1997) investigated the effect of hypohydration, dehydration and water intake on thermal and circulatory responses during treadmill walking for 90 min in 33°C and similarly observed that subjects were

dehydrated (3% of their body weight) towards the end of exercise,  $T_{re}$ , plasma osmolality and sweat sensitivity were similar compared with a control trial (euhydrated + water *ad libitum*) (Armstrong *et al.*, 1997). However, when >5% BW loss was attained, the increase of plasma osmolality depressed the sweat sensitivity associated with an increase of  $T_{re}$ .

Nielsen *et al.* (1993) proposed that an elevated body temperature provides peripheral feedback from thermal-sensitive sites to the central nervous system (CNS), resulting in reduced central neural drive to the exercising muscles in hot, dry environments. The authors reported that each cyclist in their study discontinued the daily cycle bout because they had reached their own intrinsic critical core temperature. The results of the current study do not support the view that prolonged exercise performance in the heat is compromised by hypohydration and an associated high  $T_{core}$  (Nybo *et al.*, 2001).

If  $T_{re}$  is not the primary factor affecting prolonged exercise performance in the heat, what are the underlying mechanisms impairing exercise performance? Our data clearly demonstrates that “cool skin” in **D10** is advantageous during prolonged exercise irrespective of a mild to moderate level of hypohydration (3 to 4.5% BW loss). Our data shows every 10°C of ambient temperature increment was in proportion with a ~ 2°C increase in  $\bar{T}_{sk}$ .  $\bar{T}_{sk}$  averaged 28.8°C throughout the 60 min **D10** (Figure 7.6). The decrement in time to fatigue in **D10** was small (-2.1%) compared with **E20** and no significant difference in performance time was found between trials (Figure 7.2). Our cool condition findings are similar to those of Kenefick *et al.* (2010), who undertook the hypohydration trial in 10°C with a different exercise mode (running *vs.* cycling), duration (60 min *vs.* 30 min) and intensity (65%  $\dot{V}O_{2max}$  *vs.* 50%  $\dot{V}O_{2max}$ ) compared

with our study. They reported that the performance time trial in both hypohydration and euhydration trials at 10°C were similar.

A vast range of protocols such as active or passive heat exposure, food restriction (reduced salt) various forms of exercise with overnight fluid restriction and sauna have been used in studies to induce BW loss prior to the commencement of experimental trials (Caldwell *et al.*, 1984; Armstrong *et al.*, 1997; Roy *et al.*, 2000; Ikegawa *et al.*, 2011). The present study employed a diuretic method to achieve 3% BW loss and reduce the effect of potential confounders associated with other methods, such as elevated body temperature, muscle fatigue, energy deficits and time of day for conducting experiments. Our data demonstrated that  $T_{re}$ , HR and  $\dot{V}O_2$  prior to the commencement of dehydration trials did not vary from euhydration trials, which indicates that the diuretic-induced dehydration protocol was successful in eliminating the main confounding factors.

We are not aware of any study that has investigated the effect of diuretic-induced dehydration during prolonged running performance in cool conditions (10°C). Most studies have used a cycle ergometer or treadmill walking as the mode of exercise for investigating the effect of dehydration on prolonged exercise performance. Chevront *et al.* (2005) have shown that there is no effect of moderate hypohydration (~3% BW loss) and no independent effect of ambient temperature (2°C vs. 20°C) on cycling performance (Chevront *et al.*, 2005). These results were consistent with a similar hypohydration study using a walking protocol at 50%  $\dot{V}O_{2max}$  for 60 min in either a 4°C or a 25°C environment (Kenefick *et al.*, 2004). In our study we found that hypohydration has no effect on thermoregulation during exercise in cool conditions

(D10) compared with thermoneutral conditions (E20). The cool environment limited an increase in  $T_{re}$  despite subjects being dehydrated. The  $T_{re}$  in D10 was approximately 0.4°C lower than D35 at 45-min of steady state run, as well as at the point of exhaustion. Given that no change was found in  $T_{re}$  between D10 and E20 trials and the hot condition trials (E35 and D35) throughout the PETs (Figure 7.6), it is concluded that hypohydration (<4.5% of BW loss) does not mediate the increase in thermoregulatory strain especially in cool conditions.

## Conclusion

In summary, our study shows that dehydration had a marked effect on prolonged exercise performance in the heat whereas there was a limited effect of dehydration on exercise performance in cool conditions. The results of the current study do not support the “critical core temperature hypothesis” as the subjects stopped exercising at different level of  $T_{re}$ . Our results provide evidence against the existence of a threshold  $T_{re}$  of ~40°C being associated with fatigue. In turn, our results show that the significant reductions in plasma volume and stroke volume, associated with an increase in HR, ultimately compromised the  $\dot{Q}$  and  $\dot{V} O_{2max}$  during prolonged exercise in hot and dehydrated conditions. Meanwhile, the increase in circulatory strain was further exacerbated by the rise in  $SkBF$  and plasma viscosity which might contribute to a reduction in central blood volume. It was shown that further dehydration (from 3 to 4.5% of BW loss) and the maintenance of plasma volume (~17% reduction in plasma volume) throughout exercise in cool conditions did not adversely affected prolonged exercise performance. It appears that ambient temperature and “cool skin” play an important role in assisting the convective heat transfer from the body to the environment, evidenced by a widening of the  $T_{re}-\bar{T}_{sk}$  gradient during exercise in cool conditions.

The findings of this study have important practical implications for the efficacy of fluid ingestion by competitive athletes competing across a range of climatic conditions. A ~3% BW loss impairs prolonged exercise in hot conditions but not in cool conditions. Therefore coaches and athletes should take note of the climatic conditions during competitions when preparing a hydration regimen to avoid “overdrinking” or dehydration.

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## **CHAPTER 8**

### **STUDY FOUR: EFFECT OF ALANYL-GLUTAMINE INGESTION ON PROLONGED RUNNING PERFORMANCE IN HOT AND HYPOHYDRATED CONDITIONS**

## **8.0 EFFECT OF ALANYL-GLUTAMINE INGESTION ON PROLONGED RUNNING PERFORMANCE IN HOT AND HYPOHYDRATED CONDITIONS**

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## 8.1 ABSTRACT

This study addressed the question of whether enhanced HSP expression induced via glutamine supplementation is beneficial in offsetting the deleterious effect of hypohydration on exercise performance. The study further investigates whether alanyl glutamine administration offsets the reported prolonged exercise-induced decrease in plasma glutamine concentration. Seven well trained male endurance runners (age:  $32.4 \pm 6.3$  yr; height:  $177.3 \pm 4.8$  cm; weight:  $70.5 \pm 8.2$  kg;  $8.4 \pm 2.3\%$  body fat; maximal oxygen uptake,  $\dot{V}O_{2\max}$ :  $63.8 \pm 5.5$  mL.  $\text{kg}^{-1}\cdot\text{min}^{-1}$ ; training volume:  $84.9 \pm 20.5$  km. $\text{week}^{-1}$ ) participated in this study. They undertook three  $1^+$  hr at  $65\% \dot{V}O_{2\max}$  treadmill running trials spaced over 2-3 weeks with the first trial (**CON**) while in a euhydrated state in hot conditions ( $35^\circ\text{C}$ , 40% rh); followed by either the **GLUT** trial when dehydrated in hot conditions ( $35^\circ\text{C}$ , 40% rh); or the **PCB** trial when dehydrated in hot conditions ( $35^\circ\text{C}$ , 40% rh). The order of undertaking the **GLUT** and **PCB** trials was randomised. In the **GLUT** trial glutamine ( $0.2 \text{ g}\cdot\text{kg}^{-1}$  body mass per liter) was ingested with water while in the placebo trial (**PCB**) sugars were ingested (2g of dextrose with maltodextrin) with water, which was equivalent to 70% of the volume of sweat loss during the **CON** trial. Time to exhaustion during **GLUT** was similar to **CON** ( $p=0.112$ ) but a significant reduction was found during **PCB** trial ( $p=0.044$ ). Similarly, there were no significant differences between **CON** and **GLUT** in plasma [glutamine] but a lower plasma [glutamine] was found in **PCB** when compared with **GLUT** ( $p=0.003$ ). Supplement drinks with glutamine significantly enhanced HSP 72 expression in **GLUT** when compared with **PCB** ( $72.2 \pm 14.0\%$  vs.  $67.2 \pm 8.4\%$ ;  $p=0.046$ ). The present study demonstrates alanyl-glutamine ingestion confers protection and enhances plasma HSP 72 expression. Furthermore, ingestion of alanyl-glutamine was associated with an increased time to exhaustion during hot and hypohydrated conditions.

## 8.2 INTRODUCTION

During prolonged exercise in hot climatic conditions, metabolic heat production is dissipated to the surrounding environment via increased skin blood flow and sweat evaporation to achieve heat balance. However, when metabolic heat production exceeds the capacity for heat dissipation body core temperature increases, which further leads to thermal and circulatory strain (Sonna *et al.*, 2007). Sweating during exercise elicits an increase in body fluid losses, which may exacerbate a pre-existing body fluid deficit whereby the athlete is dehydrated (Sawka & Noakes, 2007). Dehydration associated with a reduction in central blood volume (Rowell, 1974) indirectly impairs heat transfer and is recognised as one of the contributing risk factors for heat illness (Epstein & Roberts, 2011).

The early reported observation of Robinson (1963) showed that highly trained and well-monitored athletes attained a rectal temperature ( $T_{re}$ ) over 40°C at the conclusion of 3 and 6 mile track races equivalent metric distances (5 and 10 km). Similarly Pugh *et al.* (1967) reported that the winner of the marathon race completed the race with a rectal temperature greater than 40°C and bodyweight deficit of 6.7% as a result of sweating. The research undertaken by Davies and Thompson (1979) shows that highly trained and well-motivated distance runners race at an intensity greater than 96%  $\dot{V}O_{2max}$  over 5 to 10 km, while marathon runners sustain an average race pace equivalent to 85%  $\dot{V}O_{2max}$ . At these exercise intensities there is a significant metabolic heat load which is associated with considerable storage of heat, as reflected in the studies of Robinson (1966) and Pugh *et al.* (1967).

The intensity of exercise for elite distance runners also limits their capacity to ingest and absorb fluid at a rate that will offset sweat loss. With sweat rates during marathon races exceeding 2.0-3.0 L.hr<sup>-1</sup> (Armstrong, 1986) it is not uncommon for these athletes to experience significant levels of dehydration over the course of the race. A recent field study (Zouhal *et al.*, 2010) found that the fastest runners (less than 3 hours finishers) completing a marathon race were the most dehydrated when compared with slower runners with a > 3 hours finishing time. Moreover, a 7 days dietary monitoring study on elite Kenyan runners who were preparing for the Kenyan Olympic selection trials demonstrated that these runners had a low fluid intake during this period of intense training (Fudge *et al.*, 2006). It thus appears that the more elite distance runners do not practise the advice of the American College of Sports Medicine (ACSM) on Exercise and Fluid Replacement (Sawka *et al.*, 2007). The elite distances runners endure a significant level of dehydration and success in marathon races irrespective of ACSM guidelines raises the question of whether they are able to adapt to dehydration and the associated high metabolic heat loads, or whether they are able to acquire a tolerance to dehydration. Heat acclimatisation provides a range of adaptation which serves to reduce the metabolic heat load inducing earlier onset and more profuse sweating which paradoxically may lead to greater body fluid losses. At a cellular level, hot climatic conditions trigger a range of responses, including the “heat shock response”, which enables the stressed cells to restore homeostasis by synthesizing a small set of proteins called heat shock proteins (HSPs) (Parsell & Lindquist, 1993). The expression of HSPs which located in various cellular compartments plays an important role in the development of thermotolerance and protection from cellular damage (Moseley, 1997; Kregel, 2002; Wischmeyer, 2006; Ruell *et al.*, 2007; Horowitz & Robinson, 2007).

Glutamine is an amino acid essential for homeostatic functions in the human body. Prolonged exhaustive exercise is associated with a decrease in plasma glutamine level possibly due to impaired immune function (Walsh *et al.*, 1998; Newsholme *et al.*, 2011). Glutamine supplementation has been shown to be beneficial in providing a prophylactic effect and thus reducing the occurrence of upper respiratory tract infection in athletes following intense training sessions (Castell & Newsholme, 1997). Furthermore, research studies have reported that glutamine ingestion enhances lymphocyte proliferation (Parry-Billings, 1990), improved blood acid-base balance with an increase in plasma bicarbonate concentration (Welbourne, 1995) and enhanced sport performance with reduced perception of fatigue during exercise (Favano *et al.*, 2008). Lima *et al.* (2002) also demonstrated that an alanyl-glutamine-based oral rehydration fluid increased water and electrolyte intestinal absorption in a rat model of secretory diarrhea induced by cholera toxin. This result is supported by a recent study by Hoffman *et al.* (2010) which showed that an oral rehydration beverage containing L-alanyl-L-glutamine (0.05 and 0.2 g.kg<sup>-1</sup> body mass per liter) provided an ergogenic effect by increasing fluid and electrolyte (Na<sup>+</sup>) uptake in ten physically active males. Based on the aforementioned studies, glutamine supplementation is likely to be an ergogenic aid, increasing performance time to exhaustion during mild dehydration.

It has been found that glutamine can enhance stress-induced HSP expression *in vitro* and *in vivo* and improve cell survival against a variety of stimuli in humans as well as animals (Wischmeyer *et al.*, 2003; Ziegler *et al.*, 2005; Singleton & Wischmeyer, 2006). Singleton and Wischmeyer (2006) demonstrated that oral glutamine administration in rats significantly enhanced the expression of HSP and improved survival following hyperthermia. Further, a clinical trial on critically ill patients has shown that parenteral alanyl-glutamine dipeptide administration 0.5 g/kg per day for 7 days upregulates serum

HSP 70 concentrations and reduces the patients' length of stay in an Intensive Care Unit (ICU) and days on a ventilator (Ziegler *et al.*, 2005). It is worth noting that the patients in Ziegler *et al.* (2005) were in a hypohydrated state which has relevance to our investigation. We therefore speculate that enhanced HSP expression induced via glutamine supplementation is beneficial in offsetting the deleterious effect of hypohydrated on exercise performance.

To date, the underlying mechanism explaining the effects of glutamine ingestion on induction of heat shock protein expression during prolonged exercise in a hypohydrated state have not been clearly elucidated. We hypothesized that (1) the administration of alanyl-glutamine would attenuate exercise-induced decrease in plasma glutamine concentration; and (2) alanyl-glutamine ingestion would induce plasma HSP 72 expression during prolonged exercise in hot conditions and in a hypohydrated state. Therefore, the purpose of this study was (1) to determine whether alanyl glutamine administration attenuates exercise-induced decrease in plasma glutamine concentration, and (2) to investigate the relationship between alanyl-glutamine ingestion and the induction of plasma HSP 72 expression during prolonged exercise in hot conditions and in a hypohydrated state.

## **8.3 METHODS**

### **8.3.1 Subjects**

Seven competitive male endurance runners (age:  $32.4 \pm 6.3$  yr; height:  $177.3 \pm 4.8$  cm; weight:  $70.5 \pm 8.2$  kg;  $8.4 \pm 2.3\%$  body fat; maximal oxygen uptake,  $\dot{V}O_{2\max}$ :  $63.8 \pm 5.5$  mL. kg<sup>-1</sup>.min<sup>-1</sup>; training volume:  $84.9 \pm 20.5$  km.week<sup>-1</sup>; 10 km performance time:  $35.5 \pm 2.7$  min; 21.1 km performance time:  $78.0 \pm 5.6$  min) participated in this study. Before completing a written informed consent document, subjects were fully informed of the experimental procedures and risks and benefits associated with participation in the study. They completed the Physical Activity Readiness Questionnaire (PAR-Q) and a Medical History & Health Screening Questionnaire. Subjects were instructed to cease consuming nutritional supplements for at least 4 weeks prior to the study. This study conformed with the Declaration of Helsinki guidelines and was approved by the Human Research Ethics Committee of The University of Sydney (Ref No.13880).

### **8.3.2 Anthropometric Measurements**

Anthropometric data was collected during the preliminary testing session. Height and body weight with running shorts and socks only were determined using a height stadiometer (Harpندن Stadiometer, Holtain Limited, UK) and an electronic weighing scale (Mettler Toledo ID1 Multi Range Scale, Columbus, OH, USA), respectively. Seven sites of skinfolds thickness: triceps, chest, midaxillary, subscapular, suprailiac, abdomen, and anterior thigh were measured twice to the nearest 0.5 mm using a skinfold calliper (Holtain, Crymych, UK) to determine body density (Jackson & Pollock, 1978). The relative percentage of body fat was estimated using Siri's equation (Siri & Lukaski, 1993).

### 8.3.3 Preliminary Testing

A familiarisation trial was undertaken prior to the submaximal exercise test. A submaximal exercise test was undertaken on a motor driven treadmill (Payne Engineering, Lidcombe, Australia) with the treadmill speed ranging from 10, 12, 14, 16 km.h<sup>-1</sup> (4 min at each speed in a continuous incremental protocol) in thermoneutral conditions [20°C, 40% relative humidity (rh)]. Subjects then completed an incremental gradient run (12 km.hr<sup>-1</sup> with the gradient increased by 2% every 2 min) to volitional fatigue to determine maximal oxygen uptake ( $\dot{V}O_{2max}$ ) and maximal heart rate ( $HR_{max}$ ). The linear regression equation correlating treadmill velocity with the corresponding oxygen uptake and heart rate was extrapolated to measured  $HR_{max}$  to verify the  $\dot{V}O_{2max}$  and to determine the treadmill velocity corresponding to 65%  $\dot{V}O_{2max}$  for each subject's subsequent series of prolonged experimental runs.

### 8.3.4 Experimental Design

This study was a double blind, randomised and crossover experimental design. To examine the effect of glutamine on prolonged exercise in hot and hypohydrated conditions, subjects performed three experimental trials: (1) **CON** (Control): euhydrated in hot conditions (35°C, 40% rh); (2) **GLUT** (Glutamine): dehydrated in hot conditions (35°C, 40% rh); (3) **PCB** (Placebo): dehydrated in hot conditions (35°C, 40% rh). **CON** trial was undertaken to determine sweat rate during exercise and fluid regimen for the next set of tests. **GLUT** and **PCB** trials were undertaken in randomised order, separated by at least 7 days between each trial.

#### **8.3.4.1 Experimental Protocol**

Subjects were instructed to drink a prescribed volume of approximately  $6 \text{ mL} \cdot \text{kg BW}^{-1}$  (every 2-3 hours, 4-5 sessions per day) of water on the day before and during the day of testing to maintain euhydration prior to all experimental trials. Subjects were asked to refrain from strenuous physical activity, nutritional supplements, alcohol and caffeinated drinks within 24 hours before arrival at the laboratory for the experimental trials. Subjects were also required to maintain a similar record of food and fluid intake by recording this in a food diary for 24 hours before arrival at the laboratory for the experimental trials. Subjects were then required to follow the same diet before each of the following experimental trials to ensure minimal variance and maximal repeatability of the data.

Upon arrival at the laboratory, subjects were asked to void their bladder of urine and a mid-stream sample of urine was collected. A sterilised disposable thermistor probe (YSI 400 series, Mallinckrodt Medical, USA) secured by a sterilised bead positioned 12 cm from the tip of the probe was then inserted to a depth of 12 cm beyond the anal sphincter to monitor rectal temperature ( $T_{re}$ ). A Polar Trainer™ heart rate monitor (Polar Electro Oy, Kemele, Finland) which was secured on the chest was used to record heart rate. A resting blood sample was collected after maintaining a seated position for 15 min in a thermoneutral environment ( $21^{\circ}\text{C}$  and  $\sim 45\% \text{ rh}$ ) to allow equilibration following insertion of a venous cannula at the cephalic vein in the forearm. An extension tube of 20 cm was attached to the venous cannula and kept patent with heparinised saline at rest and during exercise to allow repeated blood sampling.

As the subjects proceeded to the climate chamber, they were weighed with the pre-weighed sports attire (shorts, socks and shoes) and all instrumentation using the same

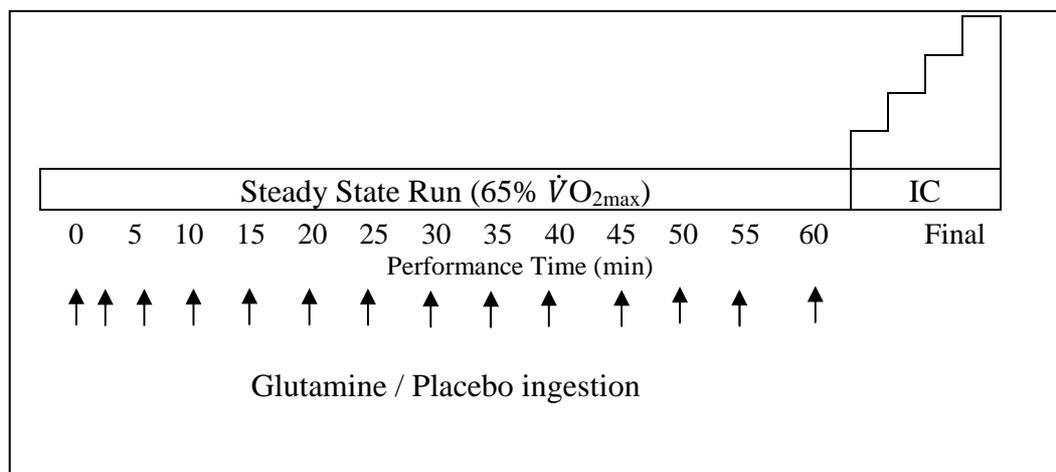
electronic weighing scale throughout each experimental trial. Resting HR and  $\dot{V}O_2$  were measured and subjective reporting of RPE, thermal comfort and thirst sensation was recorded prior to the commencement of each experimental trial.

### ***Exercise-Heat Exposure Protocol***

For **GLUT** and **PCB** trials, subjects performed a walking protocol prior to the experimental runs. Subjects walked at an exercise-to-rest ratio of 25 min exercise to 5 min rest in an environmental chamber set to 37°C, 60% rh. The treadmill was set to 5.5 km.hr<sup>-1</sup> with 4% inclination for 90 min.  $T_{re}$  and heart rate (HR) were monitored continuously and the 15-point Borg scale (Borg, 1982) for rating perceived exertion (RPE) was used to record RPE every 25 min. Body mass was weighed during each rest interval. To increase evaporative sweat loss during the walking protocol, air flow of 2.44 m.s<sup>-1</sup> was generated by a large electric fan situated 1 m in front of the subject's position on the treadmill. The fan switched on 30 min after commencing the walking protocol. No fluid or food was provided throughout the exercise-heat exposure protocol. After completion of the dehydration protocol, all instrumentation was removed (except the rectal probe) and subjects were positioned supine while four ice-packs were placed on the chest and inguinal crease areas at both left and right sides to enhance body cooling in thermoneutral conditions.  $T_{re}$  was monitored throughout the cooling process to determine when  $T_{re}$  had decreased below 37.5°C. Thereafter, subjects were requested to take a shower, change into their pre-weighed sports attire and rest for 40 min. Urine specific gravity and body mass with all instrumentation were weighed again and 2 cups of glutamine or placebo solution (see below "*Experimental Runs*") ( $207.3 \pm 26.1$  mL) were given to the subjects prior to the experimental runs.

### **Experimental Runs**

During each experimental run, subjects were required to run at 65%  $\dot{V}O_{2\max}$  for an hour followed by an incremental gradient protocol (+1% every 3 min) until volitional fatigue. A fan was positioned at approximately 1 m distance in front of the treadmill which created an artificial wind resistance of  $\sim 12 \text{ km}\cdot\text{hr}^{-1}$ . All subjects were instructed and encouraged to run for as long as possible. A total of 12 cups of Glutamine ( $0.2 \text{ g}\cdot\text{kg}^{-1}$  body mass per litre, Trans-Alanyl-Glutamine, Metabolic Nutrition<sup>®</sup>;  $13.4 \pm 5.0\text{g}$ ) or placebo (2g of dextrose with maltodextrin, Equal Classic<sup>®</sup>, Chicago, Illinois) were mixed with water, which was 70% of fluid replacement based on sweat loss during the CON trial were given to the subjects. The first 2 cups of supplement drinks were ingested immediately prior to commencing the trial followed by 1 cup every 5 min and subjects were encouraged to rapidly finish each cup of solution. In compliance with ethical approval, exercise was terminated if a  $T_{re}$  of  $39.9^{\circ}\text{C}$  was attained. Time to exhaustion was recorded as a measure of exercise performance. Following completion of the experimental trials, clothed body weight, sports attire and water cups were weighed to determine sweat loss. Subjects were instructed to wear the same sports attire during each experimental trial.



**Figure 8.1** Schematic representation of the experimental trials protocol  
IC: Incremental gradient protocol (+1% every 3 min)

#### **8.3.4.2 Hydration Measurements**

Urine samples were collected upon arrival at the laboratory, and after completion of each experimental run. Urine specific gravity (USG) was analysed using a digital urine specific gravity refractometer (Atago® UG- $\alpha$ , Japan) to determine the hydration status: USG <1.020 was considered to be euhydration. The same electronic weighing scale ( $\pm 0.001$  kg; Mettler Toledo ID1 Multi Range Scale, Columbus, OH, USA) was used to measure subjects' body mass and sports attire throughout the study. These body mass measurements established a baseline from which hydration status was determined on the morning of each experimental trial.

Sweat loss was calculated as the difference in pre- and post-exercise nude body mass, corrected for fluid intake, urine output, respiratory water loss, and sweat contained in sports clothing and running shoes (Pugh *et al.*, 1967; Mitchell *et al.*, 1972). Whole body sweat rate was estimated as:  $\text{Sweat Rate (L/min)} = \text{Sweat loss (L)} / \text{Performance time (min)}$

#### **8.3.4.3 Cardiorespiratory Measurements**

Heart rate (HR) was monitored at rest and every 5 min interval during each experimental run. Oxygen uptake ( $\dot{V}O_2$ ) was measured at 0, 10, 30, 60-min and in the final minutes prior to the cessation of exercise. A 60 s collection of expired respiratory gas was collected in a Douglas Bag for later analysis of O<sub>2</sub> and CO<sub>2</sub> fraction. Expired gas fractions were measured using O<sub>2</sub> and CO<sub>2</sub> analysers (Model 2-3A and Model CD-3A respectively, Ametek, Thermo Instruments, Pittsburg, PA) calibrated with a known gas (O<sub>2</sub>:15.9%; CO<sub>2</sub>: 4.03%) and outside air (O<sub>2</sub>: 20.93%; CO<sub>2</sub>: 0.03%). Gas analyzers were calibrated immediately before the analysis of the expired respiratory gas samples.

Expired gas volumes were determined using a dry gas flow meter (Parkinson-Cowan, England) and standard temperature, pressure and dry gas values were calculated according to corrected barometric pressure and temperature.

#### **8.3.4.4 Thermoregulatory Measurements**

Rectal ( $T_{re}$ ) temperature was monitored continuously and  $T_{re}$  data was logged on a portable temperature data logger (Digi-Sense<sup>®</sup> Thermistor Thermometer, Chemopharm, US) every 5 min. The data logger was calibrated before the study in a water bath and platinum resistance probe (T1091) within a temperature range of 30 to 45°C.  $T_{sk}$  was measured at four sites using iButton<sup>™</sup> temperature sensors: (i) halfway between the acromium process and nipple (chest), (ii) deltoid, (iii) anterior mid-thigh and (iv) right lateral calf; which have an inbuilt data logger for subsequent data downloading (van Marken Lichtenbelt et al., 2006). These were attached to the skin using narrow strips of Opsite<sup>™</sup> surgical bandage.  $\bar{T}_{sk}$  was calculated using the equation:  $\bar{T}_{sk} = 0.3 (T_{chest} + T_{arm}) + 0.2 (T_{thigh} + T_{leg})$  (Ramanathan, 1964) .

#### **8.3.4.5 Haematological Measurements**

A total volume of 12 mL of venous blood samples were collected upon arrival to the laboratory (baseline measurement), 0-min, and during the exhaustion point of exercise, whereas a total of 8 mL of venous blood samples were collected at 10, 30, and 60-min of exercise. A 1 mL blood sample was collected into EDTA coated tubes for haemoglobin and haematocrit analysis. Haemoglobin concentration was analysed (Sysmex KX-21N Hematology Analyser, Kobe, Japan) and haematocrit percentage was estimated using a microcentrifuge (Hawksley) and micro haematocrit reader (Hawksley

CE/15006, UK). Haemoglobin and haematocrit values were used to calculate the percentage change in plasma volume (Dill & Costill, 1974). Triplicate measurements were made of haematocrit and haemoglobin.

A 4 mL venous blood sample was collected into lithium-heparin coated tubes, centrifuged and stored at -20°C for subsequent determination of plasma viscosity, total protein, glucose and lactate concentrations. Plasma viscosity was analysed using a viscometer (Cone and plate Viscometer, LVT with CP-40, Wells-Brookfield). Plasma total protein was analysed using a total protein kit (Stanbio Total Protein LiquiColor®, Texas). Plasma glucose was analysed using a *glucose oxidase colorimetric analysis kit* (TR-1511-200 Thermo Electron Noble Park, Victoria, Australia) and lactate dehydrogenase (LDH) was assayed using the method of Annan (1975).

Another 4 mL sample of venous blood was were collected into clot activator coated tubes, centrifuged and stored at -80°C for subsequent analysis of serum glutamine, osmolality and electrolytes concentrations. Fluorometric analysis was used to determine glutamine concentration, as described by Grossie *et al.* (1993). Serum osmolality and serum electrolytes were analysed using an osmometer (OSMOMAT 030, Gonotec GmbH) and a radiometer (ABL80 Flex, Denmark), respectively.

An additional 4 mL of venous blood were collected into EDTA coated tubes (2 mL in each aliquot), centrifuged and stored at -80°C for plasma renin and plasma HSP 72 analysis upon arrival to the laboratory (baseline measurement), 0-min, and during the exhaustion point of exercise. Plasma renin was analysed using a Renin active ELISA kit (IBL International GMBH, Germany) with samples diluted 1:2, as described by the Renin active ELISA kit instruction manual. Plasma HSP 72 was analysed with samples

diluted 1:5 using a commercial ELISA Kit (Stressgen, Canada). All samples were analysed in the same assay run to eliminate inter-assay variance.

#### **8.3.4.6 Subjective Reporting**

Perceived thirst sensation was identified using a nine-point thirst scale with verbal anchors (Engell *et al.*, 1987). Ratings of perceived exertion (RPE) were obtained using the 15-point Borg scale (Borg, 1982) and thermal comfort was recorded from a seven point scale (Bedford, 1936). Thirst sensations, RPE and thermal comfort were recorded at 10-min intervals throughout the testing and at the point of exhaustion. Subjects were familiarized with the scales prior to testing and these measurements were obtained in a random order to eliminate an ordering effect.

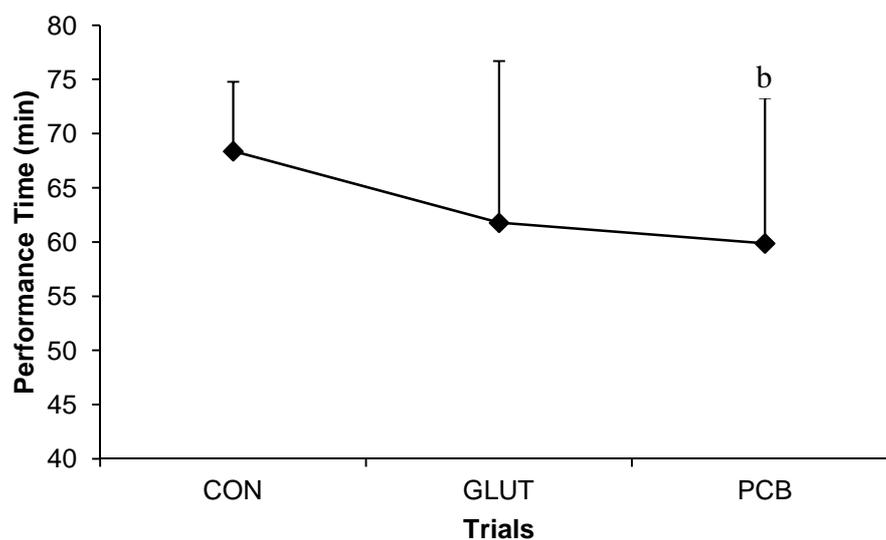
#### **8.3.5 Statistical Analysis**

Power and Sample Size Calculation (PS) software by Dupont and Plummer (1997) was used to determine the sample size. A two-way (time x trial) repeated ANOVA was performed using Statistical Package for Social Sciences (SPSS 18.0, Chicago, IL) to compare significance difference within and between treatments. Where significant interaction effects were established, pairwise differences were identified using Tukey's HSD post hoc analysis procedure. Where appropriate, differences between trials were also identified using one-way analysis of variance with repeated measures. Linear regression analysis was used to determine relationship between  $T_{re}$  and HR response. All values are expressed as means  $\pm$  SD and the significance level was accepted at  $p < 0.05$ .

## 8.4 RESULTS

### 8.4.1 Performance Time and Plasma [Glutamine]

Four out of seven subjects successfully completed the 60 min steady state run in each of the three conditions with different hydration status and supplement drinks in the heat. Mean running performance time for **CON**, **GLUT** and **PCB** trials were  $68.4 \pm 6.4$ ,  $61.8 \pm 14.9$  and  $59.9 \pm 13.4$  min, respectively. Time to exhaustion during **GLUT** was similar with **CON** ( $p=0.112$ ) but a significant reduction was found during **PCB** trial (Figure 8.2;  $p=0.044$ ). Similarly, there were no significant differences between **CON** and **GLUT** in plasma [glutamine] but a lower plasma [glutamine] was found in **PCB** when compared with **GLUT** ( $p=0.003$ ).



Plasma [Glutamine] ( $\mu\text{mol.L}^{-1}$ )	Trials		
	CON	GLUT	PCB
Baseline		$494.2 \pm 98.4$	$510.5 \pm 55.7$
0 min	$542.6 \pm 50.1$	$537.7 \pm 91.8$	$531.2 \pm 48.3$
Final	$596.2 \pm 64.6$	$633.0 \pm 45.0$	$547.6 \pm 68.0^b$

**Figure 8.2** Performance time and plasma [Glutamine] mean  $\pm$ SD during three experimental trials (**CON**, euhydrated in 35°C; **GLUT**, dehydrated in 35°C; **PCB**, dehydrated in 35°C)

<sup>b</sup> indicate a significant difference from **GLUT** ( $p<0.05$ )

#### 8.4.2 Hydration Status

There was no significant difference in body mass and USG measurements prior to the experimental trials (Table 8.1;  $p>0.05$ ). Approximately 2.4% body mass loss was induced by the 90 min of exercise-heat exposure protocol. As the subjects completed the experimental runs, a greater % body mass change was found in **CON** when compared with **GLUT** and **PCB** ( $p=0.005$  and  $p=0.001$ , respectively). The fixed supplement drinks intake regimen resulted in a similar % body mass loss in **GLUT** and **PCB** trials. Sweat rate was similar in all trials  $\sim 1.73 - 1.81 \text{ L}\cdot\text{min}^{-1}$  regardless of different hydration status.

#### 8.4.3 Cardiorespiratory Responses

Subjects ran at  $\sim 66.3\% \dot{V}O_{2\text{max}}$  during the first 10 min of steady state exercise (Table 8.2). At 30 min of running,  $\dot{V}O_2$  was significantly higher in **GLUT** and **PCB** when compared with **CON**, but a significant difference in metabolic drift was found in **PCB** at the end of 60 min steady state run. All subjects stopped running at a similar relative percentage of  $\dot{V}O_{2\text{max}}$ : **CON**:  $78.8 \pm 7.6\% \dot{V}O_{2\text{max}}$ ; **GLUT**:  $78.1 \pm 7.4\% \dot{V}O_{2\text{max}}$  and **PCB**:  $76.3 \pm 5.5\% \dot{V}O_{2\text{max}}$  ( $p>0.05$ ).

HR increased dramatically over 60 min of exercise from 10-min to 60-min in all experimental trials ( $p<0.001$ ; Table 8.2). Subjects stopped exercise at similar HR responses ( $\sim 186 \text{ beats}\cdot\text{min}^{-1}$ ), which were  $97 \pm 3$ ,  $98 \pm 5$  and  $99 \pm 4\%$  of their  $\text{HR}_{\text{max}}$  in **CON**, **GLUT** and **PCB** respectively (Table 8.2).

**Table 8.1:** Hydration status determined by percentage changes of body mass, urine specific gravity (USG), sweat rate, and fluid intake during **CON**, **GLUT** and **PCB** trials

Variable and Time	Trials		
	CON	GLUT	PCB
<b>Body Mass (kg)</b>			
Baseline		70.54 ± 7.72	70.82 ± 8.00
0 min	70.46 ± 8.09	68.83 ± 7.64 <sup>#</sup>	69.04 ± 7.82 <sup>#</sup>
Final	68.86 ± 8.01 <sup>*</sup>	67.79 ± 7.52 <sup>#,*, a</sup>	68.14 ± 7.85 <sup>#,*, a</sup>
<b>% Δ Body Mass</b>			
Baseline – 0 min		-2.43 ± 0.35	-2.51 ± 0.23
0 min – Final	-2.09 ± 0.79	-0.94 ± 0.56 <sup>a</sup>	-1.14 ± 0.75 <sup>a</sup>
Baseline – Final	-2.09 ± 0.79	-3.91 ± 1.01 <sup>a</sup>	-3.80 ± 0.76 <sup>a</sup>
<b>USG</b>			
Baseline		1.011 ± 0.007	1.011 ± 0.006
0 min	1.009 ± 0.006	1.019 ± 0.008 <sup>a</sup>	1.017 ± 0.007 <sup>a</sup>
Final	1.014 ± 0.004	1.024 ± 0.003 <sup>a</sup>	1.023 ± 0.003 <sup>a</sup>
<b>Sweat Rate (L.min<sup>-1</sup>)</b>			
0 min - Final	1.79 ± 0.23	1.81 ± 0.50	1.73 ± 0.33
<b>Fluid Intake (mL)</b>			
0 min – Final	424 ± 287	973 ± 249 <sup>a</sup>	873 ± 306 <sup>a</sup>

Baseline: Prior to the exercise-heat exposure protocol

<sup>a, b, c</sup> Significantly different from CON, GLUT and PCB respectively

<sup>#</sup> Significantly different from baseline value

<sup>\*</sup> Significantly different from 0 min value

**Table 8.2:** Oxygen uptake and heart rate measurements during **CON**, **GLUT** and **PCB** trials

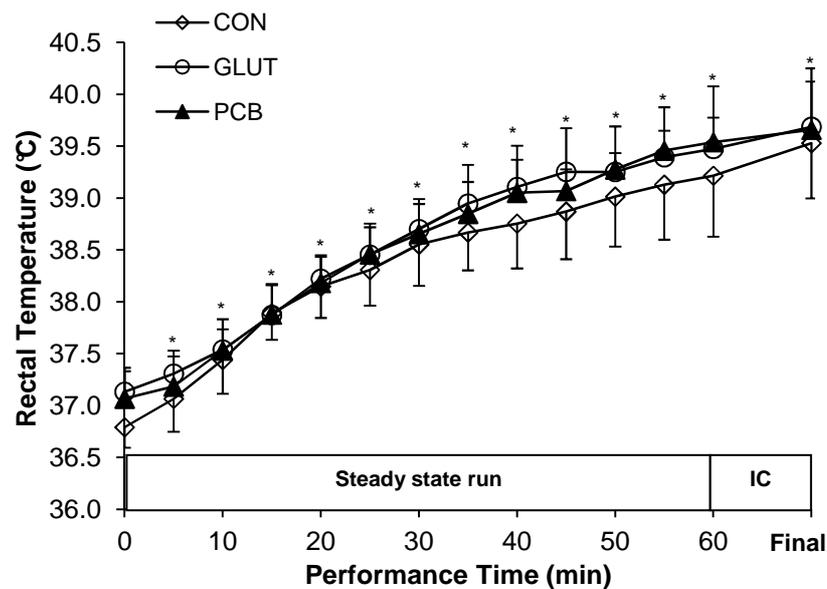
Variable	Trials	Performance Time				
		0 min	10 min	30 min	60 min	Final
$\dot{V}O_2$ (mL.min <sup>-1</sup> .kg <sup>-1</sup> )	<b>CON</b>	4.2 ± 1.7	41.7 ± 5.1	42.1 ± 4.4	42.0 ± 4.4	50.2 ± 5.5
	<b>GLUT</b>	4.8 ± 1.7	42.4 ± 3.9	44.2 ± 4.2 <sup>b</sup>	42.0 ± 4.7	49.6 ± 3.6
	<b>PCB</b>	4.6 ± 2.1	42.8 ± 4.0	44.4 ± 4.3 <sup>b</sup>	43.7 ± 5.1 <sup>b</sup>	48.6 ± 4.3
<b>HR</b> (beats.min <sup>-1</sup> )	<b>CON</b>	69 ± 8	148 ± 9	160 ± 8 <sup>*</sup>	166 ± 9 <sup>*</sup>	185 ± 7 <sup>*</sup>
	<b>GLUT</b>	77 ± 11	156 ± 8	167 ± 9 <sup>*</sup>	173 ± 13 <sup>*</sup>	187 ± 10 <sup>*</sup>
	<b>PCB</b>	78 ± 12	154 ± 8	170 ± 9 <sup>*,b</sup>	178 ± 8 <sup>*,b</sup>	187 ± 8 <sup>*</sup>

<sup>b</sup> Significantly different from **CON** trial

<sup>\*</sup> Significantly different from 10 min

#### 8.4.4 Thermoregulatory Responses

$T_{re}$  increased significantly from 5-min of running until exhaustion in all trials ( $p < 0.05$ ; Figure 8.3). Subjects stopped exercise with  $T_{re}$ :  $39.5 \pm 0.5^\circ\text{C}$ ,  $39.7 \pm 0.6^\circ\text{C}$  and  $39.7 \pm 0.5^\circ\text{C}$  in **CON**, **GLUT** and **PCB** trials, respectively. A significant relationship was found between  $T_{re}$  and HR response in all trials: **CON**,  $r = 0.81$ ; **GLUT**,  $r = 0.76$  and **PCB**,  $r = 0.76$ ;  $p < 0.001$ ).  $\bar{T}_{sk}$  remained similar  $\sim 35^\circ\text{C}$  throughout the trials except a significant increase of  $\sim 1^\circ\text{C}$  was found at 5 to 10-min in **GLUT** and **PCB**. There was no significant difference in  $T_{re}$  and  $\bar{T}_{sk}$  between trials throughout the experimental runs.



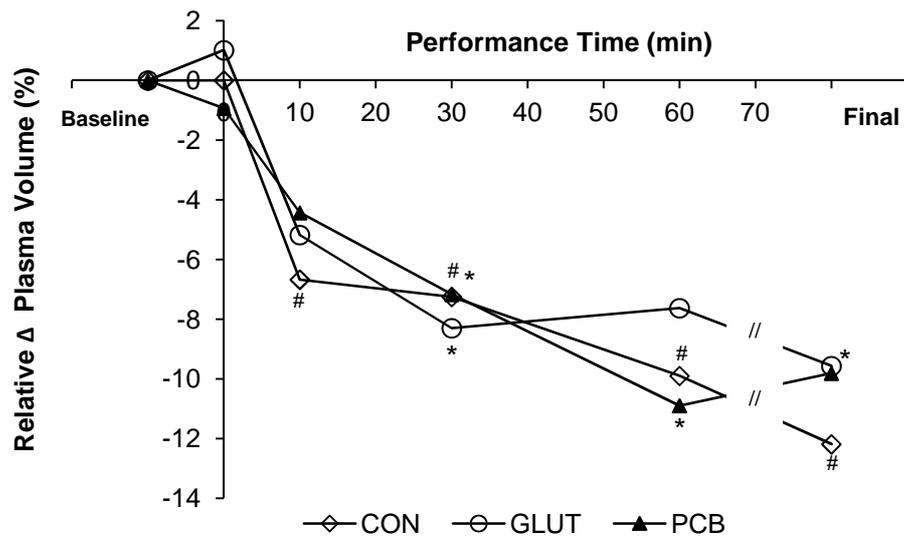
**Figure 8.3** Rectal temperature ( $T_{re}$ ) measurements during exercise in **CON**, **GLUT** and **PCB** trials

# Significantly different from 0 min

IC: Incremental gradient protocol

### 8.4.5 Haematological Responses

No significant difference was observed in plasma volume with ~ 2.4% body mass loss after the exercise heat-exposure protocol ( $p>0.05$ ; Figure 8.4). During experimental runs, plasma volume in **CON** was reduced over time:  $-12.2 \pm 5.0\%$  from 0 min to the point of exhaustion; while plasma volume in **GLUT** and **PCB** decreased significantly after 30 min of exercise until exhaustion ( $-9.6 \pm 5.8\%$  and  $-9.8 \pm 6.3\%$ , respectively). No significant difference was noted between trials ( $p>0.05$ ). The initial decrease in PV is thought to be result of increased metabolites within muscle fibres drawing water into the fibre.

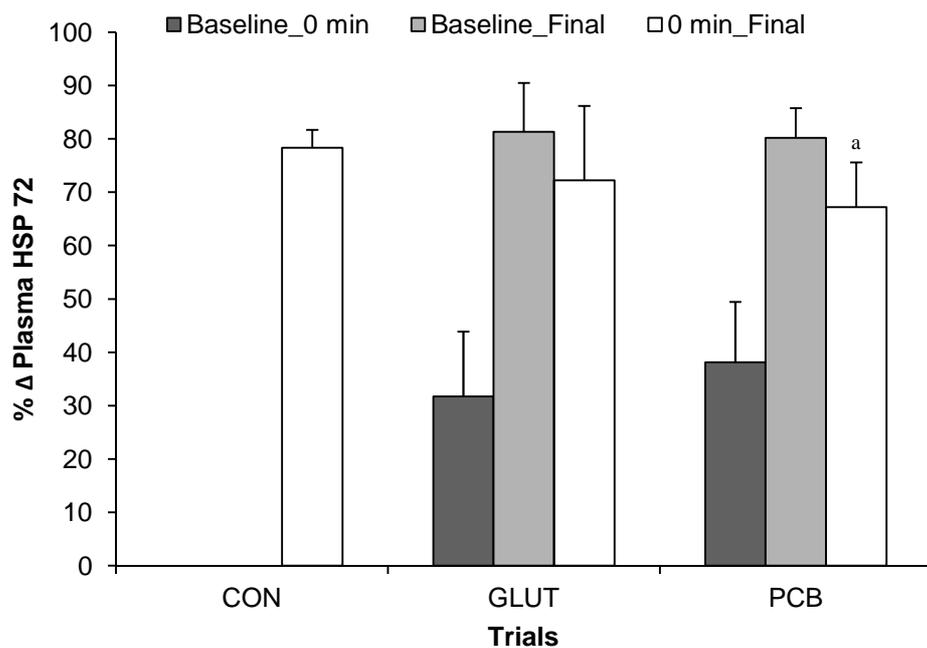


**Figure 8.4** Plasma volume changes after the exercise-heat exposure protocol and during exercise in **CON**, **GLUT** and **PCB**

\* Significantly different from baseline value

# Significantly different from 0 min

Percentage changes of HSP 72 from baseline and immediately prior to the commencement of exercise were examined during all trials. The exercise-heat exposure dehydration protocol induced an increase of HSP 72 expression in both **GLUT** and **PCB** ( $31.7 \pm 12.2\%$  and  $38.1 \pm 11.4\%$ , respectively; Figure 8.5). In order to examine the effect of glutamine ingestion on plasma HSP expression and the possible protective role of HSP 72 against heat stress and dehydration, either glutamine or a placebo was given to the subjects during the **GLUT** and **PCB** experimental runs. Supplement drinks with glutamine significantly enhanced HSP 72 expression in **GLUT** when compared with **PCB** ( $72.2 \pm 14.0\%$  vs.  $67.2 \pm 8.4\%$ ;  $p=0.046$ ).



**Figure 8.5** Percentage changes of plasma heat shock protein (HSP) 72 in **CON**, **GLUT** and **PCB** trials

<sup>a</sup> Significantly lower than **CON** trial ( $p<0.01$ )

Serum osmolality, plasma viscosity and plasma [total protein] were similar during baseline measurements between trials and increased significantly throughout the experimental runs until exhaustion ( $p < 0.05$ ; Table 8.3). Serum osmolality and plasma viscosity in **GLUT** and **PCB** were significantly greater than **CON** during exercise ( $p < 0.05$ ) except similar values of serum osmolality were found at exhaustion. Similarly, greater plasma [renin] was found in the blood sample taken immediately prior to the commencement of exercise and at exhaustion in **GLUT** and **PCB** when compared **CON** ( $p < 0.05$ ).

No significant difference was noted in plasma glucose  $\sim 4.8 \text{ mmol.L}^{-1}$  and plasma [lactate]  $\sim 2.0 \text{ mmol.L}^{-1}$  during baseline, 0 min and 10 min measurements in all trials ( $p > 0.05$ ; Table 8.3). Plasma glucose between trials was similar during the first 10-min of experimental runs. However, as subjects progressed to 30-min of running, plasma glucose was significantly elevated in **PCB** when compared with **CON** ( $p = 0.036$ ). A slight increase was found in plasma [lactate] at 30-min in **PCB** trial when compared with other trials. At the point of exhaustion, no significant differences were observed in plasma [glucose] and plasma [lactate] between trials ( $p > 0.05$ ).

Serum [ $\text{Na}^+$ ] and [ $\text{Cl}^-$ ] were significantly elevated as  $2\text{-}3 \text{ mmol.L}^{-1}$  after the exercise heat-exposure dehydration protocol in **GLUT** and **PCB** trials. After 10-min of the experimental runs, serum [ $\text{Na}^+$ ] and [ $\text{Cl}^-$ ] increased significantly in **GLUT** and **PCB** trials when compared with **CON** ( $p < 0.05$ ; Table 8.3), but no significant difference was noted in serum [ $\text{K}^+$ ] between trials.

**Table 8.3:** Haematological Responses during PETS

Variable	Trials	Performance Time				
		Baseline	0 min	10 min	30 min	Final
Serum Osmolality (mosmol.kg <sup>-1</sup> )	CON		287 ± 5	291 ± 5	291 ± 5 <sup>#</sup>	300 ± 9 <sup>#</sup>
	GLUT	288 ± 6	292 ± 5 <sup>*</sup>	299 ± 6 <sup>a,*,#</sup>	299 ± 5 <sup>a,*,#</sup>	301 ± 9 <sup>*,#</sup>
	PCB	288 ± 5	294 ± 5 <sup>*</sup>	297 ± 5 <sup>a,*</sup>	297 ± 4 <sup>a,*,#</sup>	299 ± 5
Plasma Viscosity (mPa.s)	CON		1.46 ± 0.12	1.56 ± 0.12 <sup>*</sup>	1.58 ± 0.12 <sup>*</sup>	1.73 ± 0.14 <sup>*</sup>
	GLUT	1.52 ± 0.22	1.74 ± 0.20 <sup>a,*,#</sup>	1.82 ± 0.16 <sup>a,*</sup>	1.83 ± 0.20 <sup>a,*</sup>	2.04 ± 0.19 <sup>a,*,#</sup>
	PCB	1.55 ± 0.16	1.80 ± 0.15 <sup>a</sup>	1.84 ± 0.19 <sup>a,*</sup>	1.85 ± 0.22 <sup>a</sup>	1.89 ± 0.18 <sup>a,*</sup>
Plasma [Glucose] (mmol.L <sup>-1</sup> )	CON		4.6 ± 0.4	4.4 ± 0.7	4.9 ± 0.9	6.5 ± 1.8 <sup>#</sup>
	GLUT	4.9 ± 0.4	4.6 ± 0.5	4.5 ± 0.5	5.6 ± 0.8 <sup>#</sup>	7.3 ± 1.5 <sup>*,#</sup>
	PCB	5.1 ± 0.9	4.6 ± 0.4	4.9 ± 1.2	6.0 ± 1.7 <sup>a</sup>	7.0 ± 1.6 <sup>*,#</sup>
Plasma [Lactate] (mmol.L <sup>-1</sup> )	CON		1.9 ± 1.2	2.6 ± 1.2 <sup>#</sup>	2.4 ± 1.4 <sup>#</sup>	6.1 ± 3.7 <sup>#</sup>
	GLUT	1.9 ± 1.4	2.0 ± 1.3	2.2 ± 1.2 <sup>*,#</sup>	2.5 ± 1.1 <sup>*</sup>	5.1 ± 2.0 <sup>*,#</sup>
	PCB	2.2 ± 1.5	2.0 ± 1.4	2.3 ± 1.4	2.6 ± 1.0 <sup>#</sup>	4.1 ± 2.5 <sup>#</sup>
Serum [Na <sup>+</sup> ] (mmol.L <sup>-1</sup> )	CON		139.0 ± 1.6	140.7 ± 0.8 <sup>#</sup>	140.4 ± 1.3 <sup>#</sup>	141.4 ± 1.7 <sup>#</sup>
	GLUT		141.4 ± 1.6 <sup>a,*,#</sup>	143.4 ± 1.8 <sup>a,*</sup>	142.7 ± 2.5 <sup>a,*</sup>	142.4 ± 2.6 <sup>*</sup>
	PCB	139.0 ± 1.4	142.0 ± 2.9 <sup>a,*</sup>	142.7 ± 2.2 <sup>a,*</sup>	142.3 ± 2.0 <sup>a,*</sup>	141.9 ± 2.2 <sup>*</sup>

<b>Serum [K<sup>+</sup>] (mmol.L<sup>-1</sup>)</b>	CON		4.6 ± 0.6	5.0 ± 0.4 <sup>*,#</sup>	5.1 ± 0.4	4.6 ± 0.6
	GLUT	4.9 ± 0.5	4.6 ± 0.5	5.2 ± 0.3 <sup>*,#</sup>	5.2 ± 0.3 <sup>#</sup>	4.7 ± 0.6
	PCB	4.9 ± 0.3	4.6 ± 0.3	5.2 ± 0.3 <sup>#</sup>	5.2 ± 0.2 <sup>#</sup>	4.4 ± 0.4
<b>Serum [Cl<sup>-</sup>] (mmol.L<sup>-1</sup>)</b>	CON		107.7 ± 1.3	111.0 ± 2.1	111.0 ± 2.1	109.9 ± 1.1
	GLUT	107.9 ± 2.0	109.4 ± 1.5 <sup>a</sup>	112.3 ± 2.8 <sup>a</sup>	112.9 ± 3.4	111.6 ± 2.9
	PCB	107.0 ± 2.5	110.1 ± 3.6	111.9 ± 2.4 <sup>a</sup>	112.0 ± 3.4	111.0 ± 2.9
<b>Plasma [Protein] (g.dL<sup>-1</sup>)</b>	CON		7.44 ± 0.41	7.80 ± 0.53	8.02 ± 0.45 <sup>#</sup>	8.36 ± 0.68 <sup>#</sup>
	GLUT	7.25 ± 0.58	7.59 ± 0.61 <sup>*</sup>	7.92 ± 0.39 <sup>*</sup>	8.18 ± 0.42 <sup>*,#</sup>	8.19 ± 0.41 <sup>*,#</sup>
	PCB	7.34 ± 0.38	7.88 ± 0.55 <sup>*</sup>	8.17 ± 0.40 <sup>*</sup>	8.16 ± 0.51 <sup>*</sup>	8.28 ± 0.34 <sup>*,#</sup>
<b>Plasma [Renin] (pg.mL<sup>-1</sup>)</b>	CON		15.9 ± 8.5	-	-	134.5 ± 95.0 <sup>#</sup>
	GLUT	29.9 ± 25.7	40.1 ± 20.4 <sup>a</sup>	-	-	254.1 ± 144.4 <sup>a,*,#</sup>
	PCB	42.3 ± 34.4	49.4 ± 42.0 <sup>a</sup>	-	-	220.0 ± 132.7 <sup>a,*,#</sup>

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Baseline: Prior to the exercise-heat exposure protocol

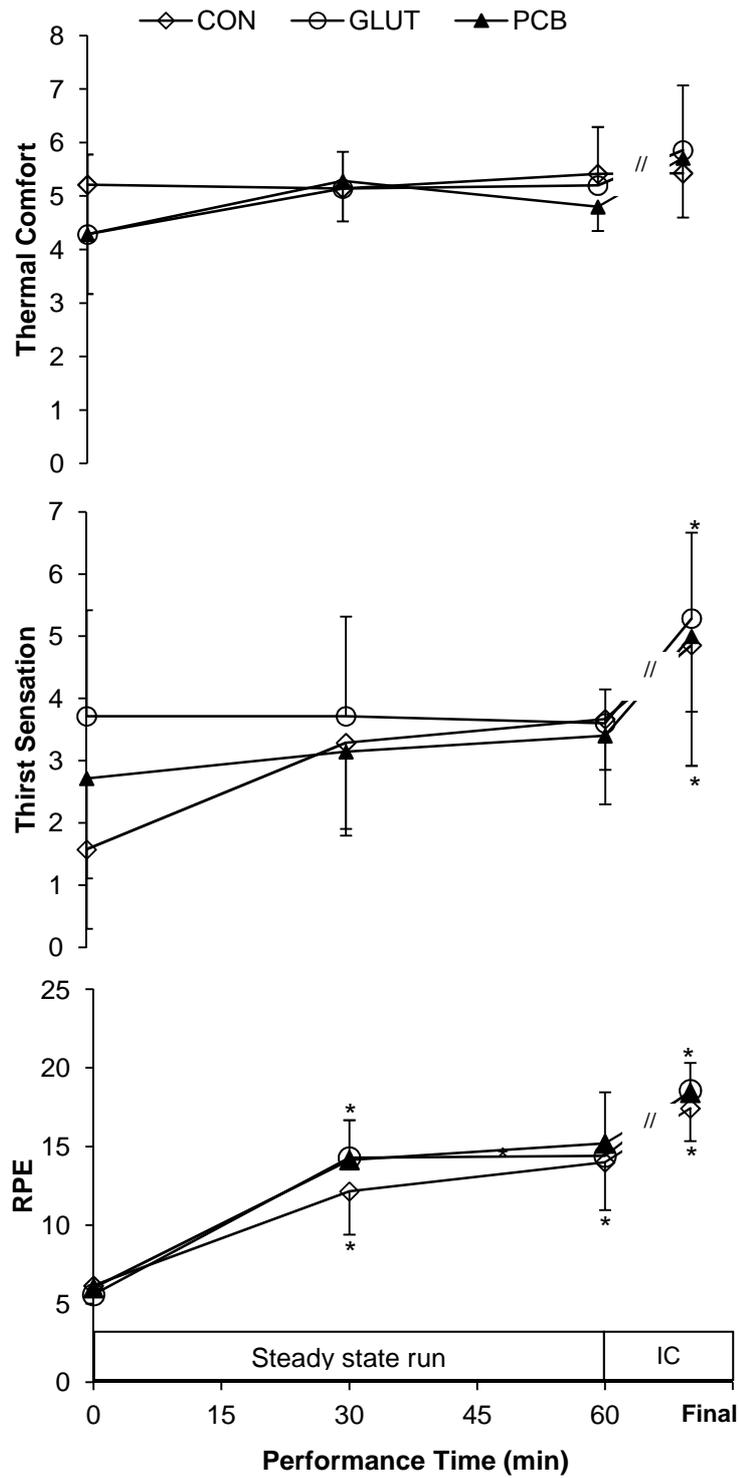
<sup>a</sup> Significantly different from CON

<sup>\*</sup> Significantly different from baseline value

<sup>#</sup> Significantly different from 0 min value

#### 8.4.6 Subjective Responses

Ratings of perceived exertion (RPE) increased significantly over time in all trials ( $p < 0.01$ ; Figure 8.6) and no significant difference was found between trials. Similarly, thirst sensation and thermal comfort scores during exercise were similar across different trials ( $p > 0.05$ ). At exhaustion, RPE was similar in all three conditions (**CON**:  $17.4 \pm 2.1$ , **GLUT**:  $18.6 \pm 2.1$  and **PCB**:  $18.4 \pm 0.8$ ). Meanwhile thirst sensation was greater when compared with immediately prior to the commencement of exercise across all trials and a greater thermal comfort score was found in **PCB** when compared with other trials ( $p < 0.01$ ).



**Figure 8.6** Rating of perceived exertion (RPE), thirst sensation and thermal comfort scale during exercise in CON, GLUT and PCB

<sup>a</sup> Significantly different from CON

\* Significantly different from 0 min

IC: Incremental gradient protocol

## 8.5 DISCUSSION

The purpose of this study was to investigate the effect of alanyl-glutamine ingestion in attenuating the exercise-induced decrease in plasma [glutamine] and to elucidate the relationship between alanyl-glutamine ingestion and the induction of plasma HSP 72 expression during prolonged exercise in hot climatic conditions undertaken in a hypohydrated state. This study demonstrates that alanyl-glutamine administration during exercise in hot and hypohydrated conditions successfully enhanced plasma [glutamine] and further enhanced the expression of plasma HSP 72. In addition, the magnitude of running performance time to exhaustion in the **PCB** trial was significantly 12.4% less when compared with the **CON** trial. However, when subjects ingested the supplement solution containing glutamine, running time to exhaustion in the **GLUT** trial did not differ from the **CON** trial where the subjects were in a euhydrated state prior to the exercise (Figure 8.2). The improvement in running performance occurred seemingly due to the increase in plasma [glutamine] associated with an enhanced plasma HSP 72 expression, which may be coupled with an increase in cell-stress tolerance to dehydration during exercise in hot conditions. Indeed, it has been shown that resistance to dehydration damage in some cell lines correlates with the presence of a range of heat shock proteins (Ravindran *et al.*, 2005).

Prolonged exercise is associated with a decrease in plasma [glutamine] and it has been suggested that this may be due to over-training and associated immunosuppression (Parry-Billings *et al.*, 1992). Our data did not show any decrement in plasma [glutamine], and therefore we conclude that “immunosuppression” is not necessarily a contributing factor to impaired performance when exercising in hot and hypohydrated conditions. Conversely, our data showed an increase in plasma [glutamine] during all experimental trials but with differing magnitudes of increase from immediately pre-

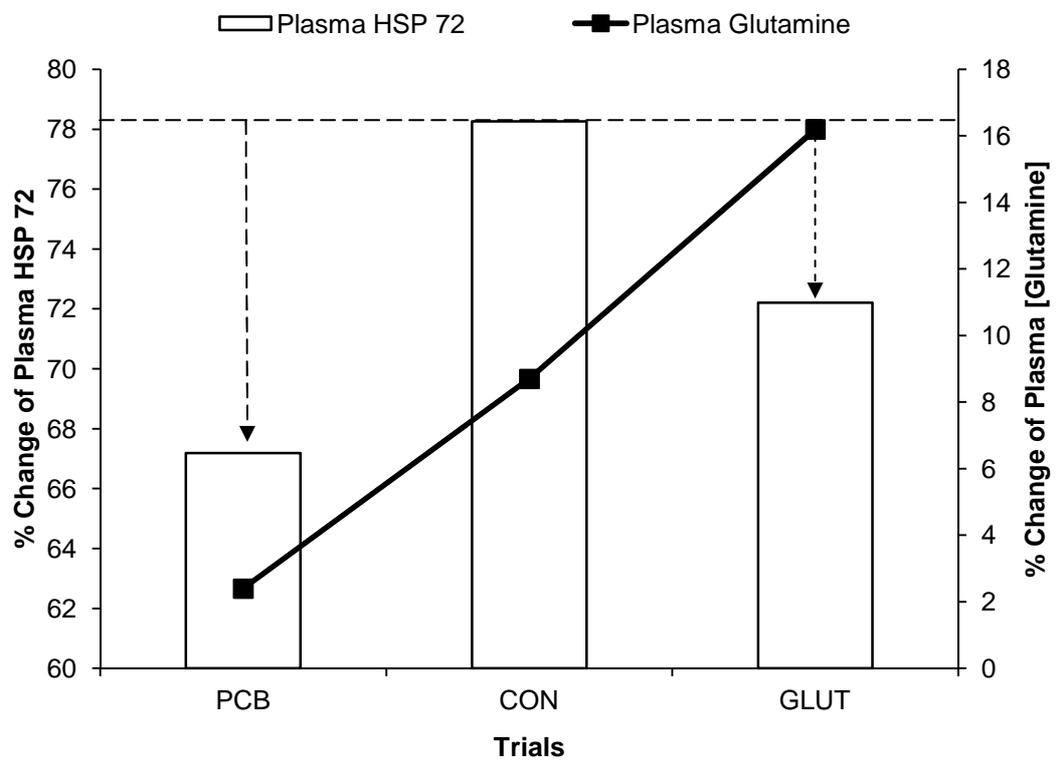
exercise to the point of exhaustion. The **GLUT** trial exhibited the greatest elevation (~16.2%) in plasma [glutamine], followed by **CON** trial with an elevation of ~8.7% in plasma [glutamine]. The **PCB** trial showed the least increment ~2.4% in plasma [glutamine]. A possible explanation for the increase in plasma [glutamine] in the **CON** trial could be the release of glutamine from skeletal muscles into the circulation (Walsh *et al.*, 1998; Newsholme *et al.*, 2011), whereas a greater elevation of plasma [glutamine] in the hypohydrated state was experienced in the **GLUT** trial due to the ingestion of the supplement solution containing glutamine when compared with no administration of exogenous glutamine in the **PCB** trial.

The potential for glutamine to be used as an oral rehydration beverage has been investigated by Hoffman *et al.* (2011). Plasma [glutamine] was significantly elevated with the ingestion of 0.2 g.kg<sup>-1</sup> body mass per liter of L-alanyl-L-glutamine (~20g) as a rehydration beverage. Hoffman *et al.* (2011) found this solution to be associated with a significant cycling performance improvement when cyclist exercised at 75%  $\dot{V}O_{2max}$  to volitional exhaustion. The authors concluded that glutamine ingestion mediated an enhanced fluid and electrolyte uptake while a significant reduction (~3 mmol.L<sup>-1</sup>) in plasma [Na<sup>+</sup>] was observed. In the present study, we employed a similar dosage of glutamine in solution with water (0.2 g.kg<sup>-1</sup> body mass per liter) during exercise in hot and hypohydrated conditions. Subjects ingested 974 ± 250 mL of supplement drinks containing 13.4 ± 5.0 g glutamine, resulting in a greater running performance time but no significant effect on plasma [Na<sup>+</sup>]. In addition, percentage changes of body mass during the glutamine supplementation trial (-0.93%) did not differ from the placebo trial (-1.14%). Similarly, urine specific gravity, serum osmolality, plasma viscosity, plasma [total protein] and plasma [renin] measurements were similar with and without the glutamine ingestion experimental trial, while body weight changes, plus haematological

and urinary indices are often used to assess hydration status (Kavouras, 2002; Armstrong, 2007), we found that the ingestion of supplement drinks with glutamine did not influence these measures. This evidence clearly shows that the greater running performance time in **GLUT** trial was not due to an increase in fluid and electrolyte absorption.

Accumulating evidence supports the observation that the induction of HSP expression is strongly correlated with the development of a tolerance to heat stress as well as other stressors including hypoxia and oxidative stress (Moseley, 1997; Kregel, 2002; Ruell *et al.*, 2009). Our findings indicate that exercise in hot conditions (35°C; 40% RH) enhanced plasma HSP 72 expression with a ~2.1% body mass loss through sweating in the **CON** trial (Figure 8.5). However, as the body mass loss approached ~3.8% in the **GLUT** and **PCB** trials, the presence of plasma HSP 72 was depressed and this seemed to be associated with the runners being significantly hypohydrated (Figure 8.7). To our knowledge, there is only one study on humans that has investigated the effect of dehydration on HSP responses during exercise in hot conditions (Hillman *et al.*, 2011). The authors of this study did not find any pronounced influence of dehydration on cellular HSP concentration following exercise in both thermoneutral and warm environments with and without dehydration. Hillman *et al.* (2011) suggested that oxidative stress was a more potent stressor than dehydration, and as such dehydration did not induce an increase in HSP concentration. Their subjects experienced a 3.8% bodyweight deficit due to dehydration during a prolonged exercise trial (90 min cycling) in warm conditions ( $33.9 \pm 0.9^{\circ}\text{C}$ ) followed by a 5-km time trial. The degree of dehydration was similar to that observed in the present study at the conclusion of the **GLUT** and **PCB** trials. Our data shows that dehydration depressed the induction of plasma [HSP] expression. However, as a consequence of ingesting supplement drinks

with the glutamine in solution, plasma [glutamine] was associated with a greater plasma HSP 72 expression in the **GLUT** trial when compared with the **PCB** trial, and exhibited greater performance times in the **GLUT** trial. It is noteworthy that Hillman et al (2011) investigated cellular HSP concentrations (monocyte HSP 72 and lymphocyte HSP 32) in their study, whereas our study measured plasma [HSP 72], which makes comparisons difficult.



**Figure 8.7** Relationship between percentage change of plasma HSP and percentage change of plasma [Glutamine] from 0 min to point of exhaustion during exercise in **CON**, **GLUT** and **PCB** trials

Increased expression of plasma HSP is associated with a rise in core temperature during both passive and active heat exposure (Ruell *et al.*, 2006). The release of plasma HSP 72 may elicit a sensation of fatigue to reduce the effort of exercise, which acts as a CNS fatigue-signalling mechanism to prevent life threatening situations (Heck *et al.*, 2011).

In the current study,  $T_{re}$  in all trials was similar and consistently increased during exercise in the heat, despite subjects having started their exercise with a different level of hydration and either a glutamine or placebo solution was ingested during experimental runs. Subjects stopped exercise with a similar  $T_{re}$  at  $\sim 39.7^{\circ}\text{C}$  but with different levels of plasma HSP expression. These results demonstrate that glutamine seems to be responsible for an enhanced plasma HSP 72 expression during exercise in a hot and hypohydrated state. Previous reviews have shown that glutamine depletion following critical illness or injury is likely to affect the induction of heat shock protein expression (Wischmeyer, 2006). Therefore we speculate that without glutamine supplementation during the **PCB** trial there was an associated depression in plasma [HSP 72].

Oxygen uptake and heart rate responses in both the **GLUT** and **PCB** trial were similar, thus indicating that subjects ran at similar efforts to exhaustion during the experimental trial. Similarly our results showed that L-alanyl-L-glutamine ingestion did not affect perceptual ratings of fatigue and none of our subjects reported gastrointestinal distress after ingesting supplement drinks with glutamine during exercise. To the contrary, a study evaluating peptide glutamine ingestion 30 min prior to soccer players commencing exercise demonstrated that subjects who ingested carbohydrate with peptide glutamine exhibited reduced feelings of fatigue compared with the use of carbohydrate alone (Favano *et al.*, 2008). Favano *et al.* (2008) also found that there was an improvement in distance run and exercise tolerance. It is possible that combining carbohydrate and glutamine ingestion may have an influence on blood glucose concentration and increase time to fatigue during prolonged submaximal exercise. Further research is required to elucidate the relationship between combined drinks (glucose and glutamine) and the induction of plasma HSP 72 expression.

In conclusion, the present study demonstrates alanyl-glutamine ingestion confers protection and enhances plasma HSP 72 expression. Furthermore, ingestion of alanyl-glutamine was associated with an improved time to exhaustion during hot and hypohydrated conditions. This observation suggests that supplementation drinks with alanyl-glutamine are likely to provide an ergogenic benefit for runners who are competing in hot climatic conditions in a hypohydrated state.

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## **CHAPTER 9**

### **KEY FINDINGS & RECOMMENDATIONS**

## **9.0 KEY FINDINGS**

### **9.0.1 Hypohydration and Prolonged Exercise Performance (Study One)**

1. Elite Kenyan distance runners completed the half and full marathons during SCKL Marathon 2009 with a BW loss (up to 3%) in warm humid climatic conditions (26°C, 90% rh). They completed the races as the fast finishers but ran slower than their previous best performance due to the adverse impact of environmental factors.
2. Elite Kenyan distance runners are able to compensate well by increasing the sweating rate regardless of the volume of fluid ingested or percentage of BW loss in the warm and humid conditions (26°C, 90%rh).

### **9.0.2 Running Economy with Hypohydrated and Simulated Hyperhydrated State (Study Two)**

1.  $\dot{V}O_2$  ( $\text{mL.kg}^{-1}.\text{min}^{-1}$  and  $\text{mL.kg}^{-0.75}.\text{min}^{-1}$ ), caloric unit cost,  $C_R$  ( $\text{kcal.kg}^{-1}.\text{km}^{-1}$ ) and gross oxygen cost of running ( $\text{mL.kg}^{-1}.\text{km}^{-1}$ ) in hypohydrated state (-3 and -4% BW) are significantly greater than when in a euhydrated state.
2. Simulated hyperhydration does not increase the oxygen cost of running proportionally with the added gross weight (+3 and +4% BW).

### 9.0.3 Hypohydration and Hyperthermia: Circulatory and thermoregulatory responses (Study Three)

1. Hypohydration (~ 4% BW loss) significantly increases cardiovascular strain (i.e. increases HR and decreases SV,  $\dot{Q}$  and MAP) during prolonged exercise in hot climatic conditions (35°C, 40% rh) whereas there is a limited effect of hypohydration on exercise performance in cool conditions (10°C, 35% rh).
2. The reduction in  $\dot{V}O_{2\max}$  at the point of exhaustion immediately following prolonged exercise in the heat with progressive hypohydration is associated with a lower HR<sub>max</sub>, which is tightly coupled with the decline in SV and  $\dot{Q}$ .
3. A marked increase in thermal strain (i.e. T<sub>re</sub>,  $\bar{T}_{sk}$  and SkBF) during prolonged exercise in the heat with mild and moderate hypohydration (~2.2% BW loss and ~ 4.4% BW loss, respectively) significantly impairs running capacity. The time to exhaustion in hot conditions was significantly shorter than thermoneutral and cool conditions.
4. The running performance time in the heat is impaired with ~4.5% BW loss of hypohydration (~25 min shorter than thermoneutral with euhydrated conditions trial). However, this decrement of performance (-46.4%) is not associated with a high T<sub>re</sub> (~39.2°C).
5. The reduction in plasma volume (-19.8%) due to the diuresis effect and sweat loss during prolonged exercise is associated with a significant increase in plasma viscosity, consequently exacerbating the cardiovascular strain.
6. A cool environment limits the increase in T<sub>re</sub> (i.e. ~38.8 °C at fatigue) and  $\bar{T}_{sk}$  (i.e. ~ 28°C at fatigue) despite hypohydration (~4% BW loss) is being attained, consequently maintaining the performance time of prolonged exercise.
7. Every 10°C of ambient temperature increment is in proportion with a ~2°C

increase in  $\bar{T}_{sk}$ , which impairs prolonged exercise performance in the heat irrespective of a mild to moderate level of hypohydration (3 to 4.5% BW loss).

#### **9.0.4 Hypohydration and Thermotolerance (Study Four)**

1. The administration of alanyl-glutamine (0.2 g.kg<sup>-1</sup> body mass per litre) has attenuated the exercise-induced decrease in plasma glutamine concentration during prolonged exercise in the heat (35°C, 40% rh) while in a hypohydrated state (~4% BW loss).
2. The induction of plasma [HSP] expression is depressed by hypohydration. However, with the alanyl-glutamine ingestion a greater plasma HSP 72 expression is induced during prolonged exercise in the heat.
3. The ingestion of alanyl-glutamine as an oral rehydration beverage has no significant effect on plasma [Na<sup>+</sup>], urine specific gravity, serum osmolality, plasma viscosity, plasma [total protein] and plasma [renin].

## **9.1 RECOMMENDATIONS**

1. Further studies should investigate whether there is any adaptive metabolic response to hypohydration in elite runners with regular training in hot and hypohydrated conditions. The adaptive responses in elite runners may increase their tolerance to high body temperatures and body water deficits during prolonged exercise in warm / hot conditions.
2. Investigating the relationship between physiological responses and behavioural perception (i.e. thirst and RPE) may help in understanding the factors which elicit a sensation of fatigue during prolonged exercise in the heat.
3. The combining carbohydrate and glutamine ingestion may have an influence on blood glucose concentration and increase time to fatigue during prolonged submaximal exercise. Further research is required to elucidate the relationship between combined drinks (glucose and glutamine) and the induction of plasma HSP 72 expression, which may enhance thermotolerance during prolonged exercise in the heat.

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