THE UNIVERSITY OF HULL

The Effect of Short-Term Heat Acclimation with a Permissive Dehydration Stimulus in Female Team Sport Players

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ABSTRACT

Introduction: Repeated heat exposure can facilitate physiological adaptation to heat during intermittent exercise; however, there is limited information available regarding female cohorts. This has implications on the health and safety guidelines for females during heat exposure. Studies using short-term heat acclimation (STHA) with permissive dehydration have reported improved physiological response and performance during heat exposure but have tended to use male participants. Therefore, the aim of this study was to investigate the efficacy of STHA over 5-days using the controlled hyperthermia technique (with permissive dehydration), on an intermittent heat stress test (HST), using a female cohort and controlling for menstrual cycle.

Methods: Eight healthy, active, moderately trained females (mean [SD]; age 22.6 [3.0] y; height 166.2 [6.0] cm; body mass 62.3 [9.0] kg; $\dot{V}O_{2\,max}$ 43.2 [8.2] $mL \cdot kg^{-1} \cdot min^{-1}$). The HST (31.0˚C, 50% RH) consisted of 9x5min (45 mins) of intermittent exercise (individualised standing, walking, jogging, low-, medium-, and high-intensity running on a motorised treadmill) finishing with a 6s maximal sprint on a cycle ergometer. The exercise intensities were adapted from the match-play dynamics of female collegiate football players. Participants completed two HSTs (HST1 and HST2), separated by one week, with no STHA, as a control (C) trial. This was followed by 90 mins dehydration (no fluid), heat acclimation for 5 consecutive days (39.5˚C, 60% RH), using the controlled hyperthermia technique (rectal temperature [T_{re}] 38.5˚C). Participants completed a final HST (HST3), within one week of the STHA. The HST2 and HST3 trials were in the same week of each participant's menstrual phase determined by self-reported menstrual cycle and plasma 17β-estradiol.

Results: Post (HST3) vs. Pre- (HST2) STHA, showed a reduced rectal temperature (T_{re}) at 45-min by 0.20˚C (95%CI: -0.31 to -0.05˚C; p = 0.01; *d* = 1.13); mean skin temperature ($\overline{T}_{sk}$) (-0.47; -0.82 to -0.12°C; P = 0.02; *d* = 1.06) and mean body temperature ($\overline{T}_b$) (-0.21; -0.31 to -0.11°C; P = 0.001; *d* = 1.28). There was limited change (P > 0.05) for these measures in the HST1 vs. HST2 C trial. Resting cardiac frequency decreased by 11 $b \cdot min^{-1}$ (-16 to -5 $b \cdot min^{-1}$; P = 0.004; *d* = 0.81) and by 3 $b \cdot min^{-1}$ at 45-min (-8 to 0 $b \cdot min^{-1}$; P = 0.07; *d* = 0.65). There was an increase in percentage plasma volume (%PV) change post-STHA by 8.65% (-1.21 to 18.51%; P = 0.007; *d* = 1.26) but limited change in C (P > 0.05). There was an increase in mean average power (MAvP) across all 9 sprints by 41W (3 to 80W; P = 0.04; *d* = 0.18) but limited change in C (P > 0.05).

Discussion and conclusion: Short-term heat acclimation (5 days), with permissive dehydration, using the controlled-hyperthermia technique leads to physiological adaptation during intermittent exercise in the heat, in moderately trained females when controlling for menstrual cycle.

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LIST OF ABBREVIATIONS

RH	Relative humidity [%]
T_a	Ambient Temperature [^{o}C]
T_{db}	Dry Bulb Temperature [^{o}C]
WBGT	Wet Bulb Globe Temperature [^{o}C]
T_b	Mean body temperature [^{o}C]
T_c	Core temperature [^{o}C]
T_{re}	Rectal temperature [^{o}C]
$\overline{T}_{sk}$	Mean skin temperature [^{o}C]
BV	Blood volume [mL·kg^{-1}]
PV	Plasma volume [mL·kg^{-1}]
$\dot{m}_{sw}$	Mean sweat rate [mg·cm^{-2}·min^{-1}]
f_c	Cardiac frequency [b·min^{-1}]
$\dot{V}O_2$	Oxygen consumption [L·min^{-1} or mL·kg^{-1}·min^{-1}]
$\dot{V}O_{2\,max}$	Maximum oxygen uptake [L·min^{-1} or mL·kg^{-1}·min^{-1}]
$\dot{Q}$	Cardiac output [L·min^{-1}]
V_s	Stroke volume [mL]
PSI	Physiological strain index
$[TP]_p$	Total protein concentration (plasma)

$[alb]_p$	Albumin concentration (plasma)
EHRI	Exertional heat-related illness
STHA	Short-term heat acclimation
MTHA	Medium-term heat acclimation
LTHA	Long-term heat acclimation
E_{req}	Required evaporation for heat balance
E_{max}	Maximal evaporative potential within given environment
$E_{sk,max}$	Individuals maximum evaporative capacity

1 Introduction

Women's sport is becoming increasingly popular, especially that of football (Hong, 2003). The requirements of the female game are similar to that of the male game characterised by short bursts of high intensity and near maximal running interspersed with periods of lower intensity activity (Burke & Hawley, 1997). Several competitions such as the 2016 Olympic Games and the 2019 FIFA World Cup occurring in hot, humid environments (Hong, 2003). It is well documented that as environmental conditions become hotter and more humid athletic performance is attenuated (Galloway & Maughan, 1997; Gregson, Drust, Batterham, & Cable, 2002; Parkin, Carey, Zhao, & Febbraio, 1999).

Heat acclimation can be described as the physiological changes that occur as a result of repeated heat exposure in an artificial environment (Aoyagi, McLellan, & Shephard, 1997). Heat acclimation has been shown to yield a similar response to that of acclimatisation, the adaption to heat in a natural environment (L. E. Armstrong & Maresh, 2012), and it has been suggested that acclimatisation could provide athletes with a more complete response (J. D. Périard, Racinais, & Sawka, 2015). Heat acclimation, however, is widely regarded as one of the most successful means of physically adapting to hot environments in which heat significantly impacts upon the ability to maintain a high level of physical performance (Garrett, Creasy, Rehrer, Patterson, & Cotter, 2012; Kelly, Gastin, Dwyer, Sostaric, & Snow, 2016; Lorenzo, Halliwill, Sawka, & Minson, 2010; Ruddock et al., 2016; Sunderland, Morris, & Nevill, 2008) when athletes and workers such as those in the military are relocated to environments in which heat exposure is inevitable under short notification (Garrett, Rehrer, & Patterson, 2011).

It is recognised that a majority of significant cardiovascular adaptations, ~75% , can be attained in the short-term (> 7days) (Racinais, Alonso, Coutts, Flouris, Girard, González-Alonso, et al., 2015) using the controlled hyperthermia stimulus, that is elevating core

temperature to a set point, usually 38.5˚C, and maintaining said temperature for a set duration of time, usually 90 minutes (Garrett et al., 2011; N. A. S. Taylor, 2000). Most literature however focusses on the utilisation of constant workload techniques in which participants work at a set velocity based upon $\dot{V}O_{2\,max}$ or %HR (Gibson et al., 2015; C. J. Greenleaf & Greenleaf, 1970; Pandolf, Burse, & Goldman, 1977; Pandolf et al., 1988; Radakovic et al., 2007). Whilst such a technique has its merits it dose pose a differing heat strain from participant to participant constant workload techniques result in reduced strain as time elapses (N. A. S. Taylor, 2000). The controlled hyperthermia technique achieves an equal strain amongst participants for the duration of the acclimation period (Garrett et al., 2011) which could be of great benefit as elevated core temperature during exposure is a key consideration during heat acclimation (Hessemer, Zeh, & Brück, 1986).

It has recently been evidenced that males and females may acclimate differently (Mee, Gibson, Doust, & Maxwell, 2015). Most literature has focussed solely on male populations however in modern society there is a requirement to ensure that females are given equal opportunity to succeed in all professions. Data from male cohorts should therefore not be deemed sufficient to draw conclusions to how best acclimate females to the heat.

2 Literature Review

It is commonly recognised that the female body thermoregulates differently throughout the menstrual cycle (Grucza et al., 1993; Rogers & Baker, 1997; Stephenson & Kolka, 1985) and thus athletic performance can vary as a result (Avellini et al., 1979; Janse de Jonge et al., 2012; Tenaglia et al., 1999). Although in a more well-trained population these performance differences are often minor (Sunderland & Nevill, 2003), one factor still significantly affects athletic performance between menstrual cycle phases regardless of training status: heat. One way to offset such declines in performance in hot and/or humid environments is heat

acclimation (Chalmers, Esterman, Eston, Bowering, & Norton, 2014; Garrett et al., 2011; N. A. S. Taylor & Cotter, 2006). Repeated heat exposure can elicit thermoregulatory and cardiovascular adaptation that can improve performance in such environments and decrease the risk of EHRI. This section aims to review the effect of the menstrual cycle on performance in the heat, and how heat acclimation can maximise athletic performance in such conditions.

2.1 Dynamics of women's football

Despite its mass popularity amongst the male population, it wasn't until 1971 that the first FIFA-recognised women's football match was played (FIFA, 2012). Since then, the growth of female football has been phenomenal and rapid. In 2012, a 32% increase in participation was reported for the 10 year period from 2002 with 29 million female footballers worldwide (FIFA., 2012; Hong, 2003). This growth has made football one of the most popular team sports amongst the female population. In Britain alone football has surpassed netball in popularity, traditionally the most popular female team sport. This is mirrored in other nations such as Canada; traditionally known as a hockey-playing nation, participation in football has surpassed it (Hong, 2003).

Football is habitually a game that is intermittent in nature, demanding a high level of physical fitness, and characterised by short bouts of high-intensity and near maximal running interspersed with lower intensity activity caused by the movements and skills involved in gameplay. The female game requirements are similar to the male game; a multitude of interactions between motor skills such as muscular strength, endurance and power (Cometti, Maffiuletti, Pousson, Chatard, & Maffulli, 2001; Requena et al., 2009; Wisloeff, Helgerud, & Hoff, 1998), speed (Young, Benton, & Pryor, 2001), acceleration (Spinks, Murphy, Spinks, & Lockie, 2007), repeated sprint ability (Bishop, Girard, & Mendez-Villanueva, 2011;

Girard, Mendez-Villanueva, & Bishop, 2011; Impellizzeri et al., 2008), agility (Little & Williams, 2005; Sheppard, Young, Doyle, Sheppard, & Newton, 2006) and flexibility (Gleim & McHugh, 1997), as well as a high level of cognitive functioning are required for successful performance (Burke & Hawley, 1997).

During match play, elite female football players typically cover ~10km (Andersson et al., 2010; Datson et al., 2014; Gabbett & Mulvey, 2008; Hewitt, 2007; Krustrup et al., 2005). High speed running is considered to be of particular interest due to its relationship with training status (Krustrup et al., 2003) and is a distinguishing factor between playing standards (Mohr et al., 2008; Mohr et al., 2004), with elite females covering ~1.53-1.68km (Andersson et al., 2010; Datson et al., 2014; Krustrup et al., 2009; Mohr et al., 2008) at such a level.

Typically, high intensity running distances increase as the level of competition increases. For example in one study top class players covered 28% more high-intensity running distance and 24% more sprinting distance, despite a similar distance overall (10.33 ± 0.15 vs. 10.44 ± 0.15km), than high-level players (Mohr et al., 2008). Likewise, when observing the same players at domestic (DOM) and international (INT) levels, it was found again that overall match play distance covered was similar (DOM: 9.7 ± 1.4; INT: 9.9 ± 1.8km), yet distance covered at high-intensity running and sprinting were respectively 13% and 14% greater in INT than in DOM competition (Andersson et al., 2010).

It is well documented that exercise performance is attenuated at high ambient temperatures (Galloway & Maughan, 1997; Gregson et al., 2002; Parkin et al., 1999) as a result of a greater increase in core (González-Alonso et al., 1999) and muscle (Drust et al., 2005) temperatures. The worldwide popularity of football is huge, resulting in competitive matches, such as the 2016 Olympic Games in Brazil and the upcoming 2019 FIFA Women's World Cup in

France, being held in a whole host of environmental conditions, some of which can be in excess of 30°C, with or without high levels of relative humidity (Özgünen et al., 2010).

Fatigue in football is potentiated by hot environmental conditions, as is evidenced by decreased high-intensity running (Mohr et al., 2012), maximum sprint performance (Mohr et al., 2010) and jump ability (Mohr & Krustrup, 2013). Furthermore, female hockey players sport specific skill was also shown to decline following high intensity activity in a hot environment compared with a cool one (Sunderland & Nevill, 2003).

During match play, exercise intensity is driven by the dynamics of play, often determined by tactics It is commonly reported that the most intense 5 minutes of play is followed by a period of low intensity play (Mohr et al., 2003). It is therefore likely that pacing strategies are used to ensure game completion, particularly in the heat (Aughey et al., 2014; Mohr et al., 2012; Morante & Brotherhood, 2008).

In the heat, it has been observed that during a hot (HOT; 43˚C) vs. a thermoneutral (CON; 21˚C) football match, players work rate was greater in the first half of the CON match than the HOT with 41% less high-intensity running in HOT (Mohr et al., 2012). This led to greater cumulative fatigue in CON and a better maintenance of exercise intensity in the HOT match (Mohr et al., 2012). Similarly, in Australian Rules Football, it has been observed during hot conditions (27 ± 2˚C) vs. cool conditions (17 ± 4˚C) that players reduced running volume thus preserving the performance of high-intensity activities (Aughey et al., 2014).

2.2 Physiology of thermoregulation

Through a complex interplay between the central nervous system (CNS), cardiovascular system and the skin (Casa et al., 2015), the human body aims to regulate its core temperature within a very narrow range (36-37.5˚C) despite large variations in ambient temperatures (Grubenhoff, du Ford, & Roosevelt, 2007).

The preoptic anterior hypothalamus houses the CNS temperature-regulation centre in which thermal integration occurs (Boulant, 2000). Reacting to thermal stimulation, peripheral thermoreceptors relay thermoafferent information to the CNS (Gagnon & Kenny, 2012). After integration of thermal information, thermoefferent signals are sent via the sympathetic nervous system in an open feedback loop to the appropriate effector organs which control the body's heat exchange with the environment (Casa et al., 2015; Gagnon & Kenny, 2012). Peripheral feedback to the hypothalamus allows the body to adjust its heat-transfer responses as necessary according to the environmental conditions faced (Casa et al., 2015).

Core body temperature is the product of metabolic heat production and the transfer of heat between the body its external environment. The following heat balance equation allows us to determine heat storage rate (L. E. Armstrong, 2003):

$$S = M - (\pm Work) \pm E \ \pm R \ \pm C \ \pm K$$

Where S = heat storage, M = metabolic heat production, E = evaporation, R = radiation, C = convection and K = conduction.

Heat can be exchanged between the body and its external environment via four main mechanisms, the success and direction of heat exchange being dependent upon the heat and water vapour pressure gradients present:

- Conduction represents direct heat transfer from a warmer surface to a cooler surface (Casa et al., 2015; Grubenhoff et al., 2007; Howe & Boden, 2007). The conductive properties of the objects, the percentage of the contact surface and the heat gradient influence conductive heat exchange (L. E. Armstrong et al., 2007; Becker & Stewart, 2011).
- Convection represents the transfer of heat by circulating air or water. Air velocity plays a role in the rate of convective heat transfer since a greater air flow will more

quickly displace the warmer air surrounding the body with cooler air (Becker & Stewart, 2011). For this reason, sports associated with a high velocity, such as cycling, are less affected by high temperatures and humidity (Bergeron et al., 2012; Nybo, 2010). Similarly, the rate of convection in water is determined by the speed of the water and the temperature gradient between the water and skin. Therefore in water temperatures below 31˚C heat illness is unlikely (Bergeron et al., 2012).

- Radiation represents the direct exchange of heat between the body and the external environment via electromagnetic waves (Howe & Boden, 2007; Nichols, 2014). Radiative heat loss is most profound when ambient temperatures are below 20˚C as the temperature gradient favours heat loss. As radiant temperature rises, heat transfer direction can change with radiation causing heat gain rather than dissipation (Barrow & Clark, 1998).
- Evaporation represents the vaporisation of sweat from the skin's surface which provides the most effective means of heat dissipation during exercise (Casa et al., 2015). In optimal conditions, up to 600kcal of heat is dissipated via evaporation (Glazer, 2005). High humidity represents a high water vapour pressure of the surrounding air and, in such conditions, evaporative heat loss is inhibited (Bergeron et al., 2012; Maughan, Otani, & Watson, 2012). Like convection, wind speed influences evaporative heat transfer via the displacement of saturated air surrounding the skin's surface (Bergeron et al., 2012).

Mean body temperature (T_b) is a function of both core and skin temperature, calculated using a weighted summation of core and mean skin temperature, which is heavily weighted towards core temperature in heat stress. The effector responses, cutaneous vasodilation and sweating occur when T_b reaches an onset threshold for their activation, here a proportional increase in thermoeffector output will occur until a plateau is reached. The thernosensitivity of the

response is the increase in in the effector response per unit rise in T_b, thus the greater the relationship the greater the sensitivity. Both cutaneous vasodilation and sweating (effector responses) have onset thresholds, which are described as the mean body temperature at which a sustained increase in thermoeffector output occurs, and thermosensitivities, which are described as the rate at which thermoeffector output changes as a function of the increase in body temperature.

2.3 Thermoregulation during heat stress

At the onset of physical exercise there is a rapid increase in the rate of metabolic heat production. This is not immediately matched by whole-body heat loss (Webb, 1995) and results in an increase in body temperature. Whole-body heat loss mechanisms, cutaneous vasodilation and sweating, are activated when body temperature exceeds their respective thresholds and act to maintain thermal homeostasis.

As T_a approaches that of the skin's surface, heat gradients are reduced, which decreases the effectiveness of the heat loss mechanisms conduction, convection and radiation. If T_a exceeds that of the skin surface, the heat gradient is reversed which can result in heat being gained, rather than lost, by the body. At this point evaporation of sweat becomes the only mechanism for heat dissipation and thus whole-body cooling.

The environmental conditions in which one is exposed dictate rate of the rate of heat production, this presents an evaporative requirement to maintain heat balance (E_{req}), as well as a maximal evaporative capacity (E_{max}). When $E_{max} > E_{req}$ one can successfully dissipate the necessary heat to maintain heat balance. In such a compensable environment, sudomotor activity is driven by E_{req} (Jay et al., 2011; Shapiro, Pandolf, & Goldman, 1982).

If, however, the environmental conditions (i.e. temperature and/or humidity) and metabolic heat production (from increased physical activity) (E_{req}) exceed one's maximal evaporative

capacity ($E_{sk,max}$), i.e. $E_{sk,max} < E_{req}$, sudomotor activity is driven by one's maximal sweat rate (Gagnon & Kenny, 2012). Thus, an uncompensable heat stress scenario arises and heat balance cannot be maintained (Gagnon & Kenny, 2012).

In reality, sweat efficiency decreases as E_{req} approaches that of $E_{sk,\,req}$ in a given environment (Candas, Libert, & Vogt, 1979). Therefore, it is suggested that the ratio between E_{req} and $E_{sk,max}$ best describes the level of sudomotor activity with a given environment (Bain, Deren, & Jay, 2011).

2.3.1 Critical temperature theory

The critical temperature theory simply suggests that an individual's volitional exhaustion occurs at a consistent core temperature even when experimental conditions are manipulated (Cheung & Sleivert, 2004) and may be a key limiting factor for physical performance in the heat (Cheung & McLellan, 1998).

This was first observed in rats (n =8) exercising at different temperatures. Although running time differed between trials, at exhaustion, brain (40.1-40.2˚C) and abdominal (39.8-39.9˚C) temperatures were consistent between trials (Fuller et al., 1998). Cheung and McLellan (1998) then observed this phenomenon in highly fit (HF) and moderately fit (MF) participants. The HF group had a lower initial T_{re} and a greater end-exercise T_{re} than the MF group. Despite differences in each group's temperature at volitional exhaustion, these temperatures remained the same even after a 10-day heat acclimation protocol (1 hour treadmill walk, 4.8km/h, 3-7% gradient; 40˚C, 30% RH) (Cheung & McLellan, 1998).

Another investigation looked at whether starting temperature affected temperature at volitional exhaustion (González-Alonso et al., 1999). This team observed consistent thigh (40.7-40.9˚C) and core (40.1-40.2˚C) temperatures at volitional exhaustion in trained males

cycling (60% $\dot{V}O_{2\,max}$) in 40˚C, despite differing starting core temperatures (35.9 ± 0.2, 37.4 ± 0.1 and 38.2 ± 0.1˚C), a result similarly found in rats (Walters et al., 2000).

It is important to recognise that, although volitional exhaustion appears to occur at the same temperature within an individual and this temperature appears not to alter, even after acclimation (Cheung & McLellan, 1998), it is feasible to suggest that we can manipulate how long it takes to reach this critical temperature by improving heat loss during exercise.

In summary, evidence suggests that individuals will voluntarily fatigue at a critical temperature (Cheung & Sleivert, 2004), despite ambient conditions (Fuller et al., 1998), starting temperature (González-Alonso et al., 1999; Walters et al., 2000) and acclimation status (Cheung & McLellan, 1998). Fitter individuals appear to be able to cope at a higher T_c (González-Alonso et al., 1999). Methods of lengthening the time it takes to reach such a critical temperature e.g. heat acclimation, are vital to the success of athletic performance and endurance in the heat.

2.4 Heat related illness

Exertional heat related illnesses, namely heat stroke, represent the third most common cause of death amongst athletes, behind only cardiac disorders and severe neck/head trauma (Barrow & Clark, 1998; Howe & Boden, 2007). Early detection and treatment of milder EHRI can be crucial to prevent the development of potentially fatal heat stroke (Coris, Ramirez, & Van Durme, 2004; Howe & Boden, 2007).

Exertional heat related illnesses are defined as a continuum of pathological states that occur during times of environmental stress, such as high ambient temperatures and humidity, ranging from very mild to fatal (Howe & Boden, 2007; Nichols, 2014). Female athletes exposed to considerable heat stress may be at a greater risk of developing EHRI due to their

impaired ability to evaporate sweat when the requirement for heat dissipation for heat balance is high.

In this section, a brief summary of each EHRI will be presented; full comprehensive reviews concerning the recognition and treatment of EHRI are extensively available (Barrow & Clark, 1998; Becker & Stewart, 2011; Casa et al., 2015; Coris et al., 2004; Grubenhoff et al., 2007; Howe & Boden, 2007; Nichols, 2014). Table 1-1. presents the main associated signs, symptoms and core body temperature of potential ERHI associated with athletic competition.

Table 1-1: Criteria of common exertional heat related illnesses. Adapted from Howe & Boden (2007)

Heat related illness	**Associated core temperature °C**	**Associated signs**	**Associated symptoms**
Heat Oedema	Normal (37.0)	Swelling of extremities in oedema dependent areas (e.g. hands, wrists, ankles, feet)	None
Heat rash	Normal	Papulovesicular explosion on clothed areas	Pruritic rash
Heat syncope	Normal	Loss of consciousness, rapid return of neurological status upon return to supine position	Dizziness, generalised weakness
Heat cramps	37-40°C	Possible firm affected muscles upon palpitation	Painful contractions in affected muscle
Heat exhaustion	37-40°C	Cold clammy skin, flushed, normal mental status, profuse sweating	Dizziness, fatigue, headache, malaise, nausea, vomiting
Exercise associated hyponatremia	37-40°C	Na^+ < 135 mg/dL, CNS disturbance (confusion, ataxia, irritability, coma)	Peripheral oedema, possible history of heat exhaustion symptoms before onset of mental status changes
Exertional rhabdomyolysis	37 to >40°C	Increased creatine kinase levels, cardiac arrhythmias, myoglobinuria, increased K^+, renal injury	Severe muscular pain, exertional heat stroke
Exertional heat stroke	>40°C	Hot skin with or without sweating, CNS disturbance	Possible history of heat exhaustion symptoms before mental status change

2.4.1 Heat oedema

Heat oedema is most commonly associated with unacclimated (Barrow & Clark, 1998) and aged (Grubenhoff et al., 2007) populations, and is recognised as the mildest EHRI (Coris et al., 2004). Transient peripheral dilation from prolonged sitting or standing in the heat is complicated by orthostatic pooling resulting in a swelling of the extremities (Barrow & Clark, 1998; Coris et al., 2004).

2.4.2 Heat rash

Heat rash, or miliaria rubra (prickly heat) is the result of profuse sweating (Howe & Boden, 2007). Sweat saturates the skin, thus clogging sweat ducts, and then leaks into the epidermis producing a pruritic papulovesicular rash over the clotted area (Howe & Boden, 2007; Nichols, 2014).

2.4.3 Heat syncope

Heat syncope is the result of peripheral vasodilation and venous blood pooling during heat stress (Howe & Boden, 2007). A sudden change in posture, or prolonged standing after physical exertion in the heat results in orthostatic hypotension (Casa et al., 2015; Coris et al., 2004). Heat syncope often occurs in unacclimated individuals upon acute exposure to heat stress before plasma volume expansion occurs (Casa et al., 2015).

2.4.4 Heat cramps

Heat cramps are described as ‘painful sporadic involuntary muscle contractions of skeletal muscle occurring during or after exercise (Schwellnus, Derman, & Noakes, 1997).

Heat cramps are associated with a triad of underpinning states (L. E. Armstrong et al., 2007; Eichner, 2007); electrolyte depletion, dehydration and muscular fatigue and it is reported that the onset of cramps can be felt (Bergeron, 1996). Heat cramps are associated therefore with prolonged strenuous activity in which high sweat rates are consistent and duration is

unpredictable, such as American football (Stofan et al., 2005), tennis (Bergeron, 1996, 2003) and endurance/ultra-endurance events (Maughan, 1986; Schwellnus, 2007; Schwellnus, Allie, Derman, & Collins, 2011; Schwellnus, Drew, & Collins, 2011; Schwellnus, Nicol, Laubscher, & Noakes, 2004; Sulzer, Schwellnus, & Noakes, 2005).

2.4.5 Heat exhaustion

A failure of cardiovascular response to workload, typically potentiated by heat stress and dehydration, causes heat exhaustion, the most common form of EHRI. By definition $T_c <$ 40.5˚C (Casa et al., 2015; Coris et al., 2004) and normal mental status is observed (Casa et al., 2015; Howe & Boden, 2007). The detection and immediate treatment of heat exhaustion is critical to prevent the development of potentially fatal heat stroke (Nichols, 2014).

2.4.6 Exercise associated hyponatremia

Exercise associated hyponatremia (EAH) describes pathological hyponatremia either during exercise, immediately after it, or up to 24-hours afterwards. The main characteristic of EAH is lowered plasma Na^+ concentration which can be either asymptomatic (<135mmol/L) (Hew-Butler et al., 2015) or symptomatic (<125mmol/L) (Rosner & Kirven, 2007).

Typically dilutional in nature (Hew-Butler et al., 2015; Rosner, 2009), EAH results from the consumption of hypotonic fluids in excess of fluid losses (Noakes et al., 2005; Noakes, Wilson, Gray, Lambert, & Dennis, 2001; Speedy, Noakes, Boswell, et al., 2001; Speedy et al., 1999). Positive fluid balance from excess fluid consumption usually results in the maintenance or net gain in body mass, which has a negative correlation with serum sodium concentration (Almond et al., 2005; Mettler et al., 2008; Noakes et al., 2005; Sharwood, Collins, Goedecke, Wilson, & Noakes, 2002; Speedy et al., 1999).

Since it is recommended that 0.8-1.6L/h fluid should be consumed (Gisolfi & Duchman, 1992), event organisers and coaches should stress that these recommendations are not a one-

size-fits-all. In fact, in longer endurance events as much as 0.5L/h may present a fluid overload for many female athletes (Knechtle et al., 2010; Speedy, Noakes, Boswell, et al., 2001; Speedy, Noakes, Kimber, et al., 2001).

2.4.7 Exertional rhabdomyolysis

Exertional rhabdomyolysis (ER) is the severe breakdown of skeletal muscle tissue. In unacclimated individuals, unaccustomed high intensity exercise such as those completed in trials (Harriston, 2004) and pre-season trials (Cleary, Ruiz, Eberman, Mitchell, & Binkley, 2007; Cleary, Sadowski, Lee, Miller, & Nichols, 2011; Moeckel-Cole & Clarkson, 2009; O'Connor, 2006; Oh, Laidler, Fiala, & Hedberg, 2012; Ruiz, Mitchell, Eberman, & Cleary, 2013) such as excessive press ups, chair dips (Lin, Lin, Wang, & Leu, 2005; Oh et al., 2012) and squat jumps (Clarkson, 2006) in the heat, results in ATP depletion which, if unreversed, can cause muscle necrosis and cell death (Harriston, 2004). As myocytes are degenerated, large amounts of electrolytes, creatine kinase (CK), myoglobin and other substances leak into the circulatory system (Luck & Verbin, 2008).

2.4.8 Exertional heat stroke

Exertional heat stroke (EHS) is a potentially fatal medical condition characterised by core body temperature > 40.5˚C and CNS dysfunction (Glazer, 2005), and is often accompanied by skeletal muscle injury and multiple organ failure (Casa et al., 2015; Nichols, 2014). Exertional heat stroke is associated with thermoregulatory failure, only reversible by aggressive whole-body cooling in which cold-water immersion is considered ‘gold standard’ (Casa, Armstrong, Kenny, O'Connor, & Huggins, 2012; Casa, Kenny, & Taylor, 2012; Casa et al., 2007; N. A. Taylor, Caldwell, Van den Heuvel , & Patterson, 2008). Although typically associated with exercise in significant heat stress, EHS can develop in more temperate environments (Casa et al., 2015; Nichols, 2014).

2.5 The effect of the menstrual cycle on athletic performance in the heat

It has long been recognised that the menstrual cycle plays a significant role in athletic performance (Avellini et al., 1979; Janse de Jonge et al., 2012; Tenaglia et al., 1999). During the luteal phase of the menstrual cycle and OCP usage, when progesterone concentrations are at their highest, T_c is regulated ~0.2-0.6˚C above that of the follicular phase (Grucza et al., 1993; Kolka & Stephenson, 1989; Rogers & Baker, 1997; Stephenson & Kolka, 1985). A parallel shift in the temperature threshold for effector responses cutaneous vasodilation and sweating is also present (Stachenfeld et al., 2000; Sunderland & Nevill, 2003). Such a shift could lead to a quicker elevation of core temperature to the 'critical temperature' in which volitional exhaustion when exercising in the heat (Tenaglia et al., 1999).

During exercise in the heat, such changes in thermoregulatory control have been shown to significantly hinder athletic performance, although only a few studies have compared exercise performance during multiple phases of normally menstruating females in the heat.

A ~16% reduction in tolerance time has been observed during the luteal phase vs the follicular phase when females alternated between walking at 4km/h and resting for 15-minute blocks in nuclear, biological and chemical (NBC) protective clothing in the heat (Tenaglia et al., 1999). Similarly, when walking at 5.6km for up to 3 hours, females lasted for ~11% less time than they did in the follicular phase (Avellini et al., 1979). This finding during constant power exercise is not universal however. A third constant exercise stress test, using high thermal resistance clothing to produce heat stress, showed no difference in tolerance time to exercise at 40% $\dot{V}O_{2\,max}$ (Kolka & Stephenson, 1997).

Further observations that menstrual cycle phase does not affect athletic performance has been observed during high-intensity exercise in the heat (Sunderland & Nevill, 2003). In this study, the Loughborough Intermittent Shuttle Test (LIST) (Nicholas, Nuttall, & Williams,

2000; Nicholas, Williams, Lakomy, Phillips, & Nowitz, 1995) was employed until exhaustion. No difference was observed in distance between the menstrual phase (follicular: 6257 ± 1401m vs. luteal: 5861 ± 1035m).

In an endurance test to exhaustion in hot, humid conditions (32˚C, 60% RH), exercise time was ~6% greater in the follicular phase vs. the luteal phase (Janse de Jonge et al., 2012). Interestingly, the same decrement in endurance time was not observed during the same test to volitional exhaustion in temperate (20˚C, 45% RH) conditions (Janse de Jonge et al., 2012), which agrees with the notion that menstrual cycle phase doesn't affect athletic performance in temperate conditions but does in heat stress (Constantini et al., 2005).

A recent and significant finding concerning the effect of the menstrual cycle on athletic performance suggests that it is not heat *per se* that attenuates athletic performance during the luteal phase, but humidity (Lei et al., 2017). In this study, well-trained female athletes cycled at a fixed rate for 12 minutes, then as far as they could in 30 minutes on a stationary cycle ergometer in hot/dry (~35˚C, 40% RH) and hot/humid (~30˚C, 80% RH) conditions during the follicular and luteal phase. Participants could increase/decrease intensity at will after the fixed work rate period. In this respect, participants could behave in a way in which thermoregulatory strain is reduced. This was previously observed in males (Schlader, Raman, Morton, Stannard, & Mündel, 2011; Schlader, Simmons, Stannard, & Mündel, 2011).

Importantly, like previous observations, although menstrual cycle and environmental conditions affected thermoregulatory activity during fixed work exercise, this disappeared during self-paced exercise. A voluntary decline in power output reduced the metabolic heat production and thus lowered E_{req} (Gagnon, Crandall, & Kenny, 2013).

Due to the significantly varying methodological issues in performance studies, several considerations should be taken into account when determining whether menstrual cycle phase

will affect performance when competing in the heat. Elite female football players display a maximal aerobic capacity of 49-58ml/kg/min (Datson et al., 2014), and it is these highly trained athletes who tend to have less fluctuation between menstrual cycle phase and thus smaller differences in T_c between phases (Bullen et al., 1984; Kuwahara, Inoue, Abe, et al., 2005; Kuwahara, Inoue, Taniguchi, et al., 2005). Of the studies mentioned, only three can be considered to have used a well-trained cohort (Avellini et al., 1979; Lei et al., 2017; Sunderland & Nevill, 2003).

The work of Avellini et al (1979), only used 4 female participants, one of whom completed the 180 min walk without T_{re} or f_c reaching the point of ethical cut-off in either phase, while another terminated testing ~90 min during both phases due to dizziness.

Determination of menstrual cycle phase is key to ensure testing occurs in the correct phase. Commonly employed progesterone concentrations > 16nmol/L (Landgren, Undén, & Diczfalusy, 1980) and > 9.5nmol/L (Hatcher, 1988) have been cited as evidence that ovulation has occurred and thus the participant is in the luteal phase (Janse de Jonge et al., 2012; Lei et al., 2017; Tenaglia et al., 1999). Others (Avellini et al., 1979; Kolka & Stephenson, 1997) have used the elevation in T_c to determine that ovulation has occurred, although it has been suggested that, although elevated T_c correlates well with the elevated plasma progesterone in the luteal phase (Cargille, Ross, & Yoshimi, 1969), such a method is not entirely sufficient (Kolka & Stephenson, 1997). Given the range of 6.7-72.8nmol/L reported by Sunderland et al (2003), it is likely that at least one of their participants displayed progesterone concentrations below that suggestive of ovulation (Hatcher, 1988; Landgren et al., 1980) and therefore the thermoregulatory properties of progesterone may have been masked (Janse de Jonge et al., 2012).

In summary, it is likely that the most highly trained females indeed have a smaller bi-phasic alteration in T_c and no phase-related difference in the temperature onset threshold for effector responses during exercise. There is mixed evidence in the notion of reduced sex hormones in well-trained female athletes. When exercising at a fixed load, endurance tolerance time is probably reduced during the luteal phase because of a greater resting T_c, which would more quickly rise to its critical temperature. When participants can self-pace, there is a marked reduction in work rate, regardless of menstrual phase, as a result of impaired evaporate heat loss. This response is probably an anticipatory response, which will allow completion of the athletic performance and reduce the risk of heat related illness (Lei et al., 2017; Schlader, Raman, et al., 2011; Schlader, Simmons, et al., 2011).

2.6 Heat Acclimation

Heat acclimation represents the process in which one is repeatedly exposed to unaccustomed hot environments, with the aim of achieving physiological adaptation to improve exercise tolerance and reduce the risk of developing EHRI (Aoyagi et al., 1997). Such a process provides travelling athletes and military personnel with the necessary adaptations when time and financial restraints do not allow the period of acclimatisation necessary for adaptation within the natural environment (Garrett et al., 2011).

2.6.1 Thermal Environment

To sufficiently adapt to the conditions in which one will compete, it is necessary to acclimate in comparable conditions. In studies in which a direct comparison has been made between acclimation to dry heat and humid heat, it is wet-bulb globe temperature (WBGT) that has been used to equalise environments (Griefahn, 1997; Shvartz, Saar, Meyerstein, & Benor, 1973).

Shvartz and colleagues (1973) acclimated participants to either a hot-dry or hot-wet environment both of which elicited WBGT 35˚C for 6 days. In response to a work-heat stress test in a hot-dry environment, those acclimated in the hot-dry environment showed a significant difference in most parameters compared with a control group, whilst those acclimated in hot-wet only, showed a difference in skin temperature compared to the control group. In a longer acclimation process (15 days), Griefahn (1997) similarly used hot-dry and hot-wet acclimations with equal WBGT. Participants then alternated environments for two days, which imposed no additional strain.

Using WBGT to equalise stress level does not however necessarily equalise physiological strain (Griefahn, 1997), with humid heat providing a greater physiological strain than dry heat. Another approach to compare humid and dry heat acclimation exposures used the same rate of rise in T_c (Nielsen, Strange, Christensen, Warberg, & Saltin, 1997).

In hot-dry conditions (40˚C, 10% RH), exercise duration was extended by ~67% (Nielsen et al., 1993), whereas in hot-humid conditions (35˚C, 87% RH), exercise was increased by only ~17% (Nielsen et al., 1997). This lack of improvement in exercise tolerance time occurred despite both acclimation protocol eliciting a similar plasma volume expansion and increase in sweat rate. The deterioration in evaporative capacity with hot-humid environments, even after a period of acclimation, still limits evaporation of sweat, resulting in a quicker elevation to the critical core temperature at which volitional fatigue occurs.

In summary, athletes must acclimate to the climate in which they will be competing, due to the differing strains that hot-dry and hot-humid environments pose.

2.6.2 Exercise Intensity

For complete acclimation to occur it is typically recognised that at least 10 days of exposure are required (L. E. Armstrong & Maresh, 1991). However, as few as 4 heat exposures have

elicited partial heat acclimation (Sunderland et al., 2008). It has been suggested by Garrett and colleagues (2011), that STHA should represent < 7 days of exposure, MTHA 8-14 days and LTHA > 15 days.

2.6.2.1 Constant work-rate

The constant work-rate method uses a fixed exercise intensity protocol based upon a percentage of $\dot{V}O_{2\,max}$, f_c or peak power. Constant work-rate is a popular acclimation method amongst the military, where personnel are expected to share an equal workload independent of fitness (L. E. Armstrong & Maresh, 1991). Since the relative load placed upon participants varies this method provides a great deal of variability in physiological strain and thus in adaptive response amongst individuals (Garrett et al., 2011).

2.6.2.1.1 High-intensity work-rate

There is evidence to suggest that a shorter duration high-intensity acclimation protocol can provide similar adaptation to a longer duration low-intensity protocol. Participants undertaking 7 days' acclimation either exercising at 50% $\dot{V}O_{2\,max}$ for 60mins/day (T50) or 75% $\dot{V}O_{2\,max}$ for 30-35 mins/day (T75) demonstrated significant reduction in pre-exercise T_{re} and caloric expenditure (L. E. Armstrong & Maresh, 1991; Houmard et al., 1990). These results must be interpreted with some caution as many other markers of acclimation such as plasma volume, osmolality and protein or exercise sweat rates, were unchanged for either T50 or T75.

It has been shown that high-intensity intermittent (HIIT) exercise provides a greater thermal strain than continuous exercise (Nevill, Garrett, Maxwell, Parsons, & Norwitz, 1995; Nielsen et al., 1997) and that a rapid > 1.3˚C elevation in females' core temperature occurs within 30 minutes of HIIT running in the heat (Morris, Nevill, & Williams, 2000; Sunderland & Nevill, 2003). As HIIT elicits a large increase in T_c which is a key component to elicit heat

adaptation (L. E. Armstrong & Maresh, 1991; Houmard et al., 1990), and team sports are intermittent in nature, there has been some interest in the notion of using HIIT to elicit heat adaptation (Brade, Dawson, & Wallman, 2013; Kelly et al., 2016; Petersen et al., 2010; Sunderland et al., 2008).

Four studies have used HIIT protocol as a means of acclimation using 4-5 sessions (~30-39˚C, ~24-60% RH) either on consecutive (Petersen et al., 2010) or non-consecutive (Brade et al., 2013; Kelly et al., 2016; Sunderland et al., 2008) days.

In a group of female hockey players, a 33% increase in running distance, lower f_c and improved thermal comfort were observed after 4 sessions of the Loughborough Intermittent Shuttle Test (LIST) in the heat (Sunderland et al., 2008). Interestingly, T_{re} was lower post-acclimation in sets 1 and 2 of LIST and higher at the end of exercise indicating that the acclimation process increased the tolerable deep body temperature at which the athletes could work.

Partial heat acclimation, indicated by lower f_c, tympanic temperature and $\overline{T}_{sk}$ in conjunction with a greater sweat loss and sensitivity, was observed in moderately trained male team sports players (Brade et al., 2013). Acclimation followed repeated cycling sets of 3-minutes 80% PPO, with 1-minute passive rest, starting with 8 reps on day one (32 minutes) and adding a set a day, so that 12 reps were performed on day 5 (48 mins). No statistically significant performance improvements were observed in a repeated-sprint exercise protocol, although there were moderate effect sizes (d = 0.56-0.73) for work and 'possible' to 'very likely' benefits (Batterham & Hopkins, 2006) for all other performance measures (Brade et al., 2013).

Following a similar repeated sprint protocol (4-6 sets of 8 x 20 second maximal sprints, 10 seconds rest), male cricket players showed a moderate decrease in f_c and moderate to large

decrease in sweat electrolytes, but no other classic signs of heat acclimation were present (Petersen et al., 2010). Finally, 5 days of HIIT cycling (3 x 5 minutes' work alternating between 30 seconds 90% and 30% $\dot{V}O_{2\,peak}$) elicited limited acclimation benefits to Australian Rules footballers. A decrease in post-HIIT f_c was the only observed effect of such acclimation (Kelly et al., 2016).

The limited responses observed during HIIT acclimation sessions is not surprising given the recommendation of T_c elevation of > 1.0˚C (N. A. S. Taylor, 2000) and 60 minute acclimation duration (Racinais, Alonso, Coutts, Flouris, Girard, González-Alonso, et al., 2015). Elevation of T_c > 1.0˚C probably only occurred for ~15 mins in the 27-minute protocol used by Kelly et al (2016). Furthermore, Sunderland et al (2008) suggest HIIT training better represents the requirements of team sport yet three of the four HIIT studies (Brade et al., 2013; Kelly et al., 2016; Petersen et al., 2010) use a non-specific cycling exercise modality. Greater adaptation may have been observed if a protocol more specific to the given team sport had been used.

2.6.2.1.2 Multiple daily bouts of heat acclimation

The efficacy of STHA over 4-5 days has been well established (Garrett et al., 2012; Garrett, Goosens, Rehrer, Patterson, & Cotter, 2009; Garrett et al., 2014; Patterson, Stocks, & Taylor, 2004b; Sunderland et al., 2008). One of the fundamental advantages of STHA for military personnel and travelling athletes is the significant effects that can be induced when deployment or competition will be within days.

The notion of less than 4 days heat acclimation has been explored via multiple daily bouts of acclimation (Willmott, Gibson, Hayes, & Maxwell, 2016). 21 participants were randomly assigned to three groups who cycled for 45 minutes (50% $\dot{V}O_{2\,peak}$) once daily for 4 days (SDHA), twice daily for two days (TDHA) in 35˚C, 60% RH; or once daily for 4 days in

21˚C, 50% RH (CON). A 5km treadmill run (2km fixed 40% $\dot{V}O_{2\,peak}$, 3km self-paced) was used as a performance evaluation pre- and post-acclimation.

In the initial 2km, both heat acclimation groups displayed lower thermoregulatory and cardiovascular strain and improved comfort in the heat. However, no pre- to post-intervention differences were observed either within- or between-groups, suggesting partial acclimation only. Time to complete the self-paced 3km TT improved above the pre-defined typical error of measurement (2.1%) (Atkinson & Nevill, 1998), for both SDHA (2.8%) and TDHA (3.5%). Since there was no differentiation between SDHA and TDHA, the authors conclude that TDHA is as effective as SDHA (Willmott et al., 2016). Future research incorporating TDHA and using other acclimation methods such as the controlled hyperthermia model (see section 2.7.2.3), may show greater adaptation.

2.6.2.1.3 Effect of pre-cooling

Pre-cooling is an acute method of reducing T_c that can be comparable to heat acclimation (Duffield & Marino, 2007) during submaximal exercise in the heat (Duffield, Green, Castle, & Maxwell, 2010), with effects lasting from 70 seconds (Marsh & Sleivert, 1999) to 40 minutes (Castle et al., 2006). Two studies have evaluated whether pre-cooling methods confer any additional benefits when applied to heat acclimated participants.

Firstly Castle et al. (2011) conducted 3 repeated sprint protocols pre- and post-acclimation, one in control conditions (21.8 ± 2.2˚C, 42.8 ± 6.7% RH), and two in hot, humid conditions (33.3 ± 0.6˚C, 52.2 ±6.8% RH), one of which was preceded by 20 minutes pre-cooling with ice packs. Acclimation was 10 days cycling at 80rev/min, 50% $\dot{V}O_{2\,max}$ for 60 minutes. Pre-cooling prevented a decline in PPO during pre-acclimation trials, whereas a negative correlation between physiological strain index (PSI)and PPO (r = -0.46) was observed in the hot trial (Castle, Mackenzie, Maxwell, Webborn, & Watt, 2011). Post-acclimation, PPO was

significantly increased in the hot trial and no negative correlation was observed between PSI and PPO. More importantly, the pre-cooling trial conferred no additional performance enhancement on acclimated participants (Castle et al., 2011).

Later a similar experiment was conducted using an STHA method (Brade et al., 2013). The pre- and post-exercise trials were 70 minutes repeated sprints, acclimation was 3-minutes cycling at 80% PPO with one-minute rest between sets, starting on day one with 8 reps with one additional set per day. Both pre- and post-acclimation tests and acclimation were in ~35˚C, 60% RH. All physiological variables were improved after heat acclimation, although like (Castle et al., 2011), sprint dynamics were not enhanced further by additional pre-cooling (Brade et al., 2013).

It can therefore be suggested that both STHA (Brade et al., 2013) and MTHA (Castle et al., 2011) are both potent enough stimuli to improve repeated sprint performance in hot, humid environments. Whilst in unacclimated persons, pre-cooling can offset the detrimental effects of such environments, they offer no further performance enhancement in heat acclimated persons (Brade et al., 2013; Castle et al., 2011).

2.6.2.2 Self-paced exercise

The self-paced heat acclimation (SPHA) model has been used primarily in a military setting to enable large numbers of soldiers (~1600) to be acclimated at once (Miller, 1984). En route to a hot weather operation where individual monitoring was not feasible, participants could self-regulate their running workload (9 work periods, 5-10 minutes with 2-10 mins rest between sets = 100 minutes). It was anticipated that all participants would work at ~45% $\dot{V}O_{2\,max}$ and that intensity rather than duration would provide safeguarding measures against hyperthermia and other heat related illnesses (Miller, 1984). In environmentally controlled laboratory conditions, the same research team, using T_{re} and f_c as indices, observed

successful acclimation after just 6 days which could be maintained with one additional exposure every 3 days (Miller, 1984).

Further military research used 8 days heat acclimation (~41˚C, 39% RH). Days 1 and 8 were control, in which the participant ran for 100 mins in 9 blocks (block 1-2: ~3.2km/h, block 3-4: 5.7km/h, block 5-9: self-paced), replicated on both days. Days 2-7 (days 4-5 rest) consisted of the same procedure as described by Miller. (1984). During the controlled trials (days 1 and 8), $T_{re,}$ ΔT_{re}, f_c, Δf_c, $\overline{T}_{sk}$ and final $\overline{T}_{sk}$ were measured to assess whether participants were sufficiently adapted by SPHA. All parameters were significantly reduced on day 8 compared with day 1. However, no significant changes in any parameter were observed on days 2-7 (L. E. Armstrong et al., 1986). More importantly, symptoms of minor heat illness became less frequent as heat acclimation proceeded (L. E. Armstrong et al., 1986).

2.6.2.3 Controlled hyperthermia model

The controlled hyperthermia model aims to elevate and maintain T_c above that of the sweating threshold (Garrett et al., 2011; N. A. S. Taylor, 2000). Since the review of Garrett et al. (2011), much more emphasis has been placed on the controlled hyperthermia technique. Table 2-2 extends upon that of a previous review (Garrett et al., 2011), illustrating the physiological adaptations at rest and to exercise following short-, medium- and long-term heat acclimation via the controlled hyperthermia technique.

Core temperature has been elevated and controlled using vapour barrier suits (Fox, Kidd, Lewis, & Goldsmith, 1961) and in the military (Turk & Worsely, 1974). With a large (n=51) cohort, Turk & Worsely (1974) held T_{re} at 38.8˚C for 120 minutes a day for 5 days (WBGT 36˚C). 80 ± 5% participants improved cardiovascular stability to a satisfactory level. During an MTHA (10 days), participants maintained an aural temperature of 38.0-38.5˚C during a 70

minute water immersion and cycling protocol, reducing both thermal strain and increasing work capacity (Weller & Harrison, 2001).

Several studies employing the controlled hyperthermia model have performed heat stress tests after a period of STHA and MTHA (Gibson et al., 2015; Mee et al., 2015) and LTHA (Patterson, Stocks, & Taylor, 2014; Patterson et al., 2004b). It must be noted that despite Mee et al. (2015) and Gibson et al. (2015) defining their protocol as LTHA, by definition set by Garrett et al. (2011), a 10-day protocol signifies MTHA.

Perhaps the most significant study for review is that of Mee et al. (2015), who compares male and female adaptation to STHA (5 days) and MTHA (10 days). Three running heat tolerance tests (RHTT; 9km/h, 2% gradient, 30 minutes) were performed at baseline, after 5- and 10-days heat acclimation (90 mins, 65% $\dot{V}O_{2\,peak}$ and adjusted to maintain T_{re} ~38.5˚C, 40˚C, 40% RH). During RHTT2 (5 days) male participants had reduced resting T_{re}, (-0.24 ± 0.17˚C), peak T_{re} (-0.39 ± 0.36˚C) and peak f_c (-14 ± 12bpm), but not females (-0.02 ± 0.08˚C, -0.07 ± 0.18˚C and -5 ± 3bpm, , respectively). Females had improved sweat rate (691 ± 412g/h) and sweat rate relative to body area (428 ± 269g/h) but not males (-22 ± 533g/h and -11 ± 286g/h, respectively). Following MTHA (RHTT2-RHTT3), sweat rate (583 ± 638g/h) and sweat rate relative to body surface area (308 ± 346g/h/m²) increased in males but not females (85 ± 564g/h and 44 ± 373g/h/m², respectively) whereas resting T_{re}, (-22 ± 0.12˚C) and peak T_{re} (-0.41 ± 0.24˚C) improved in females but not males (0.04 ± 0.15˚C and -0.05 ± 0.26˚C), respectively.

Using an identical protocol (ISO_{cont}) vs. a progressive controlled hyperthermia model (ISO_{prog}: 5 days maintaining T_{re} 38.5˚C and 5 days maintaining T_{re} 39.0˚C) and a fixed-rate protocol (FIXED: 50% $\dot{V}O_{2\,peak}$), with 24 male participants, the same pattern in cardiovascular, thermoregulatory and sudomotor adaptation was observed. Both ISO_{cont} and

ISO_{prog} heat acclimation protocols elicited lower session and exercise intensity (% $\dot{V}O_{2\,peak}$) and time to reach target T_{re} and thus shorter exercise duration than FIXED in both STHA and MTHA (Gibson et al., 2015).

Lack of sudomotor response was also observed after 5 days of the controlled hyperthermia technique by Cotter et al. (1997). Participants cycled at 50% $\dot{V}O_{2\,max}$ until T_c was elevated by 1.2˚C. Thereafter the intensity was modified to maintain 1.4˚C elevation (6 days). Regional sweat response at 8 sites was measured (forehead, scapula, arm, forearm, hand, thigh, leg and foot) on days 1 and 6 of acclimation. Sweat onset threshold was lowered by heat acclimation, however no evidence of further sudomotor adaptation, nor a redistribution of local sweat response was observed (Cotter, Patterson, & Taylor, 1997).

Garrett et al. (2009, 2012 and 2014) used the controlled hyperthermia (40˚C, 60% RH) STHA (5 days) with aerobically fit (Garrett et al., 2014), moderately trained (Garrett et al., 2009) and highly trained (Garrett et al., 2012) cohorts, with (Garrett et al., 2012; Garrett et al., 2014) and without (Garrett et al., 2009; Garrett et al., 2014) permissive dehydration. In all populations, including the highly trained who behave physiologically as if already acclimated (N. A. S. Taylor, 2000), the key characteristic adaptations of decreased exercising f_c and T_{re} and improved exercise performance (see table 2-2) were observed. The employment of permissive dehydration (Garrett et al., 2012; Garrett et al., 2014), that is the cessation of fluid intake during acclimation sessions, had a profound effect on PV expansion such that, compared to euhydration trials, PV was expanded ~50% further (Garrett et al., 2014).

Two more recent studies employing the permissive dehydration stimulus to the controlled hyperthermia technique (James et al., 2016; Neal, Corbett, Massey, & Tipton, 2015) have found similar improvements in the key components of heat acclimation. Neal et al. (2015) acclimated trained participants to 40˚C, 50% RH (RPE = 15 until desired T_{re} thereafter

adjusted to maintain T_{re}) and observed a significant improvement (21.2%) in a 20km cycling TT as well as an improved lactate threshold (6.3%, $P < 0.05$) and PPO (1.8%, $P < 0.05$) in a graded exercise test (20 minutes at 85-110W thereafter 25W/3mins increments until volitional exhaustion) in temperate (22˚C, 60% RH) conditions. James et al. (2016) included a control (CON) as well as an experimental (STHA) group. The STHA exercised at 2.7W/kg, rather than a % $\dot{V}O_{2\,max}$ as employed by others (Garrett et al., 2012; Garrett et al., 2009; Garrett et al., 2014; Patterson et al., 2004b), until reaching desired T_{re} and adjusted thereafter to maintain T_{re}. 5,000m performance (5,000m treadmill running, self-paced, fixed 1% gradient) was subsequently enhanced to a greater extent for STHA (-6.2 ± 5.5%) than CON (-0.6 ± 1.7%) whilst endurance performance (part 1: 3-minute running stages, 1-minute rest, 1km/h increments, part 2: test to exhaustion, start 2km/h below final stage of part 1, 1% grade increase per minute) was enhanced more in STHA (20.8 ± 12.7%, $P \leq 0.001$) than CON (9.8 ± 1.2%, $P = 1.000$).

In a very short-term heat acclimation study (3 days), no improvements were observed in young, healthy female participants (Daanen & Herweijer, 2015). Several methodological limitations should be noted: (i) level of heat stress was lower than previous studies (35˚C, 40% RH), (ii) acclimation sessions lasted significantly less time than previous studies (60 minutes) and (iii) T_c target was less than previous studies (38.0˚C). Furthermore, only four (50%) participants exceeded T_c 38.0˚C on all three acclimation days while three exceeded the threshold for two days and one exceeded the threshold on just one day. It is plausible that less than 5 days acclimation can elicit significant adaptation. Certainly 4 days has merit (Sunderland et al., 2008), although future research needs to address the aforementioned methodological limitations if we are to see adaptation to 3 days heat acclimation.

Table 2-2: Physiological adaptations at rest and end-exercise, induced by short-, medium- and long-term heat acclimation using the controlled hyperthermia technique[a]. Adapted from Garrett et al. (2011)

Study	Plasma volume (%)	Core temperature (%)	Mean skin temperature (%)	Mean sweat rate (%)	Work capacity (%)
STHA (<7 days)					
Turk & Worsley (1974)		↓2.7			↔
Cotter et al. (1997)		↓0.6 R		↔	↔
Patterson et al. (2004b)	↑10.9	↓0.5R	↔	↑25.0	↔
Garrett et al. (2009)[b]	↑4.2	↓0.8			↑14.2
Garrett et al. (2012)[cd]	↑4.5R	↓0.8			↑1.5
Garrett et al (2014)[c]	↑4.0	↓0.7	↓2.0		↑14.0
Garret et al. (2014)[cd]	↑8.0	↓1.1	↓1.0		↑19.0
Patterson et al. (2014)	↑13.1R	↓0.5			

Daanen & Herweijer (2015)[e]		↔	↔		
Gibson at al. (2015)		↓0.5	↓1.2	↑21.1	
Mee et al. (2015)		↓1.0	↔	↔	
Mee et al. (2015)[e]		↔	↔	↑140.5	
Neal et al. (2015)[cd]	↔	↓0.5	↔	↑12.0	↑21.2
James et al. (2016)[d]	↑5.7	↓0.6	↓0.9	↔	↑20.8
MTHA					
(8-14 days)					
Weller & Harrison (2001)	↓2.3	↓0.5	↔		↑11.6
Gibson et al. (2015)		↓0.6	↓3.2	↑34.8	
Mee et al. (2015)		↓1.1	↔	↑35.4	
Mee et al. (2015)[e]		↓1.2	↓2.9	↑144.8	
LTHA					
(> 14 days)					
Patterson et al. (2004b)	↑10.9	↓0.9R	↔	↑33	↑15.4

Patterson et al. (2014)	12.6R	↓1.5	↑41.5

[a] unless otherwise states (R), variables are a product of end-exercise

[b] participants moderately trained

[c] participants highly trained

[d] permissive dehydration

[e] female participants

R= resting; **STHA**= short-term heat acclimation; **MTHA**= medium-term heat acclimation; **LTHA**= long-term heat acclimation; ↑ indicates variable increase (%); ↓ indicates variable decrease (%); ↔ indicates no change in variable (%)

The works of Patterson et al. (2004b, 2014) were conducted to track changes in body fluid compartments. Whilst Patterson et al. (2004b) observed that STHA of 5 days-controlled hyperthermia (40˚C, 60% RH) elicited similar physiological and performance improvement to LTHA (17 days), their important finding was that PV is expanded and can be maintained during LTHA (see table 2-2), and that expansion of the extracellular compartment is primarily responsible. However, a later observation in which inter-compartmental fluid losses and movements were tracked during the same acclimation protocol (Patterson et al., 2014), observed increased resting PV following humid heat acclimation was not defended when exercising in the heat; rather a greater plasma fluid loss occurred, although during recovery volume restoration was enhanced (Patterson et al., 2014).

It is thus conceivable that STHA elicits partial heat acclimation using the controlled hyperthermia technique in both males and females. However, 10 days is required for females to elicit cardiovascular and thermoregulatory adaptation and males sudomotor adaptation (Mee et al., 2015). The controlled hyperthermia technique provides a more economical methodology of heat acclimation than fixed-intensity techniques (Gibson et al., 2015).

2.6.2.4 Mixed model

Prior to the 2014 FIFA world cup, a novel mixed method heat acclimation regimen was utilised on a single football referee in preparation for the hot/humid (20-30˚C, 80% RH) conditions in which he would face at competition (Ruddock et al., 2016). The referee had previous experience of the Brazilian climate and experienced symptoms of EHRI. Pre- and post- acclimation tests included 10 minutes running in heat (30˚C, 80% RH) at 6, 11 and 13.5km, 5 minutes at 16km/h and a repeated high-intensity running test (Weston, Castagna, Helsen, & Impellizzeri, 2009). The MTHA regime included controlled hyperthermia active heat acclimation (40˚C, 80% RH) in which 20-second high intensity (RPE =17) efforts were

followed by 40 seconds of low intensity (RPE =11) running to attain T_c 38.5˚C, adjusted to maintain for 60-80 mins with permissive dehydration (Garrett et al., 2014). Passive sessions consisted of whole-body hot water immersion (~48˚C) after typical (temperate environment) training sessions (Scoon, Hopkins, Mayhew, & Cotter, 2007; Stanley, Halliday, D'Auria, Buchheit, & Leicht, 2015; Zurawlew, Walsh, Fortes, & Potter, 2015) for 30 mins to maintain T_c 38.5˚C.

The mixed methods MTHA regime induced a 7.1% increase PV expansion, 0.9L/h increase in sweat rate, 0.6˚C reduction in T_c, 0.5˚C reduction in T_b, 29% increase in high-intensity running and an improved thermal comfort (0.3 units). Such a study demonstrates that a mixed-mode acclimation regime for team sport athletes may provide a strategy in which the logistical demands on a team sports player can be reduced while adaptation can be induced (Ruddock et al., 2016). As the literature is based solely on a single case study, such a model requires further research.

In summary, one must consider the requirements of athletes before prescribing an acclimation regime. Whist MTHA-LTHA provide the greatest adaptation to the heat, STHA elicits ~75% (Pandolf, 1979) of adaptation which includes many important cardiovascular adaptations. Due to time constraints, many athletes may not have sufficient time or resources to undertake MTHA-LTHA and, further, longer regimes may also negatively impact quality training (Garrett et al., 2011).

It would seem the controlled hyperthermia model elicits the greatest heat adaptation when time is short, requiring a mere 5 heat exposures (Garrett et al., 2012; Garrett et al., 2009; Garrett et al., 2014; Mee et al., 2015; Neal et al., 2015). Furthermore, additional voluntary dehydration elicits greater adaptation to the fluid regulatory system (Garrett et al., 2012; Garrett et al., 2014; Neal et al., 2015). Whilst MTHA using the controlled hyperthermia

technique appears to adapt both males and females to a similar extent (Mee et al., 2015), the response to STHA differs between genders (Mee et al., 2015).

There is inadequate evidence to suggest HIIT training elicits significant adaptation as T_c is inadequately elevated for long enough (Brade et al., 2013; Petersen et al., 2010; Sunderland et al., 2008). Multiple daily bouts of heat acclimation yields similar effects to STHA (Willmott et al., 2016), although future research using different heat acclimation modals (i.e. controlled hyperthermia) is warranted. Acclimation is a sufficient stimulus for improved repeated sprint performance, although the addition of pre-cooling techniques does not further enhance performance in acclimated participants (Brade et al., 2013; Castle et al., 2011).

Self-paced heat acclimation may be of benefit for the acclimation of the masses, such as military personnel, as many will select a work intensity at approximately the same % of $\dot{V}O_{2\,max}$ (L. E. Armstrong et al., 1986; Miller, 1984). However, in an athletic population one may want a degree more control of intensity in order to maintain quality training in conjunct with heat acclimation (Garrett et al., 2011).

Finally, the use of a mixed mode acclimation protocol, that is with active and passive acclimation bouts, may provide sufficient adaptation. Active heat acclimation may be used on intermittent days with regular (temperate) quality training, supplemented with passive heat exposure via hot water immersion (Scoon et al., 2007; Zurawlew et al., 2015) to maximise preparation for the heat (Ruddock et al., 2016).

2.6.3 Cognitive functioning

Cognitive function is a major part of both military and sporting activity (Burke & Hawley, 1997) and deficits in cognitive functioning appear to be well correlated with T_c (Faerevik & Reinertsen, 2003; Hancock & Vasmatzidis, 2003; L. Taylor, Watkins, Marshall, Dascombe, & Foster, 2015) and WBGT (Mazloumi et al., 2014). Simple cognitive tasks appear

relatively unaffected by heat stress (Watkins et al., 2014), although more complex tasks are significantly affected by it (Gaoua, Racinais, Grantham, & El Massioui, 2011; L. Taylor et al., 2015). Sport-specific skills are certainly attenuated (Dawson, Elliott, Pyke, & Rogers, 1985; Rico-Sanz et al., 1996; Sunderland & Nevill, 2005). During work in heat stressful conditions, an increased test duration (3.4, 3.0 and 13.6%), reduced reaction time (6.2, 6.6 and 15.4%) and increased number of errors (77.6, 132.7 and 58.9%) to Stroop tests 1, 2 and 3, respectively have been observed (Mazloumi et al., 2014).

The effect of heat acclimation on cognitive functioning has been evaluated (Radakovic et al., 2007). A group of soldiers were either passively or actively acclimated for 10 days and undertook the Computerized Cambridge Neuropsychological Test Automated Batteries, version 2.0 (Fray, Robbins, & Sahakian, 1996), before and after exercise heat stress test (EHST). Unacclimated individual's performance in complex tasks such as reaction time (RTI) and rapid visual information processing (RVP), were attenuated after EHST whereas the simple cognitive task, motor screening (MOT), was not significantly impaired by EHST. Following active or passive acclimation, such attenuation in performance following EHST was eliminated, with no between-group differences (Radakovic et al., 2007).

In summary, significant heat stress elicits a significant attenuation of complex (Gaoua et al., 2011; L. Taylor et al., 2015), but not simple (Watkins et al., 2014), cognitive tasks. Such tasks include sport-specific skill performance (Dawson et al., 1985; Rico-Sanz et al., 1996; Sunderland & Nevill, 2005). Heat acclimation, passively or actively, can help maintain cognitive functioning (Radakovic et al., 2007), however more research is required in a sports context.

2.6.4 Cardiac stability

2.6.4.1 Plasma Volume

A rapidly occurring adaptation to repeated heat exposure is PV expansion, typically occurring after 3-6 days (Garrett et al., 2012; Garrett et al., 2014; Garrett et al., 2011). Typically, PV expansion is largely variable in this period, ranging from 3 to 27% (Garrett et al., 2014; Nielsen et al., 1993; Patterson et al., 2014; Patterson et al., 2004b). It has been suggested that the expansion of the extracellular fluid compartment (ECF) during acclimation is responsible for PV expansion (Patterson et al., 2004b).

An increase in the intravascular protein, albumin (Yang, Mack, Wolfe, & Nadel, 1998), increases colloid-osmotic pressure resulting in fluid being drawn into the intravascular compartment, thus inducing PV expansion (Senay, 1979; Senay et al., 1976). Certainly, after a 5-day moderate intensity (70% $\dot{V}O_{2\,max}$, 30mins/day) STHA regime, with carbohydrate and protein supplementation (6.4ml/kg), the increase in albumin content was almost identical to the greater PV expansion observed compared with the control (no supplementation). 80-90% of PV expansion was attributable to the increased albumin content (Goto et al., 2010).

Patterson and colleagues (2004b), however, argue that an elevated intravascular protein concentration does not solely account for PV expansion, rather that a generalised EFC expansion is the primary mechanism in the early stages of PV expansion (Bass, Kleeman, Quinn, Henschel, & Hegnauer, 1955). In the study by Patterson et al (2004b), an equivalent relative PV and interstitial fluid (ISF) expansion was observed. It is likely, therefore, that the increased electrolyte retention from an increased circulating aldosterone concentration expanded the ECF compartment and thus mediated PV expansion.

It has been found that acclimation-induced PV expansion was attenuated when participants followed a low-to-moderate rather than a high-sodium diet (L. E. Armstrong, Costill, & Fink,

1987; L. E. Armstrong et al., 1993). Furthermore, administration of an aldosterone inhibitor, spironolactone also blunted exercise induced PV expansion (Luetkemeier, Flowers, & Lamb, 1994). This suggests that PV expansion doesn't occur without replacement or retention of electrolytes (Patterson et al., 2004b).

Several more traditional constant work-rate acclimation studies have observed an initial PV expansion followed by a secondary decay to baseline PV. For example, 6 men stepping at the rate of 12 steps/min to elicit an oxygen consumption ~1.0L/min (~34˚C, ~89% RH) showed significant PV expansion over the first 5 days (10.4%; $P < 0.05$) acclimation. However, by day 17, PV expansion had decreased to just 4.8% ($P > 0.05$) (Wyndham et al., 1968). Similarly, 16 males underwent either 6 (n = 8) or 12 (n = 8) days walking at ~4.8km/h (3-12% gradient) to elicit 45-55 $\dot{V}O_{2\,max}$ for 60 mins/day (40˚C, 30% RH). After 6 days, a 7% PV expansion was observed, whereas after 12 it was unchanged (Aoyagi, McLellan, & Shephard, 1995). It may therefore be that PV is a transient adaptation.

2.6.4.2 Fluid balance

Good hydration is typically recommended during acclimation (Bergeron et al., 2012). Thirst, however, has been shown to be a poor indicator of the body's water requirements, often resulting in incomplete fluid replacement or dehydration in the heat (Eichna et al., 1945; J. E. Greenleaf, 1992; J. E. Greenleaf et al., 1983). For example, a group of non-acclimated boys (Wilk & Bar-Or, 1996) and girls (Wilk et al., 2007) cycled 3x20 minute periods (25 minutes rest) in hot (~35˚C, ~40-50% RH) at 50% $\dot{V}O_{2\,peak}$. During exercise, boys dehydrated to -0.65% body mass (Wilk & Bar-Or, 1996), while girls maintained euhydration for 110 minutes before a progression in dehydration (-0.15% body mass) occurred (Wilk et al., 2007).

It is suggested thirst sensations are related to changes in plasma osmolality, plasma volume and arginine vasopressin (AVP) concentration (J. E. Greenleaf et al., 1983). Water was

drunk *ad* libitum during an 8-day heat acclimation protocol in which participants cycled for up to 2 hours each day at 75W (~45% $\dot{V}O_{2\,peak}$) in ~40˚C, ~50% RH, while a control group replicated this in temperate conditions (~23˚C, ~50% RH). Whilst water consumption was consistent (129-232mL/h) across the 8-day period in the control group, consumption increased from 450mL/h on day 1 to 1,188mL/h on day 4 and levelled off to ~1,000mL/h on days 5-8 in the acclimation group. Time to first drink reduced with acclimation (day 1: 26, day 7: 11 minutes, NS) and number of drinks increased (day 1: 4 to day 3: 9, $P < 0.05$ and days 4-8: 10-13, $P < 0.05$) (J. E. Greenleaf et al., 1983).

2.6.4.2.1 Aldosterone

Aldosterone concentration during exercise has been shown to be unchanged by acclimation (Finberg & Berlyne, 1977; Francesconi, Sawka, & Pandolf, 1983; Kirby & Convertino, 1986; Nielsen et al., 1993; Nielsen et al., 1997; Sunderland et al., 2008). However, concentrations have been shown to increase as an acute response to exercise (Finberg & Berlyne, 1977; Garrett et al., 2014; Kirby & Convertino, 1986).

Sunderland and colleagues (2008) suggest well-trained populations may already possess a high level of salt balance. Kirby & Convertino (1986), however, suggest increased sweat gland responsiveness to aldosterone results in sodium reabsorption. These authors observed a significant reduction in total sodium loss, despite a 12% increased sweat rate, and a decrease in plasma aldosterone following 10 days' heat acclimation (Kirby & Convertino, 1986). With 1.0mg/day *d*-aldosterone injections no difference in sweat sodium conservation has been observed, yet cessation of injections significantly increased sweat sodium concentration (Braun, Maher, & Byrom, 1967).

2.6.4.2.2 Arginine vasopressin

Arginine vasopressin (AVP), secreted from the posterior pituitary gland, retains water and thus solutes in the kidney. It is postulated that an increased Na^+ concentration is the major stimulus driving an increase in AVP (Convertino, Keil, Bernauer, & Greenleaf, 1981).

Thermal adaptation may be optimised by stressing the fluid regulatory system (N. A. S. Taylor & Cotter, 2006). Garrett et al (2011) suggests permissive dehydration increases fluid-electrolyte retention. In a 5-day controlled hyperthermia heat acclimation regime, Garrett et al (2014) used a randomised cross-over design to evaluate the effect of permissive dehydration (DEH) and euhydration (EUH). Permissive dehydration did not enhance AVP, possibly due to methodological limitations. It may also be feasible that, due to the increased Na^+ and therefore water retention effect of aldosterone, the permissive dehydration may have provided a greater stimulus for Na^+ conservation.

2.6.4.3 Cardiac frequency, stroke volume and cardiac output

Perhaps the greatest challenge facing athletes competing in the heat is their ability to provide a sufficient cardiac output ($\dot{Q}$) to adequately perfuse skeletal muscle to support metabolism, whilst perfusing skin to promote heat loss (Hales, 1997; J.D. Périard, Travers, Racinais, & Sawka, 2016). Heat acclimation has been shown to increase $\dot{Q}$, specifically as a product of increased stroke volume (V_s) (Nielsen et al., 1993).

During low- to moderate-intensity exercise, heat acclimation produced increased V_s with very little change in $\dot{Q}$, possibly due to decreased f_c (Rowell, Kraning, Kennedy, & Evans, 1967; Wyndham et al., 1968). Rowell et al. (1967) acclimated participants to dry, whilst Wyndham et at. (1968) acclimated participants to humid heat.

It could be that differences occur between dry and humid heat acclimation. The work of Neilsen et al. (1993, 1997) observed V_s before and after dry (40˚C, 10% RH) and humid

(35˚C, 87% RH) heat acclimation during exercise (45-50% $\dot{V}O_{2\,max}$). After dry heat acclimation, V_s and $\dot{Q}$ were respectively elevated by ~21mL/beat and ~2.0L/min (Nielsen et al., 1993). However, after humid heat acclimation, no improvements were observed in either parameter (Nielsen et al., 1997) despite PV expansion in both (Nielsen et al., 1993; Nielsen et al., 1997).

Other studies in humid heat (Wyndham, 1951; Wyndham, Rogers, Senay, & Mitchell, 1976) report a decreased $\dot{Q}$ associated with a reduction in forearm blood flow, little change in V_s and decreased exercising f_c (Wyndham, 1951), with mixed results from participant to participant (Wyndham et al., 1976). Wyndham et al. (1976) reports differences between subjects during LTHA such that V_s showed no significant changes in one participant, a steady rise in two participants and a transient increase with declines after 6 days in a fourth participant.

Two groups were studied during an STHA (5-days), moderate intensity (70% $\dot{V}O_{2\,max}$, 50% RH) exercise regime, a control (no supplementation; CNT) and a supplemented group (6.4ml/kg protein and carbohydrate supplement, Pro-CHO). Both groups had increased plasma volume (CNT: ~3.9%; Pro-CHO: ~7.4%), V_s (both $p < 0.001$) and decreased exercising f_c (both $P < 0.001$) which maintained $\dot{Q}$ (Goto et al., 2010).

Garrett et al. (2009) showed that PV adaptations to STHA decayed at a faster rate than f_c adaptation suggesting PV expansion is not solely responsible for increased cardiac stability (Chalmers et al., 2014; Garrett et al., 2009; Garrett et al., 2011). There is evidence that MTHA (8 days) constant-rate (40% $\dot{V}O_{2\,max}$) acclimation decreased norepinephrine concentration from 1.58 ± 0.22ng/ml to 1.01 ± 0.20, 0.98 ± 0.15 and 0.89 ± 0.11ng/ml on days 3, 5 and 8, respectively, whilst exercising f_c reduced from 152 ± 18 to 135 ± 15bpm

from day 1-8 (Hodge, Jones, Martinez, & Buono, 2013). Interestingly, the reduction f_c and norepinephrine were highly correlated (r=0.79), therefore reduced sympathetic nervous activity could play a significant role in the attenuation of exercising f_c (Hodge et al., 2013).

In summary, heat acclimation improves cardiovascular stability, possibly through plasma volume expansion, and maintenance of cardiac output via increased V_s and reduced exercising f_c. Plasma volume expansion is likely initially mediated through a generalised expansion of the extracellular compartment by means of electrolyte and water retention mediated by improved sensitivity to aldosterone and increased AVP activity (Bass et al., 1955; Patterson et al., 2004b).

The oncotic role of increased total protein, specifically albumin (Yang et al., 1998), is likely to play a role in PV expansion as acclimation progresses (Goto et al., 2010; Patterson et al., 2004b; Senay, 1979; Senay et al., 1976). Alterations in plasma osmolality, plasma volume and AVP concentration after acclimation, likely improve thirst sensations which serve to enable athletes to better hydrate during exercise in the heat (J. E. Greenleaf et al., 1983), as thirst is a poor indication of the body's fluid requirements in unacclimated populations (Eichna et al., 1945; Wilk & Bar-Or, 1996; Wilk et al., 2007).

Cardiac output is overall better maintained after a period of acclimation due to the net product of improved exercising V_s and reduced exercising f_c. However, the magnitude of such responses is dependent upon environmental conditions, acclimation protocol, exercise dynamics and subject population (J.D. Périard et al., 2016) with much between-subject variability (Wyndham et al., 1976).

Finally cardiovascular stability may be improved as a result of decreased sympathetic nervous activity following acclimation (Hodge et al., 2013), rather than PV expansion alone (Garrett et al., 2009; Garrett et al., 2011).

2.6.5 Menstrual Cycle

To the author's knowledge, only one paper has evaluated the effect of heat acclimation during multiple phases of the menstrual cycle. Avellini et al. (1979) assessed the response to 10 days humid (dry bulb temperature [T_{db}] = 36˚C, WBGT = 32˚C) heat acclimation (5.6km/h, 120 minutes). Participants undertook a pre- and post-acclimation humid (T_{db} = 36˚C, WBGT = 30˚C) heat stress test (5.6km/h, 180 minutes) in both a pre-ovulatory (pre-OV) and post-ovulatory state (post-OV), in a randomised order. To maintain acclimation, relatively short (50-70 minutes) acclimation bouts were administered every other day after acclimation until both post-acclimation heat stress tests were complete.

Prior to acclimation, as in much previous research (Constantini et al., 2005; Janse de Jonge et al., 2012; Tenaglia et al., 1999), participants in pre-OV group outperformed post-OV. Time to exhaustion was 143 and 112 minutes in pre- and post-OV groups respectively, final T_{re} were 38.23 and 38.40˚C and mean sweat rates were 433 ± 39 and 377 ± 26g/m/hr respectively (Avellini et al., 1979).

Crucially, after acclimation, all participants completed the 180-minute heat stress test. Rectal temperature was decreased in both groups (pre-OV: 0.12˚C, post-OV: 0.19˚C) and mean sweat rate increased in both groups (pre-OV: 1381g/m; post-OV: 1317g/m). The authors concluded that, prior to acclimation; menstrual phase affected T_{re}, sweat rate and tolerance such that pre-OV women were at an advantage. Acclimation, however, eliminated such physiological differences (Avellini et al., 1979).

The results of Avellini et al. (1979) must be taken with caution due to individual variations in results and a small sample size (n=8). Further research comparing menstrual cycle phase effects on acclimation is warranted.

To summarise, pre-ovulatory (or follicular phase) females exhibit a physiological advantage during heat stress compared with post-ovulatory (or luteal phase) females (Constantini et al., 2005; Janse de Jonge et al., 2012; Tenaglia et al., 1999). Acclimation improves physiological responses to exercise in the heat during both phases, although to a larger magnitude in the luteal phase (Avellini et al., 1979). The net effect of these improvements means no menstrual phase differences are observed during exercise in the heat following a period of acclimation.

2.6.6 Oral contraceptives

In excess of 11.2 million young women in the U.S. alone use oral contraceptives (Mosher, Martinez, Chandra, Abma, & Willson, 2004). The prevalence amongst athletes is now thought to mirror that of non-athletes (Bennell, White, & Crossley, 1999), with reports of up to 83% of elite female athletes utilising them (Rechichi, Dawson, & Goodman, 2009).

Their use in heat acclimation has been assessed (L.E. Armstrong et al., 2005). Participants were either eumenorrheic (EU-OV), took combined oestrogen-progestin pill (ORAL) or received contraceptive injections of depot medroxyprogesterone acetate (DEPO) and participated in a 6-7-week heat acclimation and physical training (HAPT) programme. The HAPT programme consisted of 6 sessions per week (3 x heat acclimation and 3 x physical training). Heat acclimation consisted of a 90-minute circuit (36-37˚C, 33-37% RH).

Acclimation was confirmed in all three groups with an increased total exercise time (70.9%), decreased final f_c, T_{re}, $\overline{T}_{sk}$ and RPE with no between-group differences. This study therefore suggests heat acclimation regimes are not affected by oral contraceptive use (L.E. Armstrong et al., 2005). However, this is based upon the combined oestrogen-progestin pill; therefore further research is warranted regarding the progestin-only pill.

2.6.7 Decay

One of the key factors that athletes are faced with is fitting acclimation sessions in with high quality training. It is well recognised that the fastest occurring adaptations are also the fastest to decay. Since cardiovascular adaptations are recognised as the fasted to attain they are also reportedly the fastest to decay (Garrett et al., 2009; Garrett et al., 2011). For example, a greater percentage loss of heart rate was observed after 4 days' exercise in the cold (29%) and cold exposure (17%) than T_{re} (24% and 2%, respectively) (Saat, Sirisinghe, Singh, & Tochihara, 2005).

In a study of South African miners, the rate of decay was measured at 1, 2 and 3 weeks following a 16-day acclimatisation/acclimation procedure. After one week, there was a 50% decay in f_c and sweat rate ~50%, which by week three was ~100%. T_{re} response, however, was much slower to decay, with only a ~25% decay rate after one week and ~50% decay over the full three-week period (Williams, Wyndham, & Morrison, 1967). Likewise, following a 5 day controlled hyperthermia acclimation, T_{re} and f_c adaptation persisted for 1 but not 2 weeks (Garrett et al., 2009).

However, such a finding is not universal. In a more trained population, heat adaptation may be better maintained (Pandolf, 1998). For example, after one week deacclimation following a controlled hyperthermia MTHA (8 days) intervention in both a euhydration and permissive dehydration, no significant decrease in any adaptations were found (Neal, Massey, Tipton, Young, & Corbett, 2016). Likewise little decay was observed for f_c or T_{re} up to 18 days, following 9 days walking at 4.8km/h for 110mins/day for 9 days (Pandolf et al., 1977). Furthermore, retention appears greater when acclimated to dry rather than humid heat (Pandolf, 1998).

After a period of deacclimation, the rate at which reacclimation occurs is debated. It was first suggested that one day of acclimation is lost for every two days spent away from heat (Givoni & Goldman, 1973). A more recent suggestion is that one additional heat exposure is sufficient to offset the decay in acclimation for every 5 days outside the heat (N. A. S. Taylor, 2000). Following a 10-day heat exposure using the controlled hyperthermia technique, Weller and colleagues (2007) divided participants into two groups who were respectively re-exposed to work in the heat after 12 and 26 days of deacclimation. Acclimation was re-attained after 2 and 4 days of re-exposure respectively, suggesting that almost a month of deacclimation is required before a significant re-acclimation period is required (Weller, Linnane, Jonkman, & Daanen, 2007).

Whether a significant decay in adaptation does (Saat et al., 2005), or does not (Neal et al., 2016; Pandolf, 1998; Pandolf et al., 1977; Weller et al., 2007) rapidly occur after heat acclimation, travelling athletes must consider the timing of acclimation to the heat to maximise their benefits at competition. If acclimation is completed more than a week before travelling it is currently recommended that one additional heat exposure should be taken for every 5 days without exposure (N. A. S. Taylor, 2000) with up to a month of non-exposure required before a significant re-acclimation period is needed (Weller et al., 2007).

2.7 Aims and hypothesis

The aim of the current study is to evaluate the physiological and performance effects on an intermittent heat stress test of a 5-day short-term heat acclimation protocol, using the controlled hyperthermia technique with additional permissive dehydration.

It is hypothesised that STHA will improve thermotolerance, which is the body's tolerance to exercise in the heat, in a cohort of young, moderately-trained female game players when controlling for menstrual phase. As with previous research conducted with male and female

cohorts, it is hypothesised this will be achieved via reductions in body temperatures (T_{re}, $\overline{T}_{sk}$ and T_b), increased %PV and improved psychophysical feelings to intermittent exercise in the heat. The underlying theme of the proposed work will be the health and wellbeing of female players during the preparation for heat stress conditions, in order to maintain a high level of performance in a safe manner.

3 Methods

3.1 Participants

Participants were 8 female, games-playing volunteers from the University of Hull. Participant information is displayed in table 3-3. All participants were previously unacclimated to the heat and acclimations were completed outside the British summertime (2016-2017) to minimise seasonal acclimatisation effects. To minimise circadian rhythm effects acclimations occurred at the same time of day within an hour of each other from day to day. Participants completed pre-exercise medical questionnaires, gave informed consent (appendix A and B) to participate in the study and all were deemed to be in good health. The study was completed within the boundaries of the ethical approval granted by the University of Hull ethics committee.

Table 3-3: Individual and mean characteristics of participants

Participant	Age (yrs)	Body mass (kg)	Height (cm)	Cardiac Output (L/min)	VO_{2max} (ml/kg/min)
001	24	78.0	177.0	6.2	49.0
002	21	57.2	165.0	3.6	50.7
003	20	69.3	167.7	8.4	36.0
004	18	65.0	169.5	4.6	35.5
005	26	66.7	156.8	5.7	32.0
006	22	57.1	165.5	5.3	41.3
007	23	51.1	167.4	6.3	54.5
008	27	53.8	160.4	5.6	46.8
Mean	23	62.3	166.2	5.7	43.2
SD	3	9.0	6.0	1.4	8.2

$\dot{V}O_{2\,max}$ = peak oxygen uptake; SD = standard deviation

3.2 Experimental design and overview

Participants undertook a 5-day heat acclimation regimen using the controlled hyperthermia model, with permissive dehydration. A standard individualised heat stress test (HST) was completed in response to no heat acclimation (control) and the 5-day heat acclimation protocol. Figure 3-1 displays the experimental timeline. Cardiovascular and thermoregulatory responses were measured during the HSTs. Fluid-regulatory measures were taken pre- and post-heat acclimation on days 1 and 5 of the acclimation regimen. Relative changes in plasma volume was measured pre- and post- HST and pre- and post-heat acclimation on days 1 and 5 of the acclimation regimen.

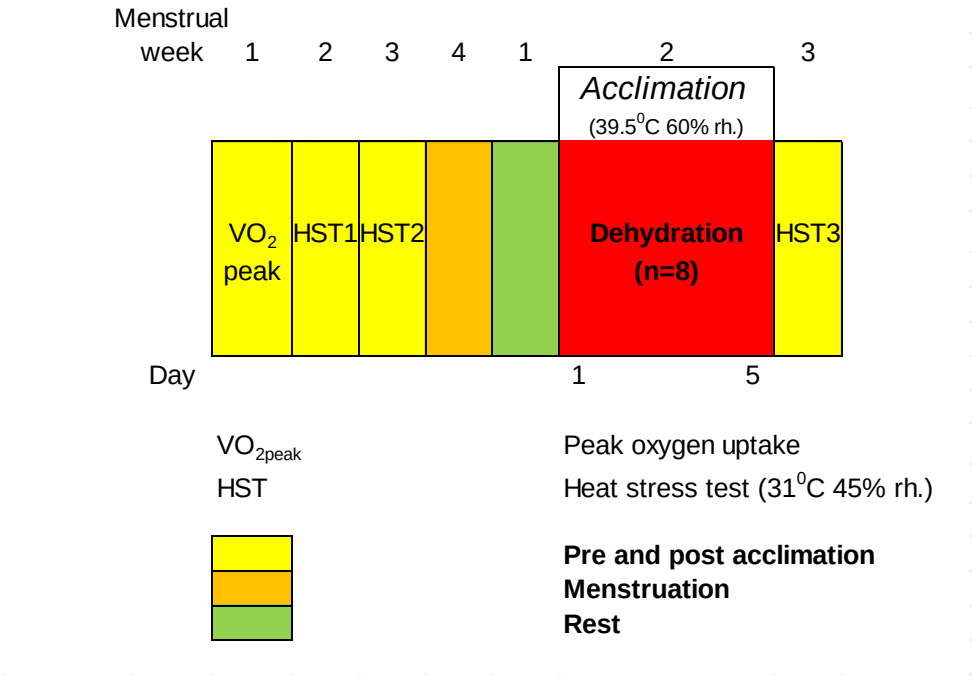

Figure 3-1: Schematic timeline of experimental proceedings for the examination of STHA with permissive dehydration on heat adaptations.

3.3 Protocol

3.3.1 Experimental standardisation

All participants were fully informed of all experimental procedures (appendix A). Prior to experimental testing, participants completed pre-exercise medical questionnaires and signed informed consent forms (Appendix A and B). Participants were asked to refrain from strenuous exercise for 24 hrs prior to HSTs and caffeine and alcohol consumption for 12 hrs before all testing procedures. Participants were advised to maintain habitual exercise habits between HST1 and HST2 but not to maintain habitual exercise habits during acclimation week.

Participants were advised to consume a high carbohydrate meal, 2-3 hours before exercise and to ensure good hydration the night before, prior to and at the conclusion of all exercise sessions. Each participant undertook HSTs and acclimation bouts at the same time of day

(within an hour of each other). On arrival at the laboratory, all participants' measures were taken in the same order.

Participants completed HST2 and HST3 in the same phase of their own menstrual cycle as reported by menstrual cycle questionnaires (Appendix C) and confirmed by plasma 17β-estradiol.

3.3.2 Aerobic fitness testing

Participants performed an incremental ramp exercise test to volitional exhaustion on a treadmill (h/p/Cosmos, Model Pulsar 3p, Traunstein, Germany), for determination of peak oxygen uptake $\dot{V}O_{2\,max}$ and velocities for individualisation of the HSTs. This procedure involved a starting velocity of 5km/hr with workload increments of 0.1km/hr every 6 secs (1km/hr/min), until volitional exhaustion. Participants were strapped into a safety harness hung from the top of the treadmill before initiating testing. Breath by breath expired air was collected via a metabolic cart system (Cortex Metalyzer 3B, Cortex Biophysic, Leipzig, Germany). Participants RPE (Borg, 1982) and f_c were recorded every minute. All participants were given strong verbal encouragement in the latter stages of the incremental test.

3.3.3 Heat stress test (HST)

The HST took place in an environmental chamber (Type SSR 60-20H, Design Environment, Gwent, Wales) set to ambient conditions of 31°C (T_{db}) and 50% RH. The experimental procedure was identical between all three HSTs.

Pre-exercise urine and blood measures were taken outside the chamber. All other measures were taken inside the chamber. Skin thermistors were fitted inside the chamber during a 10-minute stabilisation period.

The HST consisted of 9 identical 5 min blocks of intermittent exercise on a treadmill and cycle ergometer. Each 5-minute block consisted of intermittent treadmill activity defined as:

standing, walking, jogging, low-, moderate- and high-intensity running, at, respectively: standstill, 50, 60, 70, 85 and 95% HR_{max} determined by linear regression of $\dot{V}O_{2\,max}$ data, ending with a 6-second maximal cycle ergometer sprint. Treadmill velocity changed every 5-11 seconds and the percentage of time spent at each velocity was calculated from collegiate level football match play characteristics (Vescovi & Favero, 2014).

Treadmill speed and distance remained uniform between all three HSTs. Cycle sprint characteristics; PPO, MPO and distance sprinted were used as performance measures.

Prior to acclimation, two HSTs were performed separated by a week, to act as a control. Post acclimation HSTs were all performed within a week of the final acclimation day to prevent the dissipation of acclimation effects. Participants were not permitted to drink during HST trials to prevent whole body cooling from drinking.

3.3.4 Short term heat acclimation protocol

The STHA protocol consisted of 5 consecutive days of heat exposure (39.5°C [T_{db}], 60% RH) for 90 minutes a day, using the controlled hyperthermia technique (Garrett et al., 2009) with permissive dehydration (Garrett et al., 2014). Participants cycled against a self-selected resistance at 60rpm aiming to attain a T_{re} of 38.5°C as quickly as possible. Upon attainment of target T_{re} participants sat for the remainder of the 90 mins. If T_{re} fell below 38.5°C, the participant returned to the ergometer and continued to cycle until re-attainment of target T_{re}. During the week it was it was aimed that a progressive increase in workload and exercise time would be required to achieve target T_{re} whilst workload over the whole 90-minute period still remained relatively low by not exercising upon attainment of target $T_{re.}$

3.4 Apparatus, procedures and calculations

3.4.1 Stress equipment

The environmental chamber, situated in the Department of Sports, Health and Exercise Science, was used to control for ambient temperature and relative humidity throughout the study.

During the HSTs, a treadmill (pulsar 3p, h/p/Cosmos, Traunstein, Germany) was used to control running speed, a gradient of 1% was used to replicate standard outdoor running conditions and velocity changes were set at a speed factor of 5/7 to standardise the rate of change in velocity. The final activity of each 5-minute block was a 6 second maximal sprint on a cycle ergometer (Wattbike, Wattbike Ltd, Nottingham, UK) in which participants sprinted against a standardised resistance of 4. During STHA trials, participants cycled on a friction-braked cycle ergometer (Monark 824E, Monark Exercise AB, Varberg, Sweden).

3.4.2 Body temperature

Core body temperature was measured using a sterilised rectal thermistor (U thermistor, Grant Instruments Ltd, Cambridge, UK) inserted to a depth of 10cm beyond the anal sphincter. Thermistors were tested for reliability in boiling water left to cool. Skin temperature was measured using skin thermistors (Type EUS-U-V5-V2, Grant Instruments Ltd, Cambridge, UK) placed on four, left sided, sites: chest, bicep, thigh and calf, and secured using micropore tape. Mean skin temperature ($\overline{T}_{sk}$) and mean body temperature ($\overline{T}_{b}$) were calculated as:

$$\overline{T}_{sk} = (0.3 \cdot T_{chest}) + (0.3 \cdot T_{bicep}) + (0.2 \cdot T_{thigh}) + (0.2 \cdot T_{calf})$$ (Ramanathan, 1964)

$$\overline{T}_{b} = (0.9 \cdot T_{re}) + \left(0.1 \cdot \overline{T}_{sk}\right)$$ (Sawka, Wenger, & Pandolf, 2011)

Temperature data was recorded at 5-minute intervals on a portable data logger (2020 series data logger, Grant Instruments Ltd, Cambridge, UK).

3.4.3 Oxygen consumption and cardiac frequency

Breath-by-breath respiratory data was collected throughout aerobic fitness testing using a metabolic cart system (Cortex Metalyzer 3B, Cortex Biophysic, Leipzig, Germany) calibrated using a 3L calibration syringe (Hans Rudolph 3L, Cranlea and Co., Birmingham, UK) and calibration gas (5% CO_2, 15% O_2, Cranlea and Co., Birmingham, UK).

Cardiac frequency was measured every minute throughout all test procedures using a heart rate monitor (Polar FS1, Polar Electro, OY, Finland). Furthermore, during aerobic fitness testing, MetaSoft software recorded average 6s f_c.

3.4.4 Rate of body mass loss

Whole body sweat rate (L/h) was calculated from pre- to post-exercise nude body mass using digital weighing scales (WB-100A NTEP III digital scales, Tanita Inc, Illinois, USA).

Participants were weighed pre-exercise after completely voiding bladder and post-exercise before voiding bladder. Sweat weight was calculated as follows:

$$\text{Sweat rate (L/h)} = \frac{\text{pre mass (kg)} - \text{post mass (kg)}}{0.75}$$

Where 0.75 represents time (hours).

Net body mass loss was also calculated as a percentage according to the formula:

$$\text{Body mass loss (\%)} = \frac{pre\ mass\ (kg) - post\ mass\ (kg)}{pre\ mass\ (kg)} \times 100$$

3.4.5 Cardiac output and stroke volume

Baseline cardiac output ($\dot{Q}$) was measured using a breath-by-breath cardiac output analyser (Innocor, Innovision, Odense, Denmark). Prior to measurement, calibration of the cardiac output analyser was completed. Each participant had a fresh mouthpiece connected to a bacterial filter (Innovision, Odense, Denmark). A nose clip (Innovision, Odense, Denmark)

was then placed over the participant's nose to prevent any expired air escaping. Participants were instructed to breathe in synchronisation (~5 breaths, ~15 secs) with the on-screen demonstration until measurement was complete.

3.4.6 Blood measures

. On days 1 and 5 of STHA, at both rest and 90 mins, venous blood was obtained for the subsequent assay of various blood markers: total protein and albumin; electrolytes: Na^+, K^+ and Cl^-, fluid regulatory hormone: aldosterone, and stress hormone: cortisol.

Venous blood was collected using a Vacuette Safety blood collection kit + Luer adapter (Greiner Bio-One Gmbh, Kremsmunster, Austria); the protective cap was removed, and needle placed into an antecubital vein. During HST trials, a 5ml Z serum separator clot activator (SST) and 6ml K3E K3EDTA tube (EDTA) were collected. During acclimation protocol an SST, EDTA and a 6ml LH lithium heparin tube were collected (Greiner Bio-One GmbH, Kremsmunster, Austria).

Immediately after obtaining blood, the tubes were inverted. The EDTA plasma tubes were spun in a centrifuge at 2118g for 10 mins at 4°C; the SST tubes were allowed to stand for 30 mins at room temperature and spun at 2118g for 10 mins at 4°C. Plasma and serum were then aliquoted, respectively, into CryoPure tubes (Sarstedt AG & Co, Germany) and stored at -80°C until analysis.

Whole blood was immediately analysed in a blood, gas and electrolytes analyser (ABL800 Basic, Radiometer, West Sussex, UK) for determination of electrolyte concentrations.

Capillary blood samples from the finger were obtained pre- and post-HSTs and pre- and post-acclimation on days 1 and 5 for determination of haemoglobin (Hb) and haematocrit (Hct) for calculation of PV change via the Dill & Costill method:

$$\Delta PV\ (\%)\ =\ 100\ \times \frac{PVpost\ -\ PVpre}{PVpre}$$

A single-use lancet (Accu-Chek Safe-T-Pro Plus, Roche Diagnostics, Mannheim, Germany) was used to administer a finger prick, blood was collected in microcuvettes (HemoCue Hb 201 Microcuvettes, Radiometer, West Sussex, UK) and analysed in a haemoglobin analyser (HemoCue 201, Radiometer, West Sussex, UK). Sodium heparinised micro-haematocrit tubes (Hawsley & Sons, Lancing, UK) were filled and spun in a centrifuge (HaematoSpin 1400, Hawsley & Sons, Lancing, UK), then analysed with a tube reader (Hawsley & Sons, Lancing, UK).

3.4.7 Urinary measures

Urine samples were collected in 50ml centrifuge tubes (Scientific Laboratory Supplies, Nottingham, UK) pre- and post- HST and acclimation bouts on days 1 and 5. Urine samples were measure against a urine colour chart (Appendix E) (L. E. Armstrong et al., 1994) for determination of urine colour ($Colour_u$). Urine specific gravity (SG_u) was measured using a calibrated digital pen refractometer (Atago Co., Tokyo, Japan). Finally, urine osmolality (Osm_u) was measured by placing a sample of urine into an osmometer (modal 3320, Advanced Instruments, Massachusetts, USA). It has been suggested that USG and osmolality are valid measures (L. E. Armstrong et al., 1998) that can be used interchangeably as appropriate makers of hydration (L. E. Armstrong et al., 1994).

3.4.8 Psychophysical measures

In order to understand participants interpretation of the exercise and thermal stress being imposed, participants were asked to rate perceived exertion on the 16-point scale (Borg, 1982), thermal sensation (TS; 1-13) and thermal comfort (TC; 1-5) (Appendix D) (Gagge, Stolwijk, & Hardy, 1967). These psychophysical variables were recorded every 5 minutes

after completion of the maximal sprint. Participants were asked to base their interpretations on how each 5-minute exercise block felt.

3.4.9 Physiological strain index

To determine heat strain on a simple scale, we employed the method of Moran, Shitzer & Pandolf (1998). This method provides a comparable measure of heat strain between any combination of climate and clothing based upon f_c and T_{re} (Moran, Montain, & Pandolf, 1998) which is sensitive to acclimation (Moran, Horowitz, Meiri, Laor, & Pandolf, 1999) and hydration status (Moran, Shitzer, & Pandolf, 1998).

This PSI methodology has not had validation against the current population. Physiological strain during exercise varies between gender and age (Pandolf, 1997) and therefore the assumptions made by Moran and colleagues (1998) may not be representative of the current cohort. The following calculation was used to calculate PSI at 5-minute intervals throughout HSTs:

$$\text{PSI} = 5(T_{re\text{t}} - T_{re0}) \cdot (39.5 - T_{re0})^{-1} + 5(f_{\text{c}t} - f_{\text{c}0}) \cdot (180 - f_{\text{c}0})^{-1}$$

Where $T_{re\text{t}}$ and $f_{\text{c}t}$ represent rectal temperature and heart rate meaurements, respectively, taken simultaneously at a given point during the exposure T_{re0} and $f_{\text{c}0}$ represent rectal temperature and heart rate, respectively, at rest (Moran, Shitzer, et al., 1998).

3.5 Data analysis

The primary aim of the current study was to evaluate the physiological adaptations of a STHA regimen with permissive dehydration in a cohort of moderately-trained, female games players. We also sought, as a secondary aim, to determine whether sprint performance would be improved after the STHA with permissive dehydration regimen.

Statistical analysis was performed using SigmaPlot 14.0 (Systat Software, San Jose, CA). Data was checked for normal distribution using the Shapiro-Wilk test. When normal distribution failed data was analysed using median values. Data was collected at rest, during exercise and at end-exercise, during three HSTs and on days 1 and 5 of the heat acclimation regimen.

Data are presented as median differences ± interquartile range with upper (75%) and lower (25%) quartiles (95% CI) and Cohen's *d* effect sizes (ES; where 0.2 - 0.59 small; 0.6 - 1.19 moderate; 1.2 - 1.99 large; 2.0 - 4.0 very large).

4 Results

All 8 participants completed the 5-day STHA protocol and the three HSTs. Due to complications in venepuncture blood collection, a full data set was lost for one participant, while during day one of acclimation, baseline blood for a second participant was not collected and therefore this participant was removed from blood data analysis. Thus, blood data was analysed for 6 participants. One participant lacks several data components - $\overline{T}_{sk}$ (and thus $\overline{T}_b$) as well as urine data - from HST1, therefore in the control study 7 participants are used in the statistical makeup for the aforementioned measures.

Data from the acclimation period are presented first, followed by data at rest, and in response to the individualised HST protocol from before and after the heat acclimation period.

4.1 Acclimation

Data from the acclimation bouts are shown for an n=8 with blood markers being presented for n=6.

4.1.1 Thermal stress and strain

Table 3-4 presents parameters of thermal stress and strain from days 1 and 5 of heat acclimation. Environmental conditions, as measured from ambient temperature and relative humidity, were similar between days, indicating that the participants received a similar thermal stressor between acclimation bouts. Mean T_{re} indicates thermal strain was less during day 5 than day 1, whilst mean f_c suggests otherwise.

On day 5, participants cycled for 7.9 mins longer (0.48 to 15.35 min, $P < 0.05$, $d = 0.88$), producing 4.1kJ more work (0.8 to 7.5kJ; $P < 0.05$, $d = 0.74$) to reach T_{re} 38.5°C (Table 3-4 and Figure 3-2), compared with day 1 of the acclimation period.

Table 3-4: Thermal stress and strain

	Day 1	Day 5	P-value
Ta **(°C)**	39.6 ± 0.1	39.7 ± 0.2	0.14
RH (%)	60.0 ± 0.2	60.1 ± 0.1	0.72
Resting f_c **(b·min^{-1})**	95 ± 16	93 ± 11	0.14
Mean f_c **(b·min^{-1})**	144 ± 22	141 ± 19	0.11
Resting ***Tre*** **(°C)**	37.35 ± 0.33	37.33 ± 0.39	**0.35**
Mean ***Tre*** **(°C)**	38.29 ± 0.46	38.24 ± 0.47	**0.00**
Time to ***Tre*** **38.5°C (min)**	36.70 ± 6.36	44.62 ± 11.04	**0.04**
Work (kJ)	18.98 ± 5.94	23.03 ± 5.14	**0.02**
Body mass change (%)	-1.7 ± 0.6	-1.8 ± 0.7	0.80
PV% change	0.9 ± 13.1	0.7 ± 12.0	0.98

Ambient temperature (*Ta*), relative humidity (RH), cardiac frequency (f_c), rectal temperature (T*re*), time to *Tre* 38.5°C and work on day one and five of acclimation undertaken with permissive dehydration. Data presented as mean ± SD for eight female participants. Significant differences by paired sample t-test analysis presented in **bold**.

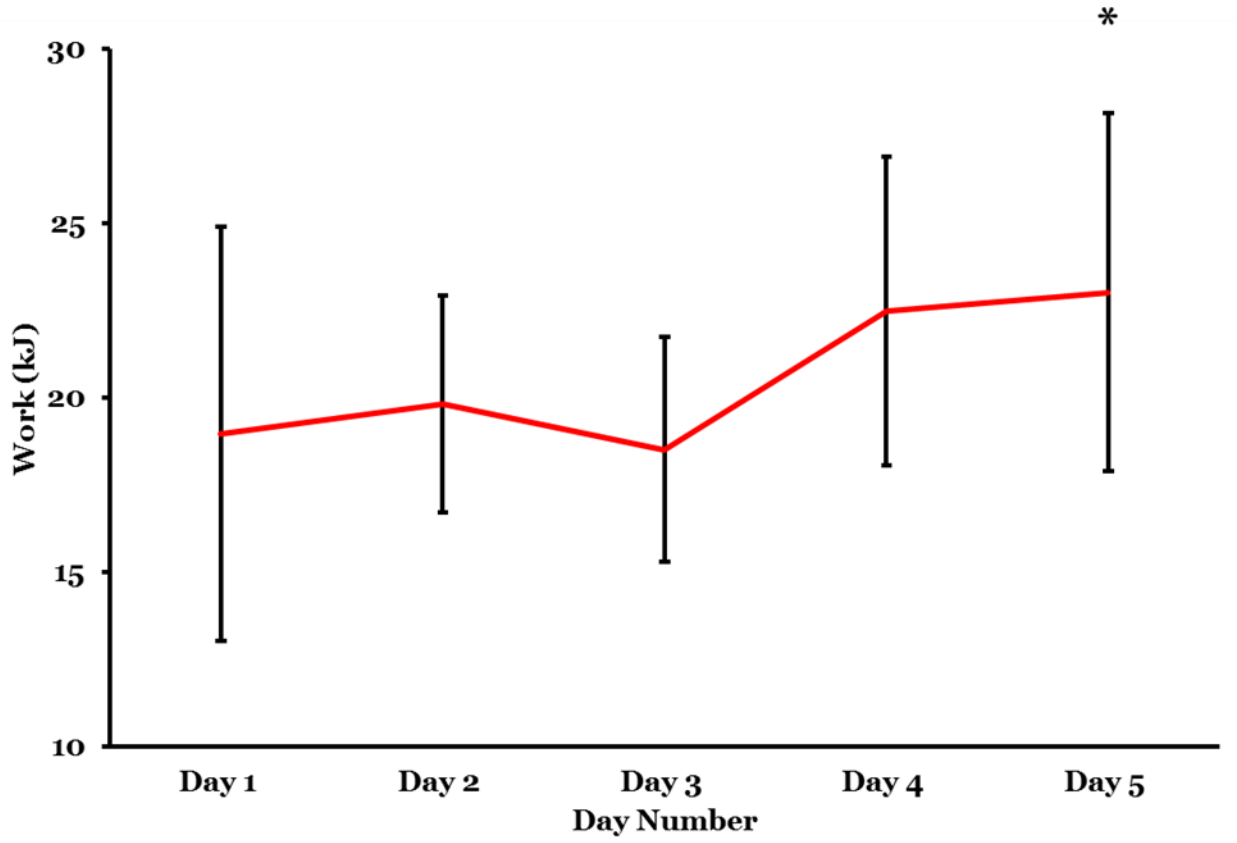

Figure 3-2: *Mean ± SD work (kJ) performed for each day of heat acclimation (n=8). * denotes significant difference (P < 0.05) from day 1.*

4.1.2 Urinary markers

To determine hydration status, each participant provided a nude body mass and urine sample prior to and upon completion of acclimation on days 1 and 5. Measures of urinary hydration; $colour_u$, SG_u and Osm_u were completed for all participants at each time point and are presented in Table 3-5.

Body mass changed significantly during day 1 (-1.1 ± 0.4; -1.4 to -0.7kg; $P \leq 0.001$) and day 5 (-1.1 ± 0.4; -1.5 to -0.8kg; $P \leq 0.001$), but no significant difference was seen between BM% loss on day 5 compared with day 1 (-0.1 ± 1.1; -1.0 to 0.8%; $P = 0.80$).

Neither Osm_u nor SG_u changed significantly during heat exposures. However, $colour_u$ did increase significantly on day 1 (2 ± 1; 1 to 3, $P \leq 0.001$) but not on day 5 (1 ± 2; 0 to 2, $P = 0.14$; Table 3-5).

Table 3-5 Urinary hydration measures

	Day 1: rest	**Day 1: 90 mins**	**P-value**	**Day 5: rest**	**Day 5: 90 mins**	**P-value**
$Colour_u$	2 ± 1	4 ± 2	**0.00**	3 ± 1	4 ± 2	0.14
Osm_u (mOsm/kg)	379 ± 292	447 ± 181	0.48	379 ± 267	396 ± 271	0.87
SG_u	1.0087 ± 0.0072	1.0123 ± 0.0073	0.27	1.0089 ± 0.0068	1.0106 ± 0.0088	0.63

Urine colour ($colour_u$), urine osmolality (osm_u), urine specific gravity (SG_u) and body mass (BM) changes from rest to 90 mins on days 1 and 5 of STHA with permissive dehydration. Data presented as mean ± SD for 8 female participants. Significant differences (rest to 90 mins) by paired sample t-test analysis presented in **bold**.

4.1.3 Total protein

To determine the effect of STHA on plasma total protein concentration, $[TP]_p$ was measured at rest and at 90 mins on day 1 and day 5 of the heat acclimation week (Figure 3-3).

There was no significant difference in $[TP]_p$ concentration at rest ($P = 0.33$) or 90 mins ($P = 0.99$) between days 1 and 5. However, a significant increase in $[TP]_p$ occurred from rest to 90 mins on days 1 (5.5 ± 2.6; 2.7 to 8.2mg/mL; $P < 0.05$) and day 5 (6.0 ± 2.7; 3.2 to 8.9mg/mL; $P < 0.05$).

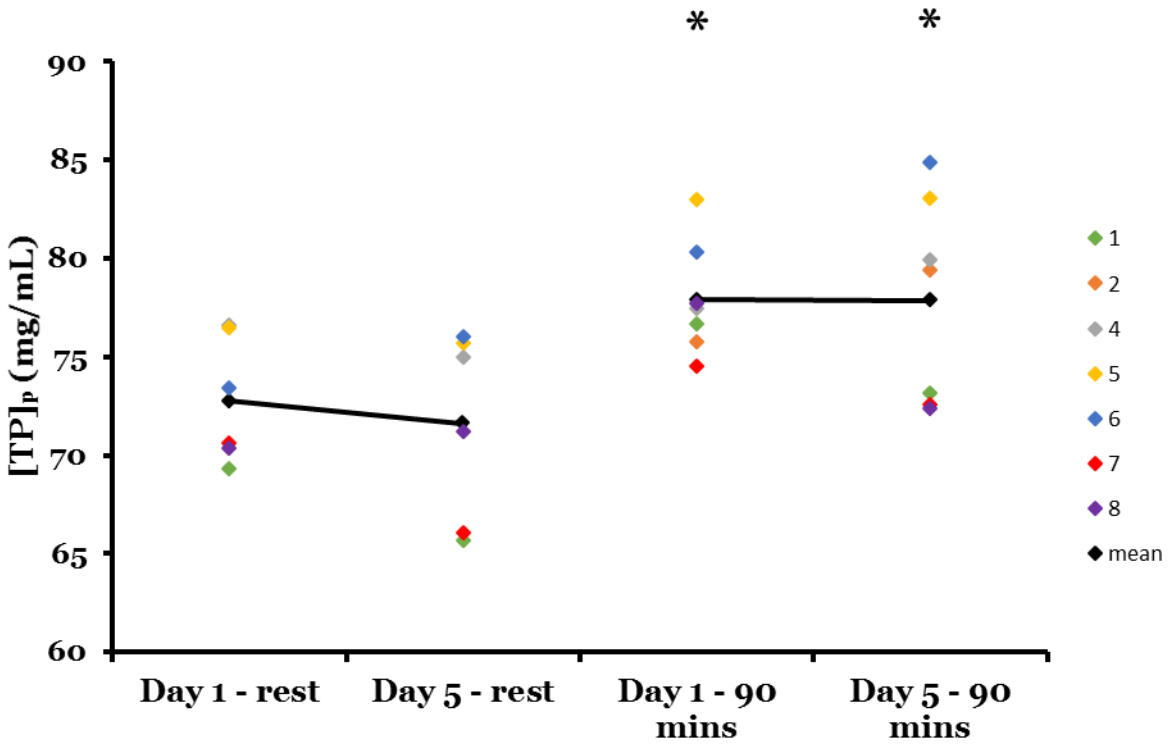

Figure 3-3: Plasma total protein ($[TP]_p$) concentration on day 1 and day 5 at rest and at 90 mins of STHA with permissive dehydration. Data presented as individual and mean (black line) for six female participants at rest and seven female participants at 90 mins. ***** *denotes significant difference ($P < 0.05$) between rest and 90 mins on the same day.*

4.1.4 Albumin

Albumin and globulin are the two main components of $[TP]_p$. We sought to observe the change in the $[alb]_p$ component of $[TP]_p$ (figure 3-4). There was no significant difference in $[alb]_p$ concentration at rest ($P = 0.61$) or 90 mins ($P = 0.66$) between days 1 and 5. There was, however, a significant increase in $[alb]_p$ concentration from rest to 90 mins on day 1 (3.0 ± 1.7; 1.3 to 4.8mg/mL; $P < 0.05$) and day 5 (3.4 ± 1.1; 2.3 to 4.5mg/mL; $P \leq 0.001$).

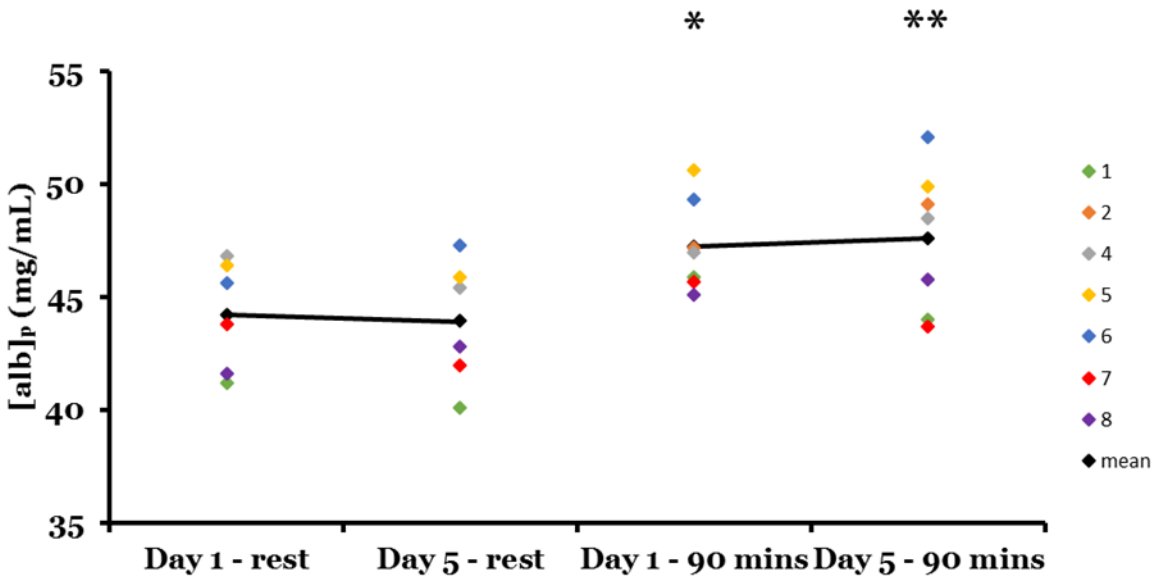

Figure 3-4: Plasma albumin ($[alb]_p$) concentration on day 1 and 5 of STHA with permissive dehydration. Data presented as individual and mean (black line) for six female participants at rest and seven female participants at 90 mins. ***** *denotes significant difference ($P < 0.05$) between rest and 90 mins on same day,* ****** *denotes significant difference ($P \leq 0.001$) between rest and 90 mins on same day.*

4.1.5 Aldosterone

Aldosterone concentration did not change either at rest (P = 0.54) or at 90 mins (P = 0.27) from days 1 and 5 of heat acclimation (fig 3-5). Aldosterone concentration did, however, significantly increase from rest to 90 mins on day 1 (201.2 ± 97.0; 111.5 to 290.8pg/mL; P < 0.05) and day 5 (127.4 ± 152.4; 0.0 to 254.8pg/mL; P < 0.05)

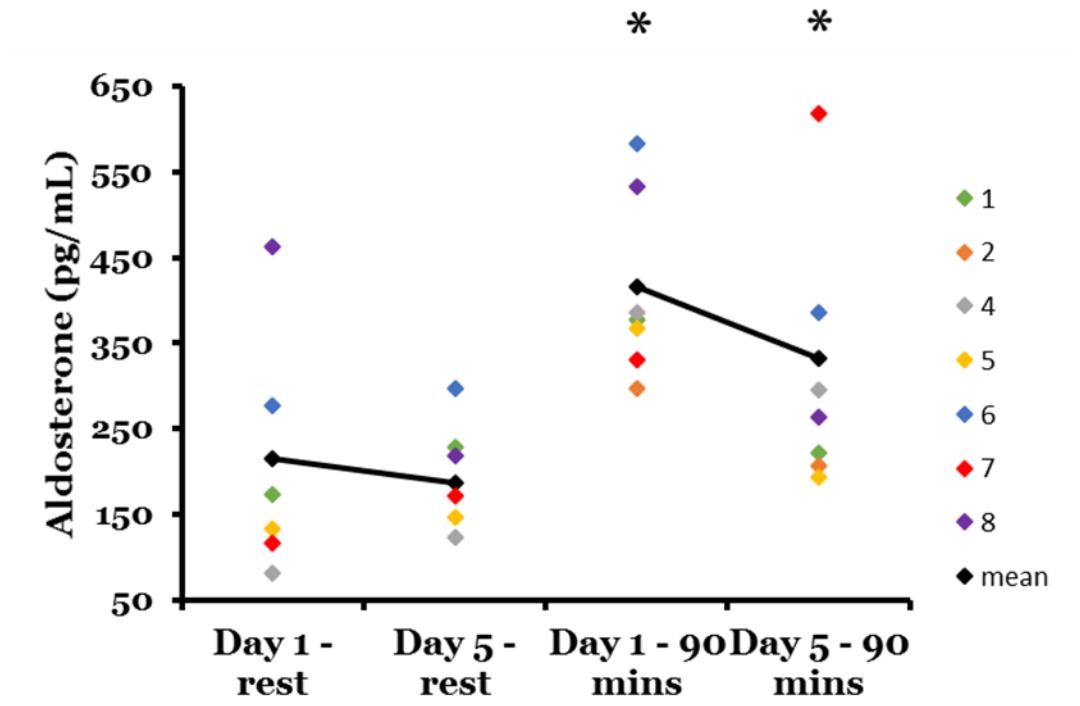

*Figure 3-5: Aldosterone concentration on day 1 and 5 of STHA with permissive dehydration. Data presented as individual and mean (black line) for seven female participants at rest and seven female participants at 90 mins. * denotes significant difference (P < 0.05) between rest and 90 mins on same day.*

4.1.6 Cortisol

Cortisol concentration (fig 3-6) did not significantly change at rest between days 1 and 5 of heat acclimation (P = 0.43) but was significantly lower at 90 mins on day 5 than day 1 (-106.24 ± 55.67; -157.31 to 55.16nmol/L; P < 0.05). Cortisol concentration did increase significantly from rest to 90 mins on day 1 (158.86 ± 92.63; 73.19 to 244.52nmol/L; P <

0.05) but not on day 5 (37.57 ± 103.24; -57.91 to 133.06; P = 0.78).

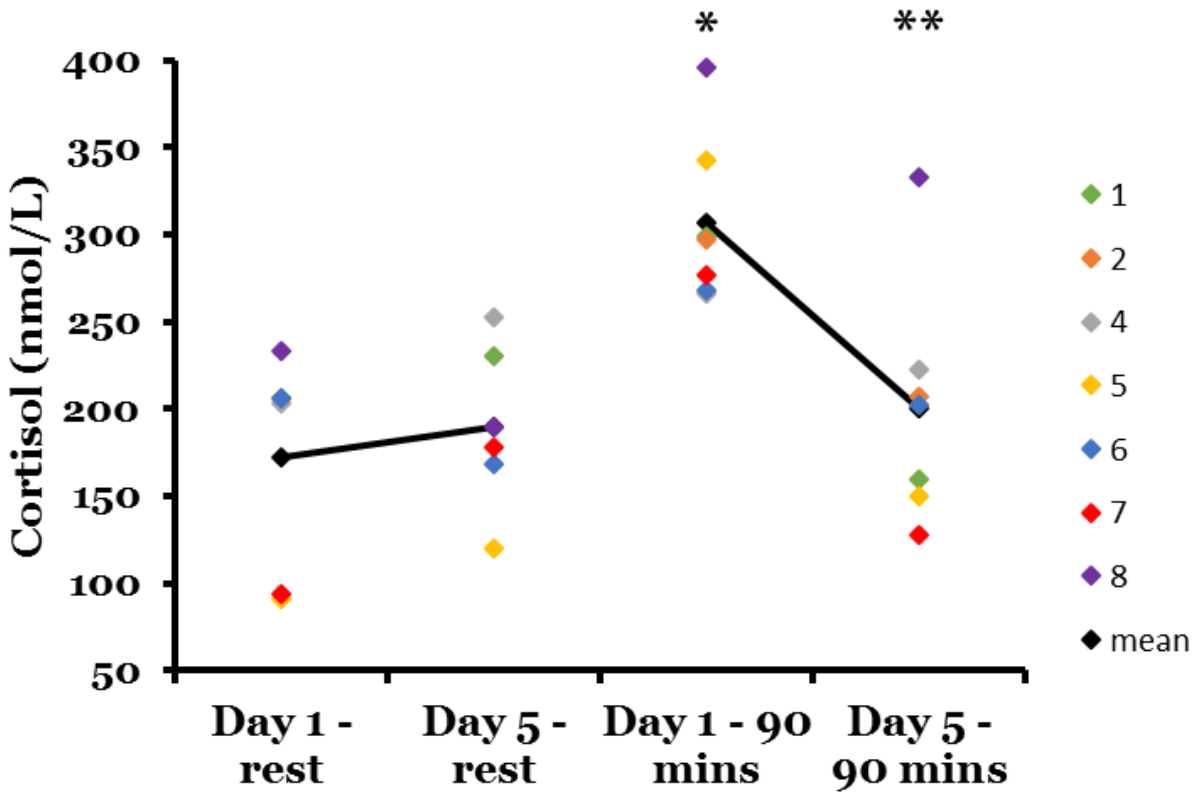

*Figure 3-6: Cortisol concentration on day 1 and 5 of STHA with permissive dehydration. Data presented as individual and mean (black line) for six female participants at rest and seven female participants at 90 mins. * denotes significant difference (P < 0.05) between rest and 90 mins on same day. ** denotes significant difference (P < 0.05) from day 1 to day 5.*

4.1.7 Plasma volume

Plasma volume was measured at baseline and at 90 mins on both acclimation days 1 and 5. Using the method of Dill and Costill (1974), plasma volume was calculated from Hb and HCT. Plasma volume was not significantly altered over 90 mins on day 1 or 5 (Table 3-4). However, the variability in individual change between days was quite broad (-18.2 to 17.7%; P = 0.98).

4.1.8 Electrolytes

Electrolytes Na^+, K^+ and Cl^- were analysed at rest and at 90 mins during day 1 and day 5 of STHA with permissive dehydration (Table 3-6).

Table 3-6 Electrolyte concentrations

Electrolyte (mmol/L)	Day 1 pre	Day 1 post	p-value	Day 5 pre	Day 5 post	p-value
Na^+	140 ± 2	141 ± 1	0.10	139 ± 1	142 ± 1	**0.00**
K^+	4.1 ± 0.6	4.1 ± 0.4	0.91	3.9 ± 0.3	4.1 ± 0.4	0.18
Cl^-	107 ± 3	109 ± 2	0.18	106 ± 2	109 ± 1	**0.00**

Electrolytes; sodium (Na^+), potassium (K^+) and chloride (Cl^-) change from rest to 90 mins on days 1 and 5 of STHA with permissive dehydration. Data presented as mean ± SD for 8 female participants. Significant differences (rest to 90 mins) by paired sample t-test analysis presented in **bold**.

On day 1, neither Na^+ ($P = 0.10$), K^+ ($P = 0.91$) or Cl^- ($P = 0.18$) concentration changed over the 90-minute heat stress period. On day 5 however, there was a significant increase in Na^+ (3 ± 1; 2 to 4mmol/L; $P \leq 0.001$) and Cl^- (3 ± 1; 2 to 4mmol/L; $P \leq 0.001$) but not K+ ($P = 0.08$). There was no significant difference for Na^+, K^+ or Cl^- at rest or at 90-minutes between days 1 and 5 (all $P > 0.05$).

4.2 Heat stress test

Physiological measures in response to the individualised HSTs were measured at rest and in response to exercise before and after STHA with permissive dehydration. The same physiological measures were also measured at rest and in response to the HSTs before and after a week of habitual activity with no heat acclimation intervention. Data is presented for 8 female participants unless otherwise stated.

4.2.1 Cardiac frequency

Cardiac frequency was measured at rest and every minute during the HSTs. Median f_c for each 5-minute period in HST2 and HST3 is displayed in Figure 3-7. Minimal difference was observed in resting f_c between HST1 and HST2 (1 ± 6; -1 to 5 b.min^{-1}; P = 0.31; d = 0.1) but was lowered by 12 b/min from HST2 to HST3 (-14 to -7 b.min^{-1}; P < 0.05; d = 1.12).

Participants worked at a similar percentage of their maximum f_c during HST1 and HST2 (HST1: 87 ± 4; 84 to 91%, HST2: 86 ± 2; 84 to 88%, P = 0.15; d = 0.48) but worked at a significantly lower percentage of peak f_c in HST3 (84 ± 3; 82 to 87%) than HST2 (P < 0.05, d = 0.64).

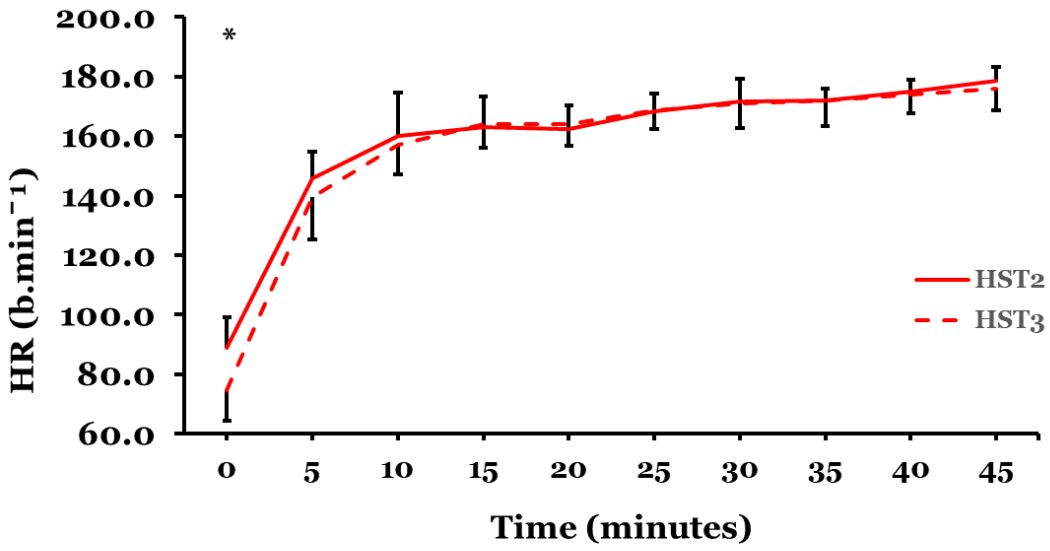

*Figure 3-7: f_c during HST2 and HST3. Data presented as median ± IQR resting f_c and median ± IQR f_c for each 5 minutes of exercise for 8 female participants. Statistical analysis performed at rest and for each 5 minutes of exercise. * denotes significant difference (P < 0.05) between HST2 and HST3.*

4.2.2 Body Temperatures

During exercise, rectal temperature (T_{re}, Figure 3-8 upper panel), mean skin temperature ($\overline{T}_{sk}$, fig 3-8 middle panel) and mean body temperature ($\overline{T}_b$, fig 3-8 lower panel) were measured at 5-minute intervals during the HSTs.

No significant change in resting T_{re} was observed between HST1 and HST2 (-0.07 ± 0.37; -0.15 to 0.22˚C; P = 0.74; *d* = 0.61) or between HST2 and HST3 (-0.03 ± 0.19; -0.11 to 0.08˚C; P = 0.76; *d* = 0.00).

No differences in exercising T_{re} were found during any period of exercise between HST1 and HST2, whereas between HST2 and HST3 there was a significant decrease in T_{re} at 40 (-0.17 ± 0.22; -0.25 to -0.03°C; P < 0.05; *d* = 0.98) and 45minutes of exercise (-0.13 ± 0.20; -0.28 to -0.07˚C; P < 0.05; *d* = 1.09).

As for T_{re}, no changes were observed in $\overline{T}_{sk}$ between HST1 and HST2 at rest (-0.03 ± 0.71; -0.64 to 0.07˚C; P = 0.38; *d* = 0.68) or at any time point during exercise (all P > 0.05). No change in $\overline{T}_{sk}$ was observed at rest (0.09 ± 0.66; -0.36 to 0.30; P = 0.99, *d* = 0.89). A significant reduction of $\overline{T}_{sk}$ was present at 40- (-0.44 ± 0.47; -0.69 to -0.22°C; P < 0.05; *d* = 0.94) and 45- (-0.53 ± 0.57; -0.74 to -0.16°C; P < 0.05; *d* = 0.86) minutes of exercise between HST2 and HST3.

Since $\overline{T}_{b}$ was calculated from T_{re} and $\overline{T}_{sk}$ there was no difference found between HST1 and HST2 at rest or at any point during exercise. There was no difference in $\overline{T}_{b}$ at rest between HST2 and HST3 (-0.15 ± 0.23; -0.17 to 0.06˚C; P = 0.54, *d* = 0.36), but a significant decrease in $\overline{T}_{b}$ was observed at 35- (-0.05 ± 0.09; -0.13 to -0.03°C; P < 0.05; *d* = 0.80), 40- (-0.14 ± 0.13; -0.23 to -0.10; P ≤ 0.001. *d* = 0.94) and 45- (-0.16 ± 0.17; -0.28 to -0.12°C; P ≤ 0.001; *d* = 1.28) minutes of exercise between HST2 and HST3.

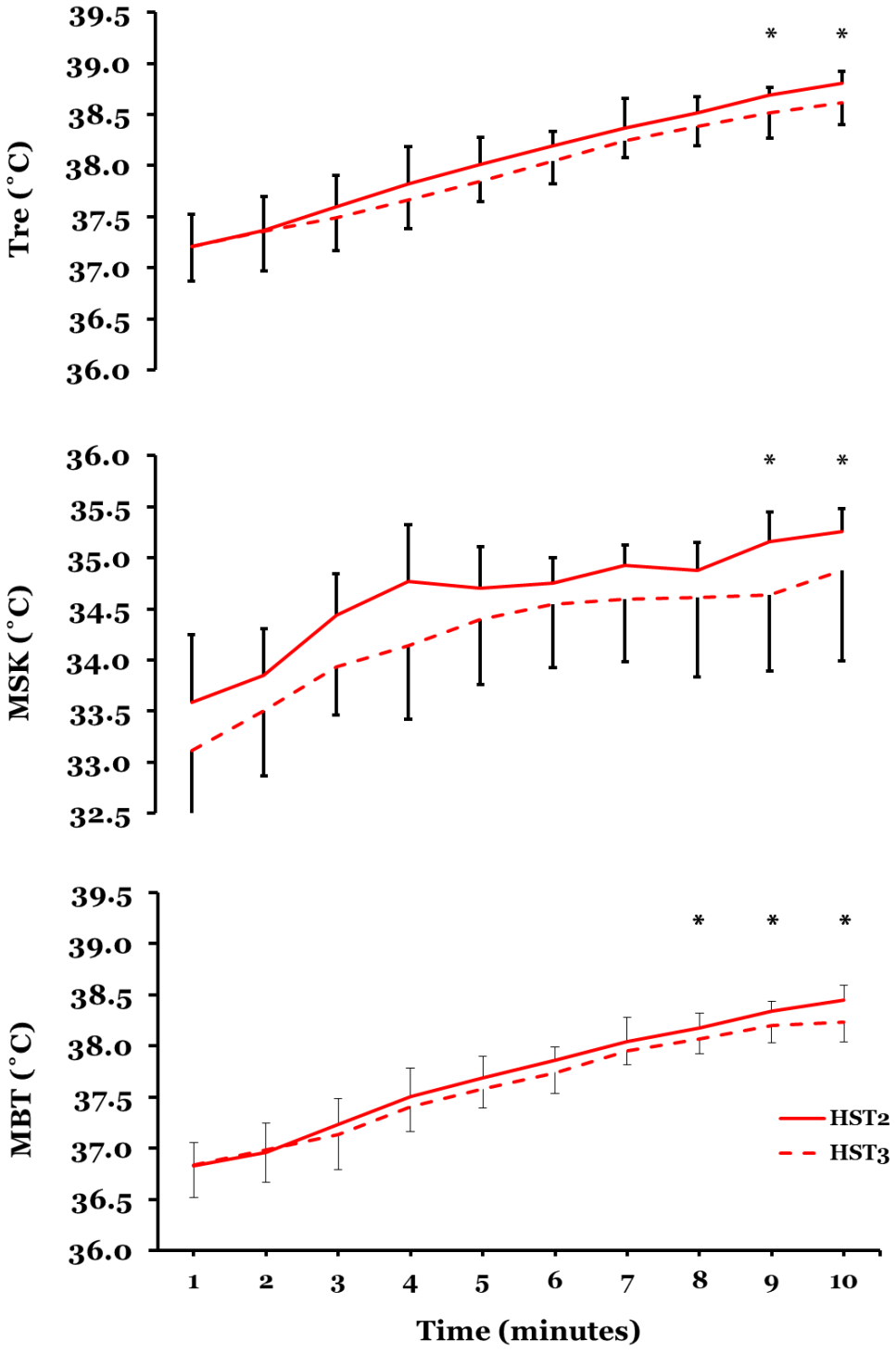

*Figure 3-8: Mean body temperature (MBT, bottom panel), mean skin temperature (MSK, middle panel) and mean rectal temperature (Tre, top panel) during HST2 and HST3. Data presented as median ± IQR for each 5 minutes of exercise for 8 female participants. * denotes significant difference ($P < 0.05$) between HST2 and HST3, ** denotes significant difference ($P \leq 0.001$) between HST2 and HST3.*

4.2.3 Physiological strain index

Physiological strain index (PSI) was calculated and analysed every 5 minutes (fig 3-9). There was no difference at any time point for PSI between HST1 and HST2 (all P > 0.05). Between HST2 and HST3 there was a significant decrease in PSI at 40- (-0.30 ± 0.44; -0.55 to -0.11; P < 0.05; *d* = 1.36) and 45- (-0.36 ± 0.39; -0.69 to 0.29; P ≤ 0.001; *d* = 2.00) minutes of exercise.

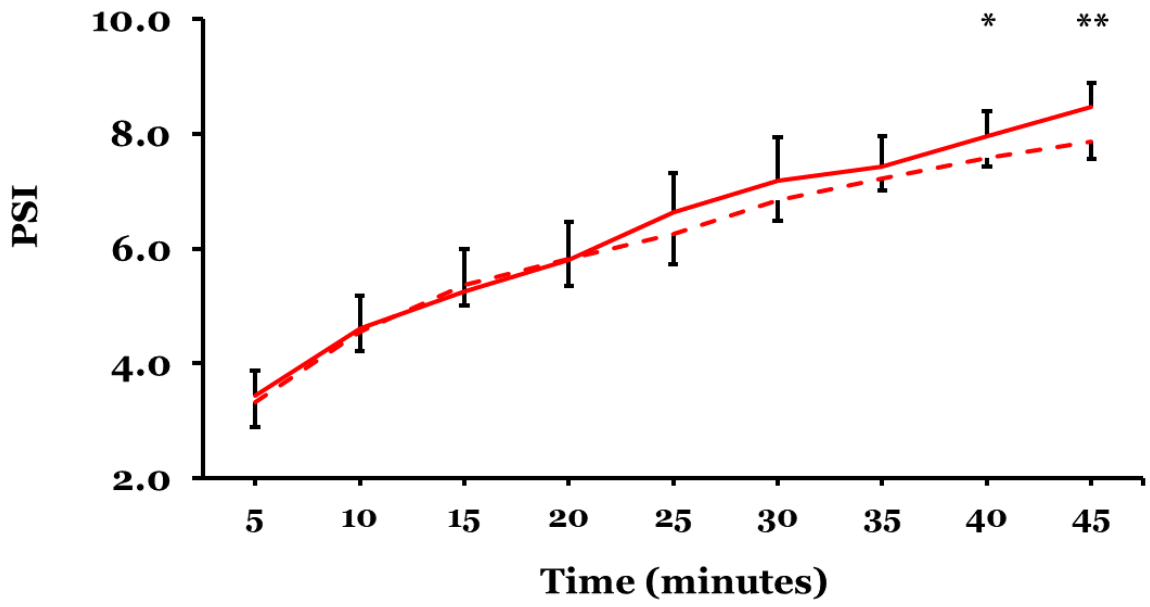

*Figure 3-9: PSI during HST2 and HST3. Data presented as median ± IQR PSI for each 5 minutes of exercise for 8 female participants. * denotes significant difference (P < 0.05) between HST2 and HST3, ** denotes significant difference (P ≤ 0.001) between HST2 and HST3.*

4.2.4 Sudomotor responses

Sweat rate (Figure. 3-10), as calculated from pre- and post- exercise body mass, did not differ between either HST1 and HST2 (0.2 ± 0.4; -0.1 to 0.3L/hr; P = 0.38, *d* = 0.2) or between HST2 and HST3 (0.1 ± 0.4; -0.3 to 0.2L/hr; P = 0.86, *d* = 0.0) as a result of acclimation.

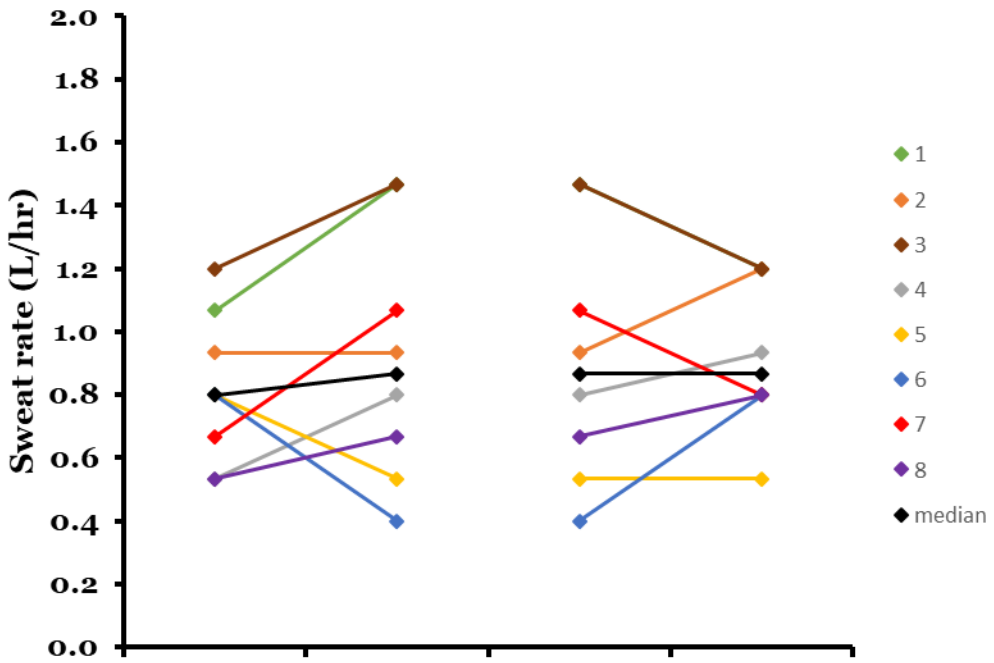

Figure 3-10: Individual and median (solid black line) change in sweat rate between HST1 and HST2 (left) and HST and HST3 (right). Data presented for 8 female participants.

4.2.5 Psychophysical responses

Participants RPE, TS, and TC (Figure 3-11) were recorded at rest and after every 5 minutes of exercise. Mean RPE was not changed between HST1 (fig 3-11 yellow bars) and HST2 (fig 3-11 orange bars) (-0.6 ± 2.0; -1.9 to 0.1; P = 0.18; *d* = 0.14) or HST2 and HST3 (fig 3-11 red bars) (-1.1 ± 1.0; -1.5 to -0.5; P = 12; *d* = 0.12). There appeared to be no change in RPE at any point between any of the trials except during at the 15-minute point of HST2 compared with HST1 (-1.0 ± 3.0; -3.0 to 0.0; P < 0.05; *d* = 0.24).

Mean TS was lowered from HST1 to HST2 (-0.4 ± 0.3; -0.5 to -0.2, P < 0.05; *d* = 0.08) and from HST2 to HST3 (-0.8 ± 0.9; -1.0 to -0.1; P < 0.05; *d* = 0.08). Participants felt cooler during the first 5 minutes of HST2 compared to HST1 with a reduction in TS (-1.0 ± 1.0; -1.0 to 0.0; P < 0.05, *d* = 0.17), and during HST3 compared with HST2 at rest (-1.0 ± 01.0; -1.0 to -0.0; P < 0.05; *d* = 0.20), 10- (-1.0 ± 01.0; -1.0 to -0.0; P < 0.05, *d* = 0.17), 20- (-1.0 ± 0.3; -1.0 to -0.8, P ≤ 0.001; *d* = 0.08), 25- (-1.0 ± 0.0; -1.0 to -1.0; P ≤ 0.001; *d* = 0.16), 30- (-1.0 ±

1.0; -1.0 to 0.0; P < 0.05; *d* = 0.00) and 45- (-0.5 ± 1.0; -1.0 to 0.0; P < 0.05; *d* = 0.08) minutes of exercise.

Mean comfort level did not improve from HST1 to HST2 (-0.2 ± 0.4; -0.4 to 0.0; P = 0.14; *d* = 0.09) but was significantly improved between HST2 and HST3 (-0.6 ± 0.7; -0.9 to -0.2, P < 0.05, *d* = 0.34). Participants did not feel more comfortable in the heat at any point during exercise between HST1 and HST2. However, between HST2 and HST3, participants felt more comfortable in the heat at 10- (-0.5 ± 1.0; -1.0 to 0.0, P < 0.05, *d* = 0.00), 15- (-0.5 ± 1.0; -1.0 to 0.0, P < 0.05, *d* = 0.56), 20- (-0.5 ± 1.0; -1.0 to 0.0, P < 0.05, *d* = 0.56), 25- (-1.0 ± 1.0; -1.0 to 00, P < 0.05, *d* = 0.00), 30- (-1.0 ± 0.3; -1.0 to -0.8, P < 0.05, *d* = 0.50), 35- (-1.0 ± 0.3; -1.0 to -0.8, P < 0.05, *d* = 0.50), 40- (-1.0 ± 1.0; -1.0 to 0.0, P < 0.05, *d* = 0.48) and 45- (-1.0 ± 1.3; -1.3 to 0.0, P < 0.05, *d* = 0.45) minutes of exercise.

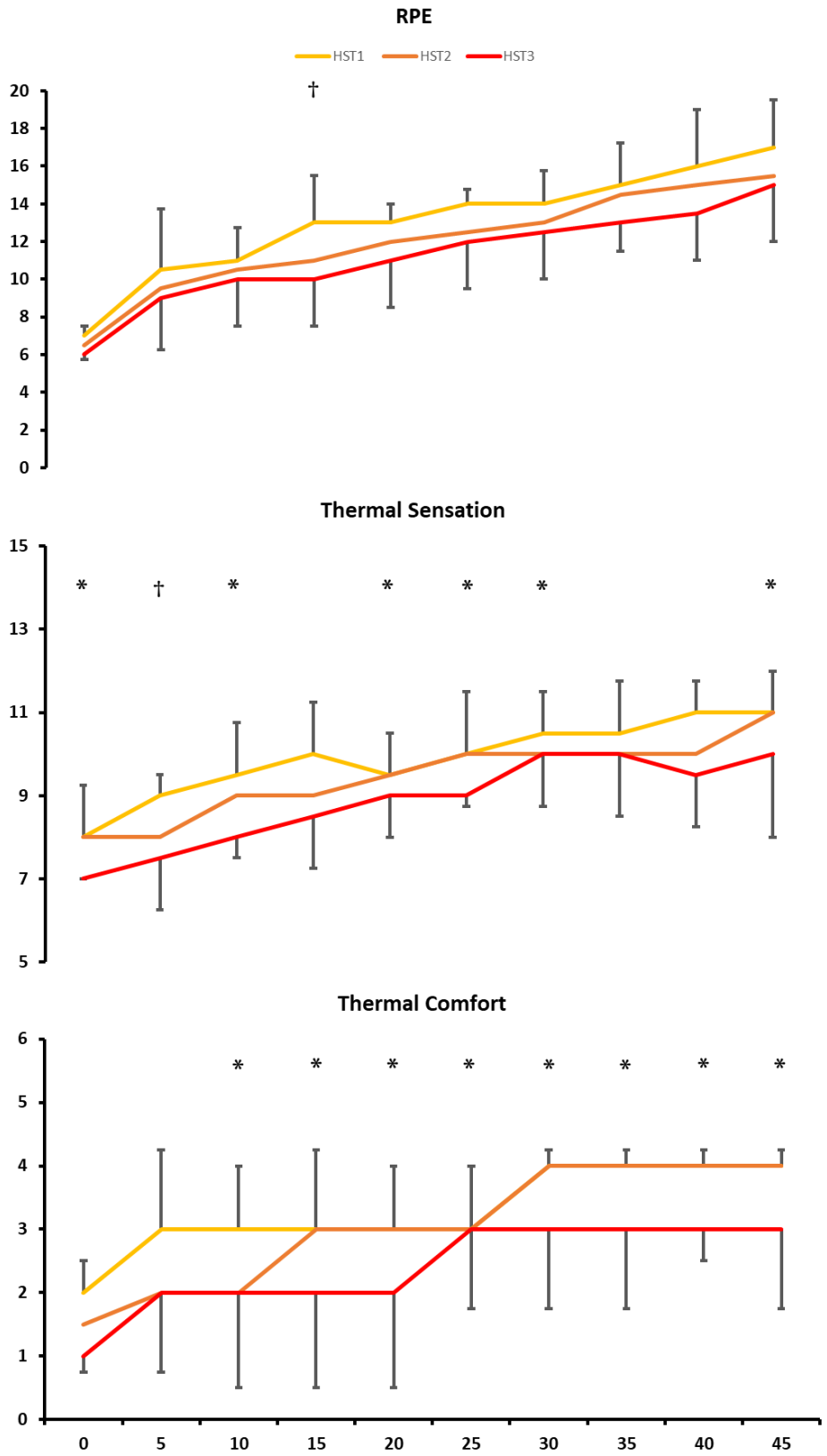

*Figure 3-11: Rate of perceived exertion (RPE, top panel), thermal sensation (TS, middle panel) and thermal comfort (TC, bottom panel) during HST1 (yellow), HST2 (orange) and HST3 (red). Data presented as median ± IQR for 8 female participants † denotes significant difference ($P < 0.05$) between HST1 and HST2, * denotes significant difference ($P < 0.05$) between HST2 and HST3.*

4.2.6 Urinary hydration measures

Participants were sufficiently hydrated prior to exercise during all HSTs. There were no differences in any hydration measures at rest or end exercise between HST1 and HST2 or between HST2 and HST3. From rest to end exercise there were some significant changes in hydration measures during each HST. During HST1, $colour_u$ increased significantly during the exercise period (0.9 ± 0.8; 0.0 to 1.7, $P < 0.05$; $d = 0.62$) and body mass reduced by 0.8 ± 0.6% (1.3 to -0.3%; $P < 0.05$; $d = 0.05$). During HST2 body mass decreased by 1.1 ± 0.4% (-1.44 to -0.76%; $P < 0.001$, $d = 0.07$). During HST3 $colour_u$ increased throughout exercise (1.0 ± 1.1; 0.0 to 2.0; $P < 0.05$; $d = 0.74$) and body mass decreased by 1.1 ± 0.3% (-1.35 to -0.91%; $P < 0.001$; $d = 0.07$). Neither SG_u nor Osm_u was significantly affected during exercise in heat stress.

4.2.7 Plasma volume

Plasma volume change was calculated from haematocrit and haemoglobin using the Dill and Costill method (1978). There was no difference in % change in plasma volume in HST2 compared with HST1 (-3.7 ± 7.3; -10.5 to 3.0%, $P = 22.0$, $d = 0.71$). A significant plasma volume expansion was present in HST3 compared with HST2 (8.7 ± 7.9%; -1.1 to 18.5; $P < 0.001$; $d = 1.26$).

4.2.8 Distance covered

Distance covered during treadmill running was 4840 ± 743m (4219 to 5461m). Participants covered the same distance on the treadmill during each HST. Cycle sprint distance remained the same between HST1 and HST2 (0 ± 2; -1 to 1m; P = 0.32 *d* = 0.28) and between HST2 and HST3 (2 ± 3; 1 to 3m; P = 0.06; *d* = 0.22).

4.2.9 Power output during sprinting power

Peak power output (PPO, fig 3-12 lower panel) and average power (MAvP, fig 3-12 upper panel) were measured during each 6 second maximal sprint bout. No differences in PPO were observed at any time point between HST1 and HST2 (all P > 0.05). Between HST2 and HST3 PPO increased at the 40-minute mark (29 ± 29; 15 to 44W; P < 0.05; *d* = 0.29) only.

Similarly, MAvP did not differ at any time point between HST1 and HST2 (all P > 0.05). However, MAvP did improve from HST2 to HST3 at 20- (30 ± 32; 13 to 45W; P < 0.05; *d* = 0.34), 25- (30 ± 67; -2 to 64W; P < 0.05; *d* = 0.23) and 40- (31 ± 59; -2 to 56W; P < 0.05; *d* = 0.29) minutes of exercise.

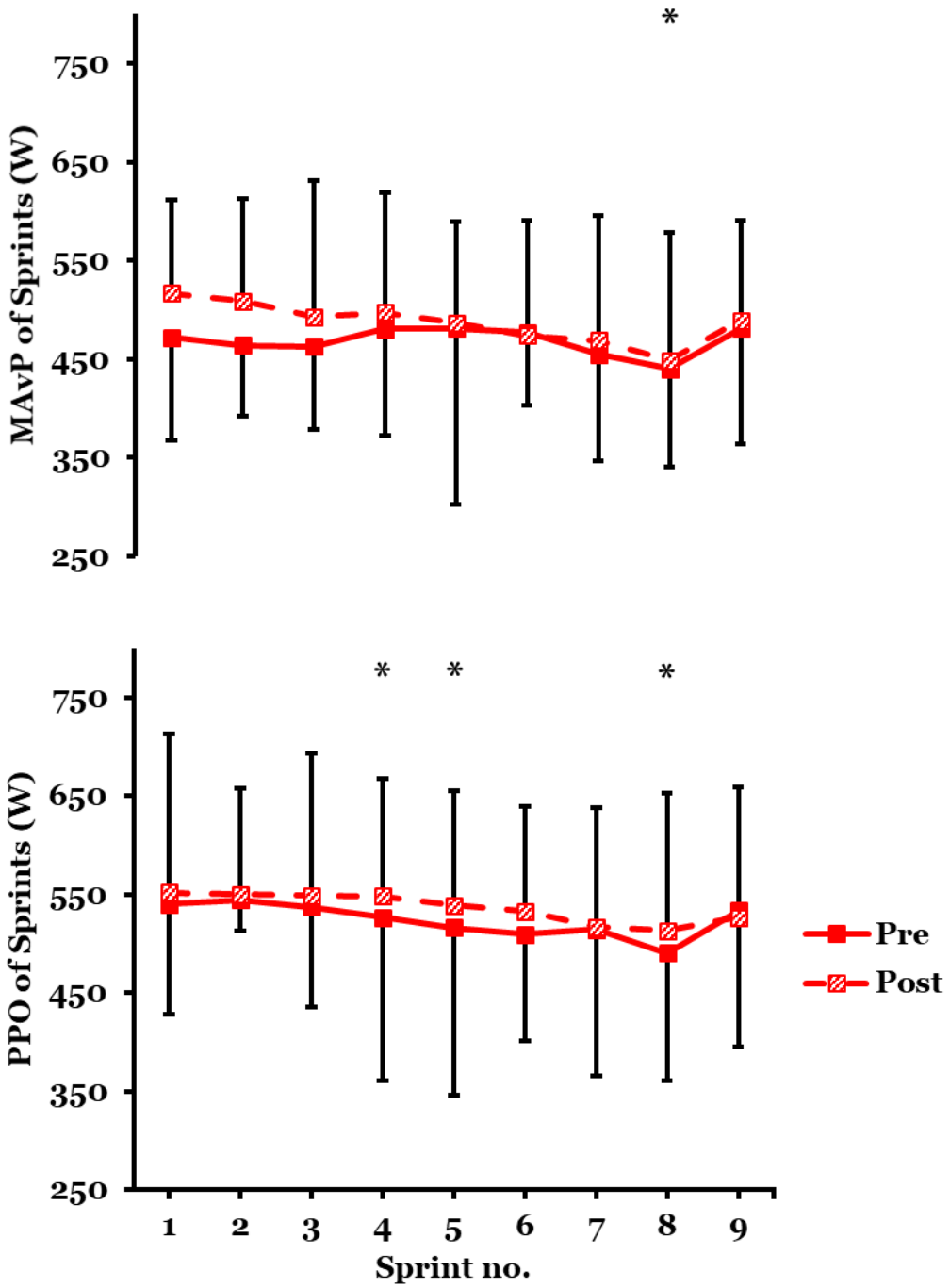

*Figure 3-12: Peak power output (PPO, lower panel) and mean average power output (MAvP, upper panel) for 9 sprints thermal during HST2 and HST3. Data presented as median ± IQR for 8 female participants. * denotes significant difference (P < 0.05) between HST2 and HST3.*

5 Discussion

The aim of the present study was to evaluate whether a 5-day STHA regimen using the controlled hyperthermia technique with permissive dehydration and controlling for menstrual cycle phase would elicit heat adaptive responses in a healthy, team sports, female population. This work has demonstrated an enhancement in thermoregulatory, cardiovascular, psychophysical and repeated sprint performance with this female cohort.

5.1 Evaluation of short term heat acclimation protocol

The purpose of the controlled hyperthermia model is to elevate and maintain T_{re} at a predefined level. Most protocols to maintain this T_{re} have participants continuing exercise by adjusting workload (Garrett et al., 2012; Garrett et al., 2009; Garrett et al., 2014). A recent study with male participants in our laboratory has shown that such continuation of exercise is unnecessary to maintain T_{re}. Once at target temperature, male participants ceased exercise and all but remained at or above target temperature for the remainder of the 90 minutes (Nation et al., 2015). If temperature fell below target, participants returned to cycling until elevated again (Nation et al., 2015). As travelling athletes will be in a state of tapering prior to competition, it is important to ensure that heat acclimation is achieved with minimal exercise stress, thus being able to maintain quality training (Garrett et al., 2011). In the current study, several of the participants had to return to cycling during the latter stages of the week. Such an observation is suggestive that evaporative cooling was enhanced. Certainly, this would agree with the previous work with females that observed a significant sudomotor response to STHA (40˚C, 40% RH) (Mee et al., 2015). The greater relative humidity of our environment (60%) would add significant thermal strain (Gagnon & Kenny, 2012) and thus provide a greater stimulus for enhancing sudomotor response. As sweat rate did not increase significantly in response to acclimation it is possible a redistribution of local sweat rate

increased the evaporative efficiency of the sweat response. Further research is warranted to draw firmer conclusions on such a phenomenon.

Time to reach 38.5˚C significantly increased (21.6%) from day 1 to 5 in the present study, resulting in a parallel increase in work done (21.3%). Average time to reach 38.5˚C has been shown to be longer during STHA for females (51 ± 7 minutes) than males (48 ± 9 minutes) (Mee et al., 2015). However, such times seem comparatively long in the study by Mee et al. (2015) when compared with other studies. Neal et al. (2015) reports time to 38.5˚C as 22.9 ± 5.0 minutes on day 1 and 24.3 ± 5.6 minutes on day 5, whilst in the works of Garrett et al (2012 and 2014) the time is consistently ~31 ± 4 (day 1) and ~33 ± 3 minutes (day 5). In the present study, a much larger % of individual variability in time to 38.5˚C was observed than that of previous studies (Garrett et al., 2012; Garrett et al., 2014; Neal et al., 2015). It is likely that the self-selection of resistance, which was frequently altered during the exercising period, influenced the rate in which temperature was elevated. By self-selecting resistance, participants could exercise at a comfortable rate thus minimising exercise strain.

A finding of particular interest is the stress which the acclimation imposed upon the participants. Cortisol, released as a response to stress, significantly increased during acclimation on day 1 (fig 3-6), and, although this response was not seen during day 5, this certainly agrees with previous observations suggesting heat acclimation reduces cortisol levels during exercise in the heat (L. E. Armstrong et al., 1989; Francesconi et al., 1983). However, such a finding is not universal (Finberg & Berlyne, 1977; Sunderland et al., 2008). It has been suggested that increases in plasma cortisol are only significantly elevated with feelings of ‘annoyance’ and ‘discomfort’ (Follenius, Brandenberger, Reinhardt, & Simeoni, 1979). In the present study participants after acclimation felt cooler (Fig 3-11 middle panel)

and more comfortable (Fig 3-11 bottom panel) during intermittent exercise in the heat (31˚C, 50% RH) indicating reduced psychophysical stress.

In response to hot-wet (35˚C, 79% RH) and hot-dry (49˚C, 20% RH) exercise before and after acclimation in both euhydrated and hypohydrated conditions, Francesconiet al (1989) observed that hypohydration in a pre-acclimated state significantly increased plasma cortisol levels. This effect was significantly attenuated post-acclimation, especially in the hot-wet condition (Francesconi et al., 1983). Improved fluid dynamics in the present study could therefore have played a significant role in the reduction in plasma cortisol concentration. It remains unclear whether this is a direct response of improved fluid dynamics or an indirect response in which improved fluid dynamics result in greater psychophysical comfort and thus decreased plasma cortisol concentration.

5.2 Adaptation to exercise in the heat

To evaluate the success of STHA on heat strain and exercise performance, a repeated HST was completed by all participants after a week of no acclimation and a week of STHA using the controlled hyperthermia model and permissive dehydration. Participants completed menstrual cycle questionnaires and provided venous blood samples prior to HST2 and HST3 to allow the determination of plasma E2. It has previously been evidenced that heat acclimation in females is not affected by menstrual cycle phase (Avellini et al., 1979) or oral contraceptive pills (L.E. Armstrong et al., 2005), therefore in the present study we did not exclude people based upon whether they were oral contraceptive users or not. We did, however, ensure that HST2 and HST3 were performed during the same phase of the menstrual cycle or pill phase, confirmed by E2. At time of writing, E2 data has not been analysed and thus mapping from self-reported menstrual cycle provides determination of menstrual phase.

Match play dynamics have been shown to vary with different playing positions (Gabbett & Mulvey, 2008; Hewitt, 2007; Mohr et al., 2008) and levels of play (Andersson et al., 2010; Krustrup et al., 2009; Mohr et al., 2008). The most up to date review of the physiology of female football suggests that during a 90 minute match players will cover ~10km and maintain an average f_c at ~86% $f_{c\,max}$ (Datson et al., 2014).

Locomotive activity is usually defined using velocity thresholds. However, in the present study we defined commonly identified locomotor activities as a percentage of $f_{c\,max}$ determined during a $\dot{V}O_{2\,peak}$ test. The percentage of time spent at each locomotion for collegiate female footballers has been previously described (Vescovi & Favero, 2014).

We aimed to ensure that participants mimicked the typical match play intensity of a collegiate female football match during pre-acclimation HSTs. Participants mean f_c (HST1: ~87 and HST2: ~86%) and distance covered (HST1: ~5.6 and HST2: ~5.6km) did not differ between HST1 and HST2, and were representative of that of a female collegiate football match (Datson et al., 2014; Vescovi & Favero, 2014)

5.2.1 Body temperatures and cardiac frequency

Increased cardiovascular stability is recognised as one of the most rapidly occurring adaptations to the heat and thus one of the quickest to decay (Garrett et al., 2009; Garrett et al., 2011). During the early stages of intermittent treadmill exercise, a lower f_c response was observed during HST3 vs HST2. Although this was not continued into the later stages of the treadmill protocol it is likely that the increased efforts put into the maximal sprints at the end of each 5-minute period served to elevate f_c and thus minimal differences were observed.

One key adaptation elicited by successful heat acclimation is a lower $\overline{T}_b$ for a given exercise workload (Brade et al., 2013; Garrett et al., 2012; Sunderland et al., 2008) calculated from T_{re}

and $\overline{T}_{sk}$ (Sawka et al., 2011). In a female population, previous literature would indicate that such a response requires a longer-term intervention than that we employed (Mee et al., 2015).

Whilst one STHA protocol of 4 sessions did lower T_{re} in the early stages of exercise (Sunderland et al., 2008), no responses were observed after a 3 day intervention (Dannen & Herweijer, 2015). Interestingly, the employment of the controlled hyperthermia model successfully attenuated T_{re} during a 30-minute run in the heat after 5 days in males (-0.39 ± 0.36˚C) but not in females (-0.07 ± 0.18˚C), yet after a further 5 days of acclimation, the females T_{re} response to the run were reduced by 0.48 ± 0.27˚C (Mee et al., 2015).

The present study contradicts previous studies which observed a decrease in T_{re} in response to a 5-day STHA regimen during the latter 15 mins (-0.16 ± 0.16˚C) of treadmill running designed to elicit the intensity of collegiate football in the heat.

No reduction in resting T_{re} was observed in the present study, although this has previously been observed with STHA in males (Garrett et al., 2012; Garrett et al., 2009; Garrett et al., 2014). Although the best efforts were made to ensure all test trials were performed at the same time of day, we had to fit testing around participants' personal schedules and therefore trials occurred no more than an hour of each other. It is recognised that resting T_{re} is an adaptive response, sensitive to the time of day of heat exposures (Shido, Sugimoto, Tanabe, & Sakurada, 1999), and, when time of the day is controlled for, decreases in T_{re} have been noted (Buono, Heaney, & Canine, 1998).

In the present study, $\overline{T}_{sk}$ decreased (-1.1%) in a manner comparable with several studies in male populations (Garrett et al., 2014; Gibson et al., 2015; James et al., 2016). Peak $\overline{T}_{sk}$ after 5 days STHA in a female population was decreased by 1.1% in the study be Mee et al. (2015) which was probably due to the significantly improved sudomotor response exhibited by females after STHA (Mee et al., 2015).

Due to the absence of a decrease in resting $\overline{T}_b$ and $T_{re,}$ it is likely that the lowered $\overline{T}_b$ during the latter stages of exercise was a result of improved heat loss rather than a lower initial heat content. The observed lower $\overline{T}_{sk}$ observed in the present study is probably indicative of more effective evaporative cooling.

While it has been suggested that redistribution of sweat to the limbs results in greater post-acclimation evaporative heat loss (Höfler, 1968; Regan, Macfarlane, & Taylor, 1996; Shvartz et al., 1979) this has not always been shown to be the case (Cotter et al., 1997). It has been suggested that if sweat glands can adapt, it is those farthest from their maximal sweat capacity that will undergo the greatest adaptation, such as the forearm (Patterson et al., 2004a). In the present study, sweat rate was measured as a function of body mass loss during the each HST and therefore conclusions regarding local sweat rate cannot be made.

No improved sweat rate was observed in the present study. It could be that during all three HSTs, metabolic heat production (unmeasured) was below the participants maximum sweat capacity in the given environment ($E_{sk,max}$) and thus sweat rate was driven by the required evaporative cooling rate (E_{req}) to maintain heat balance (Gagnon & Kenny, 2012). A reduction in $\overline{T}_{sk}$ would therefore be suggestive of changes in local sweat rates.

5.2.2 Plasma volume

While a significant ~8.5% PV expansion occurred between HST2 and HST3, this was widely variable (-1.2 to 18.5%). Such variability is not a novel finding. Previous research suggests large variability in PV expansion ranging 3-27% (Garrett et al., 2014; Nielsen et al., 1993; Patterson et al., 2014; Patterson et al., 2004b). Increased resting albumin concentration probably increased colloid-osmotic pressure (Yang et al., 1998). Certainly, previous research suggests that intravascular fluid expansion, resulting from such increases in colloid-osmotic pressure (Senay, 1979; Senay et al., 1976), explains the majority of PV expansion (Goto et

al., 2010). Such increases were not apparent following acclimation in the present study suggesting that, during exercise, another mechanism was involved in the enhanced PV expansion.

A significant increase in post acclimation Na^+ seen in the present study probably enhanced expansion of the entire extracellular compartment (Patterson et al., 2004b). It is probable that the increased Na^+ retention is a result of increased circulating aldosterone (C. G. Armstrong & Kenney, 1993; L. E. Armstrong et al., 1987; Patterson et al., 2004b). It may be that the hormone AVP also contributed to PV expansion by increasing the reabsorption of water in the distal tubules. Convertinho et al. (1981) suggests it is the conservation of Na^+ that drives an increase in circulating AVP, perhaps as a supportive mechanism to prevent hypernatremia.

5.2.3 Performance

It has been suggested that an elevated muscle temperature of 35-40.5˚C may in fact augment repeated sprint ability during match play (Mohr et al., 2004). Although muscle temperature was not measured in the present study, it is recognised that T_c is ~0.5-1.0˚C cooler than muscle temperature (Mohr et al., 2004). In the present study during pre-acclimation trials, especially in the latter stages of the exercise, T_m approached and may even have exceeded this optimal range. During HST3, T_{re} significantly decreased. Assuming muscle temperature was ~1.0˚C greater than T_{re}, the participants would have been in the optimal T_m range and this could explain the significant improvement in PPO (3.4%) and MAvP (4.7%) during the last 15 minutes from HST2.

An improvement in sprint dynamics is a valuable asset in team sport situations. Work-rate during team sport matches are largely determined by the playing style of the opposing team and individuals (Özgünen et al., 2010). The ability to maintain repeated sprint performance can therefore determine which player gets to the ball first and whether a player can outrun the

opposition. The improved sprint dynamics observed in the current study may be effective in achieving these goals in a match situation.

5.3 Limitations

One of the key limitations to the present study is our participation number (n = 8). Whilst several other studies report similar numbers of participants (Garrett et al., 2012; Garrett et al., 2009; Garrett et al., 2014; Mee et al., 2015; Neal et al., 2015), others (Aoyagi et al., 1995; Kelly et al., 2016; Nielsen et al., 1993; Petersen et al., 2010; Sawka, Young, Cadarette, Levine, & Pandolf, 1985) have used larger sample sizes and thus more reliable conclusions can be formed. Our participants' fitness levels were very varied within this small sample size and therefore the physiological strain placed on participants during our HSTs also varied. Adopting a more established protocol such as the Loughborough Intermittent Shuttle Test (LIST) or the YoYo intermittent recovery test, although not feasible in our laboratory, may have provided more valid results. The use of a maximal test to exhaustion, as adopted by Nation et al. (2015), repeated jump test (Mohr & Krustrup, 2013; Mohr et al., 2010) or repeated sprint test (Mohr et al., 2010; Mohr et al., 2012) would perhaps have provided a better performance outcome measure than the 6s maximal sprints employed in the current study. The detection of heat stroke and other heat related illnesses can be characterised by changes in mental status. Thus, the adoption of a cognitive task such the Stroop Coloured-Word Test would provide valuable information on cognitive functioning adaptations in response to heat acclimation. Finally, the use of treadmill running, despite being intermittent in nature and individualised to each participant, neither wholly replicates the unpredictability of team sports nor does it incorporate the multitude of movements such as tackling, jumping, dribbling and side-stepping which are integral components of team sports. The addition of countermovement jumps or squat jumps may add an extra component but addressing all components would require an extremely sophisticated protocol.

In summary, this work has established the effectiveness of short-term heat acclimation (STHA) for 5 days, using the controlled-hyperthermia technique with dehydration (Garrett et al. 2009, 2012, 2014 & 2015), on intermittent activity replicating female football match play, in a hot, humid environment with a female cohort, controlling for menstrual cycle phase.

Therefore, the underlying theme of this work is the health and well-being of female players during the preparation for heat stress conditions. The current research suggests these methods of acclimation in female games players indeed enhance thermoregulation and cardiovascular stability during intermittent exercise in the heat. Such improvements allow performance enhancements and comfort within the heat and thus may provide protection from exertional heat related illnesses associated with exercise performance in the heat. This work adds to the limited body of literature available and indicates the need to provide health and safety exercise guidelines for females in the heat that is based upon research with female cohorts.

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Table of Appendices

Appendix A

Participant information sheet and informed consent for participants

Participant Letter of Invitation 

Project title	Health and well-being of female football players in the preparation for competition in the heat
Principal investigator	Name: Dr. Andrew Garrett Email address: A.Garrett@hull.ac.uk Contact telephone number: 01482 463866
Student investigator (if applicable)	Name: Jarrod Gritt and Victoria Biddlecombe Email address: J.Gritt@2011.hull.ac.uk and V.Biddlecombe@2014.hull.ac.uk Contact telephone number: 07824775060 and 07910659851

Dear Madam

This is a letter of invitation to enquire if you would like to take part in a research project at The University of Hull

Before you decide if you would like to take part it is important for you to understand why the project is being done and what it will involve. Please take time to carefully read the Participant Information Sheet on the following pages and discuss it with others if you wish. Ask me if there is anything that is not clear, or if you would like more information.

If you would like to take part please complete and return the Informed Consent Declaration form.

Please do not hesitate to contact me if you have any questions.

Yours faithfully,

Jarrod Gritt

Participant Information Sheet

Project title	Health and well-being of female football players in the preparation for competition in the heat
Principal investigator	Name: Dr. Andrew Garrett Email address: A.Garrett@hull.ac.uk Contact telephone number: 01482 463866.
Student investigator (if applicable)	Name: Jarrod Gritt and Victoria Biddlecombe Email address: J.Gtitt@2011.hull.ac.uk and V.Biddlecombe@2014.hull.ac.uk Contact telephone number: 07824775060 and 07910659851

What is the purpose of this project?

The purpose of this study is to observe and assess the cardiorespiratory, thermoregulatory, metabolic and perceptual effects of a 5-day short term heat acclimation period on exercise performance in a game related intermittent exercise protocol in the heat. The study will help to understand better how to prepare and protect female athletes for competition in the heat.

Why have I been chosen?

You have been chosen firstly for your initial interest. Based upon your training background we believe that you will possess the attributes necessary to complete the study in question.

What happens if I volunteer to take part in this project?

First, it is up to you to decide whether or not to take part. If you decide to take part, you will be given this Participant Information Sheet to keep and asked to complete the Informed Consent Declaration at the back. You should give the Informed Consent Declaration to the investigator at the earliest opportunity. You will also have the opportunity to ask any questions you may have about the project. If you decide to take part, you are still free to withdraw at any time and without needing to give a reason.

What will I have to do?

Your participation will require 10 visits over a six-week period. We do ask that for 24 hours before all testing procedures that you refrain from strenuous exercise, caffeine and alcohol consumption. It is also important to ensure that you are adequately hydrated prior to all testing procedures and

that you rehydrate sufficiently throughout the day after all procedures. Before any testing commences we will require you to complete a short menstrual cycle questionnaire, which like all other documents will be protected strictly under the data protection act.

Visit 1 (week 1) During this visit we will be performing a VO2max test. This test is a maximal test that will allow us to determine your performance characteristics. When you arrive at the lab we will firstly ask you to complete a pre-exercise medical questionnaire which will allow us to determine your current health status and whether there are any contraindications in your health status that affects your eligibility to participate in the study. We will also ask you to complete an informed consent form which declares you understand the procedures to be completed and that you are happy with those procedures to be carried out. On every subsequent visit to the lab we will ask you to confirm that no changes in health status have occurred and an informed consent form will be presented.

We will fit you with a heart rate monitor and a face mask which you will breathe through in order for us to collect and analyse your expired air. After a brief warm up period we will ask you to run on a treadmill which will start at 5km/h and increase every 6 seconds by 0.1km/h, we will give verbal encouragement to push you to run as long as you feel possible, the test will be terminated when you cannot run any more or you terminate it for any other reason. This test typically lasts 10-12 minutes.

Visit 2 (week 2) At this visit we will firstly ask you to privately take your nude body mass, provide a urine sample and insert a rectal thermistor. You will then be seated to rest while we take a finger capillary blood sample and fit you with 4 skin thermistors which will be placed on the chest, bicep, thigh and calf and a heart rate monitor.

From here we will move you into the environmental chamber which will be set at 31˚c and 45% relative humidity. Here we will take your blood pressure, measure skin blood flow by means of vascular occlusion plethysmography and measure cardiac output by means of CO rebreathing. Following these measurements, we will ask you to complete a 45-minute intermittent exercise protocol which will consist of 9 x 5 min blocks of treadmill activity described as; standing, walking, jogging, low-intensity, moderate-intensity and high-intensity running and cycle ergometer activity described as maximal sprinting. At 15, 30 and 45 mins we will be taking cardiac output measures as previously described. No fluid consumption will be permitted during this test period.

When the protocol has finished we will once again measure skin blood flow and we will be taking the same blood, urine and weight measurements as we did at the start.

Visit 3 (week 3) Approximately one week after your second visit, and will be exactly the same as your second visit, except before procedures begin we will ask for a sample of blood via a venepuncture, this will be taken by a qualified person

Visit 4 (week 4) A very brief visit for a venepuncture blood sample only.

Visits 5-9 (week 5) This week will be a 5-day short term heat acclimation week. Each morning (mon-fri) we will require a venepuncture blood sample before and after the acclimation period as well as nude body mass, urine, and finger capillary blood samples. Like the heat stress test, we will require you to insert a rectal thermistor and wear a heart rate monitor, no skin thermistors will be required. Days 2, 3 and 4 will require the rectal thermistor and heart rate monitor to be worn however no blood or urine samples will be required. Each day will require a 90-minute period in the environmental chamber. Upon entry to the chamber, which will be 39.5˚c and 60% relative

humidity, you will be seated on a cycle ergometer and will be asked to cycle, every 5 minutes we will add or take away weight on the bike o try and elevate your core temperature as quickly as possible to 38.5˚c at which point you will get off the bike and remain seated for the remainder of the 90-minute period. You will only be required to cycle again in the unlikely case your core temperature drops below 38.5˚c. This method of acclimation is under permissive dehydration, which means for each 90-minute acclimation period no fluid consumption will be permitted.

Visit 10 (week 6) Your final visit will follow the exact same routine as visit 2.

Will I receive any financial reward or travel expenses for taking part?

No financial reward or travel expenses will be given.

Are there any other benefits of taking part?

Taking part in this study will give you an insight to the kind of research that is carried out in the department of Sports, Health and Exercise Science at the University of Hull. You will have the opportunity to exercise in the departments state of the art environmental chamber. You will see potential improvements in fitness upon completion of acclimation, furthermore as you will perform a VO2max test, we will be able to provide an indication of your fitness level.

Will participation involve any physical discomfort or harm?

Firstly, you will be performing a VO2max test which will require you to work to maximal capacity and the heat stress tests will involve submaximal exercise in hot conditions, these tests are designed to stress you and therefore some discomfort may be experienced during these tests. Heat acclimation will elevate your core temperature significantly and again may cause some discomfort.

Will I have to provide any bodily samples (e.g. blood or saliva)?

There will be several blood and urine samples required throughout the testing period which will be used for analysis of your hydration levels, menstrual cycle phase and adaptations to the heat.

Will participation involve any embarrassment or other psychological stress?

No embarrassment or other psychological stress is foreseen in this study.

What will happen once I have completed all that is asked of me?

Once you have completed all your visits a participant debrief form will be provided, and any further questions that you may have will be answered as best possible.

How will my taking part in this project be kept confidential?

All personal information will be kept securely stored on encrypted USB drives and hard copies in secure locations either within the faculty of Sports, Health and Exercise Science or Off site. Only members of the research team will have access to personal information. All information will be stored for a maximum of 5 years before disposal or destruction.

Anonymity will be ensured by all participants having a unique code in which information will be recorded under.

How will my data be used?

Your data will be used in an analysis process at which point all your data will be anonymous, this data will be used in an MSc and a BSC thesis and potentially the research may be published in a peer reviewed scientific journal.

Who has reviewed this study?

This project has undergone full ethical scrutiny and all procedures have been risk assessed and approved by the Department of Sport, Health and Exercise Science Ethics Committee at the University of Hull.

What if I am unhappy during my participation in the project?

You are free to withdraw from the project at any time. During the study itself, if you decide that you do not wish to take any further part then please inform the person named in Section 18 and they will facilitate your withdrawal. You do not have to give a reason for your withdrawal. Any personal information or data that you have provided (both paper and electronic) will be destroyed or deleted as soon as possible after your withdrawal. After you have completed the research you can still withdraw your personal information and data by contacting the person named in Section 18. If you are concerned that regulations are being infringed, or that your interests are otherwise being ignored, neglected or denied, you should inform Dr Andrew Garrett, Chair of the Department of Sport, Health and Exercise Research Ethics Committee, who will investigate your complaint (Tel: 01482 463866; Email: a.garrett@hull.ac.uk

How do I take part?

Contact the investigator using the contact details given below. He or she will answer any queries and explain how you can get involved.

Name: Jarrod Gritt Email: J.Gritt@2011.hull.ac.uk Phone: 07824775060

Department of Sport, Health & Exercise Science

Informed Consent Declaration

Project title	Health and well-being of female football players in the preparation for competition in the heat
Principal investigator	Name: Dr. Andrew Garrett Email address: A.Garrett@hull.ac.uk Contact telephone number: 01482 463866.
Student investigator (if applicable)	Name: Jarrod Gritt and Victoria Biddlecombe Email address: J.Gtitt@2011.hull.ac.uk and V.Biddlecombe@2014.hull.ac.uk Contact telephone number: 07824775060 and 07910659851

Please Initial

☐ I confirm that I have read and understood all the information provided in the Informed Consent Form (EC2) relating to the above project and I have had the opportunity to ask questions.

☐ I understand this project is designed to further scientific knowledge and that all procedures have been risk assessed and approved by the Department of Sport, Health and Exercise Science Research Ethics Committee at the University of Hull. Any questions I have about my participation in this project have been answered to my satisfaction.

☐ I fully understand my participation is voluntary and that I am free to withdraw from this project at any time and at any stage, without giving any reason. I have read and fully understand this consent form.

☐ I agree to take part in this project.

.....	.....	.....
Name of participant	Date	Signature
.....	.....	.....
Person taking consent	Date	Signature

Appendix B

Pre-exercise medical questionnaire

Pre-Exercise Medical Questionnaire

The information in this document will be treated as strictly confidential

Name: .....

Date of Birth: ..... Age: ..... Sex: .....

Blood pressure: ..... Resting Heart Rate: .....

Height (cm): ..... Weight (Kg): .....

Please answer the following questions by putting a circle round the appropriate response or filling in the blank.

1. How would you describe your present level of **exercise** activity?

   Sedentary / Moderately active / Active / Highly active

2. Please outline a typical weeks exercise activity

.....

.....

.....

3. How would you describe your present level of **lifestyle** activity?

   Sedentary / Moderately active / Active / Highly active

4. What is your occupation? .....

5. How would you describe your present level of fitness?

   Unfit / Moderately fit / Trained / Highly trained

6. Smoking Habits Are you currently a smoker? Yes / No

How many do you smoke …….. per day

Are you a previous smoker? Yes / No

How long is it since you stopped? ..... years

How many did you smoke? ..... per day

7. Do you drink alcohol? Yes / No

If you answered **Yes** and you are male do you drink more than 28 units a week?

Yes / No

If you answered **Yes** and you are female do you drink more than 21 units a week?

Yes / No

8. Have you had to consult your doctor within the last six months? Yes / No

If you answered **Yes**, Have you been advised **not** to exercise?

Yes / No

9. Are you presently taking any form of medication? Yes / No

If you answered **Yes**, Have you been advised **not** to exercise?

Yes / No

10. Do you have a history of fainting during or following exercise? Yes / No

If **Yes**, please provide details.....

.....

.....

11. To the best of your knowledge do you, or have you ever, or have a family history:

a Diabetes? Yes / No **b** Asthma? Yes / No

c Epilepsy? Yes / No **d** Bronchitis? Yes / No

e ✱Any form of heart complaint? Yes / No **f** Raynaud's Disease Yes / No

g ✱Marfan's Syndrome? Yes / No **h** ✱Aneurysm / embolism? Yes / No

I Anaemia Yes / No

12. ✸Are you over 45, and with a history of heart disease in your family? Yes / No

13. Do you currently have any form of muscle or joint injury? Yes / No

If you answered **Yes**, please give details…….

……

……. …..

14. Have you had to suspend your normal training in the last two weeks? Yes / No

If the answer is **Yes** please give details…….

……..

……..

……..

15. ✸ Please read the following questions:

a) Are you suffering from any known serious infection? Yes / No

b) Have you had jaundice within the previous year? Yes / No

c) Have you ever had any form of hepatitis? Yes / No

d) Are you HIV antibody positive Yes / No

e) Have you had unprotected sexual intercourse with any person from an HIV high-risk population? Yes / No

f) Have you ever been involved in intravenous drug use? Yes / No

g) Are you haemophiliac? Yes / No

16. As far as you are aware, is there anything that might prevent you from successfully completing the tests that have been outlined to you? Yes / No.

IF THE ANSWER TO ANY OF THE ABOVE IS YES:

Discuss with the test administrators or another appropriate member of the department.

Questions indicated by (✸) answered yes: Please obtain written approval from your doctor before taking part in the test.

PLEASE SIGN AND DATE AS INDICATED ON THE NEXT PAGE

Participant Signature: …… Date……

Test Administrator:…….. Date……

Supervising staff member..... Date.....

Parent (if minor)…….. Date: …….

THIS SECTION IS ONLY REQUIRED FOR RETURN VISITS!

For any future testing sessions it is necessary to verify that the responses provided above are still valid, or to detail any new information. This is to ensure that you have had no new illness or injury that could unduly increase any risks from participation in the proposed physical exercise.

ANSWER THE FOLLOWING QUESTION AT EACH REPEAT VISIT.

Is the information you provided above still correct, and can you confirm that you have NOT experienced any new injury or illness which could influence your participation in this exercise session?

Repeat 1	Yes / No*	Signature:	Date:
*Additional info required:			
Repeat 2	Yes / No*	Signature:	Date:
*Additional info required:			
Repeat 3	Yes / No*	Signature:	Date:
*Additional info required:			
Repeat 4	Yes / No*	Signature:	Date:
*Additional info required:			

Repeat 5	Yes / No*	Signature:	Date:
*Additional info required:			

Appendix C

Menstrual cycle questionnaire

MENSTRUAL CYCLE DETAILS

(All information is fully confidential)

Please circle the answer where appropriate.

Name:

Age:

Date:

1) Have you had regular periods in the last two years? YES NO

2) How long in days is your menstrual cycle, from day 1 of menses/period to day 1 of the next?

_____DAYS

3) Is the above time the same between periods? YES NO

If the answer was NO, please state the irregularity:

_____
_____
_____

4) How many days does your menstrual (blood) flow last?

_____DAYS

5) Do you get pain during your period? YES NO

If YES, please state the symptoms and the days during the cycle when you suffer:

_____
_____
_____

6) Do you avoid exercise during your period? YES NO

If YES, please state your reasons for avoiding exercise:

_____
_____
_____

7) Do/ Did you take contraceptive pills? YES NO

If YES, please state what (brand name) pill you take, when you started to take the pill and for how long you have been using the pill?

What (brand name):_____

When you started:_____

How long you have been taking the pill: _____

8) If you are currently taking the contraceptive pill, will you be continuing to do so for the next 3 months?

YES NO

If NO, please state when you will stop taking contraceptive pills:

_____

_____

9) What date was day 1 of your pill taking (i.e. the date you took the first pill in your new packet for this month)?

_____

10) Do you take any medication or hormones to regulate your menstrual cycle (other than the contraceptive pill)?

YES NO

If YES, please state what you take and how often?

_____

_____

_____

11) When did you have your last period (day 1)?

_____

Appendix D

Scales for rate of perceived exertion, thermal sensation and thermal comfort

Thermal Senstaion

"How does the temperature of your body feel?"

1	**Unbearably cold**
2	**Extremely cold**
3	**Very cold**
4	**Cold**
5	**Cool**
6	**Slightly cool**
7	**Neutral**
8	**Slightly warm**
9	**Warm**
10	**Hot**
11	**Very hot**
12	**Extremely Hot**
13	**Unbearably hot**

Perceived Exertion Scale (RPE)

"How hard do you feel you are exercising?"

6	
7	**Very, very light**
8	
9	**Very light**
10	
11	**Fairly light**
12	
13	**Somewhat hard**
14	
15	**Hard**
16	
17	**Very hard**
18	
19	**Very, very hard**
20	

Thermal Comfort Scale

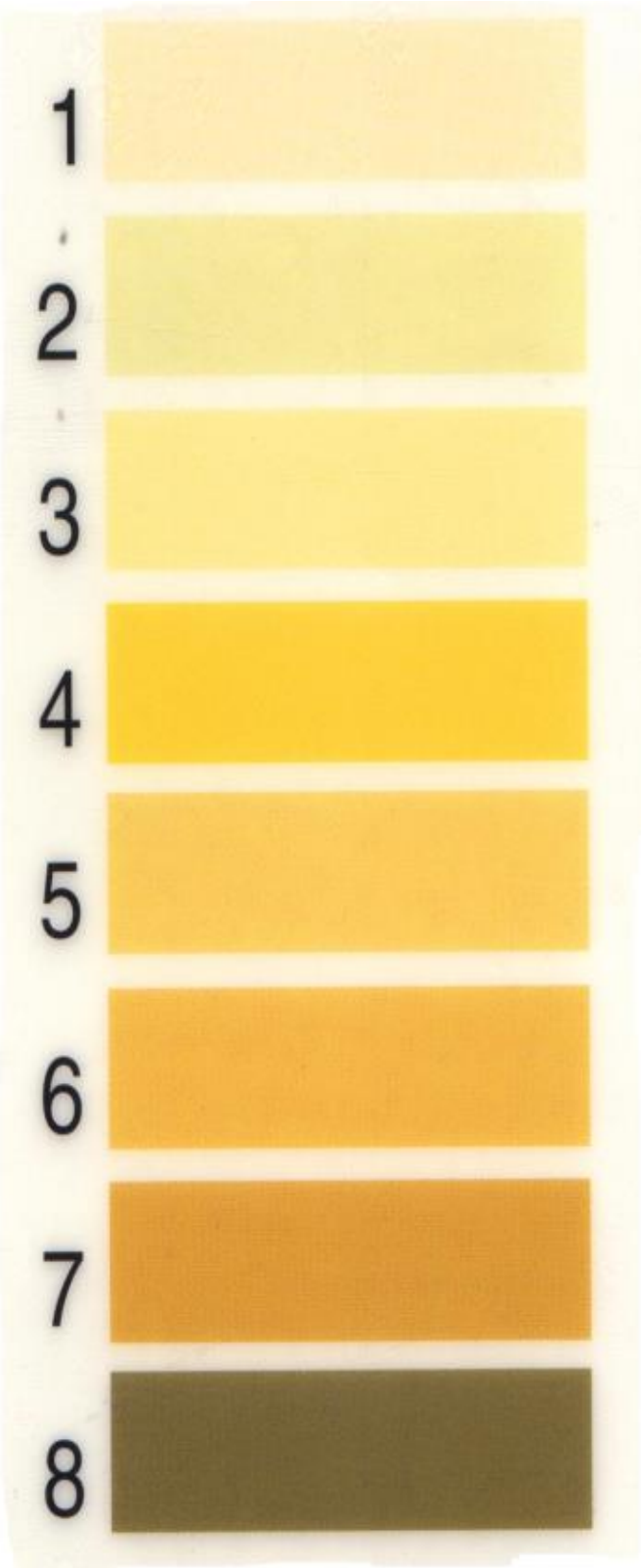
"how comfortable do you feel with the temperature of your body?"

1	**Very comfortable**
2	**Comfortable**
3	**Slightly Comfortable**
4	**Uncomfortable**
5	**Extremely uncomfortable**

Appendix E

Colour chart for the determination of urine colour

Urine Colour



The Urine Color Chart shown here will assess your hydration status (level of dehydration) in extreme environments. To use this chart, match the color of your urine sample to a color on the chart. If the urine sample matches #1, #2, or #3 on the chart, you are well hydrated. If your urine color is #7 or darker, you are dehydrated and should consume fluids.