HEAT TOLERANCE AND ACCLIMATION IN FEMALE ATHLETES

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Abstract

This thesis aimed to develop a running heat tolerance test (RHTT) to assess changes in heat tolerance and to investigate the mechanism and optimisation of heat acclimation (HA) for female athletes.

The first study introduced a RHTT and assessed its repeatability. Results demonstrate good agreement, strong correlations and small differences between repeated trials. The typical error of measure values suggested low within-participant variability. Furthermore, the RHTT was effective in differentiating between individuals' physiological responses, demonstrating that heat tolerance lies along a continuum.

The second study examined the sensitivity of the RHTT to changes in heat tolerance and to evaluate individual responses to HA. Results demonstrate that the RHTT is sensitive to changes in heat tolerance and that the magnitude of adaptation is highly individual; supporting the use of the RHTT in future investigations.

Reducing thermal strain through HA in not fully understood for a female population. The third study compared males' and females' temporal patterning to short-term HA (STHA; 5-d) and long-term HA (LTHA; 10-d). The RHTT was used to quantify changes in heat tolerance. The results confirm that whilst STHA may be effective in achieving partial adaptation in males and females, females require LTHA to establish reductions in thermoregulatory and cardiovascular strain.

Improved thermotolerance following HA, reduces disruptions to cellular homeostasis principally, but not exclusively, by increasing basal heat shock protein 72 following transcription of its gene (Hsp 72 mRNA) as part of the heat shock response (HSR). The fourth study compared males' and females' Hsp72 mRNA response during STHA and LTHA. The similar transcription of Hsp72 mRNA observed in all participants suggests that there are no differences in the endogenous criteria to elicit the HSR between sexes.

The fifth study assessed the effectiveness of preceding STHA with a passive heat exposure (HA$_{sauna}$) in females. HA$_{sauna}$ resulted in reductions in thermoregulatory, cardiovascular and perceptual strain. The adaptation pathway was likely mediated in part, by plasma volume expansion and an improved thermoeffector and thermosensitivity response of the sudomotor function.

Together, evidence in this thesis supports the notion that special considerations need to be taken when using HA to attenuate thermoregulatory strain in female athletes prior to training and competing in the heat.

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List of abbreviations

BM	Body mass
BSA	Body surface area
CI	Confidence interval
CO_2	Carbon dioxide
FSH	Follicle stimulating hormone
HA	Heat acclimation
HA_{sauna}	HA combined with a passive heat exposure
HR	Heart rate
Hsp mRNA	Heat shock protein messenger ribonucleic acid
HSP	Heat shock protein
HSR	Heat shock response
HTT	Heat tolerance test
ICC	Intra class correlation coefficient
IDF	Israeli Defence Force
LH	Luteinising hormone
LTHA	Long-term heat acclimation
NaCl	Sodium Chloride
O_2	Oxygen
PV	Plasma volume
RH	Relative humidity

RHTT	Running heat tolerance test
RPE	Rating of perceived exertion
rpm	revolutions per min
RT-qPCR	Reverse transcription polymerase chain reaction
SD	Standard deviation
SWR	Sweat rate
SWR_{BSA}	Sweat rate relative to body surface area
STHA	Short-term heat acclimation
TEM	Technical error of measurement
TE (CV %)	Technical error as a coefficient of variation
T_c	Core temperature
T_{re}	Rectal temperature
TS	Thermal sensation
T_{sk}	Mean skin temperature
USG	Urine specific gravity
$\dot{V}O_2max$	Maximal oxygen uptake
$\dot{V}O_2\ peak$	Peak oxygen uptake
WBGT	Wet bulb globe temperature

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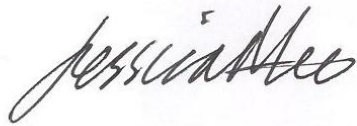
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Declaration

I declare that the research contained in this thesis, unless otherwise indicated within the text, is original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed:

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1. Introduction

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Moderate ambient temperatures are known to present sufficient heat stress that result in an elevated core temperature (T_c) and reduce endurance capacity (Galloway and Maughan 1997). During exercise in a hot environment, active muscles perform work causing an increase in body heat content. These changes represent the rate of change in body heat storage, which in turn reflects the balance between metabolic heat production, heat absorbed from the environment and total body heat loss (Jay and Kenny, 2007). Exertional heat stroke is characterised by a rise in T_c beyond 40°C coupled with central nervous system dysfunction and multiple organ system failure (Casa et al., 2012).

The work by Nielsen (1996) provides data to suggest an endurance athlete may experience up to a 1°C rise in T_c every 9-min when racing in high ambient conditions. This rate of rise in T_c would result in a runner reaching a T_c of 40°C within 25 - 30 min, with the immediate dangers of heat exhaustion. Data are limited on the incidence of exertional heat illness in long distance running, particularly the reporting of non-fatal cases. The 11.5 km Falmouth Road Race in the USA, typically run in warm humid conditions (23.3°C, 70% RH), reports on average 10 − 20 cases per 10,000 entrants per yr (DeMartini et al., 2014). In contrast, the Twin Cities Marathon in USA, run in cooler conditions (16°C, 60% RH) reports an average of 1 case of exertional heat illness per 10,000 finishers (Roberts, 2000). While high ambient temperature and RH are important factors contributing towards exertional heat illness, there are also incidences in cooler environments (Hawes et al., 2010; Roberts, 2006; Robertson and Walter, 2010). The Great North Run in the UK in 2009 (18°C) reported 55 cases of exertional heat illness among the 55,000 participants (Hawes et al., 2010), an incidence rate similar to that observed at the Falmouth Road Race where conditions are much warmer.

Martin (1997) reported a higher incidence rate in females (36 incidence; 3.63%) compared with males (26 incidence; 2.18%); with females accounting for 58% of the total number of cases (Martin, 1997). Questionably, one of the most memorable marathon finishes was of a 39-yrs. old, female Swiss athlete, Gabriela Anderson-Schiess. It was apparent Gabriela was suffering with a serious case of heat exhaustion during the latter stages of the 1984 Olympic inaugural females' marathon. She limped around the final 400 m in 5-min and 44-s, refusing medical support, eventually crossing the finish line in 2-hr 48-min and 45-s. The severity of this case is still apparent today, with similar incidences such as that of 34-yr. old, female, Namibian athlete, Beata Naigambo. During the 2014 Commonwealth Games marathon, Beata staggered down the home straight and fainted meters

from the finish line, eventually collapsing over the finish line unassisted in 2-hr 39-min 23-s. This body of evidence emphasises the severity of heat related illnesses in endurance athletes, specifically females. Further, it highlights the need to develop effective screening procedures and heat-alleviating strategies, to support endurance athletes when training and competing in thermally-challenging environments.

Heat intolerance may be permanent or acquired and refers to a syndrome describing individuals who are unable to adapt properly to exercising in a hot environment. Heat tolerance tests (HTT) are currently used to evaluate military personal that have had a previous experience of exertional heat illness, to determine whether they are able to return to duty. The Israeli Defence Force (IDF) developed a HTT which involves 120-min walking on a treadmill at a pace of 5 km.hr^{-1} and a 2% gradient in ambient conditions of 40°C and 40% relative humidity (RH). In spite of the significant role for the IDF HTT to screen military personnel for heat intolerance, the application of the IDF HTT has been questioned in reference to endurance athletes due to differences in exercise intensity and duration, the clothing, and the carrying load (Johnson et al., 2013). Endurance athletes typically race for a shorter duration and at a higher exercise intensity compared with military personnel, who most commonly perform low intensity marching for prolonged periods of time. Therefore, the rate of heat production, requirement for evaporative cooling, and changes in T_c differ substantially between these populations. The incidence of exertional heat illness in endurance athletes (Martin, 1997) raises the importance of developing a heat tolerance screening procedure that informs medical personal about an endurance athlete's current heat tolerance state, and is capable of monitoring changes in heat tolerance.

Repeated heat exposure to a stressful thermal environment initiates a phenotypic adaptation known as heat acclimation (HA), an element of which has been identified as thermotolerance (Moseley, 1997). HA improves thermal comfort, submaximal exercise performance, and increases maximal aerobic capacity in the heat (Lorenzo et al., 2010). The benefits arise from enhanced sudomotor and skin blood flow responses, plasma volume (PV) expansion, cardiovascular stability and an improved fluid balance (Poirier et al., 2015). Thermotolerance or acquired cellular thermotolerance describes the cellular adaptation accompanying systemic changes induced by successful HA (Horowitz, 2014; Magalhães et al., 2010). As a result, HA is the consensus recommendation strategy to attenuate the physiological strain associated with training and competing in the heat (Racinais et al., 2015). However, HA it is not routinely adopted by endurance athletes; UK athletics currently only offer altitude training camps as part of their world class performance programme.

Controlled hyperthermia HA ensures equal thermal strain is placed on participants during each session, as it involves elevating and maintaining a steady state T_c above the sweating threshold (Fox et al., 1963a). This method has become increasingly popular since it offers more complete adaptation due to the progressive overload approach compared with traditional, fixed-intensity protocols which use a constant overload approach (Gibson et al., 2015b). However, there remains a paucity of data concerning best practice for HA in females; consequently, female athletes are attending warm weather training camps or implementing HA strategies based on data collected from male participants. The implications of this are that females may be adopting sub-optimal strategies that provide little benefit to alleviating physiological strain.

Sex has traditionally been considered an independent modulator of temperature regulation (Kaciuba-Uscilko and Grucza, 2001; Kenney, 1985; Nunneley, 1978), however these observations may be confounded by inherent physical differences between the sexes. More recently, sex differences in temperature regulation during exercise have been reported, irrespective of confounding differences in metabolic heat production and physical characteristics (Gagnon and Kenny, 2012, 2011). Interestingly, these differences only become evident above a certain requirement for heat loss and are solely attributed to peripherally mediated control of the sudomotor function. Furthermore, fluctuations in hormonal concentrations associated with the menstrual cycle have a known effect on thermoregulation in females. During the luteal phase of the menstrual cycle there is an elevation in progesterone concentration which modifies central regulatory mechanisms resulting in alterations in basal T_c, the body temperature threshold for sweating, and the body temperature threshold for cutaneous vasodilation on the forearm, chest, back, forearm, and thigh (Inoue et al., 2005). These sex differences in thermoregulatory responses to acute exercise heat stress, raises the question of whether females adapt to chronic heat exposure differently to males. Thus, research investigating females' phenotypic and cellular responses to controlled hyperthermia HA over short- and long-term timescales is required.

Recently, novel HA strategies have been investigated in male participants. These methods involve permissive dehydration (Garrett et al., 2012; Neal et al., 2015), sauna bathing (Scoon et al., 2007; Stanley et al., 2015), and hot water immersion (Zurawlew et al., 2015). These methods aim to establish an accelerated adaptation via targeting adaptation pathways including, PV expansion, increased sudomotor function, and altered cutaneous vascular function. Once the temporal patterning to controlled hyperthermia in females has been determined, further research is required to establish novel HA strategies that accelerate adaption responses in females.

This introduction has outlined the incidence of heat related illness in runners, particularly females, the requirement to develop a running HTT (RHTT), the clear need for research into females phenotypic and cellular adaptations to controlled hyperthermia HA, and introduced the idea of investigating novel HA strategies to accelerate adaptation in females. This thesis is therefore presented in the following chapters.

Chapter 2 reviews the literature, discussing acute and chronic responses to heat stress. The first part of the literature review considers the physiological and thermoregulatory response to an acute heat exposure, discussing individual differences, with a particular focus on any potential sex differences. The second part of the literature review focuses on heat illnesses and the incidence rate in running. The third part of the literature review discusses HA protocols and the time course of adaption. The fourth section focuses on the potential sex differences in responses to HA. The final section of this chapter outlines the thesis aims, research questions and hypotheses of the experimental studies. The search was restricted to full text articles published in English language peer reviewed journals. Where possible information was limited to articles using human participants, however due to the novelty of some of research presented, animal data has been used.

Chapter 3 describes the common methods used across multiple experimental chapters.

Chapter 4 presents the first experimental chapter (Study 1) whereby the repeatability of a RHTT was assessed. The findings from this study informs future experimental chapters by quantifying the measurement error associated with the RHTT to support accurate conclusions regarding the magnitude of adaptation observed.

Chapter 5 presents the second experimental chapter (Study 2) whereby the sensitivity of the RHTT to quantify changes in heat tolerance following short-term HA (STHA) was assessed.

Chapter 6 uses the RHTT to quantify the changes in physiological and thermoregulatory responses following controlled hyperthermia HA over short- and long-term time scales in males and females (Study 3). This chapter provides novel HA data on females and describes the differences in the temporal patterning between males and females.

Chapter 7 presents the fourth experimental chapter (Study 4) describing the heat shock protein gene responses (Hsp72 mRNA) to controlled hyperthermia HA over short- and long-term time scales in males and females.

Chapter 8 describes the findings from the final experimental chapter (Study 5). Following the identification that females required long-term HA (LTHA) to establish a reduction in cardiovascular and thermoregulatory responses in chapter 6 (Study 3), a novel method of combining a sauna-like exposure with controlled hyperthermia HA was investigated to establish its effectiveness in accelerating adaptation responses in females over a short-term time scale.

Chapter 9 discusses the individual and cumulative findings of all the experimental chapters to form the general discussion. The first part of the general discussion will review the principle findings from the experimental chapters presented. The second part of the general discussion will provide a mechanistic overview, focusing specifically on heat tolerance, the temporal patterning of HA, and accelerating the adaption in females; involving retrospective analyses of pooled data from within the thesis. This chapter closes with discussing potential future directions for research in the field, in addition to reviewing the practical application of the presented data.

2. Literature review

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2.1.   Environmental heat stress and exchange

Heat stress refers to both the environmental and metabolic conditions that typically increase T_c (Sawka et al., 2011a). Heat stress is categorised as compensable heat stress, or uncompensable heat stress. Compensable heat stress refers to a steady state T_c, whereby the heat loss matches the heat production (Sawka et al., 2011a). Uncompensable heat stress refers to an imbalance between the evaporative requirement and the evaporative capacity of an individual (Cheung et al., 2000). During uncompensable heat stress, an individual is unable to dissipate the metabolic heat, causing a continual rise in T_c, until either exhaustion occurs, or the severity of the environmental conditions decreases (Cheung et al., 2000).

To quantify environmental heat stress in occupational, military and sports applications a user-friendly measure known as wet bulb globe temperature (WBGT) is commonly used (Budd, 2008). WBGT is an empirical index of climatic heat stress which can be used to quantify the heat stress (Sawka et al., 2011a). WBGT can be calculated using Equation 2.1.

Equation 2.1 Calculation for wet bulb globe temperature (WBGT) (Yaglou and Minard, 1957)

$$WBGT = 0.7\ T_w + 0.2\ T_g + 0.1\ T_d$$

Where: T_w = wet bulb temperature, T_g = black globe temperature, T_d = dry bulb temperature

The WBGT has a number of limitations which questions its validity, accuracy and applicability. Specifically, the WBGT does not make any considerations for clothing or metabolic rate, as a result, it cannot predict heat exchange between a person and the climate (Budd, 2008). Despite its limitations, the WBGT is the most widely used index, since it is easy to use and is a reliable measure of heat stress (Budd, 2008). Many geographical regions experience WBGT above 29°C during the summer months. This is caused by a high humidity and/ or a high air temperature and solar load, as reflected by a high wet bulb temperature and black globe temperature, respectively (Sawka et al., 2011a). The universal thermal climate index (Fiala et al., 2012)provides an alternative to the WBGT, by integrating predictive formulas of heat strain based on activities conducted in different

temperatures at varying constant metabolic heat productions. While the universal thermal climate index may ultimately offer a more comprehensive and robust evaluation of the risk of physiological heat strain, it is more complex and expensive to adopt. To ensure that any heat stress monitoring system is widely adopted across all settings, solutions must be relatively simple to use and implement, while being affordable (Périard et al., 2016). Additional research is warranted to develop a more appropriate tool to support governing bodies in ensuring the health and safety of athletes in hot environmental conditions.

Physical exercise increases metabolic heat production above the resting rate (<100 W). Depending upon the type of exercise, approximately 80 to 90% of energy produced is released as heat and then dispersed around the body, mostly the blood stream (Jay and Kenny, 2007). It is necessary that the heat is dissipated from the body to avoid heat storage, with a resultant increase in T_c. Cardiovascular adjustments occur in order to redirect the blood flow from the body's core to the periphery for subsequent heat dissipation (Nybo et al., 2014). The heat delivered to the periphery is transferred to the environment by dry (radiative, conductive and convective) and wet (evaporative) heat transfer pathways. The effectiveness of the heat transfer pathways is dependent upon the biophysical properties of the environment. This includes the temperature differences between the skin surface and the environment, the evaporative potential and the solar conditions (Sawka et al., 2011a).

2.1.1. Dry heat transfer pathways

The non-evaporative avenues of heat exchanges are collectively called dry heat exchange; these include conduction, convection and radiation (figure 2.1). Conductive heat transfer occurs when two solid objects are in direct contact with each other and there is a temperature gradient between the body's surface and a contacting solid object. When performing exercise in a standing position such as walking and running the conductive heat exchanges is minimal since the contact point between the body's surface and the ground is very small (Kenny and Jay, 2013). Convection is the heat exchange between the surface of a solid object and a fluid, including air and water. Heat transfer via convection to air or fluid occurs when the air or fluid temperature is below the temperature of the body. An increase in wind or fluid current facilitates a greater rate of convective heat transfer; furthermore, the heat capacity of water is far greater than air increasing convective heat transfer. Radiation is the heat exchange between a solid object and a large natural, or manufactured object such as the sun or a heater (Sawka et al., 2011a).

2.1.1. Wet heat transfer pathways

Evaporative heat loss occurs when liquid changes into water vapour. The evaporative heat loss capacity is determined primarily by the water vapour pressure gradient between the body's surface and the environment. This can be modified by the environment and clothing (Sawka et al., 2011a), in addition to physiological alteration in sweat gland activity and output (Taylor, 2014). Evaporative cooling is the primary means of heat removal during exercise. Evaporative cooling is the only means for heat removal during exercise in an ambient temperature that is equal to, or greater than skin temperature (T_{sk}).
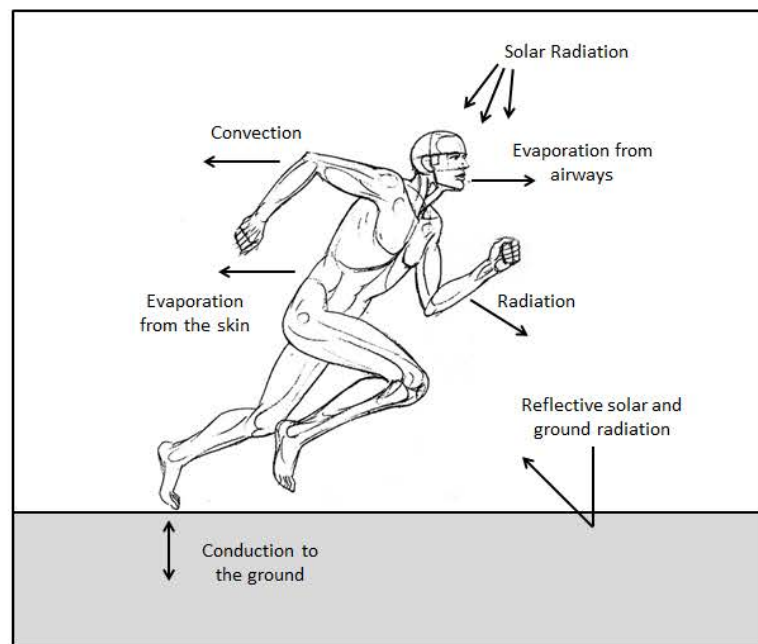
Figure 2.1 Avenues for heat exchange when performing exercise. Adapted from Gisolfi and Wenger, (1984)

2.1.2. Heat balance

The interaction between factors that increase or decrease heat production and heat dissipation and the resultant impact upon body heat storage is evident in the heat balance equation (Equation 2.2). Metabolic heat production is typically < 100 W at rest and > 1000 W during high intensity exercise. The rate of body heat storage represents heat gain if positive and heat loss if negative.

Equation 2.2. Heat balance equation

$$S \text{ (watts)} = M - (\pm W) \pm (R + C) \pm K - E$$

Where: S = rate of body heat storage in watts, M = rate of metabolic energy production, W = mechanical work, whether concentric (positive) or eccentric (negative) exercise, R + C = rate of radiant and convective energy exchanges, K = rate of conduction, E = rate of evaporative loss.

Metabolic heat production is the difference between metabolic rate and the fraction of this energy that is used to create external work. The human body is inefficient at performing external work and thus 80% - 100% of the energy produced is released as heat energy inside the body. For example, if a participant is cycling at 100 W, 400 W of the 500 W of total energy production will be heat energy inside the body.

Indirect calorimetry

Indirect calorimetry is the traditional method for estimating metabolic energy expenditure. This approach requires the measurement of the rate of oxygen (O_2) consumption and carbon dioxide (CO_2) production. Using the energy equivalent for the full oxidation of carbohydrates (21.13 kJ per L of O_2 consumed) and fats (19.63 kJ per L of O_2 consumed), metabolic energy expenditure can be subsequently calculated using Equation 2.3 (Kenny and Jay, 2013).

Equation 2.3 Calculation for metabolic energy expenditure

$$M = [\ \dot{V}O_2 \ ((((RER - 0.7) / 0.3) \ e_c) + (((1.0 - RER) / 0.3) \ e_f)] / 60$$

Where: $\dot{V}O_2$ is measure in $L.min^{-1}$; e_c is the caloric equivalent per litre of oxygen for the oxidation of carbohydrates (21130 J) and e_f is the caloric equivalent per litre of oxygen for the oxidation of fat (19630 J).

When direct calorimetry is unavailable, indirect calorimetry is the most precise method of estimating rate of metabolic energy expenditure and subsequently metabolic heat production (Kenny and Jay, 2013). However, despite heat production increasing almost immediately after the start of exercise, O_2 consumption increases exponentially with a time constant of 15 - 30 s. As such > 1-min of exercise at the same external workload is required to attain an elevated steady state of

O$_2$ consumption (Astrand and Saltin, 1961; Cheuvront et al., 2010; Whipp and Wasserman, 1972). Thus, the difference between the actual rate of metabolic heat production at the tissue level and its measurement from analysing expired air should be acknowledged.

Direct calorimetry

During bouts of heat imbalance, such as during the onset of exercise, a summation of the heat exchange via convection, conduction, radiation and evaporation must be obtained and compared to concurrent measurements of metabolic energy expenditure to determine the quantity of heat stored inside the body (Kenny and Jay, 2013). The most accurate method for quantifying the rate of net heat loss is with direct whole body calorimetry (Jay and Kenny, 2007). Whole body calorimetry is the simultaneous measurements of the internal heat generated by the body as a result of anaerobic and or aerobic metabolism and the total heat lost to the environment (Jay and Kenny, 2007). Thus, if an imbalance occurs between metabolic heat production and net heat loss to the environment, the time dependent differences between these measurements provide an estimate of the change in body heat content.

2.2.   Heat strain

Heat strain refers to any physiological consequences of heat stress (Sawka et al., 2011b). Measuring T$_c$ is fundamental to the experimental study of temperature regulation in humans (Sawka et al., 1993). T$_c$ is typically controlled at 37.0 ± 0.5°C during rest. However, T$_c$ varies between different body regions depending on the metabolic rate of the local tissues, the source and the volume of local blood flow, in addition to the temperature gradients between connecting body regions (Sawka et al., 2011a).

In a rested state, the internal organs and viscera produce approximately 70% of metabolic heat, however during movement, the skeletal muscles produce approximately 80 - 95% of metabolic heat (Sawka et al., 2011a). Metabolic heat sources are altered from rest to exercise. As a result, the calculated change in body temperature may be disproportionate in one body region compared with another. For example, when humans are in a rested state their T$_c$ (37.0 ± 0.5°C) is typically higher than muscle temperature (36.0 ± 0.5°C), however during exercise, the temperature of active skeletal muscle usually exceeds that of T$_c$ (Kenny et al., 2008) (figure 2.2).
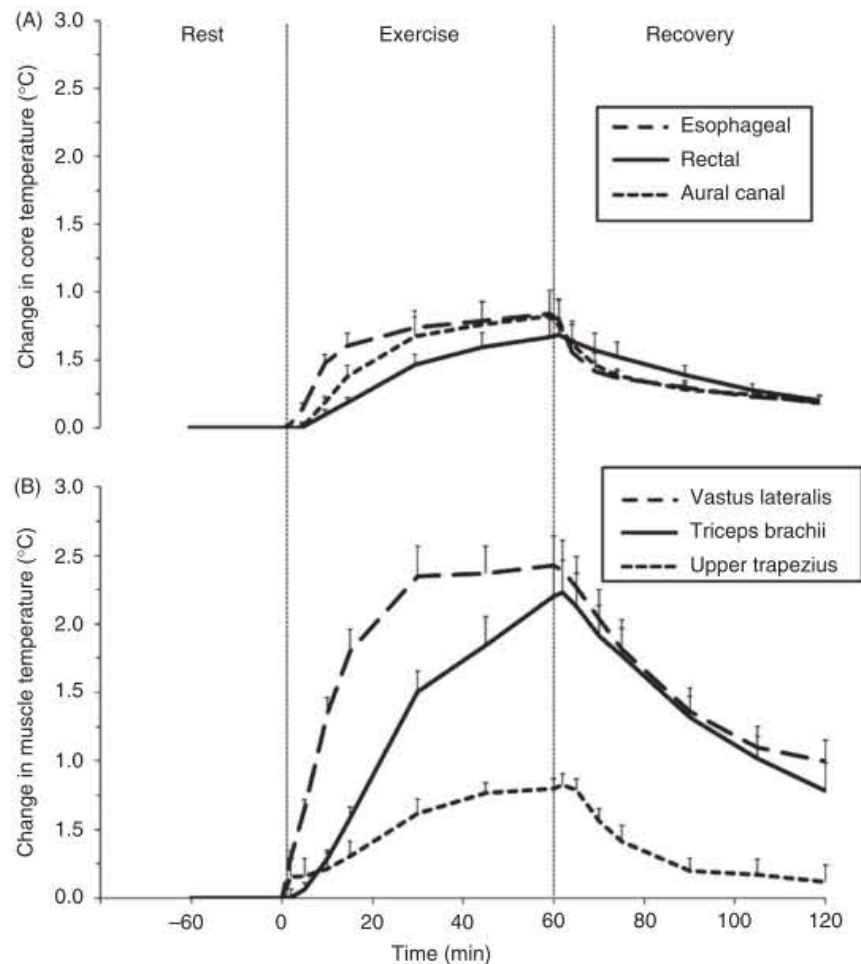
Figure 2.2 Mean changes in core temperature (A) and muscle temperature (B) during and following 60-min exercise. N = 8 (6M, 2F) Taken from Kenny et al., (2008).

2.2.1. Measurement of core temperature

The measurement of T_c varies depending on the internal measurement site (Lefrant et al., 2003). It is essential that the method adopted to monitor T_c is convenient and unbiased by environmental conditions (Sawka et al., 2011a). Furthermore, there is a requirement that the measurement technique mirrors small changes in arterial blood temperature quickly, to accurately reflect the temperature of blood perfusing the hypothalamus (Lefrant et al., 2003). The most commonly used measurement sites include, the rectum, oesophagus, and gastrointestinal tract, but also include auditory-canal. Figure 2.3 presents oesophageal, rectal, and intestinal temperature measured simultaneously during rest, submaximal supine cycling exercise at 40% O_2 uptake ($\dot{V}O_2$) peak and 65% $\dot{V}O_2$ peak, and during passive recovery.

The location of rectal temperature (T_{re}) is considered the most practical and accurate for measuring T_c (Moran and Mendal, 2002). Furthermore, T_{re} is typically very easy to administer and causes minimal discomfort to the participants. Heat exchange in the rectum is due to the neighbouring tissues, rather than the regular perfusion of hot blood; the thermal inertia and poor heat removal results in a stable measurement. However, T_{re} responds slower to the rapid changes in T_c experienced during the onset of exercise and has a mean difference of 0.4 ± 1.0°C compared with the pulmonary artery (Robinson et al., 1998). Oesophageal temperature is often considered to be the best non-invasive method to determine T_c because of its deep location, close to the left ventricle, the aorta, and to the blood flow to the hypothalamus. Furthermore, oesophageal temperature responds rapidly and quantitatively to changes in central blood temperature (Lefrant et al., 2003) and the pulmonary artery temperature (0.0 ± 0.5°C) (Robinson et al., 1998). However, oesophageal probes are sometimes deemed as undesirable in many settings because of the difficultly in inserting the thermistor, irritation to the nasal passage and general participant discomfort (Moran and Mendal, 2002). The gastrointestinal pill provides comparable steady state T_c measurements to T_{re}. However, the gastrointestinal pill responds more slowly to the changes in T_c than oesophageal temperature (Lee et al., 2000). Furthermore, the gastrointestinal pill temperature values have been shown to be more variable over time since the pill is travelling within the gastrointestinal tract (Byrne and Lim, 2007).

Auditory-canal is another established method to track T_c (Cooper et al., 1964; Edwards et al., 1978; Hayward et al., 1984) albeit with an offset and a phase delay (Taylor et al., 2014). Auditory-canal has been reported to be comparable to T_{re} (Ilsley et al., 1983). However, surface temperatures are influenced by ambient temperature resulting in a reduced accuracy of auditory-canal temperature to track T_c (Low et al., 2007)

In conclusion, it appears that T_{re}, due to its inherent stability, is the preferred method to accurately track T_c with minimal measurement artefact, practicality, and minimal participant discomfort.
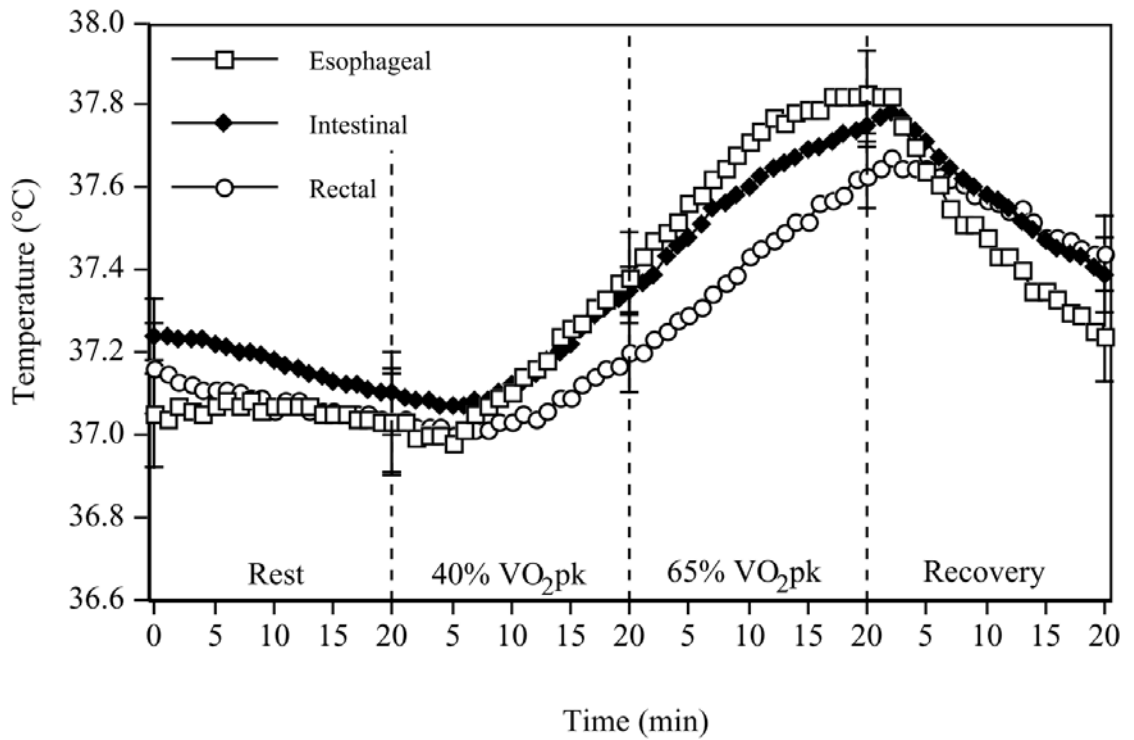
Figure 2.3 Oesophageal, rectal, and intestinal temperature measured simultaneously during rest, submaximal supine cycling exercise at 40% $\dot{V}O_2$ peak and 65% $\dot{V}O_2$ peak, and during passive recovery. N = 7 (5M, 2F).

Notes: Values are mean ± standard error. Taken from (Byrne and Lim, 2007; Lee et al., 2000).

2.2.2. Measurement of skin temperature

The measurement of T_{sk} is conducted for several reasons. Firstly, the measurement of T_{sk} enables the calculation of mean body temperature to permit the determination of heat storage (Jay and Kenny, 2007). T_{sk} measurements can also be used to estimate radiative and convective heat exchange, skin conductance, and skin blood flow requirements. Furthermore, T_{sk} measurements can be used to inform the T_{sk} input to the thermoregulatory controller (Sawka et al., 2011a). Although T_{sk} can be assessed easily, it can also be problematic, since changes in T_{sk} can be caused by physiological adjustments such as alterations in the cutaneous blood flow, secretion of sweat and evaporation, or changes to the environmental conditions (Sawka et al., 2011a). Ensuring that the thermistors stay in thermal contact with the skin is paramount to the accuracy of T_{sk} measurement (Smith et al., 2010). Thermistors have been reported to be a robust and accurate to 0.05°C across a range of water bath temperature (10 - 40°C) (Harper-Smith et al., 2010) but more variable when exercising (technical error of measure [TEM]= 0.3°C) (James et al., 2014).

Mean T_{sk} is often calculated based on multiple measurements to better reflect the changes in T_{sk}. Mean T_{sk} represents the sum of the weighted individual measurements of T_{sk}. The weighting of mean T_{sk} is established based on the percentage of body surface area (BSA) that is represented by the measurement site (Mitchell and Wyndham, 1969). Twelve to fifteen T_{sk} measurement sites have been used to calculate mean T_{sk}. However, investigators reduced the number of T_{sk} measurement sites required to obtain a valid estimate of mean T_{sk}. When it is problematic to measure accurately a large number of sites, the equation (Equation 2.4) introduced by Ramanathan should be considered (Ramanathan, 1964).

Equation 2.4. Calculation for mean skin temperature (T_{sk})

$$T_{sk} = 0.3 \cdot (T_{chest} + T_{arm}) + 0.2 \cdot (T_{upper\ leg} + T_{lower\ leg})$$

Where: T_{chest}, the midpoint of the pectoralis major; T_{arm}, the midpoint of the triceps brachii lateral head; $T_{upper\ leg}$, rectus femoris; $T_{lower\ leg}$, gastrocnemius lateral head.

In contrast, mean T_{sk} can be calculated based on the regional weighting of the skins thermal sensitivity. The thermal receptors within the skin are not evenly distributed. As a result, the warming of body regions which have the most thermal sensors, ultimately has the largest influence on the effector responses (Sawka et al., 2011a). Thus, it is suggested that calculating mean T_{sk} using thermal sensitivity weightings may provide a superior index, which more accurately reflects the peripheral input to the thermoregulatory controller. However, there remains some level of disagreement as to whether the mean T_{sk} values actually differ if calculated based on regional thermal sensitivity weightings or regional BSA weightings (Libert et al., 1984).

2.2.3. Body heat content

In the event of an imbalance between heat production and heat loss there is a rate of change in body heat storage and subsequently, a change in body heat content. Body heat content is the product of mean body temperature and the average heat capacity of the body tissues (Jay and Kenny, 2007). The determination of body heat content is of fundamental importance when assessing the exposure of the human body to a hot environment that may result in a thermal imbalance. The measurement of body heat exchange using simultaneous measures of total heat production and heat loss, in theory is the only method whereby body heat content can be directly determined (Jay and Kenny, 2007). Therefore, by definition the difference between metabolic heat

production using indirect calorimetry and the total heat lost from the body using direct calorimetry is the only method to calculate the rate of change in body heat storage and body heat content accurately (Jay and Kenny, 2007). Changes in body heat content are rarely measured directly using calorimetry since this approach requires very sophisticated and expensive equipment and instrumentation (Jay and Kenny, 2007). Typically, changes in body heat content are estimated using the two compartment thermometry model which involves the measurement of changes in mean body temperature estimated from weighted T_c and mean T_{sk} measurements (Jay and Kenny, 2007) (figure 2.4). For this method the T_c and mean T_{sk} values are weighted by their relative size which varies reciprocally with cutaneous vasodilation and vasoconstriction with core/skin weightings of 0.90/0.10, 0.79/0.21, and 0.66/0.34 in hot, temperate, and cool conditions (Sawka et al., 2011a).

The three-compartment thermometry model (figure 2.4) is reported to be a superior model compared with the traditional two-compartment model during exercise in temperate and warm conditions (Jay and Kenny, 2007). The three-compartment thermometry model uses measures of T_c, T_{sk}, and muscle temperature collectively, to predict changes in mean body temperature. However, even when including invasive muscle temperature measurements, the three-compartment thermometry model only accounts for approximately 50% of the variance of mean body temperature (Jay and Kenny, 2007). This evidence suggests that temperature measurements of more tissues intermediate to the core and shell, are required to provide a more accurate thermometric derivation of body heat content. Subsequently, a two-compartmental thermometry model was introduced (Jay and Kenny, 2007). This model only accounts for approximately 56% of the variance of mean body temperature from calorimetry measurements. These findings, suggest that thermometry provides inaccurate estimates of mean body temperature changes, and therefore thermoregulatory models to determine body heat content are likely flawed. Thus, thermometry determination of body heat content should be used with caution and only employed, when using a repeated measures design.
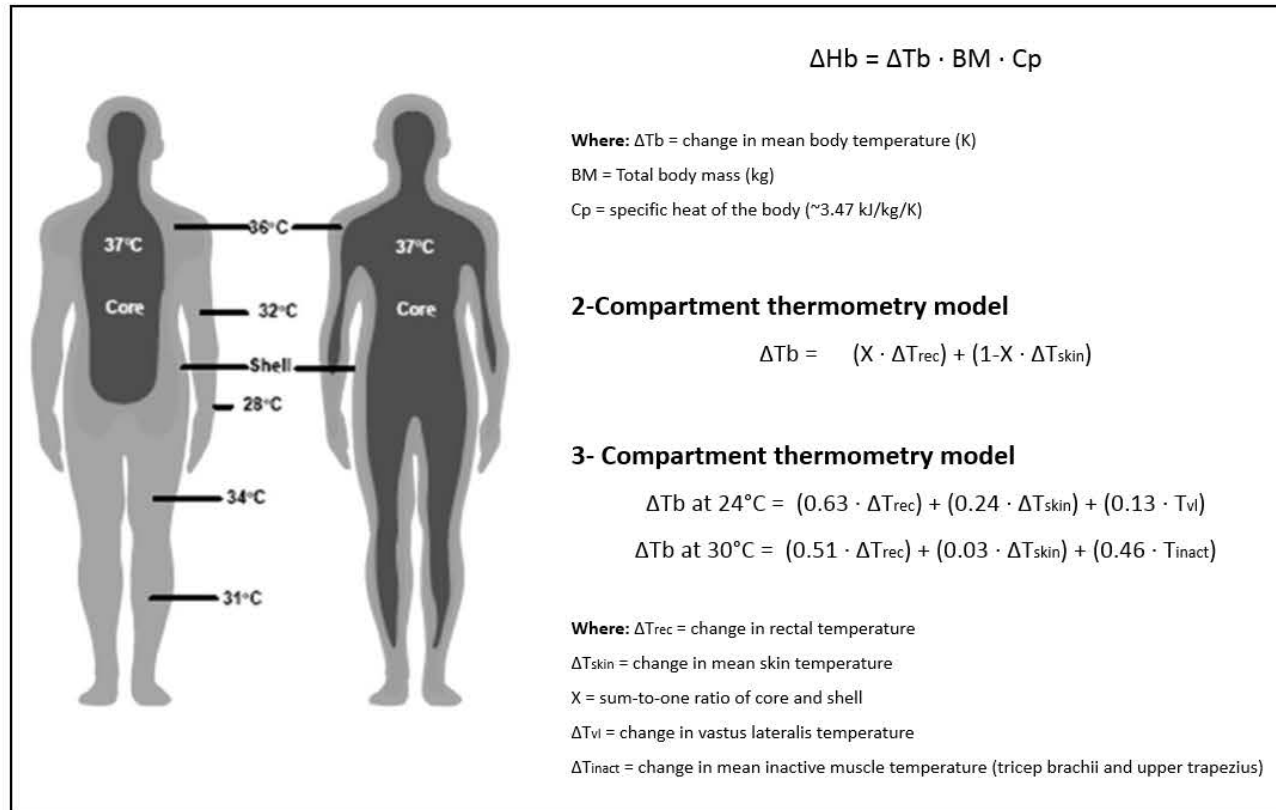
$$\Delta Hb = \Delta Tb \cdot BM \cdot Cp$$

Where: ΔTb = change in mean body temperature (K)

BM = Total body mass (kg)

Cp = specific heat of the body ($\sim$3.47 kJ/kg/K)

2-Compartment thermometry model

$$\Delta Tb = \quad (X \cdot \Delta T_{rec}) + (1\text{-}X \cdot \Delta T_{skin})$$

3- Compartment thermometry model

$$\Delta Tb \text{ at } 24°C = (0.63 \cdot \Delta T_{rec}) + (0.24 \cdot \Delta T_{skin}) + (0.13 \cdot T_{vl})$$

$$\Delta Tb \text{ at } 30°C = (0.51 \cdot \Delta T_{rec}) + (0.03 \cdot \Delta T_{skin}) + (0.46 \cdot T_{inact})$$

Where: ΔT_{rec} = change in rectal temperature

ΔT_{skin} = change in mean skin temperature

X = sum-to-one ratio of core and shell

ΔT_{vl} = change in vastus lateralis temperature

ΔT_{inact} = change in mean inactive muscle temperature (tricep brachii and upper trapezius)

Figure 2.4 The 2-compartment thermometry model and the 3-compartment thermometry model for change in mean body temperature and change in body heat content. Adapted from (Jay and Kenny, 2007; Kenny and Jay, 2013).

2.3.   Behavioural temperature regulation

Humans typically regulate their T_c within a narrow range between 35°C and 41°C using a combination of behavioural and physiological responses. Thermoregulatory behaviour, is the regulation of body temperature by a complex pattern of muscular skeletal responses to heat and cold, which modify the rates of heat production and/ or heat loss (Bligh and Johnson, 1973).

Behavioural temperature regulation is often the first line of defence in maintaining body temperature and involves a number of conscious alterations in behaviour. Examples of such behaviours include, but are not limited to, modifying the exercise intensity, adding or removing clothing, altering body position, and seeking shade or shelter (Schlader et al., 2011b; Tucker et al., 2006). Schlader et al., (2011a) reported a shorter time to exhaustion (20.3 ± 3.4 min versus 23.2 ± 4.1 min), higher peak T_{re} (39.4 ± 0.3°C versus 39.1 ± 0.4°C), and higher ratings of perceived exertion (18 versus 16) when exercise was performed at a fixed intensity compared with self-paced exercise, in an uncompensable environment. Thus, when self-paced exercise is performed in an uncompensable environment, it is less physiologically demanding since the voluntary reductions in exercise intensity reduce metabolic heat production, improve thermal compensability, and increase exercise duration. This evidence is supported by the high incidence rate of exercise induced heat stroke when high motivation and external factors dictate exercise intensity and a lower incidence when people exercise alone or self-paced (Armstrong et al., 2007; Epstein et al., 1999). Athletes often ignore behaviours designed to support thermoregulation due to a high level of motivation during competition.

2.4.   Physiological temperature regulation

Physiological temperature regulation is controlled by responses that are independent to our conscious voluntary behaviours. Examples of such responses include, but are not limited to, modifying the body heat distribution via redistributing blood flow through the core and the skin by cutaneous vasodilation and vasoconstriction, and altering the rate of metabolic heat production through involuntary behaviours such as shivering, and sweating. There are several theories developed which try to explain human thermoregulatory control; these include the adjustable set point model, the reciprocal inhibition model, and the heat regulation model.

2.4.1. Adjustable set point

The traditional model of thermoregulatory control, known as the set point model was introduced by Harold Hamel (figure 2.5). This system assumes that afferent signals from the core and periphery are combined into a thermal signal transmitted through the central nervous system and likely within the preoptic anterior hypothalamus (Hammel et al., 1963). This model is in part informed by warm and cold receptors under the human skin. These receptors are typically located at a depth of 0.3 - 0.6 mm and 0.15 - 0.17 mm, respectively (Boulant, 2006). Furthermore, neural support for this model is highlighted by the observation that the hypothalamus holds warm and cold sensitive neurons which alter their firing rate based on either warming or cooling. This thermal signal is then compared to an internally stored thermal signal, resulting in the appropriate activation of heat loss or heat gain effector responses (Hammel et al., 1963).

A common argument in contradiction of the adjustable set point model is that it does not explain any deviations from the reference temperature stored within the central nervous system. For example, during the luteal phase of the menstrual cycle, baseline temperature is regulated approximately 0.3-0.5°C higher than compared with the follicular phase (Coyne et al., 2000). Similarly, during a fever body temperature is raised and generally, appropriately defended. The set point model of temperature regulation argues that there is no requirement for a constant reference temperature; instead, the set point temperature may vary over a broad range based on the activity of warm and cold sensitive neurons (Cabanac, 2006).
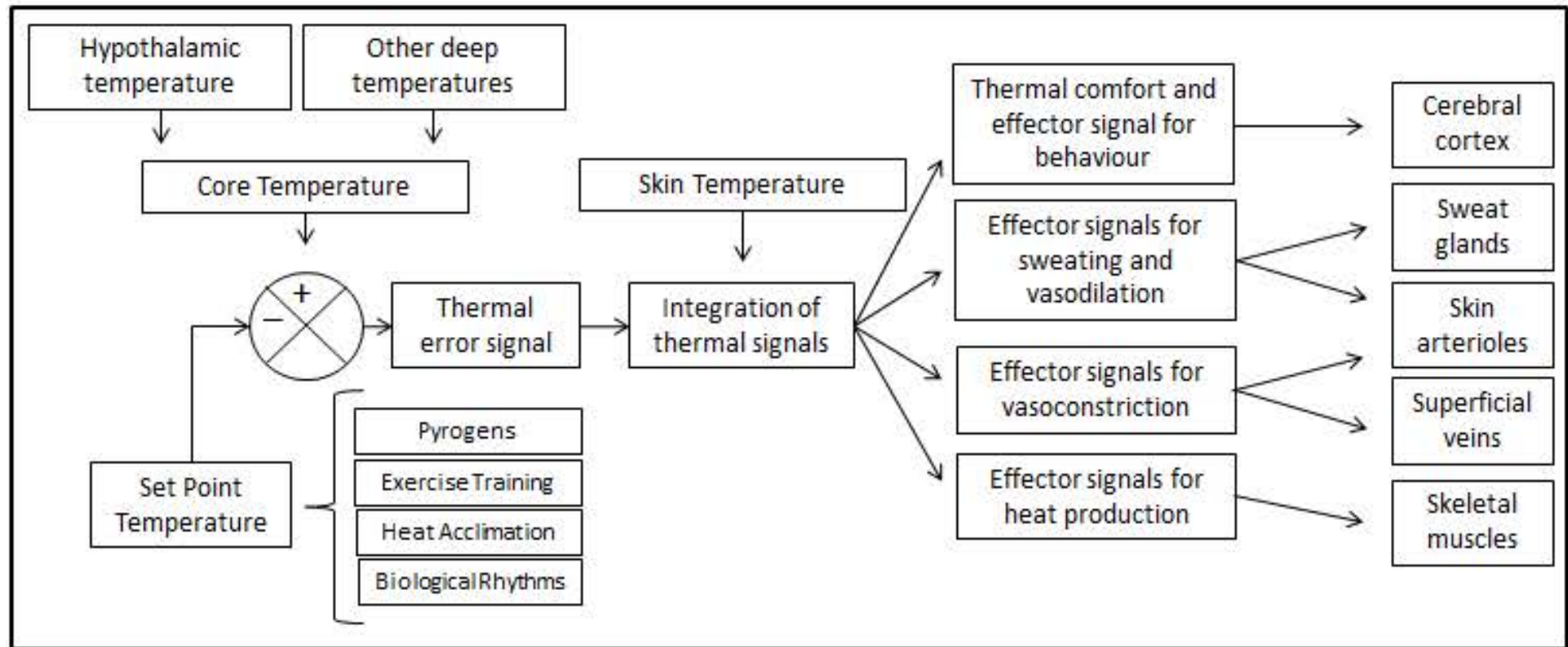
Figure 2.5 Schematic diagram of the thermoregulatory control system. Adapted from Sawka et al. (2011).

2.4.2. Reciprocal inhibition

The reciprocal inhibition model of temperature regulation utilizes a similar neural structure to the adjustable set point model, but explains temperature regulation slightly differently. The reciprocal inhibition model argues a complex integration between sensory receptors and thermal effectors for heat loss and heat gain (figure 2.6) (Bligh, 2006). As opposed to a more direct integration of a composite signal and comparison to a set signal, as suggested by the adjusted set point model (Hammel et al., 1963). This model assumes that warm and cold sensor inputs are integrated and summed to produce a net heat loss or heat gain response, respectively. However, this model assumes a "null zone" of temperature regulation where heat loss and heat gain effector responses do not sufficiently respond, since vasomotor changes maintain thermal stasis (Bligh, 2006). This model integrates temperature regulation into a large picture of whole body physiological homeostasis by assuming the ability of a variety of non-thermal factors to affect temperature control (Mekjavic, 2006).
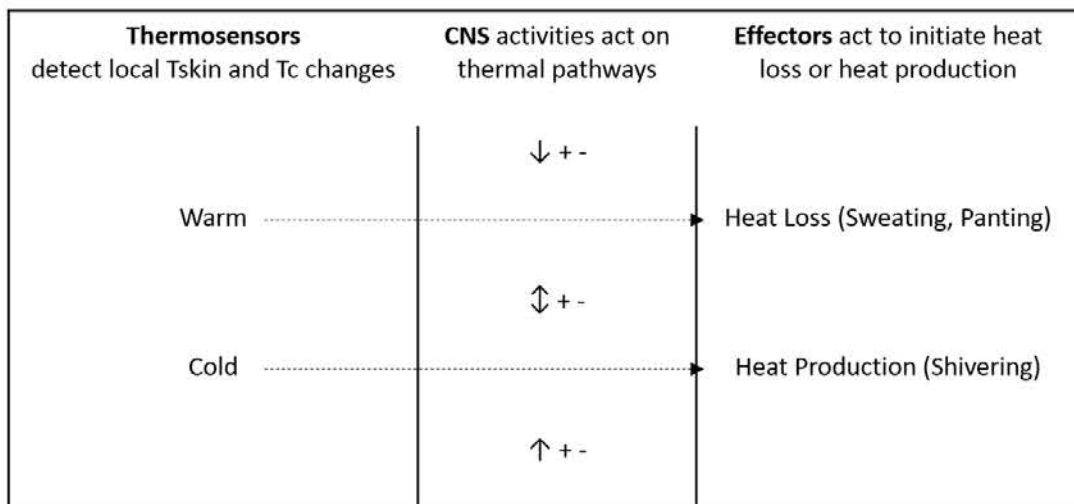
Figure 2.6 The reciprocal inhibition model of thermoregulation. Adapted from (Bligh, 2006).

The reciprocal inhibition model adds an additional layer of complexity to temperature regulation. The activity of sensors interconnects with the pathway of the other sensors, to provide an inhibitory stimulus (Bligh, 2006). For example, strong stimulation of warm responsive neurons activates the heat loss effector pathways and inhibits the cold sensitive neurons and heat gain pathways. The reciprocal inhibitory process permits the net thermal signals to evoke an effector response unless the signal is not strong enough where minor vasomotor changes occur (Bligh, 2006). Similarly, non-thermal stimulus can produce either an excitatory or inhibitory effect on either set of pathways, helping to explain the shifting of T_c thresholds with individual changes (Bligh, 2006). An implication

of this model, is that there is no central comparator signal as implied by the adjusted set point model which permits easier theoretical ability for T_c shifts (Sawka et al., 2011a). Anatomical support for this idea can be seen in clinical tests in which the hypothalamus is impaired or blocked and thermoregulatory control, although coarser, is still possible (Sawka et al., 2011a).

2.4.3. Heat regulation

The heat regulation model is different in the theoretical construct from the adjusted set point and the reciprocal inhibition models of physiological temperature regulation. The underlying principle of the heat regulation model is that the overall heat storage, or the homeostatic net balance of heat gained versus heat lost is the regulated variable. Such that body temperature is not a regulated variable, it is the result of the body's attempt to maintain a stable overall heat balance (Webb, 1995). As opposed to sensing and integrating peripheral temperature and T_c, the body uses heat flow and the temperature gradient across the skin surface as its primary afferent inputs. The central integration of the heat balance operates with feedback from heat loss and feedforward control from heat production, driving the physiological responses that defend body heat content (Webb, 1995).

The primary strength of the heat regulation model of temperature regulation is that it is sensitive to the deviations in body temperature. As a practical example, when a cyclist begins exercising there is a concurrent increase in T_c, this is followed by a stabilisation of T_c until exercise is terminated (figure 2.7). Heat production responds more quickly compared to the heat dissipation mechanism however, eventually the heat loss mechanisms matches the rate of heat production, resulting in a stable elevated T_c. When exercise is terminated, T_c remains elevated for a period of time even though heat dissipating responses are reduced rapidly (Kenny et al., 2008) (figure 2.7). This is difficult to accommodate in the temperature regulation models since it would assume that heat dissipating responses would remain elevated throughout the recovery period until T_c returns to resting levels. The primary argument against the heat regulation model is the anatomical requirement for heat flow sensors which are anatomically difficult to demonstrate, whilst accounting for the presence of temperature sensitive neurons throughout the peripheries and the central nervous system. In response supporters for the heat regulation model argue that the thermal receptors throughout the body represent multiple thermal sensors, and the difference between these thermal signals can become integrated as rates of heat flow.
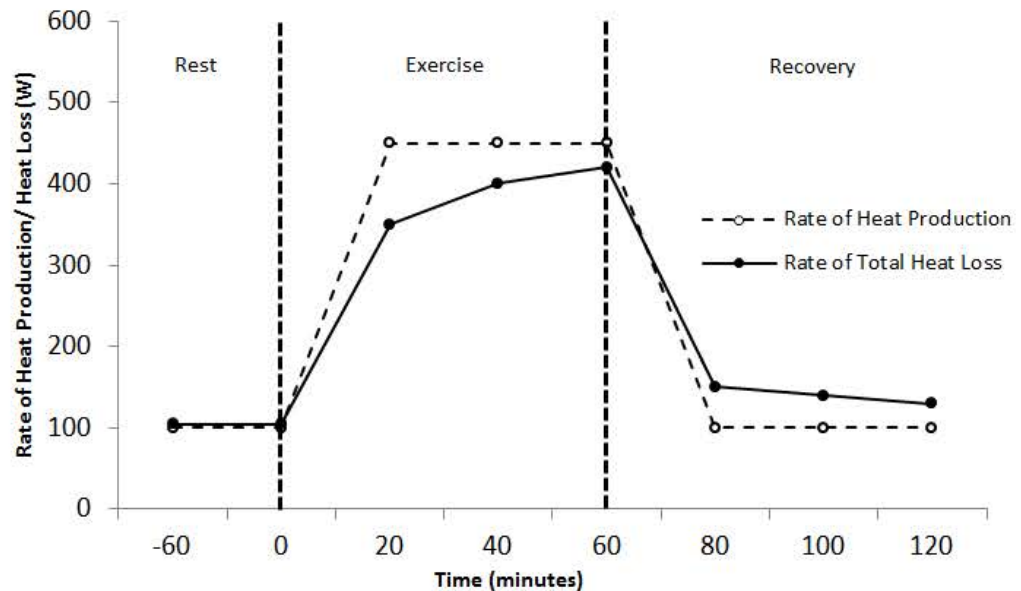
Figure 2.7 Mean whole body calorimetry data for rate of total heat production and rate of net heat loss after 60-min rest, 60-min cycling at 70 W and 60-min of inactive recovery. N = 8 (6M, 2F). Adapted from Kenny et al. (2008).

These three major models of thermoregulatory control have been advanced over the years to develop our understanding of thermal homeostasis. The first two models discussed, suggest temperature is the regulated variable and share similar ideas concerning the understanding of the neural architecture. However, the third model is fundamentally different from the first two models in that rather than temperature per se, body heat content is the regulated variable and body temperature is a by-product of that regulation. All these models have their strengths, but each remains imperfect, thus more research is required to continue the developments in our understanding of thermal homeostasis.

2.5. Physiological responses to heat stress

There are several principle thermoregulatory responses which occur during heat stress. Examples of such responses include, but are not limited to, sweating and evaporative heat loss, alterations in skin blood flow and dry heat loss, and alteration in cardiovascular function to support thermoregulation.

2.5.1. Sweating and evaporative heat loss

As ambient temperature or metabolic heat production increases, there is increasing dependence upon the sudomotor function and evaporative heat loss to dissipate body heat. There are typically two types of human sweat glands, apocrine glands and eccrine glands (Shibasaki et al., 2006). The eccrine sweat gland are primarily responsible for thermoregulatory sweating in humans (Sato et al., 1989).

Eccrine sweat glands

Eccrine sweat glands are distributed over nearly the entire surface of the human body. The number of human sweat glands typically ranges from 1.6 to 4.0 million. The eccrine sweat gland structure consists of a bulbous secretory coil which leads to a duct. This secretory coil is located in the lower dermis and the duct extends through the dermal layers and opens directly onto the skin surface. The uncoiled dimension of the secretory portion of the eccrine sweat gland is approximately 30-50 μm in diameter and 2-5 mm in length. The adult secretory coil ranges from 1 to 8×10^{-3} mm^3 (Sato and Sato, 1983). There is a positive correlation between the size of an individual sweat gland and the maximal sweat rate (SWR) of that gland (Sato and Sato, 1983).

Given the challenges of identifying neural tracts in humans, the exact neurological pathways responsible for thermoregulatory sweating are not entirely understood. Evidence from animal studies suggests that efferent signals from the preoptic hypothalamus travel via the tegmentum of the pons and the medullary raphe regions to the intermediolateral cell column of the spinal cord. In the spinal cord, neurons emerge from the ventral horn, pass through the white ramus communicans, combine with peripheral nerves and travel to sweat glands (Low, 2004; Nakamura et al., 2004).

Sweat rate and evaporative heat loss

The initial increase in sweat rate (SWR) during heat stress is a result of an increase in the activated sweat glands; however, additional increases in SWR occur through an increase in the production and secretion of sweat per gland (Kondo et al., 2011; Randall, 1946). The recruitment of sweat glands is rapid, with near maximal recruitment being achieved in as little as 8-min of exercise and passive heat stress. In contrast, the increases in sweat output per gland are more gradual (Kondo et al., 2011). The rate that sweat evaporates, is determined by the gradient between the skin and air vapour pressures, and the coefficient of evaporative heat transfer. The wider the water vapour gradient, the greater the rate of evaporation (Sawka et al., 2011a). Furthermore, warm skin

enhances the sweat response (Nadal et al., 1971), which is likely mediated by both the increased local temperature and associated increased skin blood flow (Wingo et al., 2010).

Heat loss through the evaporation of sweat is an essential process in the thermoregulatory response to exercise. For example, when an individual performs exercise at a metabolic rate of 600 W, approximately 80% of the energy consumed becomes heat. As such, 480 W (28.8 kJ.min^{-1}) must be dissipated to avoid heat storage. The specific heat of body tissue approximates 3.5 kJ.kg.°C^{-1}, so a 70 kg man or a 60 kg female has a heat capacity of 245 kJ.°C^{-1} or 210 kJ.°C^{-1}, respectively. If sweat is not secreted, T_c increases by approximately 1.0°C every 8-min 30-s for the 70 kg male (245 kJ.°C^{-1} / 28.8 kJ.min^{-1}) or 7-min 20 s for the 60kg females (210 kJ.°C^{-1} / 28.8 kJ.min^{-1}). However, as the latent heat of evaporation is 2.43 kJ.g^{-1}, secretion and evaporation of ~12 g of sweat (28.8 kJ.min^{-1}/2.43 kJ.g^{-1}) per min would enable the heat to be transferred to the environment avoiding heat storage (Sawka et al., 2011a).

When performing exercise in a high ambient condition, humans have the capacity to produce a remarkable amount of sweat. Individuals performing activities in both a desert and tropical climates have been reported to experience a mean SWR of 1.6 L.hr^{-1} when partially clothed, and 2.0 L.hr^{-1} when wearing full protective clothing (Montain et al., 1994). Factors such as exercise intensity and duration, clothing, and environmental conditions ultimately affect an athlete's SWR. Observed SWR in a variety of athletes during training and competition, range from 0.2 L.hr^{-1} to 3.4 L.hr^{-1} (Sawka et al., 2007).

2.5.2. Skin blood flow and dry heat loss

In the human body, blood transfers heat by convection from the deep body tissues to the skin. Blood flow to the cutaneous circulation has a tremendous range, from nearly zero in extreme cold to approximately 7 L.min^{-1} when T_c is high (Johnson and Kellogg, 2010). Reflexes controlling skin blood flow have the capacity to use nearly this entire range. When thermoregulatory sweating begins, skin blood flow serves as a means to deliver heat to the skin which is then removed by sweat evaporation (Sawka et al., 2011a). Thus, skin blood flow and sweating work together to dissipate the heat.

Vasoconstriction and vasodilation control of skin blood blow

Skin blood flow is ultimately affected by T_{sk}, which directly impacts on the vascular smooth muscle. Cutaneous circulation is affected by the T_{sk} and T_c, via reflexes operating through the sympathetic nervous system from the thermoregulatory control centres (Johnson and Kellogg, 2010). Certain

areas of the body, including the palm of the hand, sole of the foot, lips, ears, and nose, are predominantly innervated by adrenergic vasoconstrictor fibres (Sawka et al., 2011a). The vasodilatation activity that occurs in these regions is largely due to withdrawing the vasoconstrictor activity (Taylor et al., 1984). There is minimal vasoconstrictor activity when T_{sk} exceeds 39°C on the remaining body regions, including the arms, legs and torso. Active vasodilatation during heat exposure depends largely on sympathetic innervations (Taylor et al., 1984). Skin blood flow responses differ between rest and aerobic exercise during heat stress (González-Alonso et al., 2008). Exercise increases vasoconstrictor activity which increases the threshold temperature for vasodilatation and reduces skin blood flow at high body temperatures compared to rest (Sawka et al., 2011a). These modified skin blood flow responses occur during exercise heat stress because the cardiovascular system is challenged to support simultaneously both high skeletal muscle blood flow and skin blood flow.

Core and skin temperature effects on skin blood flow

Table 2.1. illustrates the effect of different T_c and T_{sk} on the skin blood flow requirements to preserve heat transfer (Kenefick et al., 2007). In the example, if the net heat production is 7.7 kcal.min^{-1} and T_c and T_{sk} are 38°C and 30°C, respectively, the skin blood flow requirement will be ~1.1 L.min^{-1}. When T_{sk} increases to 34°C and 36°C as a result of heat stress, the minimal skin blood flow requirement to preserve the same heat transfer rate increases to ~2.2 L.min^{-1} and ~4.4 L.min^{-1}, respectively. However, if T_c rises to 39°C the skin blood flow requirements are reduced without compromising heat transfer. This example, demonstrates how an elevated T_{sk} as a result of a warmer ambient or reduced evaporative cooling, increases skin blood flow requirements. In addition, the example demonstrates how an elevated T_c may reduce skin blood flow requirements and cardiovascular strain associated with exercise-heat stress.

Table 2.1 Estimated whole body skin blood flow requirements during prolonged, severe running exercise at different body core and skin temperatures.

Net heat production		T_c	T_{sk}	T_c-T_{sk} Gradient	Skin blood flow
watts.min^{-1}	kcal.min^{-1}	(°C)	(°C)	(°C)	(L.min^{-1})
540	7.7	38	30	8	1.1
540	7.7	38	34	4	2.2
540	7.7	38	36	2	4.4
540	7.7	39	36	3	3.0

Notes: Skin blood flow: $Qs = [((1/C) \times h)/ (T_c - T_{sk})]$. Where C = specific heat of blood ~0.87 kcal.°C.L^{-1}; h = heat production; Qs = skin blood flow T_c = core temperature, T_{sk} = skin temperature. Net heat production estimated using a body mass of 60 kg and running velocity of 325 m.min^{-1} (world class 42km pace) after subtracting for work (20% efficiency) and 50% dry and evaporative heat losses. Adapted from (Kenefick et al., 2007).

2.5.3. Cardiovascular support of thermoregulation

During exercise heat stress, the cardiovascular system is primarily required to provide sufficient cardiac output to perfuse skeletal muscle adequately to support metabolism whilst simultaneously perfusing the skin to support heat loss (Sawka et al., 2011a). These competing metabolic and thermoregulatory demands alter cardiac function and distribution of the cardiac output (Brothers et al., 2009a). When ambient temperature increases from 20°C to 36°C whilst exercising at a moderate intensity (70% maximal O_2 uptake ($\dot{V}O_2$max)), there is an increase in T_c, T_{sk}, forearm blood flow, and percentage change in PV, whereas stoke volume decreases (Nadal et al., 1979). The high skin blood flow combined with the greater reduction in PV, work to reduce venous pressure and thus, cardiac filling (Nelson et al., 2010). Furthermore, an increase in heart rate (HR) reduces diastolic filing time, contributing further to a reduced stroke volume (Fritzsche et al., 1999).

Skin temperature and cardiovascular strain

An increase in T_{sk} and a narrower T_c to T_{sk} gradient, which typically occurs during exercise heat stress, results in a greater cardiovascular strain. Cheuvront et al. (2003) demonstrated the effect of an altered T_c to T_{sk} gradient on cardiovascular strain. When T_c remains constant (37.5°C), whilst T_{sk} is increased from 32°C to 35°C, HR increased by approximately 26 beats.min^{-1}. In addition, when raising T_{sk} from 32°C to 36°C HR increased by approximately 49 beats.min^{-1}. This evidence suggests that a narrow T_c to T_{sk} gradient results in a greater strain on the cardiovascular system.

Redistribution of blood flow with exercise heat stress

During exercise heat stress, the cardiovascular systems ability to deliver adequate blood supply to the skeletal muscle and cerebrum can be compromised (González-Alonso et al., 2008; Nybo et al., 2002; Rasmussen et al., 2010). Although, cardiac output is sustained during low intensity exercise in the heat, cardiac output is compromised at moderate to high intensity exercise in the heat (Gonzalez-Alonso, 2003). To support the demands of the cardiovascular system during exercise heat stress, blood is redirected from the viscera to the skin and active skeletal muscles (Sawka et al., 2011a). The visceral blood flow reductions are graded to the exercise intensity, and the effects of exercise and heat are additive. Secondary to the reduced visceral blood flow, blood can be mobilized from the compliant splanchnic beds to help maintain cardiac filling during exercise heat stress (Sawka et al., 2011a).

Cardiovascular complications associated with exercise heat stress

The high demands placed on the cardiovascular system during exercise heat stress may lead to syncope and orthostatic intolerance (Kenefick and Sawka, 2007). Hyperthermia and dehydration are often related and may modify cardiac filling, blood pressure, and baroreceptor reflex regulation, which contribute to orthostatic intolerance (Charkoudian et al., 2003; Crandall et al., 2010; Wilson et al., 2009). In addition, hyperthermia can reduce local veno-arteriolar responses (Brothers et al., 2009b) and reduce cerebral vascular conduction (Wilson et al., 2006) and accompanying cerebral perfusion during orthostatic challenges.

2.6.   Sex differences in physiological responses to heat stress

Sex has traditionally been considered an independent modulator of temperature regulation. It has been well reported that males' and females' thermoregulatory responses to exercise in the heat differ (Kaciuba-Uscilko and Grucza, 2001; Kenney, 1985; Nunneley, 1978). The two common heat

loss avenues which have been compared between males and females are evaporative (sweating) and dry (skin blood flow) heat loss.

2.6.1. Sweat rate and evaporative heat loss

Females, have previously demonstrated a lower SWR whilst maintaining a similar T_c to that of males (Avellini et al., 1980; Keatisuwan et al., 1996). The primary concern with comparing SWR between males and females is that typically experimental designs adopt a protocol using a relative $\dot{V}O_2$max. Administering exercise at a relative percentage of $\dot{V}O_2$max will result in substantial differences in absolute O_2 consumption (Keatisuwan et al., 1996). For example, a male and female with a body mass (BM) of 70 kg and 60 kg respectively, both with a relative $\dot{V}O_2$max of 50 mL.kg^{-1}.min^{-1} will both have a relative O_2 consumption of 25 mL.kg^{-1}.min^{-1} when exercising at 50% of $\dot{V}O_2$max. However, males will have an absolute O_2 consumption of 1,750 mL.min^{-1} and females 1,500 mL.min^{-1} respectively. This 250 mL.min^{-1} difference provides a metabolic heat production that is approximately 85 W greater in males compared with females (Gagnon et al., 2008). The lower resultant rate of heat production in females would therefore, have necessitated a smaller amount of evaporation from the skin and subsequently a lower sweat production to satisfy biophysical heat balance requirements. Thus, the previously reported thermoregulatory differences observed between males' and females' SWR may be confounded by the inherent physical differences between the sexes.

Sex differences in temperature regulation during exercise have been shown to exist irrespective of confounding differences in metabolic heat production and physical characteristics. However, these differences only become evident above a certain requirement for heat loss and are solely attributed to a lower sweat gland output in females (Gagnon and Kenny, 2012). Specifically, females exhibit a lower increase in both local (Gagnon and Kenny, 2012) and whole body (Gagnon and Kenny, 2012, 2011) sudomotor activity as a function of increases in T_c (i.e., thermosensitivity), with no differences in the onset threshold of these responses. The lower thermosensitivity of sudomotor activity, combined with a lack of sex differences in the onset threshold suggests that sex differences in sudomotor activity during exercise are mediated peripherally (Gagnon and Kenny, 2011, 2012).

2.6.2. Skin blood flow and dry heat loss

To date, there is very little data published evaluating the sex differences in the sensitivity of cutaneous vasodilation using methods that eliminate the inherent limitation associated with exercise intensity prescribed when comparing males and females, as described in section 2.6.1.

Following passive heating, Inoue et al. (2005) reported that females have a relatively greater cutaneous blood flow on the thigh compared with males, however no differences were observed at any of the other sites (Inoue et al., 2005). The higher cutaneous blood flow was suggested to reflect a greater degree of cutaneous vasodilation on this site, since no sex differences were observed in the mean arterial pressure (Inoue et al., 2005). These results suggest, that females' dependence upon the cutaneous vasodilation response for heat loss is greater than the sweating response. Furthermore, it was suggested that the regional differences between sexes in vasodilatation and heat dissipation on the thigh may be relatively greater in females than in males. It is likely that heat dissipation from the limbs is more effective than heat dissipation from the trunk, because the BSA: BM ratio of the limbs is greater. Conversely, Gagnon and Kenny (2012) reported no difference in skin blood flow responses between males and females, following exercise at a fixed requirement of heat loss. These observations are further supported by Gagnon et al. (2013) who reported no differences between males and females' cutaneous vascular conductance to varying doses of acetylcholine and sodium nitroprusside. Although there remains no conclusive evaluation of the skin blood flow differences between males and females, it remains an important consideration when comparing thermoregulatory responses between sexes.

2.7.   Menstrual cycle

The menstrual cycle is a series of various hormone releases that coordinate the readiness of the female reproductive system for conception (figure 2.8). A typical menstrual cycle occurs over a 28-d period and is broken up into two phases; the follicular phase (D1 – D13) and the luteal phase (D15 – D28). Other common terminology may be used to describe the different menstrual cycle phases (table 2.2). On day one of the menstrual cycle, the hypothalamus releases gonadotropin-releasing hormone which controls stimulation and inhibition of the release of follicular-stimulating hormone (FSH) and luteinising hormone (LT) from the anterior pituitary. These hormones then regulate the release of oestrogen from the ovarian follicles and progesterone from the corpus luteum, coordinating the progression of these events during an average cycle of 28-d (Marsh and Jenkins, 2002) (figure 2.8)

An essential consideration to make when conducting physiological research within a female population is the timing of testing in relation to the menstrual cycle. The fluctuations in hormones associated with the menstrual cycle may alter the function of a number of different physiological responses associated with thermoregulation later discussed in section 2.9. The most common method to minimise the changes in hormone concentrations associated with the menstrual cycle, that may influence the dependent variable, is to study females in the same phase of their menstrual

cycle across participants, or across study days in longitudinal studies. Females are most commonly tested during the early follicular phase (D1-D7), when both oestrogen and progesterone concentrations are at their lowest levels.

Despite the convenience of studying females in the early follicular phase of the menstrual cycle, the broader clinical relevance of the findings may be limited since females are in this part of their cycle for only ~25% of their reproductive lives. Although, oestrogen and progesterone concentrations are low relative to other phases of the cycle, hormone levels remain considerably higher compared with those of men. Thus, their impact when making comparisons between the sexes is not entirely eliminated. Researchers can attempt to keep the cycle days consistent across females within a given study to investigate the physiological effects independent of cycle phase, but they must take into account the variability of these hormones across cycle phases within females. Additional considerations for hormone verification include the large variability between individuals within the same menstrual cycle phase, the diurnal variation in hormone secretion with progesterone concentrations typically higher in the morning and the known effect of exercise on increasing oestrogen and progesterone concentrations (Syrop and Hammond, 1987).
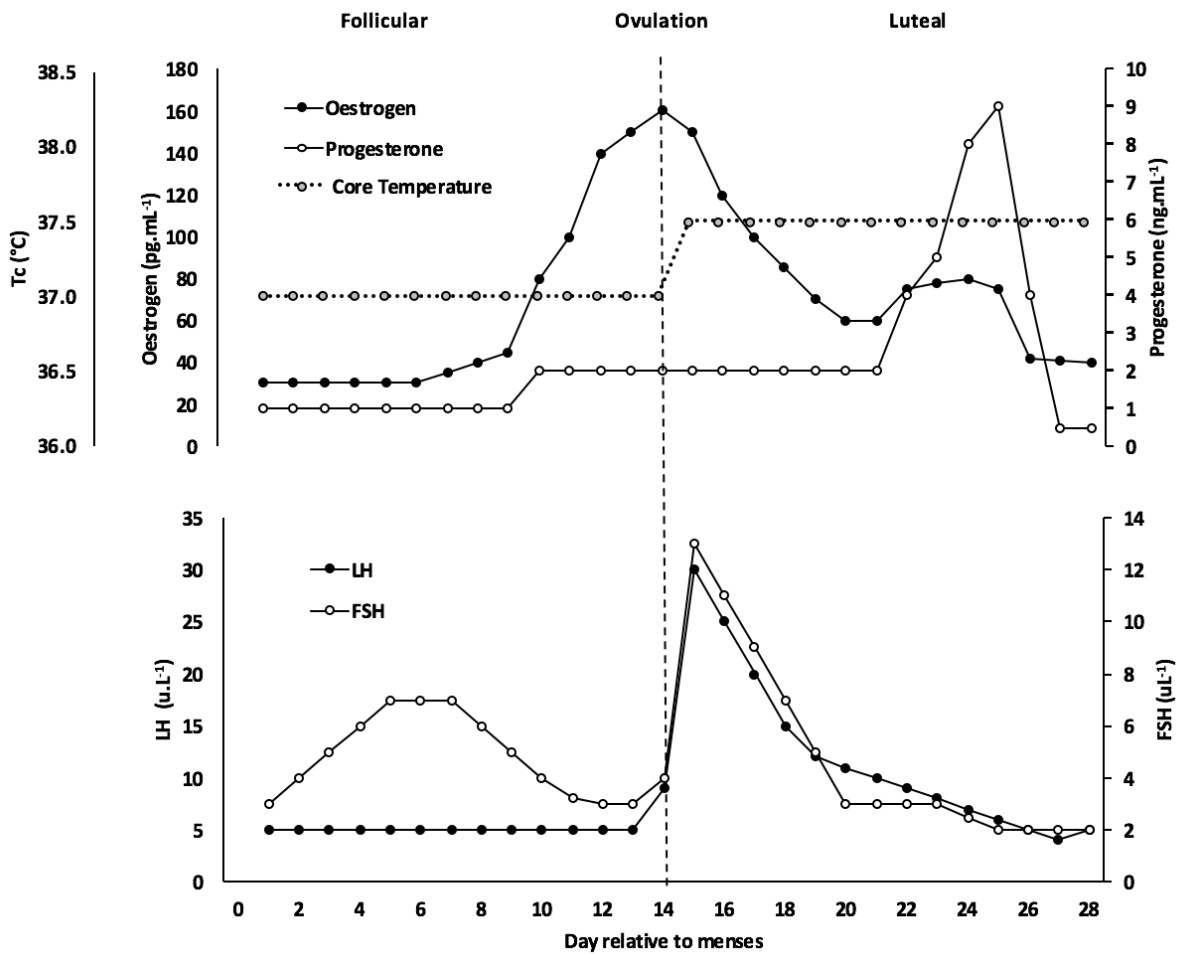
Figure 2.8 Typical core temperature (T$_c$) and hormone changes across the menstrual cycle. Note all values are approximate values since they vary greatly across individuals. LH = luteinizing hormone FSH = follicle stimulating hormone. Adapted from Stachenfeld & Taylor, (2014).

Table 2.2 Common terminology and hormone concentrations associated with a typical 28-d cycle with ovulation occurring on D14.

Menstrual cycle phase (days of menstrual cycle)	Concentrations		Additional terminology (day of menstrual cycle)
	Estradiol	Progesterone	
Early Follicular (2-7)	Low	Low	Follicular (1-13)
			Pre Ovulatory (1-13)
			Mid Follicular (6-9)
Late Follicular (9-13)	High	Low	Mid Cycle (12-18)
			Ovulatory (3-5 d around ovulation)
			Ovulation (14)
Mid Luteal (18-24)	High	High	Luteal (15-28)
			Post Ovulatory (15-28)

Notes: Adapted from Janse de Jonge, (2003).

2.8. Verification of the menstrual cycle phase

Researchers adopt a variety of methods to verify the menstrual cycle phase. These methods include counting days from the onset of menses, daily recordings of basal T_c, measuring LH concentrations for the prediction of ovulation, and the most established method, of measuring oestrogen and progesterone concentration in the blood plasma or blood serum.

2.8.1. Counting days from the onset of menses

A well established, commonly used method to determine the menstrual cycle phase, involves females counting the days from the onset of menses. This method assumes that individuals have a regular menstrual cycle that can be applied proportionally to an average menstrual cycle representation. However, active women with regular bleeding, have been reported to have a high incidence of luteal phase deficient cycles and anovulation (De Souze et al., 1998). Thus, counting days from menses does not differentiate ovulatory from anovulation and may provide inaccurate

information regarding the menstrual cycle phase. Furthermore, assumptions are made that individuals correctly report their menstrual activity.

2.8.2. Basal body temperature

Another method for menstrual cycle determination is daily recording of basal T_c. This method assumes a regular pattern of activity throughout the cycle. Typically, ovulatory women have a 0.3°C increase in basal T_c subsequent to ovulation, which is sustained throughout the luteal phase (Horvath and Drinkwater, 1982). However, the relationship between basal T_c and ovulation may vary considerably, since some females do not experience an increase in basal T_c during the luteal phase (Bauman, 1981). Although, an increase in basal T_c often reflects an increase in progesterone, a weak correlation (r ≤ 0.302) has previously been found (Forman et al., 1987). Thus, the reliability of recording daily basal T_c to determine menstrual cycle phases and its reflection of progesterone levels should be treated with caution.

2.8.3. Urinary luteinising hormone concentration

Urinary LH concentration can be determined using ovulation predictor kits, consisting of colorimetric enzyme immunoassays of urinary LH (Janse de Jonge, 2003). When the urinary LH surge has been shown, it can be assumed that ovulation will take place within the next 14-26 hr (Miller and Soules, 1996).

2.8.4. Measurement of oestrogen and progesterone

On D1 of the menstrual cycle gonadotropin-releasing hormone is secreted by the hypothalamus which controls the stimulation and inhibition of FSH and LH (Marsh and Jenkins, 2002). These hormones regulate the release of oestrogen and progesterone during an average cycle of 28 d. Consequently, the measurement of oestrogen and progesterone can be used to verify the menstrual cycle phase. The hormone concentrations can be measured in blood serum, blood plasma and saliva. In addition, their metabolites can be measured in urine. Measuring oestrogen and progesterone concentration in blood serum or blood plasma confirms the menstrual cycle phase, based on an increase in progesterone from the follicular to the luteal phase, indicating that ovulation has occurred (Janse de Jonge, 2003).

2.9. Thermoregulatory differences over the menstrual cycle

In women who have no sign of any menstrual cycle irregularity, the menstrual cycle comprises a follicular phase and a luteal phase during which oestrogen and progesterone concentrations alter. Changes in the concentrations of oestrogen and progesterone modify the basal T_c and the threshold for sweating and cutaneous vasodilation by altering the activity of central regulatory mechanisms (Charkoudian and Johnson, 2000). Specifically, the elevation of progesterone concentration during the luteal phase is correlated with an increase in the basal T_c (0.3 - 0.5°C) and the threshold for sweating and cutaneous vasodilation (Carpenter and Nunneley, 1988; Charkoudian and Johnson, 2000; Inoue et al., 2005). Whereas, the elevation of oestrogen concentration during the late follicular phase causes a decrease in the same parameters (Stephenson and Kolka, 1999). The most widely accepted explanation for an elevated T_c in the luteal phase is that the thermoregulatory set point is increased (Forman et al., 1987). Thus, this would imply that the threshold for all thermoregulatory effector responses are shifted in a similar direction and the increased luteal phase basal T_c would remain elevated throughout exercise and/ or heat stress.

The mechanisms that controls these alterations in the thermoregulatory set point associated with the menstrual cycle are not well understood in females. In animal research, progesterone administration has been shown to decrease the activity of the warm sensitive neurons and increase the activity of cold sensitive neurons in the preoptic area (Nakayama et al., 1975). These findings indicate a central effect of progesterone in the preoptic area, resulting in an increased set point temperature. In contrast, a decrease in T_c has been associated with oestrogen administration (Tankersley et al., 1992). Animal research indicates that oestrogen increases the activity of the warm sensitive neurons in the preoptic area (Silva and Boulant, 1986) causing a decrease in body temperature (Silva and Boulant, 1986). As a result, researchers have speculated that the increased thermoregulatory set point during the luteal phase, is related to the ratio between oestrogen and progesterone (Forman et al., 1987).

Alterations in the thermoregulatory set point and effector responses over the menstrual cycle have been examined in females using exercise and/ or heat stress. Some researchers have reported no differences in basal T_c between the follicular and luteal phase (Avellini et al., 1980; Wells and Horvath, 1974). In contrast, some researchers have reported differences in basal T_c, however these differences were not apparent following exercise and/ or heat stress (Horvath and Drinkwater, 1982; Senay, 1973). The majority of these studies failed to verify the menstrual cycle phase with measurements of oestrogen and progesterone concentrations accurately. However, when hormonal verification of the menstrual cycle had been conducted, the reduction in basal T_c has

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been reported to remain elevated throughout exercise and/ or heat stress (Carpenter and Nunneley, 1988; Hessemer and Bruck, 1985; Stachenfeld et al., 2000). Several of these studies also reported an increased T_c threshold for thermoregulatory effector responses during the mid-luteal phase (Hessemer and Bruck, 1985; Stachenfeld et al., 2000). Accumulatively, these findings support the increased thermoregulatory set point theory.

2.9.1. Sweating and skin blood flow

It is well established, that the threshold for onset of sweating is increased during the luteal phase relative to the follicular phase (Hessemer and Bruck, 1985; Stephenson and Kolka, 1985). In addition, an increase in whole body SWR has been observed during the luteal phase compared with the follicular phase (Grucza et al., 1993; Hessemer and Bruck, 1985). However, during intense exercise in hot environmental conditions (35°C and 50°C), where there is a greater strain on evaporative heat loss, no differences in SWR have been observed between the follicular and luteal phases (Kolka and Stephenson, 1989; Stephenson and Kolka, 1985). The potential mechanisms responsible for the increased threshold for the onset of sweating during the luteal phase could be associated with the actions of either progesterone or oestrogen, as both are elevated during this time. The stimulus to increase sweat production occurs centrally, a direct result of progesterone acting on the preoptic/anterior hypothalamus (Stephenson and Kolka, 1993).

The fluctuations in hormonal concentration of oestrogen and progesterone associated with the menstrual cycle have been shown to alter active cutaneous vasodilatory controls (Brunt et al., 2012; Charkoudian and Johnson, 2000; Charkoudian, 2010). When oestrogen and progesterone concentrations are elevated during the luteal phase, there is a concurrent increase in the T_c threshold for cutaneous vasodilation, relative to the follicular phase (Charkoudian, 2001). However, beyond the threshold for cutaneous vasodilation, further increases do not incur additional alterations (Charkoudian, 2001). Horvath and Drinkwater (1982) reported a 25% reduction in forearm blood flow at rest during exposure to ambient conditions of 28°C during the follicular phase compared with the luteal phase. However, when exercise was performed in both a warm (35°C) and hot (48°C) environment there were no differences in forearm blood flow between phases. Similar threshold shifts occur with administration of oral contraceptives that contain oestrogen and progesterone (Charkoudian and Johnson, 2000; Grucza et al., 1993; Stachenfeld et al., 2000; Stephenson and Kolka, 1993). This observed shift in thresholds is the net effect of a reduction in oestrogen and an elevation of progesterone, the latter having dominance. The impact of oestrogen and progesterone on sweating and skin blood flow responses are fairly conclusive. Consequently,

consideration of the menstrual cycle phase must be made when assessing these variables in a female population.

2.9.2. Cardiovascular

Only a few alterations in cardiovascular responses have been reported as a result of the changes in hormone concentrations during the menstrual cycle. An elevation in resting and exercising HR has previously been reported during the luteal phase of the menstrual cycle compared to the follicular phase. Kolka & Stephenson, (1997) reported an increase in HR when exercising at 80% of peak aerobic power in 35°C and 22% RH, in the mid luteal phase (161 ± 9 beats.min^{-1}) compared to the early follicular phase (150 ± 11 beats.min^{-1}). This evidence is further supported by the work of Inoue et al., (2005) who reported higher resting and exercising HR during the luteal phase (80 ± 4 beats.min^{-1}, 100 ± 5 beats.min^{-1}) compared to the follicular phase (70 ± 3 beats.min^{-1}, 93 ± 5 beats.min^{-1}) of the menstrual cycle. However, Tenaglia, McLellan, & Klentrou, (1999) reported no phase related differences in HR responses during a 300-min exposure involving 15-min intervals of walking and rest in 40°C and 30% RH. Evidence remains inconclusive regarding the menstrual cycle effect on HR responses at rest and during exercise in hot conditions.

It remains unclear whether PV is affected by menstrual cycle related fluctuations in hormone concentrations. Chapman et al. (1997) reported no differences in PV between the follicular (45 ± 3 mL.kg^{-1}) and the luteal phase (47 ± 3 mL.kg^{-1}) of the menstrual cycle. In contrast, Calzone et al. (2001) reported a lower PV in the luteal phase (2437 ± 113 mL) compared to the follicular phase (2529 ± 183 mL). Furthermore, the differences observed may be due to the procedure used to determine PV; Chapman et al., (1997) used a modified carbon monoxide rebreathing technique which directly measures blood volume and Calzone et al., (2001) used a dye dilution technique with Evans blue which directly measures PV. A lower PV in the luteal phase is further supported by Stachenfeld et al. (1999) who reported a 8.4 ± 2.5 % higher PV in the follicular phase compared to the luteal phase. The alterations are potentially due to the increased levels of oestrogen associated with the luteal phase of the menstrual cycle. An increase in oestrogen is known to enhance the actions of aldosterone in the absorption of sodium in the renal tubules (Stachenfeld et al., 1998) causing a reduction in PV.

Generally, no differences are observed in haemoglobin concentrations over the course of the menstrual cycle (Lebrun et al., 1995; Maughan et al., 1996; Tenaglia et al., 1999). In contrast, Jurkowski et al. (1981) reported an increase in resting haemoglobin during the luteal phase of the menstrual cycle, although this did not result in an increase in arterial O_2 content, or O_2 delivery

during exercise. Furthermore, haematocrit levels generally remain unchanged both at rest and during exercise over the course of the menstrual cycle (Chung et al., 1999; Tenaglia et al., 1999). However, Stachenfeld et al. (2000) reported an increase in haematocrit levels during the luteal phase (39.3 ± 0.5%) compared to the follicular phase (38.3 ± 0.7%). Evidence remains inconclusive as to whether consideration of the menstrual cycle phase must be made when investigating females' cardiovascular and haematological responses across the menstrual cycle.

2.9.3. Exercise performance in the heat

Although, female athletes train and complete during all phases of their menstrual cycle, it is of high importance to understand the effects that the hormonal fluctuations associated with the menstrual cycle may have on exercise performance. The increases in T_c typically observed during the luteal phase of the menstrual cycle may result in an increased thermoregulatory and cardiovascular strain causing, a decrease in prolonged exercise performance.

Tenaglia et al. (1999) examined the effects of the possible thermoregulatory changes over the menstrual cycle on exercise time to exhaustion in the heat. A longer time to exhaustion was reported in the early follicular phase during light intensity intermittent exercise. Tenaglia et al. (1999) also reported a higher T_c during rest in the luteal phase, however, no differences were observed in the rate of T_c rise or the T_c at the end of the time to exhaustion. These findings assume that there is a critical T_c limit for exercise performance, thus, during the luteal phase when resting T_c is elevated, exercise time to exhaustion will be limited. However, there remains limited literature conducted within this area. Future research is warranted to determine whether the thermoregulatory differences across the course of the menstrual have on effect on endurance performance.

$\dot{V}O_2$ max is a strong predictor of endurance performance. Data indicates that there is no phase related differences in $\dot{V}O_2$ or $\dot{V}O_2$max in hot conditions (Horvath and Drinkwater, 1982; Stachenfeld et al., 2000; Tenaglia et al., 1999). However, Williams & Krahenbuhl (1997) reported higher resting $\dot{V}O_2$ in the luteal phase (4.8 ± 0.1 L.min^{-1}) compared to the follicular phase (3.9 ± 0.2 L.min^{-1}). In contrast, Lebrun et al. (1995) reported a lower $\dot{V}O_2$max in the luteal phase (53 ± 1 mL.kg^{-1}.min^{-1}) compared to the follicular phase (54 ± 1 mL.kg^{-1}.min^{-1}). Williams and Krahenbuhl (1997) reported lower ventilation rate during the follicular phase (10.3 ± 0.8 L.min^{-1}) compared to the luteal phase (12.4 ± 0.7 L.min^{-1}) whilst at rest. Furthermore, a higher ventilation rate whilst exercising at 55% $\dot{V}O_2$max and 80% $\dot{V}O_2$max during the follicular (42.2 ± 1.4 L.min^{-1}; 63.6 ± 2.0 L.min^{-1}) compared to the luteal phase (46.2 ± 0.9 L.min^{-1}; 68.8 ± 3.0 L.min^{-1}) of the menstrual cycle was also reported. An

increase in progesterone concentrations during the luteal phase of the menstrual cycle increases the phrenic nerve activity. As the phrenic nerve innervates the diaphragm, elevated progesterone during the luteal phase is often associated with varying degrees of hyperventilation (Denis et al., 1976). To conclude, although progesterone has been reported to have a marked effect on phrenic nerve activity, subsequent influences of the menstrual cycle on respiratory function are not conclusive. Thus, consideration of the menstrual cycle phases should be considered when assessing thermoregulatory responses in females, as alterations in $\dot{V}O_2$ will ultimately affect the metabolic heat production.

2.10.   Individual difference in heat tolerance

Individuals vary in their ability to sustain heat strain, with some demonstrating a decreased capability to dissipate heat and greater body heat content under the same exercise heat stress. These individuals have been described as heat intolerant and are often characterised by an earlier and greater rise in T_c, a greater storage of metabolic heat, have a higher physiological strain to moderate intensity exercise in the heat and reduced sweating sensitivity (Epstein, Shapiro, & Shai, 1983; Moran, Heled, Still, Laor, & Shapiro, 2004). A heat intolerance state can be either temporary or permanent. It may stem from transient predisposing factors such as an acute injury to the thermoregulatory centre, insufficient HA, dehydration, or infectious disease. In addition, a lasting thermoregulatory dysfunction may stem from conditions such as cardiac disease, diabetes, and stoke impairment to sweat glands (Epstein, 1990), or due to differences in gene expression (Moran et al., 2006). Congenital factors such as ectodermal dysplasia may also compromise heat tolerance[1] in some individuals. For the past several decades, researchers have undertaken investigations to develop a better understanding of the various factors that contribute to the inter-individual variations observed in the human heat stress response. Historically, anthropometry and aerobic fitness have been considered to be the primary transient determinants that alter thermoregulatory responses.

2.10.1. Aerobic fitness

Traditionally, it was believed that T_c changes during exercise were closely related to the relative exercise intensity (Saltin and Hermansen, 1966). To balance the increase in metabolic heat, higher

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[1] The term heat tolerance will be used throughout this thesis to describe the phenotypic responses to heat stress, whereas the term thermotolerance will be used to describe the cellular responses to heat stress.

aerobically trained individuals would typically increase their heat loss, resulting in similar T_c response to untrained individuals. In an attempt to isolate the influence of the confounding effects of aerobic capacity between groups, many studies over the past few decades comparing thermoregulatory responses between independent groups have consequently administered exercise using percentage $\dot{V}O_2$max.

Mora-Rodriguez et al. (2010) reported that when exercise is administered relative to $\dot{V}O_2$max (40% and 60% $\dot{V}O_2$max), aerobically trained individuals (66 ± 6 mL.kg^{-1}.min^{-1}) experience similar changes in T_c to untrained individuals (44 ± 3 mL.kg^{-1}.min^{-1}). Enhanced heat dissipation in the trained participants permitted similar T_c changes compared to the untrained participants, despite a higher net metabolic heat production. However, when exercising at a higher intensity (i.e. 80% $\dot{V}O_2$max) T_c was higher in trained individuals compared with untrained individuals. Thus, the superior heat dissipation of the trained participants did not compensate for the higher net metabolic heat production when exercising at a higher percentage of $\dot{V}O_2$max.

It is widely acknowledged, that the human thermoregulatory system works to attain a steady state T_c, such that the rate of metabolic heat production is balanced by an equally elevated rate of heat dissipation (Jay and Kenny, 2007). The increase in heat production during exercise is mostly determined by the change in absolute rate of O_2 consumption, whereas the increase in heat dissipation occurs mainly due to an elevated rate of sweat evaporation from the skin (Gagnon et al., 2013). Thus, when exercising at a similar percentage of $\dot{V}O_2$max, endurance trained participants exercise at a higher absolute work rate than untrained individuals and generate more metabolic heat (Gagnon et al., 2008). In turn, cutaneous blood flow (Fritzsche and Coyle, 2000) and SWR (Kuwahara et al., 2005) are higher in the aerobically trained participants compared to the untrained participants to counterbalance the increased heat production.

Cramer & Jay (2014) reported that there are no differences in whole body SWR between aerobically fit individuals (60 mL.kg^{-1}.min^{-1}) and relatively unfit individuals (40 mL.kg^{-1}.min^{-1}) during exercise at a fixed heat production of 275 W.m^{-2}, despite large differences in percentage $\dot{V}O_2$max. Furthermore, the only fitness-related differences in local SWR were observed on the forehead, where sweating was lower in individuals with a greater aerobic capacity. This finding was potentially due to greater sympatho-adrenal activity associated with a greater cardiovascular, perceptual and ventilatory strain in the less fit individuals at a fixed heat production, potentially eliciting a greater sweat production in glaborous skin areas (Cramer et al., 2012). This evidence suggests that previous observations that have used relative $\dot{V}O_2$max to assess the influence of aerobic fitness on thermoregulatory responses, may be confounded by methodological issues. Thus, when isolating

the independent influence of physiological factors on the thermoregulatory responses to exercise using a between subject design, exercise intensity should be administered using fixed heat production.

2.10.2. Anthropometry

A high body fat percentage may alter T_c changes due to a lower average specific heat capacity of adipose tissue. Jay et al. (2011) compared the thermoregulatory responses of individuals with a high (60 ± 5 mL.kg^{-1}.min^{-1}) and low (40 ± 3 mL.kg^{-1}.min^{-1}) aerobic capacity, whilst exercising at a fixed heat production. The participants in the low aerobic capacity group had a twofold higher body fat percentage ($22.2 \pm 7.0\%$) compared with the high aerobic capacity group ($11.9 \pm 5.9\%$). The lower specific heat capacity of adipose tissue (2.97 kJ.$^{-1}$kg^{-1}.°C) would have yielded a slightly lower average specific heat of the body in the low aerobic capacity group (3.53 kJ.$^{-1}$kg^{-1}.°C) relative to the high aerobic group (3.45 kJ.$^{-1}$kg^{-1}.°C) (Geddes and Baker, 1967). Therefore, a similar body heat storage would have theoretically elevated T_c in the low group by a greater magnitude. Nevertheless, the changes in T_c were almost identical between the two groups (high: 0.87 ± 0.15°C, low: 0.87 ± 0.18°C) when exercising at a fixed heat production. Therefore, it appears that any thermoregulatory implication of the difference in adiposity between the two groups was insignificant, likely due to any insulation created from the fat layers being bypassed by blood flow following the onset of exercise (Havenith and Middendorp, 1990). Another implication of a higher adiposity is a concomitant difference in body volume and therefore BSA. The lower density of fat (0.9 kg.L^{-1}) relative to muscle (1.1 kg.L^{-1}) will allow for a greater evaporative rate in the low aerobic capacity group due to the higher body fat percentage.

Cramer & Jay (2014) compared the thermoregulatory responses of participants with a small (68 ± 6 kg) and large (92 ± 7 kg) BM whilst exercising at a fixed rate of metabolic heat production (500 W and 600 W). Results demonstrate that large differences in BM systematically altered T_c changes, with smaller participants achieving a greater T_c rise. However, when exercise intensity was prescribed using a fixed heat production relative to BM (6.5 W.kg^{-1} and 9.0 W.kg^{-1}) the systematic differences in T_c were eliminated despite differences in BM, absolute heat production, and relative exercise intensity. Thus, the higher T_c in the smaller group at the same fixed heat production is directly explained by the influence of BM per se and not by any differences in heat dissipation or body composition. When exercising at a heat production of both 500 W and 600 W, no differences in T_{sk} and therefore, dry heat loss were observed between participants with a small and large BM. This resulted in a similar absolute evaporative requirement and therefore, the same whole body SWR and presumably evaporation (Cramer and Jay, 2014). As such, it is likely that the differences

in body fat percentage between the two groups contributed to the observed differences in Tre. While it may be possible that much larger differences in body fat percentage alter changes in T_c, the independent influence of high versus low adipose tissue whilst controlling for heat production and BM has not yet been evaluated and merits further investigation. Thus, when assessing thermoregulatory responses to exercise, using a between subject design with participants of varying body sizes, exercise intensity should be administered using fixed heat production.

2.11. Methods to assess heat tolerance

Experimental procedures have been employed to raise T_c under resting and exercise conditions to challenge the thermoregulatory responses as a method of assessing thermoregulatory mechanisms (Inoue et al., 2005; Kenney & Hodgson, 1987; Montain, Sawka, Cadarette, Quigley, & McKay, 1994). A HTT can provide information to evaluate the ability to avoid the risk of exertional heatstroke and aid in decisions about readiness to return to training, competition and/or active duty. The importance of heat tolerance screening was highlighted by Schutte (2010). Following the implementation of heat tolerance screening, incidences of heat stroke and heat illness in mining were reduced to 33 cases over a 2 yr. period compared with 88 for the previous 3 yrs. (Schutte, 2010).

Exercise HTTs commonly adopt protocols of a long duration (>100 min), low intensity (≤ 50% $\dot{V}O_2$max) in environmental conditions of 35-40°C and 40-50% RH, using a variety of exercise modes including, running and bench stepping (Epstein, 1990; Moran et al., 2007). The extensive variation in test paradigms makes between study comparisons difficult. Construct validity of these protocols has been assumed since physiological measures were different between groups of individuals, although the protocols employed were not always capable of differentiating between heat tolerant and heat intolerant individuals (Moran et al., 2004). Shapiro and colleagues (1979) developed a HTT which consisted of stepping for 180-min on a 30 cm high bench in a controlled hot environment. This was adapted by the IDF to be performed on a treadmill. The protocol involves 120-min exposure to 40°C and 40% RH while walking on a treadmill at a pace of 5 km.hr^{-1} and a 2% elevation (Moran et al., 2007). Criteria to discern tolerance and intolerance to exercise heat stress were developed. At the end of the 2-hr exposure, heat tolerance was defined as a peak T_{re} ≤ 38.0°C, peak HR ≤ 120 beats·min^{-1}, and SWR ≥ 780 g·h^{-1}. Heat intolerance was defined as, peak T_{re} ≥ 38.5°C or peak HR ≥ 145 beats·min^{-1} (Moran et al., 2007) and a peak physiological strain index ≥ 6 (Moran et al., 2004).

There are several limitations associated with IDF HTT, which are more apparent when conducting the test on an athletic population (Johnson et al., 2013). Endurance runners typically run at higher intensities for shorter durations than military personnel. The intensity of the protocol of the HTT is considerably below that at which athletes would train and compete. Thus, the HTT misrepresents the metabolic heat production endurance runners may experience and potentially may misdiagnose their susceptibility to a hyperthermic state, or worse a heat-related illness. Protocols of this duration would require a significant amount of time out from training for athletes, which may result in a coach and/or athlete being reluctant to have their heat tolerance assessed. Further work is required to establish an effective short duration, running based HTT improving the ecological validity and application to endurance runners.

2.12. Exertional heat illnesses in running

The challenges of training and competing in a hot environment are complex and difficult to comprehend fully, since athletes are affected in a variety of ways during high intensity exercise in hot humid environments. Pathologic events which occur in hot environments are referred to as exertional heat illnesses and are often categorised as exertional heat stroke, heat exhaustion and heat cramps (table 2.3). Exertional heat illnesses commonly affect athletes during high intensity, or long duration exercise and the severity of the illness depends largely upon the degree of hyperthermia and its duration. Our knowledge, regarding exertional heat stroke is limited to case studies of athletes who push beyond the normal physiological limits, since laboratory studies cannot be conducted, due to the risks of severe hyperthermia are ethically unacceptable for human research. The survival of these athletes depends largely on prompt recognition and the implementation of effective cooling strategies to limit tissue exposure due to destructive hyperthermia (Howe and Boden, 2007).

Table 2.3 Heat illnesses and their associated symptoms.

Condition	T_c (°C)	Associated Symptoms
Heat edema	Normal	None.
Heat rash	Normal	Pruritic rash.
Heat syncope	Normal	Dizziness, general weakness.
Heat cramps	Normal or elevated < 40	Painful muscle contractions, affected muscles firm to palpate
Heat exhaustion	37 - 40	Low blood pressure, elevated pulse and respiratory rates, sweaty and pale, headache, weakness, dizziness, nausea, vomiting, diarrhoea, irritability and decreased muscle coordination.
Exertional heat stroke	≥ 40	Disorientation, confusion, dizziness, irritability, headache, loss of balance, and muscle function, profound fatigue, hyperventilation, vomiting, diarrhoea, delirium, seizures or coma.

Notes: Core temperature, T_c. Adapted from Howe and Boden, (2007).

A high incidence of exertional heat illness has been reported in long distance runners, with 31% and 53% of the total cases of exertional heat illnesses during the 1992 New Orleans U.S. Olympic Trials and the 1996 Atlanta Olympics respectively, occurring in long distance runners (Martin, 1997). Furthermore, during the 2004 Boston Marathon where ambient temperature averaged 22.5°C, more than 300 emergency medical calls were observed, despite changes to the race start time from 1200-hr to 1000-hr in order to decrease heat stress and related casualties (Roberts, 2010). The 2007 London Marathon experienced higher ambient temperatures than expected, with an average air temperature of 19°C, versus an average of 12°C (El Helou et al., 2012). During the London marathon in 2007, 73 hospitalisations were recorded with six cases of severe electrolyte imbalance and one

death. In contrast, the number of people treated was 20% lower at the London Marathon a year later in 2008, when conditions were cool and rainy with an average ambient temperature of 10°C (Roberts, 2010). Furthermore, the total average time to complete the marathon was 17-min slower in 2007 compared to previous years. In addition, the percentage of race withdrawals in Chicago 2007 was the highest (30.74%) among all 60 marathon races analysed by El Helou et al., (2012) when temperature were 13°C higher than the average temperature. Sixty six runners were admitted to the hospital with 12 reported to be intensive care cases with hydration disorders, heat shock syndromes and one death.

In a 12 yr. summary of marathon medical encounters, there were 1.2 cases of heat cramps per 1,000 race entrants; accounting for 6.1% of medical encounters (Roberts, 2000). Furthermore, during a 14 km road race in mild conditions (11°C - 20°C), 14 heat exhaustion cases were reported in 10,000 finishers (Richards and Richards, 1984). Exertional heat stroke has been observed most commonly during activities that involve continuous high intensity exercise. The twin cities marathon which is run in cool conditions averages one exertional heat stroke patient per 10,000 finishers (Roberts, 2000). Furthermore, during an 11.5 km road race perform in hot humid conditions there were approximately 10-20 exertional heat stroke cases per 10,000 finishers (Brodeur et al., 1989). Despite the volume of runners experiencing an exertional heat illness during running events, there are currently no evidence based recommendations regarding the return of an athlete to training and competition. The American College of Sport Medicine (Howe and Boden, 2007) recommend that exertional heat stroke casualties return to training and competition when they have re-established heat tolerance. However, there is currently no established method to evaluate a runner's heat tolerance to determine whether they have re-established heat tolerance. Establishing such a protocol may prevent athletes from returning to training and competition too soon and reduce the likelihood of experiencing another episode of a heat related illness.

2.13.   Heat acclimation

Humans are regulators, and as such strive to keep mean body temperature within a narrow range (37.0 ± 0.5°C). Accordingly, humans not only possess the capability of enduring a wide range of climatic stressors, but also have a considerable capacity to extend this tolerance, through adaptation. The physiological adaptations that occur during exposure to the heat are referred to as acclimatization responses when seen in a naturally occurring environment and HA responses if observed in a controlled environmental setting. HA is achieved by a persistent challenge to the thermoregulatory control system that improves heat dissipation and thermotolerance, prolonging endurance to heat stress.

Heat acclimation develops through frequent exposure to hot environmental conditions, which elicit responses that attenuate the negative effects of heat stress. HA adaptation are discussed in detail in section 2.15. In short, the benefits of HA include an improved thermoregulatory, cardiovascular, sweating and skin blood flow, cellular, and metabolic functioning, a reduction in perceptual strain and an improved exercise performance (Horowitz, 2014; Périard et al., 2015; Sawka et al., 2011a; Taylor, 2014) (table 2.4.). The time course of HA is typically categorized into STHA (≤ 8 d) and LTHA (≥ 10 d) (Garrett et al., 2011; Guy et al., 2015; Périard et al., 2015). The magnitude of physiological adaptation induced by HA depends largely on the intensity, duration, frequency, and number of heat exposures (Sawka et al., 2011b).

2.14.   Heat acclimation protocols

Heat acclimation is typically achieved through one of three induction pathways; fixed intensity (Castle et al., 2011; Lorenzo et al., 2010), self-paced exercise (Armstrong et al., 1986; Sunderland et al., 2008) or controlled hyperthermia (Gibson, et al., 2015; Patterson et al., 2004a, 2014; Garrett et al., 2009, 2012; Regan et al., 1996). Although, exercise in the heat is the most effective method for developing HA, passive heat exposure has also been shown to incur some adaptation (Beaudin et al., 2009; Scoon et al., 2007; Stanley et al., 2015; Takamata et al., 2001).

2.14.1. Fixed intensity heat acclimation

Traditionally, HA aimed to prepare people to perform a specific task within a known set of climatic conditions. This involved repeatedly applying a fixed endogenous and exogenous thermal load to increase T_c as a result of an imbalance between heat production and heat loss (Castle et al., 2011; Houmard et al., 1990; Kresfelder et al., 2006; Lorenzo et al., 2010; Sandström et al., 2008). Thus, during each HA session participants' work at the same absolute exercise intensity for the same duration. This model develops habituation, reduces physiological strain, and increases tolerance (Fox et al., 1963a). While this approach appears logical, there are major limitations regarding the effectiveness and interpretation of this procedure. Its capacity to induce adaptation continually will progressively decline as adaptation occurs, since the strength of the adaptation stimulus is reduced. In addition, these protocols require individuals to work at the same absolute intensity; the physiological strain they experience will therefore, be variable since individuals differ in their inherent heat tolerance (Taylor, 2014). When undertaking mechanistic research, the resulting variations in physiological strain during adaptation make data interpretation challenging.

2.14.2. Self-regulated heat acclimation

Self-regulated exercise requires participants to follow prescribed work and rest intervals, whilst self-selecting and modifying the work rate. Although, this method has considerable practical application since it can be used to rehearse pacing strategies or simulate game play, the technique has no standardised endogenous thermal loading, reducing control and possibly constraining adaptation (Taylor and Cotter, 2006).

2.14.3. Passive heat acclimation

Passive HA uses exogenous heat to incur thermoregulatory stress, with a minimal metabolic contribution. Adaptation is induced if the cumulative adaptation impulse is sufficiently large, and this can be brought about through hot-water bathing (Turk and Thomas, 1975; Zurawlew et al., 2015) and by passive exposure to an environmental chamber, steam room or sauna (Fox et al., 1963a; Scoon et al., 2007; Stanley et al., 2015). While passive techniques are beneficial, the technique has been reported to be less effective than exercise in the heat (Shapiro et al., 1981; Wyndham, 1973). The reason is that passive heating may only induce a slight-to-moderate homeostatic disturbance, so the cumulative adaptation impulse remains small.

Fox and colleagues developed a very effective passive technique to solve this limitation (Fox et al., 1963a). The method was based on elevating and clamping T_c (controlled hyperthermia). Participants were heated in a hot (43°C), humid (100% RH) condition causing a rapid rise in T_c. Participants were dressed in vapour-barrier suits that were continuously ventilated with hot air (35°C) and entered a second chamber (38°C), where they rested. Three clamped T_c (37.3°C, 37.9°C, and 38.5°C) and three exposure durations (30, 60, and 120-min) were evaluated for 12-d. Results suggested a 60-min clamp at 38.5°C was required to establish full HA (Fox et al., 1963a). These specifications have led to use of controlled hyperthermia during exercise.

2.14.4. Controlled hyperthermia heat acclimation

Controlled hyperthermia HA ensures equal thermal strain is placed upon participants as it involves elevating and maintaining a steady state body temperature above the sweating threshold using exercise (Garrett et al., 2011). The use of the controlled hyperthermia HA has become increasingly popular. This method has consistently been found to reduce thermal strain and increase work capacity during both STHA and LTHA and may offer more complete adaptation to fixed intensity HA (Neal et al., 2015; Gibson et al., 2015; Garrett et al., 2009, 2012; Patterson et al., 2004a; Regan et al., 1994).

2.15.   Heat acclimation induction

Humans have a remarkable ability to adapt to heat stress and given adequate water and protection from the sun, a healthy heat acclimated individual can tolerate extended exposure to virtually any natural, weather-related heat stress. HA results in a series of adaptations that reduce the harmful effects of heat stress. Typically, adaptation occurs through morphological, chemical, functional, and genetic adjustments that decrease physiological strain under stress. HA involves a number of complex adaptations (table 2.4), which all integrate to enhance exercise performance.

2.15.1. Sweating and skin blood flow

Sudomotor adaptations were among the first described in response to HA. By the end of the 1940s, it was widely accepted that HA increased SWR and decreased sweat sodium and chloride concentrations (Dill et al., 1933). The observed shift in the onset threshold for sweating (Patterson et al., 2004a) and an altered SWR (table 2.5) and composition, are considered the principal adaptive responses to heat exposure (Buono et al., 2009a). These responses are indicative of both central and peripheral adaptation. At the central level, HA decreases the T_c at which sweating is initiated. This adjustment in onset threshold is proposed to correspond to an absolute change in mean body temperature, rather than to the attainment of a predetermined mean body temperature (Patterson et al., 2004a). Peripheral adaptations, manifested by changes in SWR and sensitivity and occur at the level of the sweat gland (Buono et al., 2009a; Inoue et al., 1999). Following STHA and LTHA using a controlled hyperthermia model, there is on average a 0.23 L.hr^{-1} and 0.46 L.hr^{-1} increase in SWR observed (table 2.5). The potential mechanisms that explain these changes include, an increased cholinergic sensitivity of the eccrine sweat gland and increase glandular hypertrophy and efficiency (Lorenzo & Minson, 2010; Buono et al., 2009; Sato & Sato, 1983). In addition, eccrine sweat glands become resistant to hidromeiosis, resulting in the ability to sustain a higher SWR for a prolonged period of time (Fox et al., 1963b).

In addition to an enhanced SWR, sweat composition changes with HA. Electrolytes are reabsorbed and sweat sodium concentration is reduced, resulting in a more dilute sweat (Chinevere et al., 2008; Saat et al., 2005). The preservation of sodium appears to stem from increased sodium conservation within the re-absorptive duct of the sweat gland (Sato and Dobson, 1970; Sato et al., 1971), which is dependent on aldosterone, a hormone secreted in response to exercise and heat stress that facilitates the reabsorption of sodium. Evaporative heat dissipation is enhanced for a given SWR when a more dilute sweat is secreted, since it is more easily evaporated because of a widening of the water vapour gradient between the skin and ambient air (Taylor, 2014).

Table 2.4 Summary of adaptations to heat acclimation.

Adaptation		Outcome
Thermoregulatory	T_c (rest and exercise)	Reduced
	T_{sk}	Reduced
Cardiovascular	HR	Reduced
	Stroke volume	Increased
	Cardiac output	Better sustained
	Total body water	Increased
	PV	Increased
	Blood pressure	Better defended
	Myocardial compliance	Increased
	Myocardial efficiency	Increased
	Cardio-protection	Improved
Sweating and skin blood flow	Sweating (earlier onset and higher rate)	Improved
	Electrolyte losses	Reduced
	Skin blood flow (earlier onset and higher rate)	Improved
Cellular	HSP	Increased
	Acquired thermotolerance	Increased
Metabolic	Whole body metabolic rate	Lower
	Muscle glycogen	Spared
Perceptual	Thirst	More sensitive
	RPE	Improved
	TS	More accurate
Performance	Lactate threshold	Increased
	Maximal aerobic power	Increased
	Sub maximal aerobic performance	Improved
	Skeletal muscle force generation	Increased

Notes: HR, heart rate; HSP, heat shock protein; PV, plasma volume; RPE, rating of perceived exertion; T_c, core temperature; TS, thermal sensation; T_{sk}, skin temperature. Adapted from Sawka et al., (2011a).

An earlier and greater sudomotor response during HA improves evaporative cooling, assuming the climate allows evaporation, and reduces T_{sk} and thus, skin blood flow requirements. A lower T_{sk} may also reduce cutaneous venous compliance, such that the blood volume is redistributed from the peripheral to the central circulation (Rowell et al., 1967). Until recently, it was assumed that HA centrally modifies thermoregulatory responses in the skin by reducing the T_c threshold for vasodilation, without altering the slope of the blood flow – T_c relationship (Fox et al., 1963a; Roberts et al., 1977; Yamazaki and Hamasaki, 2003). However, by locally infusing an endothelium-dependent vasodilator (acetylcholine) via microdialysis, Lorenzo & Minson (2010) demonstrated that HA improves local cutaneous vascular responses. The authors suggested that this peripheral response may be associated with an increase in the number and sensitivity of muscarinic receptors, a decrease in cholinesterase activity, leading to an improved vascular response to acetylcholine, or alterations in the pathway of vasodilation within smooth muscles or the endothelial cells. Given that HA does not alter maximal skin blood flow, the modified cutaneous vascular response appears to stem from improvements in vascular function (i.e., increased sensitivity of the skin microvasculature to vasodilate), rather than structural changes that limit maximal vasodilator capacity (Lorenzo and Minson, 2010).

Table 2.5 Change in whole body sweat rate observed following short- and long-term controlled hyperthermia heat acclimation.

Citation	No. of Participants	Sex	$\dot{V}O_2$ peak (mL.kg^{-1}.min^{-1})	No. of sessions	Exposure (min)	Temp (°C)	RH (%)	Δ SWR (L.hr^{-1})
STHA ≤ 7 d								
(Gibson et al., 2015)	8	M	49	5	90	40	39	0.34
(Neal et al., 2015)	10	M	63	5	90	40	50	0.13
(Patterson et al., 2004a)	11	M	54	6	90	40	60	0.22
Mean ± SD	10 ± 2		55 ± 7	5 ± 1	90 ± 0	40 ± 0	50 ± 11	0.23 ± 0.11
LTHA ≥ 10 d								
(Gibson, et al. 2015)	8	M	49	10	90	40	39	0.56
(Gibson et al. 2015)	8	M	57	10	90	40	41	0.54
(Patterson et al., 2004a)	11	M	54	16	90	40	60	0.29
(Patterson et al., 2014)	8	M	54	17	90	40	59	0.44
Mean ± SD	9 ± 2		54 ± 3	13 ± 4	90 ± 0	40 ± 0	50 ± 11	0.46 ± 0.12

Notes: LTHA, long-term heat acclimation; M, male; RH, relative humidity; STHA, short-term heat acclimation; SWR, sweat rate; Temp, ambient temperature; $\dot{V}O_2$ Peak, peak oxygen uptake.

2.15.2. Blood volume and fluid balance

Heat acclimation has been reported to increase total body water by approximately 2–3 L, (Bass et al., 1955; Patterson et al., 2014, 2004b). This increase is well within the measurement resolution (4.9%) for total body water (Maw et al., 1996) and thus, appears to be a real physiological phenomenon. The changes in intracellular fluid and extracellular fluid that contribute towards the increase in total body water are variable; since extracellular fluid has been reported to account for greater, equal, and smaller than its percentage increase in total body water after HA (Sawka and Coyle, 1999). There is relatively high variability associated with the measurement of extracellular fluid, and therefore, trends for small changes are difficult to interpret. The changes to intracellular fluid are typically calculated as the difference between total body water and extracellular fluid, and thus results are unclear since the measurement variability inherent in both these techniques, is confounded in the calculation of intracellular fluid. The increase in secretion of fluid conserving hormones aldosterone and arginine vasopressin, and/or renal sensitivity to a given plasma concentration in part explain the increase in total body water (Périard et al., 2015). Furthermore, the conservation of sodium assists in the maintenance of osmolality in the extracellular fluid which maintains or increases extracellular fluid volume during heat adaptation (Nose et al., 1988). As a result of an increase in total body water and extracellular fluid following HA, you may expect expansion of PV.

Following 3 - 4 d of heat exposure or as a result of seasonal changes, PV expansion is present (Sawka and Coyle, 1999). A 5% expansion in resting PV occurs in the hottest months of the year and a 3% contraction in the coldest months (Sawka and Coyle, 1999). However, individuals have varying responses, with some individuals not experiencing any expansion in PV. The observed PV expansion following HA has previously been described as a transient phenomenon (Shapiro et al., 1981), however this may have been an experimental artefact related to the traditional constant work rate model of HA. Recent findings, suggest that by using the controlled hyperthermia technique, which maintains a constant adaptation stimulus by clamping T_c throughout HA, PV is elevated by 15% following 10 consecutive exposures (Gibson et al. 2015) and remains similarly expanded ($\sim$14%) after 8 - 22 d of heat exposure (Patterson et al., 2004a, 2014). Following controlled hyperthermia HA, PV expansion is approximately 7% and 14% for STHA and LTHA, respectively (table 2.6). The magnitude of increase in PV is typically dependent upon the HA day, the hydration state when measured, T_{sk} and whether the individual is at rest or performing exercise (Harrison, 1985; Kenefick et al., 2014; Sawka et al., 1983). The magnitude of PV expansion may also depend on the participants' fitness level, since endurance training will typically result in an expanded PV. PV

expansion offers a thermoregulatory advantage, since it increases vascular filling to support cardiovascular stability (Sawka et al., 2011b).

A reduction in total body water as a result of dehydration will adversely affect thermoregulation and increase cardiovascular strain (Sawka and Coyle, 1999). Thus, dehydration will counteract the adaptations to thermoregulation initiated following HA and aerobic training (Sawka et al., 1983); which the more severe the level of dehydration, the greater the strain and the greater elevation in T_c. Dehydration also impairs dry and evaporative heat loss via the development of plasma hyper-osmolality and hypovolemia (Sawka and Coyle, 1999). However, Takamata et al. (2001) provides evidence to suggest that HA might attenuate the adverse effects of hyper-osmolality on impairing sweating and skin blood flow responses. As such, total body water expansion stemming from HA may confer a protective benefit against dehydration. Although it is apparent that dehydration has clear detrimental effects on exercise performance, moderate permissive dehydration during exercise HA may accelerate the HA process, (Taylor and Cotter, 2006) by increasing fluid electrolyte retention, PV expansion, and cardiovascular responses to heat stress (Garrett et al., 2014, 2011; Neal et al., 2015).

Table 2.6 Change in plasma volume observed following short- and long-term controlled hyperthermia heat acclimation.

Citation	No. of Participants	Sex	$\dot{V}O_2$ peak (mL.kg^{-1}.min^{-1})	No. of sessions	Exposure (min)	Temp (°C)	RH (%)	Δ PV (%)
STHA ≤ 7 d								
(Garrett et al., 2012)	8	M	66	5	90	40	60	4.5
(Garrett et al., 2014)	9	M	60	5	90	40	60	4.0
(Neal et al., 2015)	10	M	63	5	90	40	50	0.0
(Patterson et al., 2004b)	12	M	54	6	90	40	59	15.0
(Patterson et al., 2014)	8	M	54	5	90	40	59	13.1
Mean ± SD	9 ± 2		59 ± 5	5 ± 1	90 ± 0	40 ± 0	58 ± 4	7 ± 6
LTHA ≥ 10 d								
(Gibson, et al., 2015)	8	M	57	10	90	40	41	15.0
(Patterson et al., 2004b)	12	M	54	16	90	40	59	14.4
(Patterson et al., 2014)	8	M	54	17	90	40	59	12.8
Mean ± SD	10 ± 2		55 ± 2	14 ± 4	90 ± 0	40 ± 0	53 ± 10	14 ± 1

Notes: LTHA, long-term heat acclimation; M, male; PV, plasma volume; RH, relative humidity; STHA, short-term heat acclimation; Temp, ambient temperature; $\dot{V}O_2$ **Peak, peak oxygen uptake**.

Eichna et al. (1950) demonstrated that HA reduces voluntary dehydration as a result of an improved relationship between thirst and body water needs. Consequently, heat-acclimated individuals are better able to maintain hydration during exercise in the heat, and thus minimize body water deficits and voluntary dehydration, provided that access to fluids is not restricted. This is an essential adaptation since following HA SWR increases and if fluid replacement is not proportionately increased, greater dehydration will occur. Along with the expansion of PV induced during HA, the ability to better maintain fluid balance through thirst represents an adaptive response that contributes to reduce cardiovascular strain during subsequent heat stress.

2.15.3. Cardiovascular stability

Following STHA and LTHA using a controlled hyperthermia model, resting HR reduces by 3 beats.min^{-1} and 12 beats.min^{-1} respectively (table 2.7). There are several potential mechanisms that explain the reduction in cardiovascular strain. These mechanisms include, an improved skin cooling and redistribution of blood volume, PV expansion, increased venous return from cutaneous and non-cutaneous vascular beds and reduced T_{sk} and T_c (table 2.8).

Following HA, central cardiac function was assessed by either walking at 5.6 km.h^{-1} (Rowell et al., 1967) or cycling at 50% of their maximal aerobic power (Nielsen et al., 1993) until volitional exhaustion. Due to the protocol administered the heat strain was variable between sessions and between the pre and post HA heat stress tests. Nevertheless, both groups observed significant elevations in stroke volume along with reduced HR following HA. These changes signify an improved cardiac function since HR and T_c were lower (Nielsen et al., 1993; Rowell et al., 1967). Wyndham, (1951) and Wyndham et al., (1976, 1968) findings are divergent to that observed by Rowell et al. (1967) and Nielsen et al. (1993). These studies all adopted a fixed intensity HA protocol and thus, the absolute stress level was identical between the pre and post heat stress test. Following HA cardiac output and HR declined, while stroke volume remained constant. These observations may in part be explained by the reduction in the HA stimulus towards the end of HA, as a result on the fixed intensity protocol. However, Wyndham et al. (1976) reported a 25% increase in cardiac output following 4 – 7 d of HA, however this was only 8% following 10 d. The observed changes to cardiac output may in part be mediated by PV expansion which was reported in a companion paper using the same participants (Senay et al., 1976). In this paper it was reported that there was a gradual elevation in PV over the first 6-d of HA, after which it remained stable. Interestingly, no changes in HR, stroke volume or cardiac output were reported during subsequent maximal exercise in the heat (40°C, 30% RH) following 10-d HA in trained cyclists (Lorenzo et al., 2010). However, when the same

exercise was performed in cool environment (13°C, 30% RH), both stroke volume and cardiac output were significantly higher.

Goto et al. (2010) reported resting stroke volume to be elevated following HA. This is potentially mediated by a better maintenance of ventricular filling pressure (Gledhill and Jamnik, 1994), and blood volume, which support venous return and cardiac preloading. Indeed, during times of multisystem stress, a small PV elevation can dictate one's ability to accommodate these stresses and to avoid hypotension and cardiovascular insufficiency. Working with a rat model, Horowitz (2003) has advanced our understanding into the cardiovascular adaptation achieved following HA. While the translation of these modifications to humans is speculative at present, they help explain some adaptations previously explained. There is rightward shift in the diastolic pressure-volume relationship of the heart following chronic heat exposure (Horowitz et al., 1986b), signifying increased cardiac compliance. This is important, since it indicates the heart can be filled more readily and without relying on the relatively small blood volume elevation accompanying heat adaptation to increase central venous pressure. This observed change in compliance enhances stroke volume, making the heart more economical and lowering the metabolic cost of pumping (Horowitz et al., 1986b). The corresponding increase in efficiency reduces metabolic heat production. Finally, since there is an increase in myocardial contractile performance following endurance training (Schaible and Scheuer, 1979) and HA typically involves endurance exercise, then overall cardiac function appears to be enhanced.

Table 2.7 Change in resting heart rate observed following short- and long-term controlled hyperthermia heat acclimation.

Citation	No. of Participants	Sex	$\dot{V}O_2$ peak (mL.kg^{-1}.min^{-1})	No. of sessions	Exposure (min)	Temp (°C)	RH (%)	Δ HR $_{rest}$ (beats.min^{-1})
STHA ≤ 7 d								
(Garrett et al., 2012)	8	M	66	5	90	40	60	-4
(Garrett et al., 2014)	9	M	60	5	90	40	60	0
(Gibson et al. 2015)	8	M	49	5	90	40	39	-5
(Patterson et al., 2004a)	11	M	54	6	90	40	60	-5
Mean ± SD	9 ± 1		57 ± 7	5 ± 1	90 ± 0	40 ± 0	55 ± 10	-3 ± 2
LTHA ≥ 10 d								
(Gibson et al. 2015)	8	M	49	10	90	40	39	-8
(Gibson et al. 2015)	8	M	57	10	90	40	41	-18
(Patterson et al., 2004a)	11	M	54	16	90	40	60	-10
Mean ± SD	9 ± 2		56 ± 2	12 ± 5	90 ± 0	40 ± 0	47 ± 12	-12 ± 5

Notes: LTHA, long-term heat acclimation; M, males; RH, relative humidity; STHA, short-term heat acclimation; HR$_{rest}$, resting heart rate; Temp, ambient temperature; $\dot{V}O_2$ Peak, peak oxygen uptake.

Table 2.8 Change in resting core temperature observed following short- and long-term controlled hyperthermia heat acclimation.

Citation	No. of Participants	Sex	$\dot{V}O_2$ Peak (mL.kg^{-1}.min^{-1})	No. of sessions	Exposure (min)	Temp (°C)	RH (%)	Δ T_c rest (°C)
STHA ≤ 7 d								
(Garrett et al., 2009)	10	M	56	5	90	40	60	0.0
(Garrett et al., 2012)	8	M	66	5	90	40	60	-0.1
(Garrett et al., 2014)	9	M	60	5	90	40	60	-0.2
(Gibson et al., 2015)	8	M	49	5	90	40	39	-0.1
(Neal et al., 2015)	10	M	63	5	90	40	50	-0.2
(Patterson et al., 2004a)	11	M	54	6	90	40	60	-0.2
(Patterson et al., 2014)	8	M	54	5	90	40	59	-0.2
Mean ± SD	9 ± 1		57 ± 6	5 ± 0	90 ± 0	40 ± 0	55 ± 8	-0.1 ± 0.1
LTHA ≥ 10 d								
(Fox et al., 1963a)	18	M	-	10	120	43	100	-0.2
(Gibson et al., 2015)	8	M	49	10	90	40	39	-0.1
(Gibson, et al., 2015)	8	M	57	10	90	40	41	-0.5
(Patterson et al., 2004a)	11	M	54	16	90	40	60	-0.3
(Patterson et al., 2014)	8	M	54	17	90	40	59	-0.3
(Weller et al., 2007)	16	M	51	10	110	46	17	-0.3
Mean ± SD	12 ± 5		53 ± 3	12 ± 3	98 ± 13	42 ± 3	52 ± 28	-0.3 ± 0.1

Notes: LTHA, long-term heat acclimation; M, males; RH, relative humidity; STHA, short-term heat acclimation; T_c rest, resting core temperature; Temp, ambient temperature; $\dot{V}O_2$ Peak, peak oxygen uptake.

2.15.4. Thermotolerance

Thermotolerance or acquired cellular thermotolerance is the nomenclature used to describe the cellular adaptation caused by a single, or repeated severe, but non-lethal heat exposure that preconditions an organism to survive a subsequent and otherwise lethal heat stress (Kuennen et al., 2011; Moseley, 1997). It has been suggested that thermal tolerance and HA share a common basis, as both may potentially be governed by the heat shock response (HSR) (Kuennen et al., 2011). As such, thermal tolerance and HA are complimentary, as HA reduces heat strain and tolerance increases survival to a given heat strain. For example, Maloyan et al. (1999) demonstrated that rodents, with fully developed thermotolerance, survive 60% more heat strain than what would have been initially lethal. Thermal tolerance is associated with heat shock proteins (HSP)[2] binding to denatured or nascent cellular polypeptides and providing protection and accelerating repair from heat stress, as well as fever, hypoxia, ischemia, viral infection, energy depletion, and acidosis (Kregel, 2002). HSP are grouped into families based upon their molecular mass (8 to 110 kDa), with HSP72 being particularly responsive to heat stress and exercise (Locke, 1997). Heat shock protein families have different cellular locations and functions. At the intracellular (iHSP) level, HSP's process stress-denatured proteins, manage protein fragments, maintain structural proteins, and chaperone other proteins across cell membranes. In the extracellular (eHSP) milieu, it is suggested that HSP act as a signal, triggering an immuno-stimulatory response (Pockley, 2003).

Heat shock protein expression increases during and following exposure to heat stress with differing responses across various tissues (e.g., brain and liver exhibit a greater response than skeletal muscle). After the initial exposure, Hsp mRNA levels peak within an hour and subsequent protein synthesis depends upon both the severity and cumulative heat stress (Maloyan et al., 1999). Passive heat exposure and physical exercise both elicit HSP synthesis (Febbraio and Koukoulas, 2000); however, the combination of exercise and heat exposure elicits a greater response than either stressor independently (Skidmore et al., 1995). During exercise in the heat, the expression of eHsp has been shown to be both duration and intensity dependent, relating to the level of hyperthermia attained and rate of rise in T_c (Périard et al., 2012).

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[2]The term heat shock protein (HSP) will be used throughout this thesis to describe the protein expression whereas heat shock protein mRNA (Hsp mRNA) will be used to describe the gene expression.

Horowitz & Robinson (2007) reported that HA increases HSP70 reserves and accelerates the HSR in animals. In humans, the HSR during HA and the concomitant expression of iHsp and eHsp remain somewhat unclear. Following 2-d exercise heat exposure a reduced basal level of eHsp72 and an increase expression immediately post-exercise has been reported (Marshall et al., 2006). However, it has also been shown that iHSP72 is unaffected over the same time course (Marshall et al., 2007). Yamada et al. (2007) demonstrated that when HA was extended to 10 d, basal iHSP72 expression increased, which blunted the post-exercise induction response. In contrast, eHSP72 remained unchanged. During a 15-d HA regimen, basal eHSP72 progressively increased and post-exercise expression decreased in a 29-yr. old male ultra-marathon runner preparing for the Marathon des Sables, suggesting that a longer HA period may induce greater cellular adaptations (Sandström et al., 2008). McClung et al. (2008) conducted a further study in which iHSP72 and iHSP90 responses to 10-d of exercise HA were correlated with physiological adaptations. Such that HA increased basal levels of both iHSP72 and iHSP90 and individuals who demonstrate the greatest physiological adaptation exhibit a reduced post-exercise expression (measured *ex vivo* via water bath incubation). 11-d of controlled hyperthermia (1.0°C T_c elevation) HA resulted in an increase in basal iHSP72 levels, while eHSP72 expression remained unchanged (Magalhães et al., 2010). Similar to the findings of McClung et al. (2008), Magalhães et al. (2010) reported a blunted expression following exercise heat exposure in both iHSP72 and eHSP72. Thus, it appears that iHSP may be more sensitive to heat stress than eHSP and an increase in basal level during HA results in a blunting of the acute response to exercise as HA develops. The measurement of Hsp72 mRNA, offers an alternative marker of the magnitude of the cellular stress response required for increased thermotolerance, allowing for the determination of where the inhibition has occurred. Gibson et al. (2015) reported equal sessional Hsp72 mRNA transcription across a 10-d HA using both fixed and controlled hyperthermia (figure 2.9) protocols. The equal Hsp72 mRNA increases occurring after equal, reduced or increased T_c following both 5 and 10-d HA, suggest that as long as a minimum endogenous criterion is surpassed, additional endogenous thermoregulatory strain is not of further benefit, nor is continual exercise load crucial so long as hyperthermia is present.
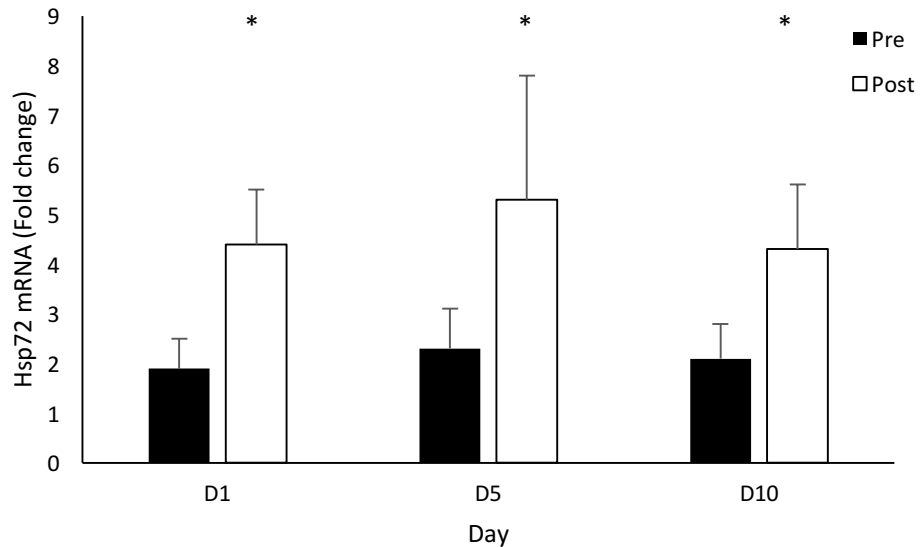
Figure 2.9 Hsp72 mRNA pre- and post-sessions on D1, D5 and D10 of controlled hyperthermia HA in male participants. Adapted from (Gibson et al., 2015a).

Notes: Values are Mean ± SD. * denotes significant pre to post difference within session ($p \leq 0.05$).

2.15.5. Exercise performance

Heat acclimation is reported to improve exercise performance in the heat. Racinais et al. (2015) reported an average time for a 43-km time trial of 66 ± 3 min and 77 ± 6 min in cool (~8°C) and hot conditions (~37°C), respectively. Following 14-d heat exposure the average time trial performance was 66 ± 4 min in a hot environment, similar to that observed in a cool environment prior to HA. Accordingly, HA mediated an improved submaximal exercise performance by reducing physiological strain and lessening a variety of other potential fatigue mechanisms (Nybo et al., 2014). Interestingly, relative to values recorded in temperate conditions, heat stress mediates a reduction in $\dot{V}O_2max$ in trained individuals that cannot be abated by HA. Lorenzo et al. (2010) reported a 5% improvement in $\dot{V}O_2max$ in a cool environment (13°C) and 8% improvement in a hot environment (38°C) following 10-d fixed intensity HA (figure 2.10). The improvement in $\dot{V}O_2max$ observed in a hot environment failed to compensate for the 20% reduction in $\dot{V}O_2max$ conferred by heat stress before and after HA. Furthermore, there was a 6% and 8% improvement in 60-min time trial performance in a cool (13°C) and hot (38°C) environment, respectively (Lorenzo et al., 2010). The observed improvements in $\dot{V}O_2max$ were similar to the improvements observed in the time trial, reinforcing the notion that relative exercise intensity strongly influences performance in the heat (Périard, 2013; Périard et al., 2011). The improvements Lorenzo et al. (2010) observed coincided

with an increase in maximal cardiac output and lactate threshold, PV expansion, lower T_{sk}, and a larger core-to-skin gradient following HA.

Interestingly, HA has been reported to have no influence on the maximal T_c an individual can tolerate during exercise in the heat; since exhaustion coincided with a T_c of ~40.0°C on each day of HA, despite exercise duration increasing throughout the HA period (Nielsen et al., 1993). However, Robinson (1963) reported that highly trained runners may reach T_c of 41.0°C during a 3 mile run (14 min, 15 s) in 30°C conditions. Furthermore, runners performing an 8 km running time trial in warm conditions (WBGT = 27°C) are able to sustain running velocity, despite a T_c exceeding 40.0°C (Ely et al., 2009). More recently, it was shown that trained cyclists reach T_c of 40.0°C at the end of a 43.3 km time trial in hot (37°C) conditions (Racinais et al., 2015). Therefore, it appears that aerobic fitness confers an increased capacity to tolerate higher T_c. However, whether HA provides a similar benefit remains to be determined.
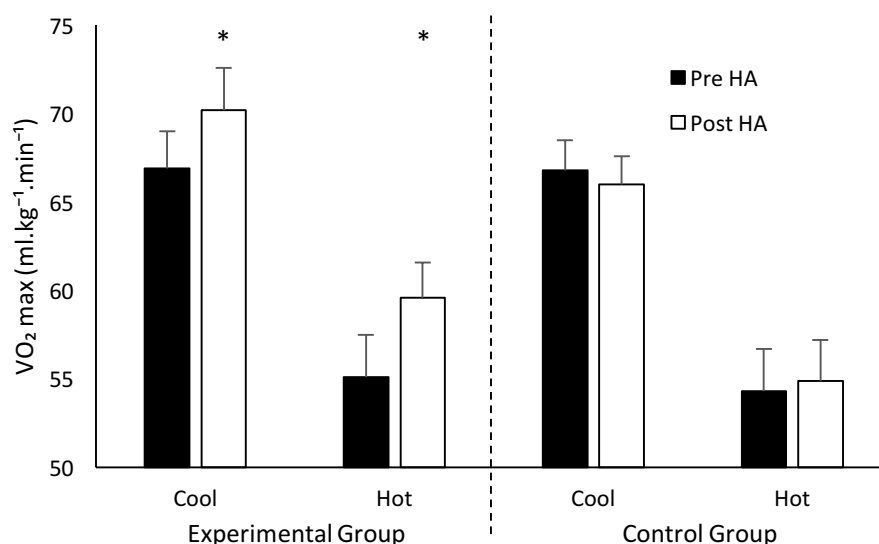
Figure 2.10 Effect of heat acclimation on $\dot{V}O_2$max in a cool (13°C) and hot (38°C) environment in male participants.

Notes: Values are means ± standard error pre and post 10-d heat acclimation (N = 12) and control training (N = 8). * denotes significant difference to pre acclimation within environmental condition. Data taken from (Lorenzo et al., 2010)

2.15.6. Perceptual responses

A reduction in thermal sensation (TS) is potentially beneficial for performance in the heat since thermal discomfort drives true behavioural thermoregulation (Flouris, 2011). Initiation of these response pathways during exercise elicits behavioural responses which reduce work rate (Tucker et al., 2006, 2004). This is undesirable with regards to optimal performance in the heat. However, HA is reported to reduce TS during exercise, performed at a set intensity in the heat. Gibson et al., (2015b) reported a significant reduction in both mean and peak TS during a 30-min fixed intensity heat stress test following 10-d, but not 5-d, of controlled hyperthermia HA. These observations are supported by Garrett et al., (2012) who observed no changes in thermal comfort following 5-d of controlled hyperthermia HA. In contrast, Neal et al., (2015) reported participants had an improved thermal comfort following 5-d controlled hyperthermia HA during a 20-min fixed intensity cycling in temperate conditions (22°C, 60%). The role of TS or thermal comfort are yet to be fully elucidated with regards to HA.

2.16.   Time course of heat acclimation

Heat acclimation is a relatively rapid process that begins on the first day of exposure to a hot climate. During the first 4-7 d of exposure 75-80% of the adaptations occur (Armstrong and Maresh, 1991; Pandolf, 1998). The Armstrong and Maresh table for plateau days of physiological adaptation during heat acclimatisation (Armstrong and Maresh, 1991) has evolved to be a temporal patterning model of HA acquisition (table 2.9). During the initial heat exposure, physiological strain is high, as demonstrated by an elevated T_c and HR. However, the physiological strain induced by heat stress progressively decreases each day of exposure (Buono et al., 2009a; Houmard et al., 1990). Cardiovascular function adapts most rapidly in 4 – 5 d and virtually complete following seven daily exposures (Gibson et al., 2015; Fujii et al., 2012; Garrett et al., 2009). In addition, majority of the thermoregulatory responses including reductions in T_{sk} and T_c typically occur during STHA (Gibson et al., 2015; Fujii et al., 2012; Garrett et al., 2009). The expansion of PV, along with thermoregulatory cardiovascular and metabolic adaptations, improves the perception of effort in athletes. Traditionally, STHA has failed to provide a strong-enough stimulus for sudomotor adaptation (Cotter et al., 1997; Sunderland et al., 2008). However, Buono et al. (2009) reported a significant increase in SWR following 4-d of HA to humid conditions. Thus, STHA appears to be sufficient at inducing performance-enhancing adaptations (Lorenzo et al., 2010), which may be more pronounced after fluid regulatory strain from a permissive dehydration HA regimen (Garrett et al., 2014, 2011; Taylor and Cotter, 2006).

Table 2.9 Plateau days of physiological adaptations (the point at which approximately 85% of the adaptation occurs) during heat acclimatisation.

Adaptation	Days of heat acclimatisation														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
HR decrease			___	___	___	___								
PV expansion			___	___	___	___								
T_{re} decrease				___	___	___	___							
RPE decrease			___	___	___	___								
Sweat NaCl decrease				___	___	___	___	___						
SWR increase								___	___	___	___	___	___	___
Renal NaCl decrease			___	___	___	___	___							

Notes: HR, heart rate; NaCl, Sodium Chloride; PV, plasma volume; Tre, rectal temperature; SWR, sweat rate. Adapted from Armstrong and Maresh, (1991).

The plateau in responses following 3-6 d HA (table 2.9) has previously been viewed as favourable. However, traditional methods of HA may constrain the adaptation and thus the plateau may simply reflect incomplete adaptation. There is an absence of information on the temporal patterning of controlled hyperthermia HA, this is problematic since it is essential that where possible the duration of HA administered elicits optimal adaptation.

2.17.   Heat acclimation decay

Heat acclimation is a transient process and will gradually disappear if not maintained by continued repeated exercise-heat exposure. Literature is consistent in reporting that the physiological adaptations associated with HA are relatively short-term and may decay only a few days or weeks after removal from heat exposure (Armstrong and Maresh, 1991). The first adaptations to occur are typically the first to decay, with the physiological adaptations that take longer to develop, such as sudomotor function, will have a slower rate of decay (Taylor, 2000). Flouris et al. (2014) assessed the decay of 14-d of fixed intensity HA. The observed changes in T_c persist for at least 2-wks after removal from heat exposure; while the changes in HR and HR variability decay faster and are only

partly evident after 2-wks of non-exposure to heat. Furthermore, Garrett et al. (2009) assessed the decay of 5-d controlled hyperthermia HA following 2, 9, 16 and 23-d without exposure to heat. STHA induced adaptations permitting increased heat loss; these adaptations persisted for one, but not 2-wks following HA. Thus, when using a cross over design to assess two HA methods, consideration needs to be taken to ensure an adequate washout period is used.

Furthermore, the re-induction of HA after its loss occurs markedly faster than during the initial induction period (Tetievsky et al., 2008). Weller et al. (2007) assessed the re-induction of 10-d controlled hyperthermia HA following 12 and 26-d without exposure to heat. It took 2-4 d respectively to restore the previous observed physiological benefits. Thus, once adaptation to the heat had been attained, the time that individuals' may spend in cooler conditions before returning to a hot environment, could be as long as one month without the need for extensive re-adaptation to heat. This observation is consistent with research concerning adaptation memory, with some acquired functional changes being rapidly restored (Tetievsky et al., 2008). Thus, when using a cross over design to assess two HA methods, consideration needs to be taken to ensure trials are successfully randomised to reduce the effect of HA memory.

2.18.   Females' responses to heat acclimation

There is a dearth of literature assessing females' responses to HA. Previously, the physiological responses of males and females to 10-d fixed intensity HA were assessed with females initially exhibiting lower T_{re} and HR, despite a lower SWR compared with males (Avellini et al., 1980). Following HA, the physiological strain was similar between males and females, although males maintained a greater SWR. This study adopted a traditional HA protocol which results in a progressive decline in the adaptation stimulus over the duration of HA. Controlled hyperthermia ensures consistent potentiating stimuli for adaptation throughout the HA period, eliciting reductions in thermal strain and increases in work capacity during both STHA (Garrett et al., 2012, 2009) and LTHA (Patterson et al., 2004a), potentially promoting more complete adaptation (Taylor and Cotter, 2006). Furthermore, Sunderland et al. (2008) assessed the responses of female games players responses to STHA. Results suggest, that females fail to establish typical phenotypic adaptations, namely reduction in T_{re} and HR despite a 33% increase in intermittent sprint performance in the heat. These typical adaptive responses have been previously observed in trained males following STHA (Buono et al., 1998; Fujii et al., 2012; Garrett et al., 2011; Poirier et al., 2015; Racinais et al., 2012); suggesting females may require LTHA to achieve adaptation. However, due to the self-paced exercise administered pre and post HA by Sunderland et al. (2008), females were exercising at a higher absolute intensity following HA, suggesting an increase in

metabolic heat production; potentially negating any improvements in thermoregulation achieved through HA. It remains unknown the extent to which females' adapt to the controlled hyperthermia model of HA. The absence of evidence concerning effective STHA protocols in females is problematic for athletes, since STHA is a preferred regime as it provides less disruption from quality training prior to competition compared with traditional HA protocols.

2.19.   Aim of thesis

A HTT using a running mode of exercise, data on females' phenotypic and cellular responses to controlled hyperthermia HA and establishing optimised HA strategies for females are required. This thesis will therefore consist of a series of experiments which will firstly assess the repeatability and the sensitivity of a RHTT. Once this has been established, the RHTT will be used to compare the phenotypic and cellular adaptation responses to controlled hyperthermia in males and females. A further experiment will then be performed to evaluate a novel HA strategy in females across a short-term time scale.

2.19.1. Research questions arising from the literature review

The literature review has identified a number of areas for investigation still outstanding. The incidence of heat-related illnesses in running is apparent, yet there is currently no standardised method to evaluate a runner's heat tolerance. The development of a RHTT is warranted. It is well understood that controlled hyperthermia HA elicits more optimal adaptation compared with fixed intensity protocols. A series of integrated physiological and cellular adaptations elicit an enhanced exercise heat tolerance. Whilst the temporal patterning and length of exposure required to elicit phenotypic and cellular adaptations following controlled hyperthermia HA are becoming well understood in males, little progression has been made with regards to females' responses. Thus, a comparison of males' and females' phenotypic and cellular responses to controlled hyperthermia HA is warranted. Once females' temporal patterning associated with controlled hyperthermia HA has been identified, it remains to be determined whether there are more optimised HA methods to establish an accelerated adaption in females. Passive heat exposures to saunas and hot water have successfully elicited phenotypic adaptation in males; consideration of these protocols is required in females.

2.19.2. Proposed research studies and hypotheses

The following research questions and hypotheses are proposed for this thesis.

Repeatability of a running heat tolerance test

Aim: To establish the repeatability of a practical running test to evaluate an individual's ability to tolerate exercise heat stress.

Hypotheses: It was primarily hypothesised that physiological responses during the running heat tolerance test will have a strong positive correlation on repeated trials. Secondly, there will be no differences in physiological responses during the running heat tolerance test between repeated trials.

Sensitivity of a running heat tolerance test

Aim: To identify the sensitivity of the running heat tolerance test to changes in heat tolerance following short-term heat acclimation.

Hypotheses: It was primarily hypothesised that there will be differences in physiological responses during the running heat tolerance test following short-term heat acclimation. Secondly, the running heat tolerance test will be sensitive to individual differences in the magnitude of adaptation.

A comparison of male and female temporal patterning to short- and long-term heat acclimation

Aim: To determine the sex differences in thermoregulatory, cardiovascular, and sudomotor adaptation to short-term and long-term heat acclimation.

Hypotheses: It was primarily hypothesised that males will achieve greater reductions in T_{re} and HR following STHA compared with females. Secondly, that there will be no differences in the reduction in T_{re} and HR between males and females following LTHA. Finally, that females will achieve a greater increase in SWR following STHA and LTHA compared with males.

Sex comparison of leukocyte Hsp72 mRNA transcription during heat acclimation

Aim: To examine the sex differences in heat shock protein 72 mRNA transcription during controlled hyperthermia heat acclimation over short- and long-term time scales.

Hypothesis: It was hypothesised that heat shock protein 72 mRNA response will be lower in females compared to males across the course of controlled hyperthermia heat acclimation.

Passive heat exposure preceding short-term heat acclimation accelerates phenotypic adaptation in females.

Aim: To determine whether short-term heat acclimation preceded by a passive heat exposure to sauna-like conditions accelerated heat adaptation in females.

Hypotheses: It was primarily hypothesised that short-term HA preceded by a passive heat exposure will result in a greater plasma volume expansion in females compared with heat acclimation alone. Secondly, short-term heat acclimation preceded by a passive heat exposure will produce a greater sudomotor adaptation in females compared with heat acclimation alone.

3. General methods

_____

This chapter describes the general methods and materials used within the experimental chapters of this thesis. When additional or modified measures were used, full descriptions are included within the methods section of the relevant experimental chapters.

3.1.   Ethics, health and safety

All experiments reported within this thesis were approved by the University of Brighton Research Ethics & Governance Committee and conducted in accordance with the guidelines of the revised Declaration of Helsinki, 2013. All experimentation was carried out in line with the University of Brighton's standard operating procedures and risk assessment laboratory guidelines.

Experimenters

There were at least two experimenters present throughout each experimental trial within this thesis. One experimenter remained within the environmental chamber attending to the participant and one experimenter remained outside the environmental chamber, ensuring the safety of individuals within the chamber for the duration of each trial. Furthermore, at least one of the experimenters present during the experimental trials was a qualified first aider.

Equipment cleaning procedure

To avoid contamination, all equipment used was cleaned before and after use. Respiratory apparatus was soaked in 1% Virkon disinfectant (Antec International, UK) for a minimum of 10-min as per manufacturer instructions. Apparatus was then thoroughly rinsed and dried prior to use. HR monitor straps were soaked for 10-min in 1% Virkon disinfectant, rinsed and dried following use. All skin thermistors were cleaned using alcohol wipes (Mölnlycke Healthcare, Sweden) before and after use. All other equipment including the cycle ergometer and the motorised treadmill were wiped down using disinfectant surface spray (Bioguard, UK) following use.

Control of substances hazardous to health

Control of substances hazardous to health forms were completed and passed onto the Welkin Laboratories Biological Safety officer for approval for every powder and solution used within all

experiments presented within this thesis. These forms outlined any known risks associated with the powder or solution and the correct storing, handling, and disposal procedures.

Waste disposal

Rectal thermistors and all other non-reusable waste were disposed of by incineration. All sharps, such as needles and lancets for the measurement of blood were disposed of in a designated sharps bin. Biological material and waste were handled and disposed of in line with relevant guidelines.

Criteria for termination of experiments

Written informed consent (Appendix 1) and a medical history (Appendix 2) was obtained from each participant prior to any testing and all participants were informed of their right to withdraw from the study at any time without obligation to give reason. Exercise was terminated if $T_{re} \geq 39.7°C$ in line with the universities ethical limits, the participant withdrew due to volitional exhaustion, the participants could no longer maintain the exercise intensity despite strong verbal encouragement, or the participant demonstrated signs of heat illness including heat syncope, exhaustion, disorientation, nausea or vomiting. Upon removal from the chamber, appropriate cooling techniques were adopted. Cooling techniques involved cold water ingestion, cold water immersion of the hands and feet and sitting in front of a fan. Participants were permitted to leave the laboratory when T_{re} returned to within 0.5°C of baseline measures obtained prior to testing.

3.2.   Participants

Recruitment

Participants were recruited via an email to University of Brighton students and through advertisement on social media, including Facebook and Twitter. This provided basic details about the study and contact details for the principal experimenter. Prospective participants were then provided with a verbal explanation of the study, in addition an explanation of the risks and benefits. A detailed participant information pack was then provided.

Medical criteria

Athletes, aged 18 – 35 yrs. old who included running and cycling within their weekly training and had a recent history of competing in an endurance event, who had no known injury, respiratory problems, diabetes, or cardiac problems were recruited. Participants were not selected to take part in any of the studies if any contraindications were identified. Participants were deemed suitable if

they had been absent from repeated external heat exposure for the previous three months and had no known previous incident of heat illness. Participants were excluded from taking part if they were taking any dietary supplements or medication other than the contraceptive pill, had taken part in other laboratory experiments in the three months preceding, had experienced anaphylactic shock symptoms to needles, probes or other medical equipment that might preclude them from inserting a rectal probe, or had a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum. Females who had irregular menstrual cycle or who were using another form of hormonal contraception other than the combination pill were excluded from taking part.

3.3.   Facilities

All testing took place in British Association of Sport and Exercise Sciences accredited laboratories, at the Welkin site of the University of Brighton, Eastbourne Campus. All preliminary testing took place in the research laboratory. All experimental heat trials were conducted in the environmental physiology laboratory which housed an environmental chamber 4.5 x 3.5 x 3m high (WatFlow control system; TISS, Hampshire, UK).

3.4.   Pre-trial diet and exercise standardisation

Experimental trials were conducted at the same time of day for all participants to avoid an effect of circadian variation on physiological variables (Atkinson and Reilly, 1996). Experimentation occurred during the UK winter (mean ambient temperature of 5°C); therefore, participants had been absent from repeated external heat exposure for the previous 3 months. Participants were instructed to refrain from hot baths, sauna rooms, steam rooms, and sun beds during the three months prior to the experimental testing and throughout the testing period. Furthermore, participants were instructed to refrain from exposure to hypobaric or hyperbaric environments during the three months prior to the experimental testing and throughout the testing period.

48-hrs prior to conducting the trials, participants were instructed to maintain normal hydration and diet, and refrain from the consumption of alcohol (Yoda et al., 2005), caffeine (Del Coso et al., 2009), glutamine, generic supplementation and exhaustive exercise. 2-hrs prior to arrival participants were instructed to slowly consume 3-5 mL.kg^{-1} (250–400 mL) of water to ensure adequate hydration (Sawka et al., 2007). Participants were recommended to consume a small breakfast consisting of 30-50 g of multigrain cereal with semi-skimmed milk, or two slices of wholemeal toast with a topping of choice, 2-hrs prior to arrival. Participants were instructed to maintain identical diet in the immediate 48-hrs prior to each experimental session.

Hydration assessment

To ensure equal and adequate hydration between trials the assessment of hydration status was performed. When two out of the following three criteria were achieved, adequate hydration to perform the trial was assumed; an osmolality value of ≤ 700 mOsm.kg^{-1}, a urine specific gravity (USG) value of ≤ 1.020, or a BM within 1% of daily average (Sawka et al., 2007). These experimental controls were not violated for any participant for any of the preliminary or experimental procedures.

Urine osmolality

Urine osmolality was measured using a handheld micro osmometer (Advanced Instruments Inc., Massachusetts, USA). The micro osmometer was calibrated prior to every sample using distilled water (Osmolality 0 mOsm.kg^{-1}). Approximately 1 mL of urine was placed into the osmometer lens, whereby the sample was measured using water freezing point depression.

Urine specific gravity

Urine specific gravity was assessed using a visual handheld refractometer (Index Instruments Ltd., Cambridge, UK). The refractometer was calibrated prior to every sample using distilled water (USG 1.000). Approximately 2 mL of urine was placed onto a glass lens of the refractometer. The refractometer was then held up to the light, while the experimenter looked through the eye lens and recorded the values from the scale within.

Repeatability[3] of urinary analysis

A urine sample was collected and the same sample analysed for osmolality and USG on ten occasions. Between analyses the osmometer and refractometer was calibrated with distilled water. Technical error as a coefficient of variation (TE (CV %)), and intra-class correlation coefficient with 95% confidence intervals (ICC 95%CI) were calculated from duplicate measures of 10 samples (table 3.1).

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[3] Reliability, repeatability, reproducibility and agreement are terms often used interchangeably. The term repeatability will be used consistently throughout this thesis since it specifically refers to the variation in repeated measurements made on the same participant under identical conditions.

Table 3.1 Repeatability of urine osmolality and urine specific gravity measurement. Mean ± SD.

	Urine osmolality (mOsm.kg^{-1})		Urine specific gravity	
	1	2	1	2
Mean ± SD	245 ± 187	245 ± 186	1.016 ± 0.030	1.016 ± 0.030
Difference	0 ± 7		0.000 ± 0.001	
TEM	5		0.001	
TE (CV %)	2		0.055	
ICC (95% CI)	0.99 (0.99,1.00); $p \leq 0.001$		1.00 (0.99, 1.00); $p \leq 0.001$	

Notes: ICC (95%CI), Intra- class correlation coefficient with 95% confidence intervals; SD, standard deviation; TEM, typical error of measure; TE (CV%), typical error as a coefficient of variation.

3.5. Anthropometric assessment

Stature

Stature was measured using a fixed stadiometer (Detecto Physians Scales, Detecto Scale Company, Missouri, USA). Participants were required to stand vertically in the anatomical position facing away from the stadiometer scale in the laboratory. The stadiometer arm was lowered until it rested horizontally on the most superior aspect of the head. The scale was then read to the nearest 0.5 cm.

Body mass

Nude BM was measured using Adam GFK 150 digital body scales (Adam Equipment Inc., Connecticut, USA) and recorded to 0.01 kg. The scales were calibrated prior to use, using a 20 kg weight. Morning BM was recorded on four consecutive occasions prior to the experimental trials. This procedure was carried out in a private room, where participants self-reported their BM to the experimenter.

Body surface area

Following measurement of both stature and BM, body surface area (BSA) was subsequently calculated using Equation 3.1 (DuBois and DuBois, 1916).

Equation 3.1 Calculation of body surface area (BSA)

$$BSA = 0.007184 \times BM^{0.425} \times H^{0.725}$$

Where: BM = body mass in kg, H = stature in meters

Skinfolds

Skin fold thickness was determined from the right side of each participant whilst stood in the anatomical position in ambient laboratory conditions of 20 ± 1°C and 40 ± 5% RH. Sum of skin folds was determined from four sites (Durnin and Womersley, 1974); the bicep, triceps, subscapular, and supra-iliac area using Harpenden skin fold callipers (Baty International, West Sussex, UK). Skin fold thickness was measured to the nearest 2 mm. Measures were taken in triplicate and the average recorded. If values were not within 0.4 mm additional measures were taken.

The bicep skin fold was located at the point on the anterior surface of the arm in the midline at the level of the mid-acromial-radiale landmark. The tricep skin fold was located at the point on the posterior surface of the arm, in the midline, at the level of the mid-acromial-radiale landmark. The subscapular skin fold was located 2 cm laterally and obliquely downwards from the under most tip of the inferior angle of the scapula. The supra-iliac skin fold was located at the point on the iliac crest where the midaxilla on the longitudinal axis of the body meets the ilium (Durnin and Womersley, 1974).

When these locations had been marked, a firm grasp was taken of the skin and subcutaneous fat ensuring no muscle or fascia was taken using the thumb and index finger. Then the contact surface of the callipers was placed at a 90-degree angle to the skin fold approximately 1 cm below the fingers. The pressure was then slightly released between the fingers, ensuring that a greater pressure was applied by the callipers. The handle of the callipers was then released and the measurement was recorded approximately 4 s after the pressure was released.

Repeatability of skinfold measurement

The skinfold thickness from four sites on eight participants was recorded on two consecutive mornings. TEM, TE (CV %), and ICC 95% CI were calculated from these duplicate measures (table 3.2).

Table 3.2 Repeatability of skinfold measurements. Mean ± SD.

	Bicep	Tricep	Subscapular	Supra-iliac
D1 (mm)	6.8 ± 2.3	7.0 ± 3.3	10.0 ± 2.5	10.5 ± 2.7
D2 (mm)	6.9 ± 2.4	7.1 ± 3.2	10.1 ± 2.5	10.4 ± 2.8
TEM	0.2	0.1	0.1	0.3
TE (CV %)	2.9	1.2	0.7	2.4
ICC (95% CI)	0.99 (0.98, 0.99); $p \leq 0.001$	0.99 (0.99, 1.00); $p \leq 0.01$	1.00 (0.99, 1.00); $p \leq 0.001$	0.99 (0.98, 0.99); $p \leq 0.001$

Notes: ICC (95% CI), Intra-class correlation coefficient with 95% confidence intervals; SD, standard deviation; TEM, typical error of measure; TE (CV %), typical error as a coefficient of variation.

3.6.   Menstrual cycle

Timing of testing

To control for hormonal effects on thermoregulation associated with the menstrual cycle, female participants filled out a menstrual cycle questionnaire (Appendix 3) for 2 months prior to the testing sessions being scheduled. Where possible, all testing was performed during the follicular phase of the menstrual cycle (3-10 d after the onset of menstruation) (Stachenfeld and Taylor, 2014). However, female participants taking oral contraceptive performed the experimental sessions during the no pill or inactive phase of oral contraceptive use where possible.

Validity of the menstrual cycle questionnaire

Data collected in our laboratory demonstrated the validity of menstrual cycle questionnaires for accurately determining the correct phase of the menstrual cycle. Nine participants completed a

menstrual cycle questionnaire for two full cycles. Participants then reported to the laboratory on five occasions across two full cycles. Participants reported to the laboratory on D3, D10, and D22 after the onset of menstruation during the first cycle and then on D3 and D10 after the onset of menstruation during the second cycle. Plasma concentrations of 17β-estradiol and progesterone were quantified to determine accurately the menstrual cycle phase using commercially available 17β-estradiol (ab108667) and Progesterone (ab108670) immunoenzymatic assay kits (Abcam plc, UK).

Plasma concentrations of 17β-estradiol and progesterone were similar on D3 and D10 across two full menstrual cycles (table 3.3). Plasma concentrations of17β-estradiol and progesterone were higher on D22 in all participants ($p \leq 0.05$), providing evidence that a menstrual cycle questionnaire is an effective method to determine menstrual cycle phase accurately.

Table 3.3 Plasma concentration of 17β-estradiol and progesterone. Mean ± SD (range).

Day	3	10	22	3	10
17β estradiol	43 ± 18*	62 ± 30*	211 ± 64	42 ± 23*	43 ± 24*
(pg.mL^{-1})	(12-75)	(32-127)	(121-279)	(23-89)	(24-97)
Progesterone	1.25 ± 0.63*	1.10 ± 0.67*	12.21 ± 16.58	1.03 ± 0.57*	1.70 ± 0.68*
(ng.mL^{-1})	(0.90 – 2.39)	(0.15 – 1.96)	(4.10 – 50.63)	(0.09 – 1.68)	(0.05 – 1.75)

Notes: * denotes significant difference to D22.

3.7.   Environmental conditions

Ambient laboratory temperature control

Seated rest for the measurement of resting values and preliminary testing were performed in ambient laboratory conditions at 20 ± 1°C and 40 ± 5% RH; WBGT = 19°C, using industrial air conditioning. Barometric pressure was determined from a portable barometer (Weather station, Oregon Scientific, Oregon, USA).

Experimental temperature and humidity

All exercise heat sessions were performed in a large, purpose-built environmental chamber with computer controlled monitoring and maintenance of desired environmental conditions with an available range of -20°C to +50°C and 20 to 95% RH. As an index of heat stress WBGT was calculated. Unless otherwise stated, experimental trials were conducted in 40°C and 40% RH (WBGT = 33°C). During exercise heat stress experimental trials, manual recording of the chamber condition was performed every 5-min to describe accurately the environment experienced by participants.

3.8. Thermoregulatory measures

Rectal temperature

Rectal temperature was measured to indicate T_c, using a general purpose disposable probe (Henley, Reading, UK) attached to a data meter (Meas Spec 4600, measurement Specialities, Virginia USA) measured to the accuracy of 0.01°C. The rectal probe was measured at a depth of 10 cm past the anal sphincter with a zinc oxide tape bung applied to the probe prior to insertion to ensure the correct depth was attained and maintained throughout testing.

Repeatability of rectal thermistor

The repeatability of the rectal thermistor was determined prior to exercise in a rested state. An individual was exposed to temperate laboratory conditions in a rested state for 20-min to stabilise Tre. Measurements of T_{re} were then taken every min for two 10-min periods. TEM, TE (CV %), and ICC 95% CI were calculated from duplicate measures (table 3.4).

Table 3.4 Repeatability of rectal thermistors. Mean ± SD.

	Resting T_{re} (°C)	
Measures	**1**	**2**
Mean ± SD	37.4 ± 0.0	37.4 ± 0.02
Difference	0.02 ± 0.02	
TEM	0.02	
TE (CV %)	0.04	
ICC (95%CI)	0.99 (0.98, 1.00); $p \leq 0.001$	

Notes: ICC, intra-class correlation coefficient; TEM, typical error of measure; TE (CV %), typical error as a coefficient of variation; Tre, rectal temperature.

Skin temperature

T_{sk} was recorded using skin thermistors (Eltek Ltd, Cambridge, UK) attached to four sites on the right side of the body connected to a Squirrel temperature logger (Squirrel 1000 series, Eltek Ltd., UK). The four sites were the chest (midpoint of the pectoralis majo), the arm (midpoint of the triceps brachii lateral head), the upper leg (midpoint of the rectus femoris), and the lower leg (gastrocnemius lateral head) Mean T_{sk} was calculated using Equation 2.4 (Ramanathan, 1964).

Repeatability of the skin thermistors

The repeatability of the skin thermistor was determined by recording the mean temperature of four skin thermistors exposed to ambient conditions. The ambient conditions were stable at 20°C and 40% RH throughout the data collection. The skin thermistors were left to stabilise for 20-min and then measurements were recorded every min for two 10-min periods. Data was then averaged over each 10-min period. TEM, TE (CV %), and ICC 95% CI were calculated across the two 10-min periods (table 3.5).

Table 3.5 Repeatability of skin thermistors. Mean ± SD.

Measures	Mean skin thermistor (°C)	
	1	2
Mean ± SD	20.3 ± 0.1	20.2 ± 0.1
TEM	0.1	
TE (CV %)	0.3	
ICC (95% CI)	0.91 (0.70, 0.98); $p \leq 0.001$	

Notes: ICC (95% CI), intra-class correlation coefficient with 95% confidence intervals; SD, standard deviation; TEM, typical error of measure; TE (CV %) technical error as a coefficient of variation.

3.9. Cardiopulmonary measures

Metabolic gas analysis

Expired metabolic gas was measured using a breath-by-breath online gas analysis system (Metalyzer Sport, Cortex, Germany). Calibration was performed as per manufacturer's instructions. Calibration was performed for pressure, volume turbine and gas sensors prior to each use. Barometric pressure was determined and calibrated to the device from a portable barometer (GA690, Castle Group Ltd, UK). Volume calibration required simulated inspiration and expiration via a manual syringe (3 L, Hans Rudolph, Germany) for five acceptable cycles eliciting a flow rate of 2 to 4 L.s^{-1}. According to the manufacturer's instructions, the flow sensor measured values to the accuracy of ± 2% with a range of 0.05-20 L.s^{-1}. Gas concentration calibration required two known O_2 and two known CO_2 concentrations, known as gas 1 and gas 2, to be sampled until stabilisation of sensors had been achieved. According to the manufacturer's instructions, the O_2 sensor and CO_2 sensors measured values to the accuracy of 0.1 Vol% with a range of 0 to 60% and 0 to 13% respectively.

In accordance with manufacturer's guidelines, gas 1 was required to represent close to the inspired fraction and gas 2 close to the expired fraction. Gas 1 was taken directly from ambient conditions (inspired fraction O_2 = 0.2093, inspired fraction CO_2 = 0.0005), Gas 2 was drawn from a contained

gas cylinder (expired fraction O_2 = 0.17, expired fraction CO_2 = 0.05) into a clamp-sealed collection bag, which was immediately affixed to the sample line. All respiratory gas exchange data were averaged over a 10 s period.

Heart rate

Heart rate was recorded using a Polar HR monitor (Polar Electro Oyo, Kempele, Finland). A chest strap transmitter was securely affixed to the participant and the sensors dampened to aid conductivity. The wrist watch receiver was also worn to enable live HR data recording. According to the manufacturer's instructions, the HR monitor is accurate during steady state conditions to ± 1% or ± 1 beat.min^{-1}, whichever is larger.

3.10.   Physiological strain index

The physiological strain index (PSI) was calculated from exercising T_{re} and HR using Equation 3.2.

Equation 3.2 Calculation of physiological strain index (PSI)

$$PSI = 5 (Tret - Treo) * (39.5 - Treo)^{-1} + 5 (HRt - HRo) * (180 - HRo)^{-1}$$

Where: Treo and HRo are the initial and Tret and HRt are simultaneous measurements taken at any time (Moran et al., 1998).

3.11.   Haematological measures

Capillary lactate sample

For capillary lactate sampling the fingertip was first cleaned using an alcohol wipe and left to air for approximately 15 s. The skin was punctured using a single use lancet (Acc-Chek Safe-T-Pro, Roche Diagnostics ltd., West Sussex, UK). Applying light pressure to the site a ~200 µl of capillary blood was collected from the fingertip into a heparin-fluoride coated microvette tube and subsequently analysed for lactate concentration using an automated analyser (YSI 2300 Stat Plus, YSI UK, Hampshire, UK). The analyser was calibrated immediately before each session using the manufacturer's 5 mmol.L^{-1} standard, set to self-calibrate every 25-min and verified after each session using the same manufacturer standard.

Repeatability of capillary blood lactate analysis

A resting capillary blood sample, combined with samples at approximately 1 mmol.L^{-1}, 2 mmol.L^{-1}, 3 mmol.L^{-1}, 4 mmol.L^{-1}, and 8 mmol.L^{-1} were analysed in duplicate. TEM, TE (CV %), and ICC 95% CI were calculated from duplicate measures (table 3.6).

Table 3.6 Repeatability of capillary blood lactate analysis. Mean ± SD.

	Lactate concentration (mmol.L^{-1})	
	1	**2**
Mean ± SD	3.30 ± 2.66	3.32 ± 2.69
TEM	0.03	
TE (CV %)	1.02	
ICC (95% CI)	1.00 (0.99, 1.00); $p < 0.001$	

Notes: ICC (95% CI), Intra-class correlation coefficient with 95% confidence intervals; SD standard deviation; TEM, typical error of measure; TE (CV %), technical error as a coefficient of variation.

3.12. Sweat rate

Towel-dried nude BM was measured and recorded to the nearest gramme before and immediately after all trials as a measure of whole body SWR. Between these two measures of nude BM, fluid intake was restricted and values were corrected for urine output. Values were uncorrected for respiratory and metabolic weight losses as they were assumed as similar between trials due to the matched exercise intensity and environmental conditions. SWR was calculated and reported in g.hr^{-1} using Equation 3.3. SWR was also calculated and reported relative to BSA (SWR$_{BSA}$) in g.hr^{-1}.m^2 using Equation 3.4.

Equation 3.3 Calculation of whole body sweat rate (SWR)

SWR (g.hr^{-1}) = [BM$_{pre}$ (g) − (BM$_{post}$ (g) + urine output (g)] / exercise duration (min) * 60

Where: BM$_{pre}$ = body mass prior to beginning the trial, BM$_{post}$ = body mass following the trial.

Equation 3.4 Calculation of whole body sweat rate relative to body surface area (SWR$_{BSA}$)

SWR$_{BSA}$ (g.hr^{-1}.m^2) = SWR (g.hr^{-1}) / BSA (m^2)

Where: BSA, body surface area; SWR, sweat rate.

3.13. Perceptual scales

Rating of perceived exertion

The rating of perceived exertion scale (RPE), also known as the Borg scale (Borg, 1962), is a subjective tool to assess subjective perception of effort during exercise. RPE ranged from 6 (very, very light), through 13 (somewhat hard), to 20 (very, very hard) along a 15-point scale (figure 3.1). RPE has been reported to be closely related to both metabolic and cardiac intensity parameters (Scherr et al., 2013). Participants were provided with standardised instructions that provided specific commands on how to report overall feelings of exertion throughout the experimental trials. The standardised instructions included clear understanding of anchoring the top and bottom ratings to previous experiences of no exertion at all (RPE = 6) and maximal exertion (RPE = 20) (Mauger et al., 2013). Within this thesis, RPE was recorded at 10-min intervals throughout the RHTT and HA sessions. During the preliminary lactate threshold and $\dot{V}O_2$ peak test, RPE was recorded in the final 15 s of each stage.

Thermal sensation

The thermal sensation (TS) scale (Toner et al., 1986) is a subjective tool to assess thermal perception. TS ranges from 0.0 (unbearably cold), through 4.0 (comfortable), to 8.0 (unbearably hot) along a 17-point scale (figure 3.1). TS has been reported to be closely related to measures of ambient air temperature, metabolic rate, Tre, and T_{sk} during exercise (Gagge et al., 1969). The standardised instructions provided to the participants included clear understanding of anchoring the top and bottom ratings to previous experiences of being unbearably cold (TS = 0) and unbearably hot (TS = 8). Within this thesis TS was recorded at 10-min intervals throughout the RHTT and HA sessions.
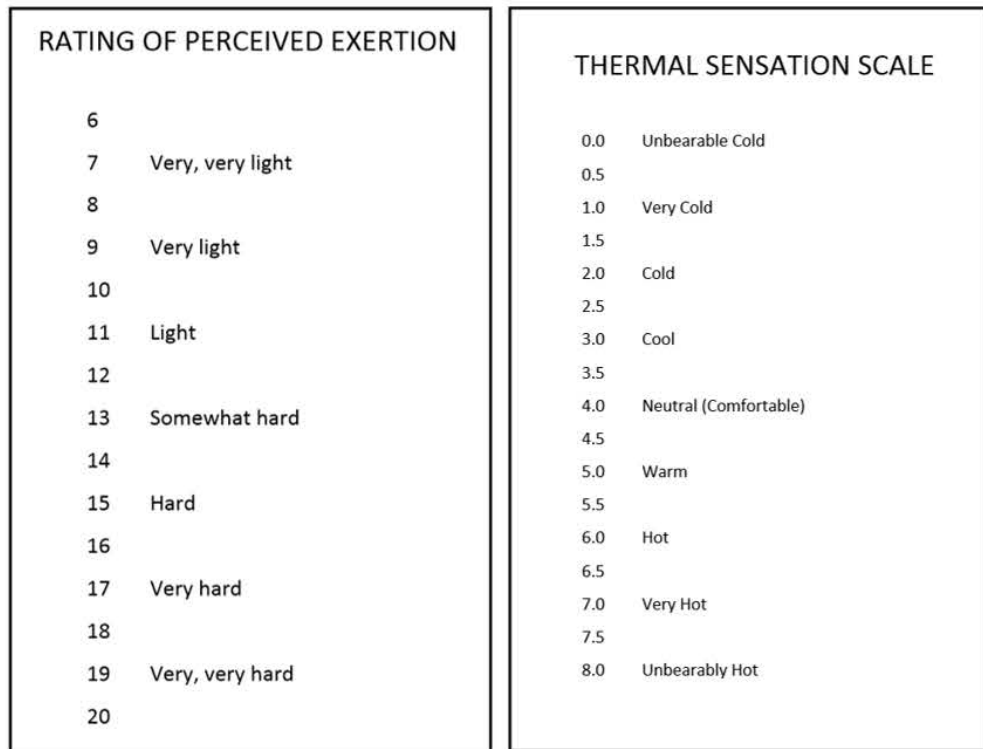
Figure 3.1 Rating of perceived exertion (RPE) and thermal sensation (TS) scales.

3.14.   Cycling exercise trials

Cycle ergometry

All cycle ergometry was performed on a cycle ergometer (Monark 874E, Monark Exercise AB, Vansbro, Sweden). For all trials the seat height was set to ensure a 25° angle in the knee flexion when at 6 o'clock pedal crank position was attained. Power was controlled by manipulating the resistance on the suspended weight pan using a fixed cadence of 80 revolutions per min (rpm). Calculations of power outputs in watts (W) during cycling ergometry were made as follows.

Equation 3.5 Calculation of work during cycle ergometry

Work (J) = Force (N) x Distance (m)

= Mass on the weight pan (kg) x Acceleration due to gravity (m.s^{-1}) x Pedal revolutions (r.s^{-1}) x Flywheel distance per revolution (m)

Equation 3.6 Calculation of power from work during cycle ergometry

Power (W) = Work (J) / Time (s)

Cycling peak oxygen uptake

A graded exercise test was performed using a cycle ergometer in temperate laboratory conditions (WBGT = 19°C) to determine participants' $\dot{V}O_2$ peak. Participants performed a 5-min warm up at 60 W and were informed to maintain a constant cadence of 80 rpm. The cycling intensity was set to 80 W and resistance was applied to the flywheel to elicit a 16 to 24 W.min^{-1} increase (selected depending on the BM of the participant). These intensities were selected to ensure volitional exhaustion was reached in 8-15 min. In the final 10 s of each stage HR was recorded. $\dot{V}O_2$, and respiratory exchange ratio (RER) were measured continuously and averaged for each 10 s period throughout each stage. The test was terminated when participants reached volitional exhaustion and/or the cadence could no longer be maintained at 80 ± 5 rpm despite strong verbal encouragement. 3-min following the completion of the test a capillary blood sample was collected for the analysis of lactate concentration. $\dot{V}O_2$ peak was identified at the power associated with the highest $\dot{V}O_2$ averaged over 10 sec. $\dot{V}O_2$ peak was determined when three out of the following four criteria where achieved; a plateau in $\dot{V}O_2$ (increase by less than 2 mL.kg^{-1}.min^{-1}), an RER ≥ 1.15, HR reached 10 beats.min^{-1} from age predicted max and blood lactate ≥ 8 mmol.L^{-1}. The protocol adopted was according to the British Association of Sport and Exercise Science Guidelines (Davison and Wooles, 2007).

Heat acclimation protocol

The daily HA sessions consisted of a 90-min exposure to 40°C, 40% RH (WBGT = 33°C). Exercise intensity was set at cycling 65% $\dot{V}O_2$ peak (Appendix 4) from the outset and adjusted with work: rest intervals to achieve and maintain a T_{re} ~38.5°C (Garrett et al., 2012; Patterson et al., 2004a), or if participants were unable to maintain a cadence of 80 ± 5 rpm despite strong verbal encouragement An example of this protocol for one participant is presented in Figure 3.2. The controlled hyperthermia model of HA was selected since it ensures a constant adaptation stimulus (Fox et al., 1963a), and is reported to result in performance and physiological improvements in highly trained athletes (Garrett et al., 2012). A cycling mode of exercise was selected since there is less variability in efficiency compared with running between participants thus, ensuring a similar stress. Furthermore, due to the consecutive nature of the HA sessions; cycling incurs less muscle

damage compared with running, subsequently reducing the chance of participants incurring an injury.
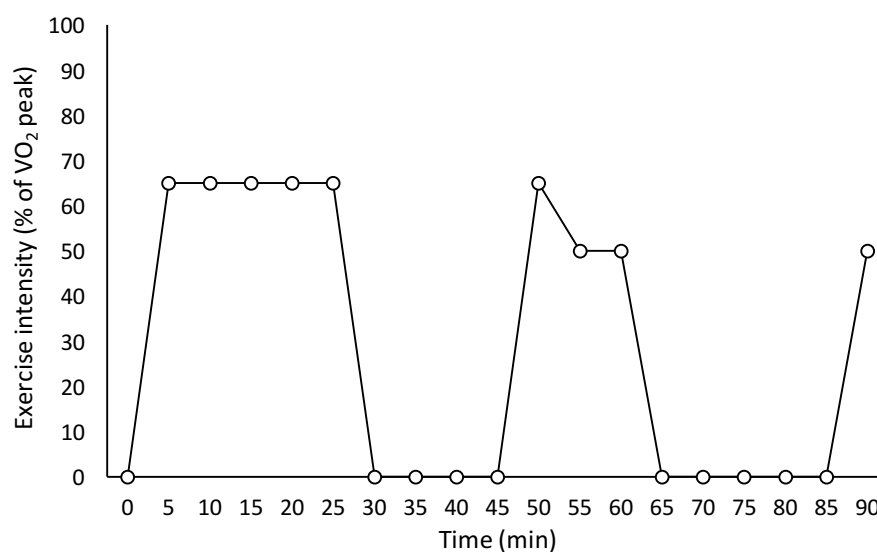
Figure 3.2. Example of relative exercise intensity elicited during a 90-min controlled hyperthermia heat acclimation session.

Heat acclimation intensity pilot study

A pilot study was carried out to determine the intensity required to elicit the required rise in Tre. The intensity needed to be high enough to increase T_c rapidly yet low enough that participants were able to sustain the intensity and not incur too much fatigue due to the consecutive days of training.

A 25-yr. old physically active female (stature, 1.78 m; BM, 70 kg, $\dot{V}O_2$ peak, 3.61 L.min^{-1} at 208 W) cycled on three occasions at 55%, 65%, and 75% $\dot{V}O_2$ peak. Tre, HR, RPE, and TS were recorded at 5-min intervals throughout the three trials. Exercise was terminated when T_{re} reached 38.5°C. Duration for T_{re} to reach 38.5°C, the rate of T_{re} increase (Equation 3.7), mean HR, and peak RPE and TS were recorded in table 3.7.

Equation 3.7 Calculation of rate of T_{re} increase

Rate of T_{re} increase = (ΔT_{re} (°C) / time taken (min)) * 60

Where: ΔT_{re} = total change in rectal temperature

The results suggested that 65% $\dot{V}O_2$ peak was of a sufficient intensity to elicit the required increase in T_{re} in 30-min. These findings are similar to the work of Périard et al. (2012) who reported physiological responses and time to exhaustion when cycling at 60% and 75% $\dot{V}O_2$ peak. During the 60% $\dot{V}O_2$ peak trial participants terminated exercise at ~59-min, having experienced a T_{re} rise of 2.1°C, HR ~96% max HR, RPE of 18 and blood lactate 4.8 ± 2.5 mmol.L^{-1}. However, during the 75% $\dot{V}O_2$ peak trial participants terminated exercise after ~27-min having experienced a T_{re} rise of 4.2°C, HR at ~99% HR max, RPE of 20 and blood lactate 10.9 ± 4.8 mmol.L^{-1}. Acknowledgment of this published data combined with the pilot work presented suggests that an initial starting intensity of 65% $\dot{V}O_2$ peak would be sufficient to elicit an increase in T_c to the desired limit of 38.5°C within approximately 30-min; providing a 60-min stimulus with $T_{re} \geq 38.5$°C.

Table 3.7 Physiological and subjective response to cycling at set percentages of $\dot{V}O_2$ peak.

	55% $\dot{V}O_2$ peak	65% $\dot{V}O_2$ peak	75% $\dot{V}O_2$ peak
Duration to T_{re} 38.5°C (min)	35	30	25
Rate of T_{re} increase (°C.hr^{-1})	2.43	3.02	3.29
Mean HR	162	167	170
RPE at 38.5°C	14	16	17
TS at 38.5°C	6.5	6.5	6.5

Notes: HR, heart rate; RPE, rating of perceived exertion; Tre, rectal temperature; TS, thermal sensation

3.15. Running exercise trials

Treadmill running

All running based trials were performed on a motorized treadmill (PPS55 sport-1, Woodway, Germany). Participants were familiarised with how to reduce the speed or stop the treadmill if they wished to terminate the trial early. Otherwise, the speed was controlled solely by the experimenter during all treadmill trials.

Running lactate threshold

A graded exercise test was performed using a motorized treadmill in temperate laboratory conditions (WBGT = 19°C) to determine participants' lactate threshold. Participants performed a 5-min warm up at a speed 1 km.hr^{-1} below the starting speed; this was typically a very light jog (7 km.hr^{-1} to 9 km.hr^{-1}). The initial running speed for the test was set between 8 km.hr^{-1} and 10 km.hr^{-1} with a 1% gradient (Jones and Doust, 1996). Participants then performed five to nine, 3-min, incremental (0.8 km.hr^{-1}) stages on a treadmill. $\dot{V}O_2$ and RER were recorded continuously throughout the stage and averaged for each 10 s period. In the final 10 s of each stage HR and RPE were recorded. On completion of each stage, exercise was momentarily paused and lactate sample was taken. The test was terminated when lactate reached 4.0 ± 0.5 mmol.L^{-1}. Lactate threshold was determined using the point at which blood lactate increased 1 mmol.L^{-1} above resting value (Jones and Doust, 1998). The protocol adopted was according to the British Association of Sport and Exercise Science Guidelines (Jones, 2007).

Running peak oxygen uptake

Following a 15-min recovery from determination of the lactate threshold, participants performed 1-min incremental (1% gradient) stages. On the completion of each stage $\dot{V}O_2$, RER and HR were recorded. The test was terminated when participants reached volitional exhaustion and/or the speed of the treadmill could no longer be maintained despite strong verbal encouragement. Immediately post and 3-min following the completion of the test, a capillary blood sample was collected for the analysis of lactate concentration. $\dot{V}O_2$ peak was identified at the intensity associated with the highest $\dot{V}O_2$ averaged over 10 s. $\dot{V}O_2$ peak was determined when three out of the following four criteria where achieved; a plateau in $\dot{V}O_2$ (increase of ≤ 2 mL.kg^{-1}.min^{-1}), an RER ≥ 1.15, HR reached 10 beats.min^{-1} from age predicted max, and blood lactate ≥ 8 mmol.L^{-1}.

3.16. Heat tolerance tests

Running heat tolerance test

The RHTT was performed on a motorized treadmill and consisted of 30-min running at 9 km.hr^{-1} and 2% gradient in ambient conditions of 40°C and 40% RH (WBGT = 33°C). Following 20-min of seated rest in temperature conditions, T_{re}, HR, T_{sk}, RPE, and TS were recorded. Participants then entered the environmental chamber to perform the RHTT; where T_{re}, HR, and T_{sk} were recorded at 5-min intervals and RPE and TS at 10-min intervals. Towel-dried nude BM was measured and recorded to the nearest gramme before and after all trials as a measure of SWR.

Running heat tolerance test pilot work

Introduction

The Israeli Defence Force (IDF) developed a walking HTT to evaluate if military personnel, who had experienced exertional heat illness, were safe to return to duty. The protocol consists of 120-min walking on a treadmill at a pace of 5 km.hr^{-1} and a 2% gradient in ambient conditions of 40°C and 40% RH (WBGT = 33°C). At the end of the exposure, heat tolerance was defined as a peak $T_{re} \leq$ 38.0°C, peak HR $\leq$ 120 beats.min^{-1}, and SWR $\geq$ 780 g.h^{-1}. Heat intolerance was defined as, peak T_{re} > 38.5°C, peak HR > 145 beats.min^{-1}, (Moran et al., 2007) and a peak PSI $\geq$ 6 (Moran et al., 2004).

There is an instant elevation in the rate of thermogenesis at the onset of physical activity. As exercise intensity increases, especially under hot environmental conditions, a thermal imbalance persists resulting in a continually positive rate of change in body heat storage, increasing body heat content and a sustained rise in T_c giving a graded increase of heat strain (Jay & Kenny, 2007). There are several limitations associated with the IDF HTT, which are more apparent when conducting the test on endurance athletes (Johnson et al., 2013). Endurance runners typically run at high intensities for shorter durations than military personnel. The intensity of the protocol of the IDF HTT is considerably below what an athlete would train and compete. Thus, the IDF HTT misrepresents the metabolic heat production endurance athletes may experience and potentially may misdiagnose their susceptibility to a hyperthermic state.

The aim of this pilot study was to determine whether there was a similar pattern of heat tolerance and heat intolerance between the IDF HTT and the RHTT. It was hypothesized that the RHTT would have a strong positive relationship for the participants' thermoregulatory and cardiovascular measures during the IDF HTT and the RHTT.

Materials and methods

Sixteen (8 males; 8 females) healthy individuals volunteered and provided written informed consent to participate in the pilot study (mean ± standard deviation (SD), age, 23 ± 5 yrs.; BM, 67.07 ± 10.96 kg; stature, 1.76 ± 0.01 m).

Participants performed two trials in a randomised order separated by 5-7 d. The IDF HTT involved 120-min walking at 5 km.hr^{-1} and 2% gradient in hot ambient conditions of 40°C and 40% RH (WBGT = 33°C). The RHTT was conducted using the previously described procedure. Nude BM was

measured pre- and post-trial as a measure of SWR. At 5-min intervals throughout the trials, HR and T_{re} were recorded.

All data were first checked for normality using the Shapiro-Wilk method. As a measure of retest correlation, ICC with 95% CI were calculated for each physiological measure during IDF HTT and the RHTT. Paired sampled t tests were calculated to identify any differences in physiological measures between the IDF HTT and the RHTT. All data was analysed using a standard statistical package (SPSS version 20.0), and reported as mean ± SD. Statistical significance was accepted at the level of $p \leq$ 0.05.

Results

Table 3.8 presents the mean ± SD data for the physiological variables measured during the IDF HTT and the RHTT. There were no observed differences between the IDF HTT and the RHTT in measures of T_{re} rest ($t_{(15)}$ = -0.597, p = 0.559) and HR rest ($t_{(15)}$ = 1.370, p = 0.191). However, there were significant differences between the IDF HTT and the RHTT in measures of peak T_{re} ($t_{(15)}$ = -5.567, $p \leq 0.001$), peak HR, ($t_{(15)}$ = -22.753, $p \leq 0.001$) and SWR ($t_{(15)}$ = -8.180, $p \leq 0.001$).

Table 3.8 Physiology variables measured during the IDF HTT and the RHTT. Mean ± SD.

Physiological Variables	IDF HTT	RHTT
T_{re} rest (°C)	37.17 ± 0.26	37.18 ± 0.26
T_{re} peak (°C)	38.30 ± 0.28	38.82 ± 0.47*
HR rest (beats.min^{-1})	65 ± 8	64 ± 8
HR peak (beats.min^{-1})	135 ± 18	182 ± 15*
SWR (g.hr^{-1})	637 ± 188	1721 ± 675*

Notes: HR, heart rate; IDF HTT, Israeli Defence Force heat tolerance test; RHTT, running heat tolerance test; T_{re}, rectal temperature; and SWR, sweat rate. * denotes significantly difference to IDF HTT ($p \leq 0.05$).

Figure 3.3 presents the raw data for T_{re} rest and HR rest. Strong correlations (ICC (95% CI)) were observed between the IDF HTT and the RHTT for T_{re} rest (r = 0.938 $_{(0.822 - 0.978)}$, $p \leq 0.001$) and HR rest ($r_{)}$ = 0.959 $_{(0.881 - 0.986)}$, $p \leq 0.001$). Figure 3.4 presents the raw data for T_{re} peak and HR peak. Strong correlations (ICC (95% CI)) were observed between the IDF HTT and the RHTT for T_{re} peak (r = 0.702

$_{(0.147 - 0.896)}$, p = 0.012) HR peak (r = 0.932 $_{(0.805 - 0.976)}$, $p \leq 0.001$). Furthermore, there was a medium correlation (ICC (95% CI)) observed between the IDF HTT and the RHTT for SWR (r = 0.599 $_{(0.147 - 0.860)}$, p = 0.043).
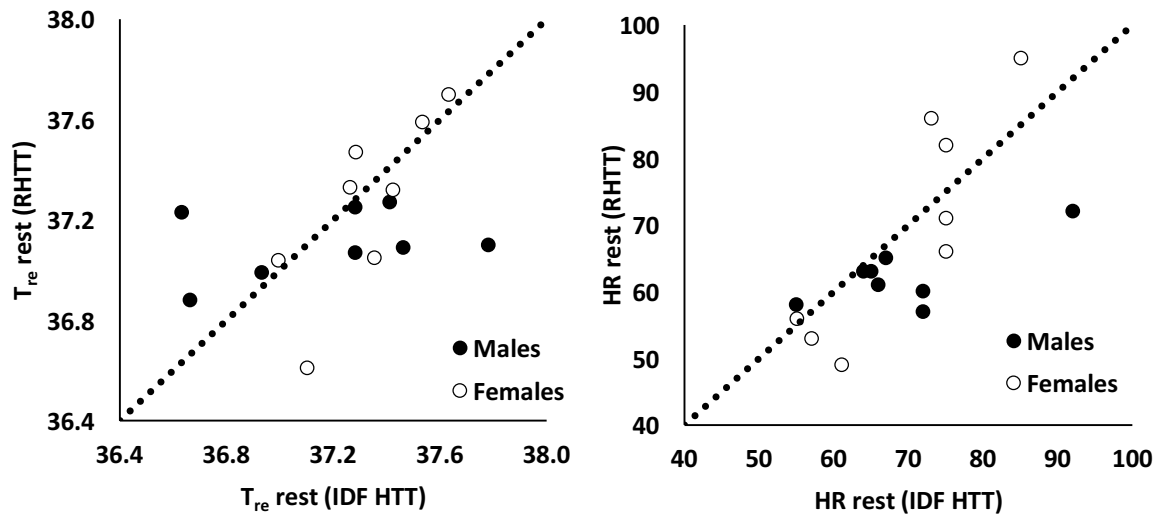
Figure 3.3 Resting rectal temperature (T_{re}) and resting heart rate (HR) during the Israeli Defence Force heat tolerance test (IDF HTT) (x-axis) and running heat tolerance test (RHTT) (y-axis) for males (closed markers) and females (open markers). Dotted line represents line of equality. N = 16 (8M, 8F).
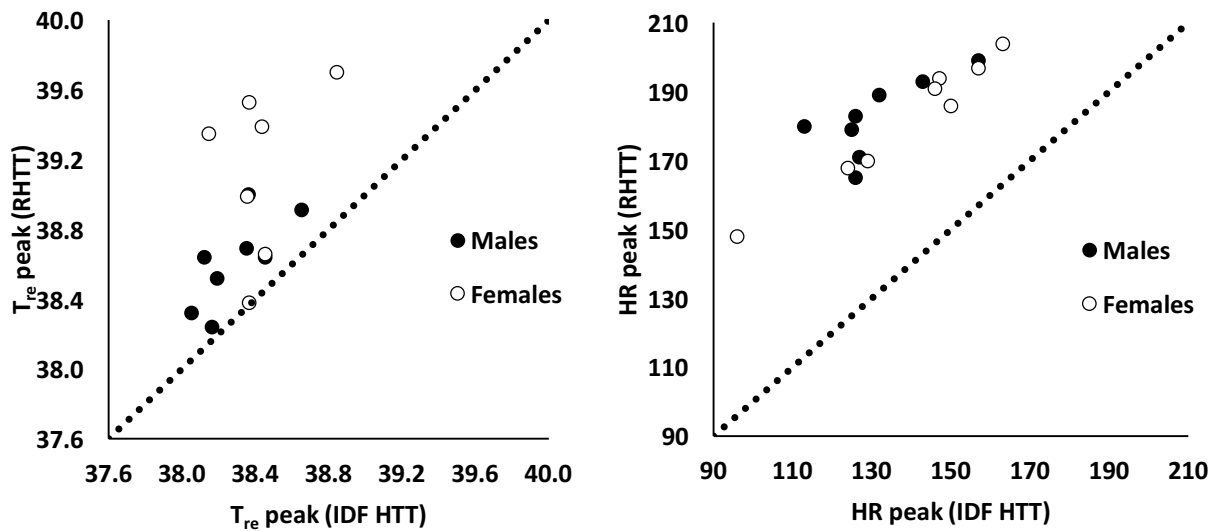
Figure 3.4 Peak rectal temperature (T_{re}) and peak heart rate (HR) during the Israeli Defence Force heat tolerance test (IDF HTT) (x-axis) and running heat tolerance test (RHTT) (y-axis) for males (closed markers) and females (open markers). Dotted line represents line of equality. N = 16 (8M, 8F).

Conclusion

The RHTT was compared against the established IDF HTT, since it has been used to differentiate between heat tolerant and heat intolerant military personal (Moran et al., 2007). The aim of this pilot study was to determine whether the physiological responses during the IDF RHTT correlated with the physiological responses during the RHTT. Classic markers of heat tolerance and HA, namely T_{re} rest, T_{re} peak, HR rest, HR peak, and SWR were used for analysis. The main finding from this pilot study was that there is a strong positive correlation between the IDF HTT and the RHTT (ICC > 0.59). In addition, data presented suggest that the RHTT is capable of differentiating between individual's heat tolerance with individual's responses sitting along a continuum, (figure 3.2 and figure 3.3); providing evidence that a 30-min HTT adopting a running mode of exercise is of a sufficient duration and intensity to establish differences in heat tolerance between individuals. Furthermore, the RHTT is a time efficient alternative to the IDF HTT that offers specificity to endurance runners.

3.17.   Statistical analysis

3.17.1. Power analysis

The number of participants required for each experimental chapter was determined using G*Power version 3.1 in accordance with established guidelines for *a priori* determination (Farrokhyar et al., 2013)The value of α and β were set as 0.05 and 0.8, respectively.

3.17.2. Normality and sphericity of data

Normal distribution

Data was checked for skewness and kurtosis to determine whether the distribution of scores was approximately normal. If values of skewness and kurtosis were between -1.96 and 1.96 data was assumed normally distributed (Field, 2013).

Furthermore, the Shapiro-Wilk method was used to compare the scores in the data set to a normally distributed set of data with an identical mean and SD. If the test was non-significant (i.e. $p \geq 0.05$), then the distribution was deemed not significantly different from the normal distribution and data was assumed normally distributed (Field, 2013).

Sphericity

Sphericity was assessed using the Mauchly's test, which tests the hypothesis that the variance of the differences between conditions is equal. If Mauchly's test statistic was significant ($p \leq 0.05$), it was concluded that there were significant differences between the variances of differences and therefore, the condition of sphericity was not met. If the assumption of sphericity was not met the degrees of freedom were adjusted using the Greenhouse-Geisser method. If Mauchly's test statistic was non-significant ($p \geq 0.05$) then it was concluded that the variance of differences were about equal (Field, 2013).

3.17.3. Repeatability statistics

Typical error of measure (TEM)

Within this thesis the TEM was calculated to represent the absolute repeatability of measurements used. TEM was calculated from the SD of the difference score by the square root of two (Hopkins, 2000a).

Equation 3.8 Calculation of typical error of the measure (TEM)

TEM = SD $_{diff}$ /√2.

Where: SD $_{diff}$ = standard deviation of the difference between the repeated measures; √2 = square root of 2.

Coefficient of variation

Within this thesis the TEM has been expressed as a percentage of its respective mean to form the technical error as a coefficient of variation (TE (CV %)) (Hopkins, 2000a).

Equation 3.9 Calculation of technical error as a coefficient of variation (TE (CV %))

TE (CV %) = (TEM/ Mean) *100

Where: TEM = typical error of measure, Mean = the mean of the mean of the paired samples

Intra class correlation coefficient

Within this thesis ICC with 95% CI were calculated using SPSS as a measure of retest correlation. Prior to conducting this test data was first checked for normality as previously described.

Equation 3.10 Calculation of intra class correlation

ICC = (SD^2 − sd^2)/ SD^2

Where: SD = between subject standard deviation, sd = within subject standard deviation

3.17.4. Statistical tests of difference

Statistical significance level

Within this thesis a statistical significance level was set to describe the probability (p) of seeing a difference as big (or bigger) than the one you saw, given that there is no difference. The threshold

p value for all experiments within this thesis was set at 5% thus, giving 95% confidence that there was a difference or association. Within this thesis, a p value ≤ 0.05 indicates that the observed effect is unlikely to have arisen purely by chance, providing evidence to reject the null hypothesis.

Type I and type II error

A statistical significance level of 0.05 was selected within this thesis to avoid the chances of type I and type II error. Type I error occurs when you incorrectly reject the null hypothesis. Thus, you would suggest a relationship or difference, when in reality none exists. This typically occurs when the statistical significance level is too high (e.g. $p ≤ 0.1$). In contrast, type II error occurs when you accept the null hypothesis when it is false. Thus, you would suggest no relationship, when a relationship exists. This typically occurs when the statistical significance level is too low (e.g. $p ≤ 0.01$).

Paired samples t test

Within this thesis paired sample t tests were used to determine statistical significance between two experimental conditions, when the same participants were used during both conditions. The ratio of these variances is known as the t-ratio. Prior to conducting the t test, data were first checked for skewness and kurtosis to determine whether

Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a way of comparing the ratio of systematic variance to unsystematic variance in an experimental study. The ratio of these variances is known as the F-ratio. ANOVA is a collection of statistical models used to analyse the difference among group means and their associated procedures. Prior to conducting an ANOVA within the respective studies data was first checked for the assumption of normal distribution and sphericity as previously described (3.17.2.).

3.17.5. Effect sizes

Within this thesis, effect sizes have been used to present the magnitude of the reported effects in a standardised metric, providing information on the practical significance of the findings. The selected effect size reported for each statistical test, was based on the recommendations from Lakens' (2013) review on calculating and reporting effect sizes.

Cohen's d

In this thesis, effect sizes for t tests have been reported as Cohen's d. Cohen's d has been used to describe the standardised mean difference of an effect. A Cohen's d value of 0.2 refers to a small effect, 0.5 as a medium effect and 0.8 as a large effect based on bench marks suggested by (Cohen, 1988). Cohen's d in between subjects' design can be readily interpreted as a percentage of the SD such that a Cohen's d of 0.5 means the difference equals to half a SD.

Equation 3.11 Calculation of Cohen's d (*d*)

$$d = (X_1 - X_2) / SD$$

Where: X_1 = mean first set of data, X_2 = mean second set of data, SD = standard deviation

Partial eta squared

In this thesis, effect sizes for ANOVA analyses, have been reported as partial eta squared (np^2) (Levine and Hullett, 2002). This was calculated by SPSS. Partial eta squared expresses the sum of squares of the effect in relation to the sum of squares of the effect and the sum of squares of the error associated with the effect.

3.17.6. Post hoc analysis

To combat the familywise error rate associated with multiple comparisons, the level of significance for individual tests was altered using Bonferroni corrections. The Bonferroni methods was selected within this thesis as it is extremely robust (although slightly conservative) and controls the alpha level regardless of the manipulation. In doing this it ensures that the cumulative type I error remains below 0.05 (Field, 2013).

Any additional statistical analyses used with this thesis are outline in the respective chapter.

4. Repeatability of a running heat tolerance test

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4.1.   Abstract

At present there is no standardised HTT procedure that uses running as a mode of exercise. The current study aimed to establish the repeatability of a practical running test to evaluate an individual's ability to tolerate exercise heat stress. Sixteen (8M, 8F) participants performed the RHTT (30-min, 9 km.hr^{-1}, 2% elevation) on two separate occasions in a hot environment (40°C and 40% RH). There were no differences in peak T_{re} (RHTT1: 38.82 ± 0.47°C, RHTT2: 38.86 ± 0.49°C, r = 0.93, TEM = 0.13°C), peak T_{sk} (RHTT1: 38.12 ± 0.45°C, RHTT2: 38.11 ± 0.45 °C, r = 0.79, TEM = 0.30°C), peak HR (RHTT1: 182 ± 15 beats.min^{-1}, RHTT2: 183 ± 15 beats.min^{-1}, r = 0.99, TEM = 2 beats.min^{-1}), or SWR (RHTT1: 1,721 ± 675 g.hr^{-1}, RHTT2: 1,716 ± 745 g.hr^{-1}, r = 0.95, TEM = 162 g.hr^{-1}) between RHTT1 and RHTT2 ($p ≥ 0.05$). Results demonstrate good agreement, strong correlations and small differences between repeated trials, and the TEM values suggest low within-participant variability. The RHTT was effective in differentiating between individuals' physiological responses; supporting a heat tolerance continuum. The findings suggest the RHTT is a repeatable measure of physiological strain in the heat and may be used to assess the effectiveness of acute and chronic heat-alleviating procedures, when using a repeated measures design.

4.2.   Introduction

During exercise in a hot environment, active muscles perform work causing an increase in body heat content. These changes are modulated by the rate of relative heat production (Cramer and Jay, 2014), and represent the rate of change in body heat storage, which in turn reflects the balance between metabolic heat production, heat absorbed from the environment and total body heat loss (Jay and Kenny, 2007). Individuals vary in their ability to withstand heat stress, with some demonstrating a decreased capability to dissipate heat and greater rise in body heat content under the same exercise heat stress (Epstein, 1990). These individuals have been described as heat intolerant and are often characterized by an earlier and greater rise in T_c, a greater storage of metabolic heat, a higher physiological strain to moderate intensity exercise in the heat, and reduced sweating sensitivity (Epstein et al., 1983; Moran et al., 2004).

An individual's heat intolerant state may be temporary or permanent (Epstein, 1990; Moran et al., 2007; Ruell et al., 2014), stemming from transient predisposing factors, such as an acute injury to

the thermoregulatory centre, insufficient HA, dehydration or infectious disease (Epstein, 1990). In addition, a lasting thermoregulatory dysfunction may stem from conditions such as cardiac disease, impairment to sweat glands (Epstein, 1990), or differences in gene expression (Moran et al., 2006). Congenital factors such as ectodermal dysplasia may also compromise heat tolerance in some individuals (Epstein, 1990). Aside from these predisposing factors, the high exercise intensity that endurance runners experience during competitions combined with extreme ambient conditions, may elicit unavoidable uncompensable heat production. The evaporative heat loss requirement to maintain a thermal steady state exceeds the maximal evaporative capacity of the individual in the given environment causing a continual rise in T_c. The work by Nielsen (1996) provides data to suggest a marathon runner may experience up to a 1.0°C rise in T_c every ~9-min when racing in high ambient conditions ($\geq$ 35°C, $\geq$ 60% RH), when radiant and convective heat loss is negligible. This rate of rise in T_c would result in the runner reaching a T_c of 40.0°C within 25-30 min, with the immediate dangers of heat exhaustion. High incidence of exertional heat illness has been reported in long distance runners, with 31% and 53% of the total number of cases of exertional heat illness during the 1992 New Orleans U.S. Olympic Trials and the 1996 Atlanta Olympics, respectively (Martin, 1997). Whether heat intolerance is permanent or acquired, the consequences of exertional heat illness among endurance athletes emphasises the value of a test to evaluate an individual's ability to withstand exercise heat stress.

Experimental procedures have been used to cause a rise in T_c under resting and exercise conditions to challenge the thermoregulatory responses (Inoue et al., 2005; Johnson et al., 2013; Kenney and Hodgson, 1987; Montain et al., 1994) and thus assess the ability of an individual to withstand heat stress and to evaluate heat dissipating mechanisms. The Israeli Defence Force (IDF) developed a HTT to evaluate whether exertional heat illness in military personnel was temporary or permanent, supporting a safe return to duty (Moran et al., 2004). The protocol involves 120-min walking on a treadmill at a pace of 5 km.hr^{-1} and a 2% gradient in hot ambient conditions of 40°C and 40% RH (WBGT = 33°C). Heat tolerance is determined at the end of the exposure, using criteria of peak T_{re} $\leq$ 38.0°C, peak HR $\leq$ 120 beats.min^{-1}, and SWR $\geq$ 780 g.h^{-1}. Deviations from the specified criteria indicate a greater state of heat intolerance, whereas a pronounced plateau in both T_{re} and HR is a definitive sign of heat tolerance.

There is an instant elevation in the rate of thermogenesis at the onset of physical activity. As exercise intensity increases, especially in an uncompensable[4] environment, a thermal imbalance persists. This results in a continually positive rate of change in body heat storage, increasing body heat content and a sustained rise in T_c, giving a graded increase of heat strain (Jay and Kenny, 2007). The IDF HTT may be appropriate for specific occupational situations due to the low-to-moderate intensity coupled with the long exposure time that is likely to be experienced in military scenarios. Acknowledging the work carried out by the IDF, limitations associated with the HTT remain when examining endurance runners. Exertional heat illness is compounded by uncompensable heat stress which is in turn, influenced by the duration and intensity of exercise. The relative work intensity of an endurance runner training and competing in the heat is markedly higher compared with occupational activities. Therefore, the IDF HTT may not be applicable to an endurance population due to the duration and intensity of the protocol. The low intensity nature of the current HTT will not reflect the metabolic heat production endurance runners experience and may misdiagnose their susceptibility to a hyperthermic state, pointing to the benefit of a RHTT. At present there is no standardised HTT procedure adopting a running mode of exercise and such a test would offer greater ecological validity for endurance runners.

Moran and colleagues (2004) assessed the heat tolerance of nineteen male participants and concluded that the duration of a HTT cannot be shorter than 120-min, since tolerance at 60-min was unable to predict tolerance at 120-min. The work by Epstein and colleagues (1983) and more recently by Moran and colleagues (2007) contradicts these findings, as the rate of increase in T_{re} and HR during the first 20-30 min was considerably different between those individuals deemed heat intolerant and those heat tolerant. This evidence suggests that it may be plausible to assess an individual's ability to withstand exercise heat stress in 30-min. A shorter RHTT requiring no prior testing would provide a more time efficient screening procedure for runners.

To assess and monitor changes in heat tolerance a protocol needs to be reliable to minimise measurement error, due to biological variation and equipment noise (Atkinson and Nevill, 1998). Typically, the assessment of repeatability uses performance markers more often than physiological markers, and especially thermoregulatory markers. When the repeatability of physiological markers has been assessed, it has often been between two pieces of equipment measuring the same

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[4] Compensable heat stress refers to a steady state T_c, whereby the heat loss matches the heat production. Uncompensable heat stress refers to an imbalance between the evaporative requirement and the evaporative capacity of an individual.

physiological variable. Consequently, there is limited evidence comparing physiological markers during repeated trials using a set intensity exercise protocol. The repeatability of mean aural temperature and mean HR during a fixed intensity cycling heat stress test, which involved three 20-min cycle bouts separated by 8-min rest was assessed in adolescents (Brokenshire et al., 2009). ICC of 0.58 and 0.95 for mean aural temperature and mean HR, respectively and a TE (CV %) of 0.1% and 3% for mean aural temperature and mean HR, respectively were reported and are indicative of strong measurement repeatability. Determining the repeatability of physiological measures during a HTT would provide confidence intervals and allow the test to be used to determine a change in heat tolerance following acute or chronic heat-alleviating interventions. Thus, it may enable specific guidance on preparation required prior to training or competing in high ambient conditions.

While acknowledging the limitations of the IDF HTT, pilot data (3.16.) established similar patterns of heat tolerance and heat intolerance providing support to examine the repeatability of the RHTT. This study aimed to establish the repeatability of a practical running test to evaluate individuals' heat tolerance. The primarily hypothesis was that physiological responses during the RHTT will have a strong positive correlation on repeated trials. The secondary hypothesis was that there will be no differences in physiological responses during the running heat tolerance test between repeated trials.

4.3. Materials and methods

Participants

Sixteen (8 males; 8 females) athletes (3.2.) volunteered and provided written informed consent to participate in the study (mean ± SD; age, 23 ± 5 yrs.; BM, 67.07 ± 10.96 kg; stature, 1.76 ± 0.10 m; BSA, 1.82 ± 0.19 m^2; sum of four skin folds, 43 ± 15 mm; speed at lactate threshold, 11.7 ± 1.8 km.hr^{-1}; and running, $\dot{V}O_2$ peak 49 ± 7 mL.kg^{-1}.min^{-1}). The study was approved by the University of Brighton Research Ethics & Governance Committee and conducted in line with the health and safety guidelines (3.1.) and in accordance with the guidelines of the revised Declaration of Helsinki, 2013.

Preliminary testing

During the first visit to the laboratory standardised anthropometric assessment was conducted (3.5.) followed by a graded exercise test to determine participants' lactate threshold and $\dot{V}O_2$ peak (3.15.). Breath-by-breath expired air was measured using online gas analysis throughout the RHTT (3.9.). A capillary lactate sample (3.11.) was take on completion of each stage during the lactate threshold test and on completion of the $\dot{V}O_2$ peak test.

Running heat tolerance test

All trials were conducted with pre-trial diet and exercise standardisation (3.4.). Participants provided a urine sample on arrival to ensure adequate hydration. The timing of testing for female participants was conducted in accordance with the menstrual cycle standardisation guidelines (3.6.). Five participants were using oral contraceptive. two used Yasmin (30 µg ethinylestradiol/ 3mg drospirenone) and three used Microgynon (30 µg ethinylestradiol/ 150 µg levonorgestrel). All testing was performed on a motorised treadmill (3.15.).

The RHTT (3.16.) was performed on two occasions. Following a 20-min stabilisation period in a temperate environment (3.7.), measures of T_{re} (3.8.), HR (3.9.), T_{sk} (3.8.), RPE, and TS (3.13.) were recorded, and participants entered the environmental chamber (3.7.). Towel dried nude BM was also recorded to calculate SWR (3.12.). Throughout the RHTT T_{re}, HR, and T_{sk} were recorded at 5-min intervals. RPE and TS were recorded at 10-min intervals. Exercise was terminated in line with university guidelines (3.1.). Participants repeated the process between 5 - 7 d later to assess the repeatability of the RHTT. Mean T_{sk} (Equation 2.4) and PSI were subsequently calculated (Equation 3.2).

Statistical analyses

All data were first checked for normality using the Shapiro-Wilk method. Physiological measures during RHTT1 and RHTT2 were examined using a battery of repeatability statistics (3.17.3). As a measure of retest correlation, ICC with 95% CI was calculated for each variable. TEM was calculated and expressed as a percentage of its respective mean to form the TE (CV %). Bland-Altman limits of agreement (LOA) plots showing the mean bias and 95% CI were produced. The individual participant differences, between the two trials for each variable were plotted against the respective individual means. Paired sampled t tests were calculated to identify any differences in physiological measures between RHTT1 and RHTT2 (3.17.4). Effect sizes (Cohens' d) were calculated to analyse the magnitude of the interaction (Lakens, 2013) (3.17.5). All data were analysed using a standard statistical package (SPSS version 20.0), and reported as mean ± SD. Statistical significance was accepted at the level of $p \leq 0.05$.

4.4.   Results

Measures of ICC, TEM, the TE (CV %) and mean bias with LOA for key markers of heat tolerance are presented in table 4.1. Strong correlations presented as ICC were observed in peak T_{re} (r = 0.93), peak T_{sk} (r = 0.95), peak HR (r = 0.99), peak PSI (r = 0.98), and SWR (r = 0.95) between RHTT1 and

RHTT2 (figure 4.1). These observed similarities are further supported by a low TEM and TE (CV %) for the key markers of heat tolerance between RHTT1 and RHTT2.

Figure 4.2 demonstrates small mean bias and LOA between trials for physiological measures between the two trials. In addition, there were no differences observed in peak T_{re} ($t_{(15)}$ = -0.785, p = 0.445, d = 0.20), peak T_{sk} ($t_{(15)}$ = 0.223, p = 0.827, d = 0.06), HR $_{peak}$ ($t_{(15)}$ = -1.005, p = 0.331, d = 0.25), peak PSI ($t_{(15)}$ = -0.969, p = 0.348, d = 0.24), and SWR ($t_{(15)}$ = 0.087, p = 0.931, d = 0.02) between RHTT1 and RHTT2.

Strong correlations presented as ICC (95% CI) were observed in T_{re} rest (r = 0.88 (0.38, 0.90), $p \leq$ 0.001), T_{re} mean (r = 0.92 (0.77, 0.97), $p \leq$ 0.001), HR rest (r = 0.91 (0.76, 0.97), $p \leq$ 0.001), and HR mean (r = 0.97 (0.98, 0.99), $p \leq$ 0.001) between RHTT1 and RHTT2. These observed similarities are further supported by a low TEM and TE (CV %) for T_{re} rest (0.17°C, 0.45%), T_{re} mean (0.15°C, 0.39%), HR rest (3 beats.min^{-1}, 5%), and HR mean (3 beats.min^{-1}, 2%). There were small mean bias and LOA for T_{re} rest [-0.04, (-0.50, 0.42)], T_{re} mean [-0.04, (-0.45, 0.37)], HR rest [0, (-8, 8)], and HR mean [1, (-8, 10)]. Furthermore, there were no differences in T_{re} rest, ($t_{(15)}$ = -0.722, p = 0.481, d = 0.18) T_{re} mean, ($t_{(15)}$ = -0.770, p = 0.453, d = 0.19) HR rest ($t_{(15)}$ = -0.301, p = 0.768, d = 0.08), and HR mean ($t_{(15)}$ = -1.003, p = 0.332, d = 0.25) between RHTT1 and RHHT2.

Strong correlations (ICC) were observed for peak RPE (r = 0.96, (0.88, 0.99), $p \leq$ 0.001) and peak TS (r = 0.92, (0.76, 0.97), $p \leq$ 0.001) between RHTT1 and RHTT2. These observed similarities are further supported by a low TEM and TE (CV %) for peak RPE (0.6, 4%), and peak TS (0.2, 3.2%). There were small mean bias and LOA for peak RPE [-0.1, (-1.7, 1.6)], peak TS [0.1, (-0.6, 0.7)] between RHTT1 and RHTT2. Furthermore, there were no differences in peak RPE ($t_{(15)}$ = -0.293, p = 0.774, d = 0.08) and peak TS ($t_{(15)}$ = 0.145, p = 0.0.27, d = 0.28) between RHTT1 and RHHT2.

Table 4.1 Repeatability statistics for physiological variables during repeated trials of the RHTT. Mean ± SD.

	T_{re} peak (°C)	T_{sk} peak (°C)	HR peak (beats.min^{-1})	PSI peak	SWR (g.hr^{-1})
RHTT1	38.82 ± 0.47	38.12 ± 0.45	182 ± 15	8.7 ± 1.5	1,721 ± 675
RHTT2	38.86 ± 0.49	38.11 ± 0.45	183 ± 15	8.8 ± 1.5	1,716 ± 745
ICC (95% CI)	0.93 (0.89, 0.99); $p \le 0.001$	0.95 (0.85, 0.98); $p \le 0.001$	0.99 (0.96, 0.99); $p \le 0.001$	0.98 (0.94, 0.99); $p \le 0.001$	0.95 (0.86, 0.98); $p \le 0.001$
TEM	0.13	0.14	2	0.3	162
TE (CV %)	0.34	0.37	1	3.0	9
Mean bias (LOA)	-0.04 (-0.41, 0.33)	0.01 (-0.38, 0.40)	-1 (-8, 6)	-0.1 (-0.9, 0.7)	-133 (-492, 227)

Notes: HR, heart rate; ICC (95% CI), intra class correlation coefficient with 95% confidence intervals; LOA, limits of agreement; PSI, physiological strain index; RHTT, running heat tolerance test; TEM, typical error of measure; TE (CV %), typical error as a coefficient of variation; T_{re}, rectal temperature; T_{sk}, skin temperature; SWR, sweat rate
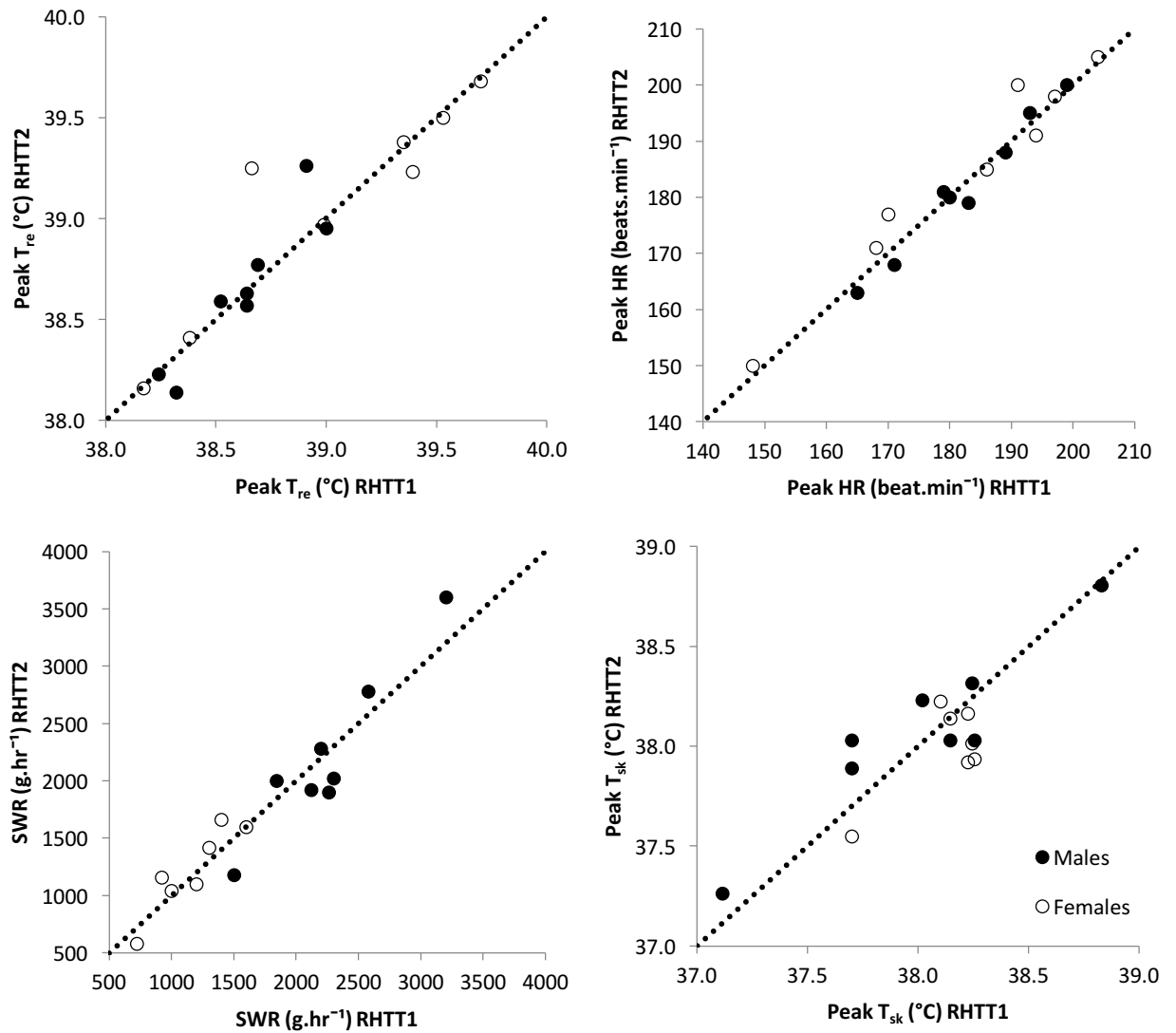
Figure 4.1 Peak rectal temperature (T_{re}), peak heart rate (HR), sweat rate (SWR), and peak skin temperature (T_{sk}) during RHTT1 (x-axis) and RHTT2 (y-axis), for males (closed markers) and females (open markers). Dotted line represents line of equality. N = 16 (8M, 8F).
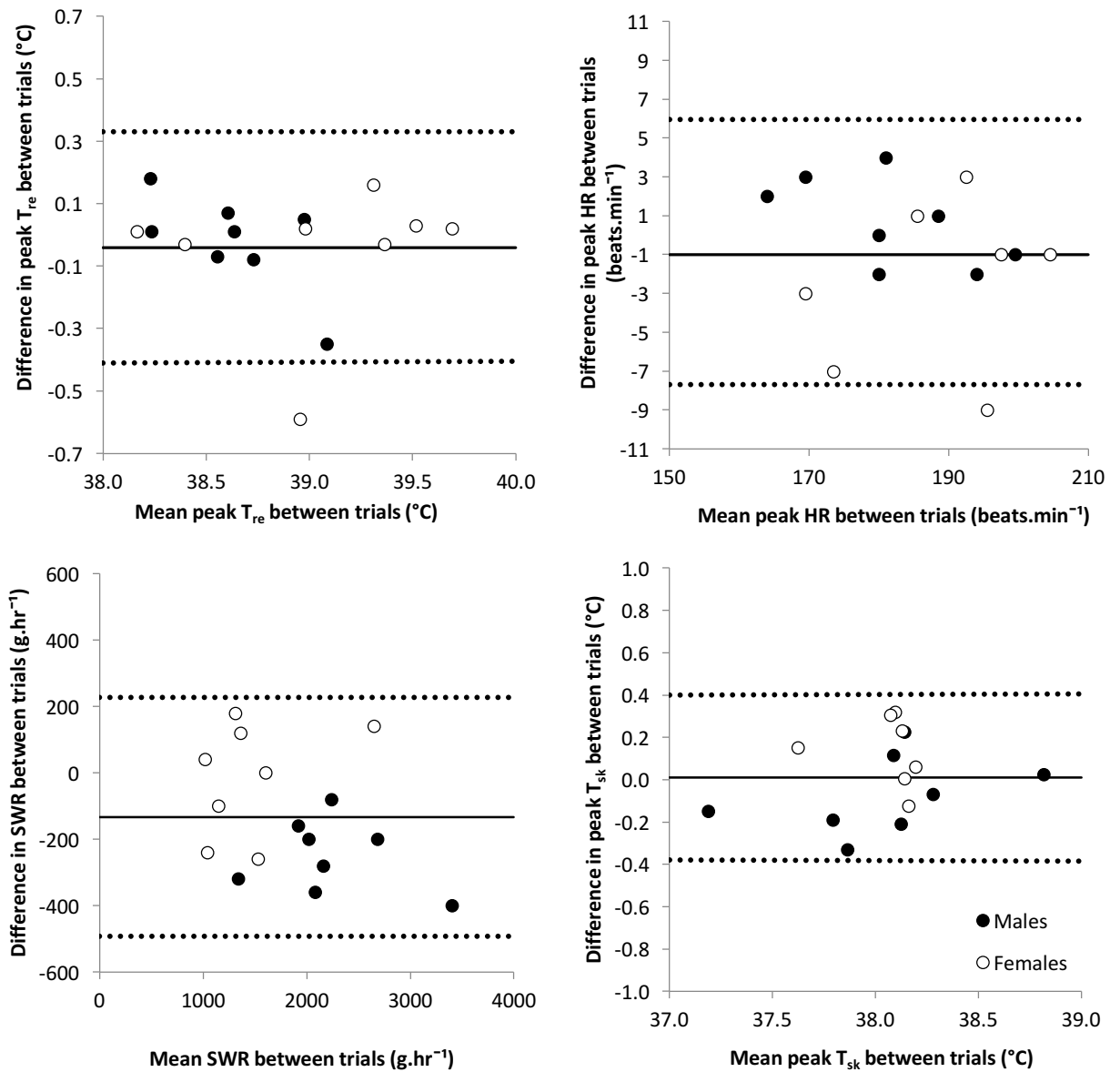
Figure 4.2 Bland-Altman plots with mean bias (solid line) and 95% limits of agreement (dotted line) for peak rectal temperature (T_{re}), peak heart rate (HR), sweat rate (SWR), and peak skin temperature (T_{sk}) for males (closed markers) and females (open markers). N = 16 (8M, 8F).

4.5.   Discussion

The principle aim of the current study was to examine the repeatability of a RHTT from two trials separated by 5-7 d. Classic markers of heat tolerance and HA, namely peak T_{re}, peak T_{sk}, peak HR, and SWR were used for analysis (Moran et al., 2007; Sawka et al., 2011a). The main finding from

the current study is that the RHTT had good agreement, strong correlations and small differences between repeated trials and the TEM values for these classic markers suggested low within-participant variability. These findings are in support of the aim of the study. Specifically, the primary and secondary hypotheses were both supported. Values of peak T_{re}, peak T_{sk}, peak HR, and SWR spread along a continuum, with a wide range of responses, challenging the previous perception that individuals are either heat heat tolerant or heat intolerant (Kresfelder et al., 2006; Moran et al., 2007). The data presented in the current study, demonstrates that the RHTT has strong repeatability and is able to differentiate between individual responses to the RHTT. These findings support the use of the RHTT in future investigations to gauge individual's running heat tolerance, and to monitor the extent of acute and chronic heat-alleviating protocols.

To the author's knowledge, there are no studies assessing the repeatability of physiological measures during a fixed intensity running protocol in the heat, making acceptable levels of repeatability *a priori* difficult to determine. Furthermore, there is no literature assessing the repeatability of the classical IDF HTT which would offer comparison. As a general rule, a correlation coefficient over 0.90 is considered to be high, between 0.70 – 0.80 moderate, and below 0.70 to be low for physiological tests (Vincent, 1995). In the current study, ICC values for peak T_{re}, peak $T_{sk,}$ peak HR, peak PSI, and SWR were all equal or greater than 0.93. Based on these predetermined criteria, it is reasonable to suggest that the physiological measures during the RHTT have an acceptable level of repeatability between repeated trials based on ICC.

The data in the current study demonstrates less variability than available data on fixed intensity cycling protocols in the heat using similar physiological variables. The TE (CV %) of 0.3% and 1% for peak T_{re} and peak HR respectively, compare favourably to the TE (CV %) of 0.3% and 3.9% for mean T_{re} and mean HR reported during a 60-min, fixed intensity cycling protocol in hot humid conditions (Hayden et al., 2004). Furthermore, Brokenshire and collegues (2009) investigated the repeatability of HR during a fixed intensity cycling heat stress test, which involved three 20-min cycle bouts separated by 8-min rest. The ICC of 0.95 and TE (CV %) of 3% for mean HR were reported and assumed indicative of strong measurement repeatability. The ICC of 0.99 and TE (CV %) of 1% reported in the current study, again compare favourably to these findings highlighting the strength of the RHTT in terms of low measurement error.

To aid interpretation and presentation of results when assessing individuals' heat tolerance, researchers have dichotomized the population and categorized individuals as either tolerant or intolerant (Kresfelder et al., 2006; Moran et al., 2007). Altman and Royston (2006) reported several problems with dichotomizing data. Statistical power to detect a relationship may be reduced, there

is an increased risk of reporting a false positive, a possibility of underestimating the extent of the variation in the outcome between groups, and using two groups conceals any non-linearity in the relationship. Altman and Royston (2006) state using multiple categories is generally favoured to dichotomizing data; with four or five groups the loss of information can be quite small but there are complexities to analysis. Indeed, Moran and colleagues (2007) acknowledge that the larger the deviations from normal values the more pronounced the state of heat intolerance, thus implying a continuum of heat tolerance. Similarly work from, Taylor and Cotter (2006) propose that heat adaptation is a continuum, with the position of an individual along the continuum representing progressive increases in heat tolerance. The findings in the present study, demonstrate clearly that an individual's heat tolerance, represented by the peak T_{re}, peak T_{sk}, peak HR, peak PSI, and SWR is a continuous variable; demonstrating that heat tolerance may be more accurately categorised on a continuum. Consequently, these findings support the use of the RHTT to track changes in individuals' ability to withstand exercise heat stress from acute and chronic heat-alleviating interventions.

Cases of exertional heat illness can occur among endurance athletes, in extreme circumstances leading to death; however, the epidemiology is not well documented in the literature. Martin (1997) reported the highest incidence of exertional heat illness occurred in long distance runners during the 1992 New Orleans U.S. Olympic Trials and the 1996 Atlanta Olympics; with long distance runners accounting for 31% and 53% respectively, of the total cases of exertional heat illness. Furthermore, Nielsen (1996) provides data to suggest the incidence of heat illness is unavoidable for endurance runners when competing in a high ambient temperature combined with high RH without a severe reduction in endurance performance; highlighting the importance of thorough preparation to prevent the incidence of a heat illness. The findings in the current study could be applied in a manner that would serve to minimise the number of athletes that suffer from hyperthermia, by supporting a more complete evaluation and subsequent preparation prior to training and competing in the heat (Johnson et al., 2013).

The TEM expressed as a coefficient of variation can be used to estimate sample size for future studies using the RHTT when a smallest worthwhile change is known; using the formula $N = 8s^2/d^2$ (where 's' = typical error expressed as a coefficient of variation and 'd' = the smallest worthwhile change (Hopkins, 2000b). A change of 0.4°C typically represents a significant reduction in T_{re} following acute and chronic heat-alleviating procedures (Buono et al. 1998; Patterson et al. 2004; Castle et al. 2006; Garrett et al. 2009). Accordingly, in the current study to detect a 0.4°C change, a sample size of six participants would be sufficient when peak T_{re} is the key variable of concern, assuming similar variability among the participants recruited.

4.6.   Limitations

The RHTT adopts a fixed absolute workload, which is an appropriate procedure to assess heat tolerance when the experimental design is a repeated measure. However, the fixed absolute workload may limit applicability of the test when comparing between groups, especially when they are not matched. The recent work by Cramer & Jay (2014) reports when comparing between individuals or groups unmatched from a biophysical perspective, exercise intensity should be administered using fixed heat production relative to BM to prevent the introduction of systematic bias. Future research is warranted to quantify the potential differences in metabolic heat production between participants of differing BM, BSA and fitness during the RHTT to ensure unbiased comparison of thermoregulatory responses between individuals (Cramer and Jay, 2014). Furthermore, the role of the RHTT in identifying individuals' susceptibility to exertional heat illness has not been identified within the current study. Collecting RHTT data on individuals who have previously incurred exertional heat illness would test the value of the RHTT in identifying an individual's susceptibility to exertional heat illness.

4.7.   Conclusion

The main finding from the current study was that the RHTT demonstrated good agreement, strong correlations and small differences between repeated trials. The TEM values showed low within-participant variability. In addition, data is presented to demonstrate that the RHTT is capable of differentiating between individuals' responses to the RHTT when adopting a running mode of exercise; providing evidence that a 30-min HTT protocol is sufficient in duration. Furthermore, the findings from the present study challenge the previous reports that heat tolerance is dichotomous, since individual responses were linearly spread over a continuum. These findings, could be used to observe individuals' thermoregulatory responses over time, providing information to physiologist, coaches, and medical staff to assist decisions about athletes' safety prior to training and competing in the heat. In addition, the RHTT could be used as a tool for investigating the effect of acute and chronic heat-alleviating procedures.

This chapter explored the repeatability of the RHTT. This established it use as a measurement tool. Chapter 5 (Study 2) will assess the sensitivity of the RHTT to changes in heat tolerance.

5. Sensitivity of a running heat tolerance test

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Study 1 (Chapter 4) of this thesis has established the RHTT as reliable, mode specific test. To assess the sensitivity of the RHTT to changes in heat tolerance, data was pooled from two further studies of which are not presented elsewhere in the thesis.

5.1.   Abstract

Establishing whether the RHTT is sensitive to changes in heat tolerance is essential to support future use of the RHTT in providing a more complete evaluation of athletes' heat tolerance, and to quantifying the effectiveness of heat-alleviating procedures. This study aimed to identify the sensitivity of the RHTT to changes in heat tolerance following STHA. Twenty-five (12M, 13F) participants performed two RHTT, 24-hr preceding HA (RHTT1) and 24 hrs following 5-d controlled hyperthermia (T_{re} = 38.5°C) HA (RHTT2). The RHTT involved 30-min running (9 km.hr^{-1}, 2% gradient) in 40°C, 40% RH (WGBT = 33°C). Following HA, values of peak T_{re} (RHTT1: 38.94 ± 0.48°C, RHTT2: 38.56 ± 0.55°C, $p \leq 0.001$), peak T_{sk} (RHTT1: 37.47 ± 0.49°C, RHTT2: 37.06 ± 0.70°C, $p = 0.003$), and peak HR (RHTT1: 185 ± 14 beats.min^{-1}, RHTT2: 175 ± 14 beats.min^{-1}, $p \leq 0.001$) reduced, and SWR increased (RHTT1: 1,070 ± 541 g.hr^{-1}, RHTT2: 1,481 ± 457 g.hr^{-1}, $p = 0.003$). The range of reductions in measures of peak T_{re} (-0.48°C, -0.03°C), peak T_{sk} (-1.74°C, 0.13°C), peak HR, (-24 beats.min^{-1}, -2 beats.min^{-1}) and SWR (-460 g.hr^{-1}, 1640 g.hr^{-1}) demonstrate marked individual differences. The data suggests the RHTT is sensitive to changes in heat tolerance and that changes in heat tolerance are highly individual.

5.2.   Introduction

During prolonged submaximal exercise in a hot environment there is a rise in body heat storage, which in turn reflects the balance between metabolic heat production, heat absorbed from the environment and total body heat loss (Jay and Kenny, 2007). There is a wide variation in individual ability to withstand exercise heat stress (Epstein, 1990). Under extreme conditions of exertion in the heat even healthy, heat acclimated, and endurance trained individuals will increase their body heat content. Individuals who experience a greater storage of metabolic heat due to a reduced sweating sensitivity and a low adaptive capacity to exercise in the heat, have been described as heat intolerant (Epstein et al., 1983; Moran et al., 2004). The ability to quantify an individual's level

of heat tolerance offers valuable information to support the prevention of future occurrence of a heat-related illness.

A RHTT was developed in Study 1 (Chapter 4). The protocol involves 30-min of running on a treadmill (9 km.hr^{-1} and a 2% gradient) in ambient conditions of 40°C and 40% RH (WBGT = 33°C). Classic markers of heat tolerance and adaptation namely, T_{re}, T_{sk}, HR, and SWR demonstrated good agreement, strong correlations, (ICC > 0.93) and small differences ($p \geq 0.05$) between repeated trials. Furthermore, the TEM values for these classic markers suggested low within participant variability. The RHTT demonstrated different degrees of heat tolerance between individuals which reinforces that the RHTT is an appropriate tool to evaluate heat tolerance. There is currently no evidence supporting the sensitivity of the RHTT to changes in heat tolerance. Establishing the sensitivity of the RHTT to changes in heat tolerance may support the use of the RHTT to monitor changes in heat tolerance following acute and chronic heat-alleviating procedures. In addition, it may support the use of the RHTT to monitor changes in heat tolerance that occur naturally to help identify periods of time when athletes are experiencing periods of low heat tolerance to support the prevention of developing a heat-related illness.

An individual's heat tolerance has been defined as the ability to withstand exercise heat stress, combined with their ability to adapt to exercising in the heat (Shapiro et al., 1979). Routinely, HTTs are used in isolation to assess heat tolerance following a heat illness incident to support the individual's return to training. Recently, Johnson and colleagues (2013) provided case study data to support the use of a combined heat tolerance screening procedure, using both acute and chronic heat exposure to assess a triathlete's exercise heat tolerance. These exploratory findings support a combined method to assess athletes' heat tolerance to facilitate optimal preparation prior to training and competing in the heat.

This study aimed to establish the sensitivity of the RHTT to changes in heat tolerance. Furthermore, this study aimed to explore the individual changes in heat tolerance following HA. The primary hypothesis was that there will be differences in physiological responses during the RHTT following STHA. The secondary hypothesis was that the RHTT will be sensitive to individual differences in the magnitude of adaptation.

5.3.   Materials and methods

Participants

Data was pooled from two data set collected at different time periods that are not presented elsewhere in the thesis. Twenty-five (12M; 13F) athletes (3.2) volunteered and provided written informed consent to participate (table 5.1). The study was approved by the University of Brighton Research Ethics & Governance Committee and conducted in line with the health and safety guidelines (3.1.) and in accordance with the guidelines of the revised declaration of Helsinki, 2013.

Preliminary testing

During the first visit to the laboratory standardised anthropometric assessment was conducted (3.5.). A graded exercise test was performed to determine participants' $\dot{V}O_2$ peak on a cycle ergometer (3.14.). Breath-by-breath expired air was measured using online gas analysis (3.9.) and a capillary lactate sample was taken on completion of the test (3.11.). A regression equation was computed from the data obtained to calculate the required intensity (65% $\dot{V}O_2$ peak) for the experimental controlled hyperthermia HA exercise sessions (Appendix 4).

Table 5.1 Participant characteristics. Mean ± SD (ranges).

	BM (kg)	Stature (m)	BSA (m^2)	Sum of 4 skin folds (mm)	$\dot{V}O_2$ peak ($mL.kg^{-1}.min^{-1}$)
All Data (N = 25)	65.67 ± 10.93	1.71 ± 0.01	1.76± 0.19	38 ± 11	48 ± 8
	(41.30 - 89.42)	(1.46 – 1.89)	(1.30 - 2.07)	(20 – 56)	(33 – 69)
Males (N = 12)	74.13 ± 7.28	1.78 ± 0.06	1.92 ± 0.10	32 ± 8	51 ± 8
	(64.70 - 89.42)	(1.72 – 1.89)	(1.79 - 2.07)	(20 – 46)	(38 – 69)
Females (N = 13)	57.86 ± 9.21	1.64 ± 0.09	1.62 ± 0.17	43 ± 11	45 ± 7
	(41.30 - 75.00)	(1.46-1.78)	(1.30 - 1.90)	(22 – 56)	(33 – 56)

Notes: BM, body mass; BSA, body surface area; $\dot{V}O_2$ peak, peak oxygen uptake

Experimental design

Testing was completed over a 7-d period. The timing of testing for female participants was conducted in line with the menstrual cycle standardisation guidelines (3.6.). Five female participants were using oral contraceptive: three used Microgynon, one used Rigevidon (30 µg ethinylestradiol/ 150 µg levonorgestrel) and one used Yasmin (30 µg ethinylestradiol/ 3mg drospirenone). All trials were conducted in line with pre-trial diet and exercise standardisation (3.4.). Participants provided a urine sample upon arrival to ensure adequate hydration (3.4.). Volunteers performed five controlled hyperthermia HA sessions on a cycle ergometer (3.14.) and two RHTTs (3.16.). The first RHTT was performed 24-hr prior to beginning HA (RHTT1), the second RHTT was performed 24-hr following 5-d HA (RHTT2). All experimental trials were performed in a hot environment (3.7.). Exercise was terminated in line with university guidelines (3.1.). There were no incidences of early termination of exercise during the RHTT within this study.

Running heat tolerance test

The RHTT (3.16.) was performed on two occasions on a motorised treadmill (3.15.). Following a 20-min stabilisation period in a temperate environment (3.7.), measures of T_{re}, T_{sk} (3.8.), HR (3.9.), RPE, and TS (3.13.) were recorded. The participants then entered the environmental chamber. Towel dried nude BM was also recorded to calculate SWR (3.12.). Throughout the test T_{re}, T_{sk}, and HR were recorded at 5-min intervals. RPE and TS were recorded at 10-min intervals. Mean T_{sk} (Equation 2.4) and PSI (Equation 3.2) were subsequently calculated.

Statistical analyses

Data were assessed for normality using the Shapiro-Wilk method. A paired sample t test (3.17.4) was calculated to identify any differences in physiological measures between D1 and D5 of HA and between RHTT1 and RHTT2. Effect sizes (Cohen's d) were calculated to analyse the magnitude of the interaction (Lakens, 2013) (3.17.5.). All data were analysed using a standard statistical package (SPSS version 20.0), and reported as mean ± SD. Statistical significance was accepted at the level of $p \leq 0.05$.

5.4.   Results

Heat acclimation

All participants completed five consecutive daily 90-min HA sessions. Participants maintained the target T_{re} of 38.5°C on average for 48 ± 9 min during each session. The mean duration of exercise was 64 ± 12 min per session. The mean session intensity (including rest periods) was 41 ± 4% $\dot{V}O_2$ peak; however, the mean exercise intensity (excluding rest periods) was 58 ± 7% $\dot{V}O_2$ peak. The mean T_{re} and HR during each HA session was 38.27 ± 0.13°C and 147 ± 9 beats.min^{-1}, respectively. Furthermore, the mean SWR during each HA session was 556 ± 153 g.hr^{-1}.

There was a reduction in T_{re} rest ($t_{(24)}$ = 2.192, p = 0.037, d = 0.408) and HR rest ($t_{(24)}$ = 3.758, $p \leq$ 0.001, d = 0.609) from D1 to D5 of HA. There was an increase in SWR during the HA session from D1 to D5 ($t_{(24)}$ = -3.030, p = 0.006, d = 0.526). Furthermore, there was an increase in SWR_{BSA} ($t_{(24)}$ = -2.917, p = 0.008, d = 0.512).

Running heat tolerance test

Table 5.2 presents the mean ± SD data for T_{re} rest, peak T_{re}, peak T_{sk}, HR rest, peak HR, and SWR during the RHTT. There was a 0.16 ± 0.29°C and 0.38 ± 0.38°C reduction in T_{re} rest ($t_{(24)}$ = 2.517, p = 0.019, d = 0.46) and peak T_{re} ($t_{(24)}$ = 4.957, $p \leq$ 0.001, d = 0.71) respectively, from RHTT1 to RHTT2. Adaptive response varied between participants with changes in T_{re} rest ranging from -0.62°C to 0.41°C and peak T_{re} ranging from -1.43°C to -0.03°C. There was a 0.41 ± 0.51°C reduction in peak T_{sk} from RHTT1 and RHTT2 ($t_{(24)}$ = 3.348, p = 0.003, d = 0.56); changes ranged from -1.74°C to 0.93°C (figure 5.1).

There was a 4 ± 9 beats.min^{-1} and 10 ± 9 beats.min^{-1} reductions in HR rest ($t_{(24)}$ = 2.195, p = 0.038, d = 0.41) and peak HR ($t_{(24)}$ = 5.788, $p \leq$ 0.001, d = 0.76) respectively, from RHTT1 to RHTT2. Adaptive responses varied between participants with changes in HR rest ranging from -19 beats.min^{-1} to 8 beats.min^{-1} and peak HR ranging from -24 beats.min^{-1} to 0 beats.min^{-1}.

These changes resulted in an average reduction in peak PSI from a very high strain to a high strain (Moran et al., 1998) ($t_{(24)}$ = 5.268, p < 0.001, d = 0.73). Adaptive responses varied between participants with changes in PSI peak ranging from -0.06 to 4.30.

Table 5.2 Peak physiological variables during the RHTT. Mean ± SD (ranges).

	RHTT1	RHTT2	Difference
T_{re} rest (°C)	37.17 ± 0.32	37.01 ± 0.34*	-0.16 (-0.65, 0.41)
T_{re} peak (°C)	38.94 ± 0.48	38.56 ± 0.55*	-0.38 (-0.48, -0.03)
T_{sk} peak (°C)	37.47 ± 0.49	37.06 ± 0.70*	-0.41 (-1.74, 0.13)
HR rest (beats.min^{-1})	69 ± 10	65 ± 9*	-4 (-28, 15)
HR peak (beats.min^{-1})	185 ± 15	175 ± 14*	-10 (-24, -2)
SWR (g.hr^{-1})	1,070 ± 541	1,481 ± 457*	+411 (-460, 1640)
SWR_{BSA} (g.hr^{-1}.m^2)	597 ± 272	797 ± 225*	+ 200 (-459, 793)

Notes: HR, heart rate; RHTT, running heat tolerance test; SWR, sweat rate; T_{re}, rectal temperature; T_{sk} skin temperature. *denotes significant difference between RHTT1 and RHTT2 ($p \leq 0.05$).

There was a 411 g.hr^{-1} increase in SWR ($t_{(24)}$ = -3.328, p = 0.003, d = 0.56) from RHTT1 to RHTT2; changes ranged from -460 g.hr^{-1} to 1640 g.hr^{-1}. Furthermore, there was an increase in SWR_{BSA} ($t_{(24)}$ = -3.505, p = 0.002, d = 0.58) from RHTT1 to RHTT2.

There was a reduction in peak RPE (-2; $t_{(24)}$ = 4.797, $p \leq 0.001$, d = 0.700) and peak TS (-0.5; $t_{(24)}$ = 2.771, $p \leq 0.001$, d = 0.492) from RHTT1 to RHTT2.
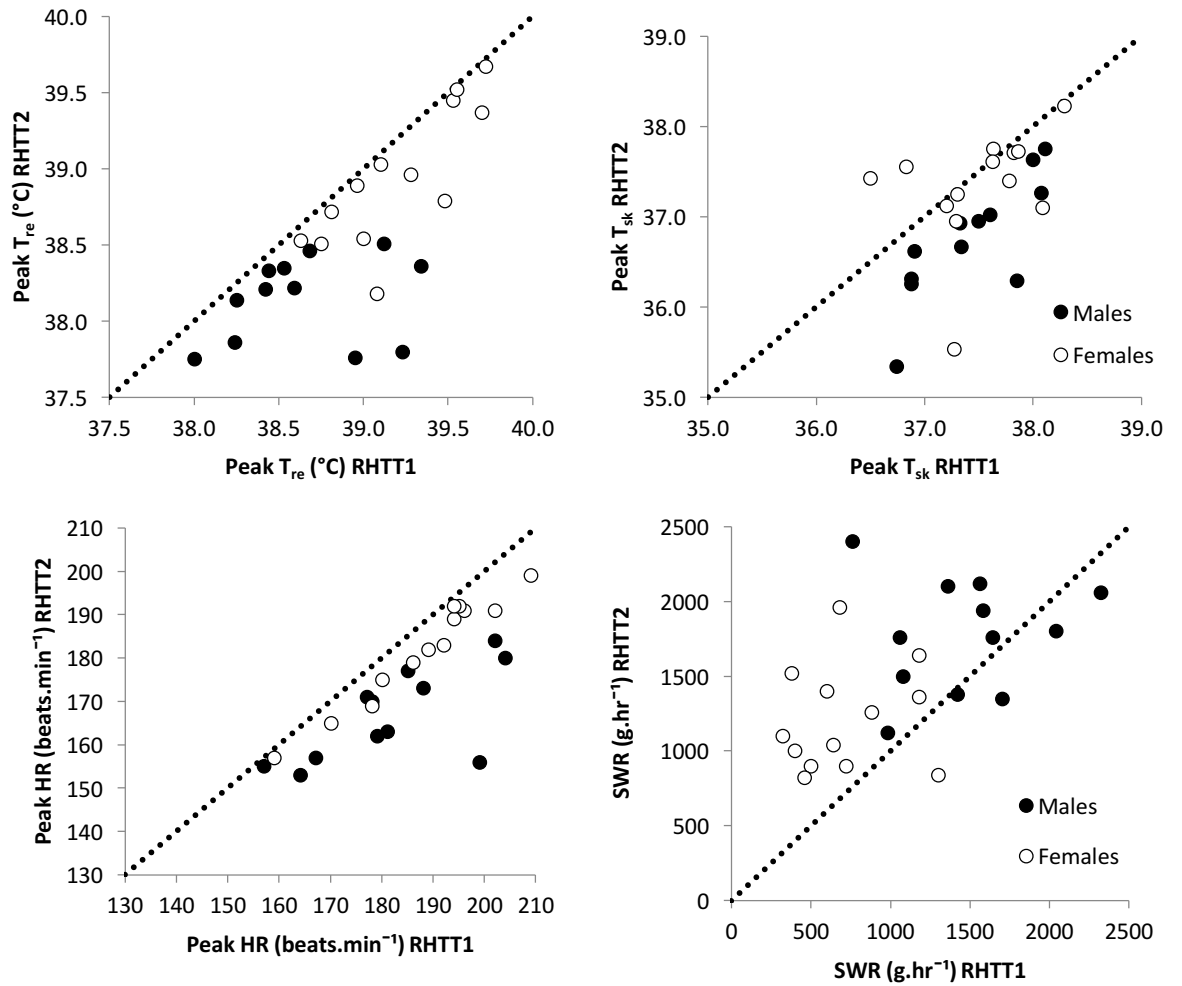
Figure 5.1 Individual data for values of peak rectal temperature (T_{re}), peak skin temperature (T_{sk}), peak heat rate (HR) and sweat rate SWR during RHTT1 (x axis) and RHTT2 (y axis) for males (closed markers) and females (open markers). Dotted line represents line of equality. N = 25 (12M, 13F).

5.5.   Discussion

The main finding in the current study was that the RHTT is sensitive to the changes in heat tolerance that are associated with STHA, evidenced by a reduction in T_{re}, T_{sk}, and HR, and an increase in SWR. These findings support the aim of the study. Specifically, the primary and secondary hypotheses were both supported. These results support the use of the RHTT to monitor changes in heat tolerance when using a repeated measures design. Furthermore, the data demonstrates marked differences between participants' changes in heat tolerance, suggesting heat adaptation is highly individual.

Heat adaptation responses

There was a 0.38°C mean reduction in peak T_{re} following STHA in the current study. Study 1 (Chapter 4) identified the TEM for peak T_{re} during the RHTT to be 0.13°C, suggesting a true reduction of > 0.25°C. These findings, are comparable to the ~0.24°C reduction, previously observed following STHA using a controlled hyperthermia model (Garrett et al., 2009; Patterson et al., 2004a). Furthermore, Study 1 (Chapter 4) identified a TEM of 0.14°C for peak T_{sk} during the RHTT, the 0.41°C reduction observed in the current study also suggests a true reduction of > 0.27°C. The observed reductions in thermoregulatory strain are potentially due to an enhanced evaporative heat loss, thus reducing heat storage. These results demonstrate the sensitivity of the RHTT to monitor changes in thermoregulatory strain associated with STHA.

There was a 10 beats.min^{-1} mean reduction in peak HR during the RHTT following STHA in the current study. Study 1 (Chapter 4) identified the TEM for peak HR during the RHTT to be 2 beats.min^{-1}, suggesting a true reduction of > 8 beats.min^{-1}. These findings are comparable to the ~12 beats.min^{-1} reduction previously observed following STHA using a controlled hyperthermia model (Garrett et al., 2009; Patterson et al., 2004a). The reduction in cardiovascular strain represent an improved myocardial efficiency and ventricular compliance (Lorenzo et al., 2010). Due to the matched exercise intensity of the RHTT, the observed reduction in cardiovascular strain demonstrates the sensitivity of the RHTT to monitor adaptation to the cardiovascular system associated with STHA.

There was a 411 g.hr^{-1} mean increase observed in SWR following STHA in the current study. Study 1 (Chapter 4) identified the TEM for SWR during the RHTT to be 162 g.hr^{-1}, suggesting a true increase of ≥ 249 g.hr^{-1}; these findings are comparable to the 220 g.hr^{-1} previously observed following STHA using a controlled hyperthermia model (Patterson et al., 2004a). An increase in SWR could be either

an altered afferent neural activity from the peripheral or central thermo-receptors causing different integration of thermal information, an altered efferent neural activity for a given level of afferent input or an altered effector response. The potential mechanisms for this include alterations in the T_c threshold for sweating onset (Shvartz et al., 1979), an increased sweating sensitivity (Shvartz et al., 1979) and/or eccrine sweat gland hypertrophy (Sato et al., 1990). Due to the matched exercise intensity during the RHTT, the repeated trials were assumed to be matched for the requirement for evaporative heat loss thus, these results demonstrate the sensitivity of the RHTT to monitor changes in sudomotor function following HA.

Individual responses

Observing individual heat tolerance using both an acute and chronic heat exposure has been suggested to offer a more comprehensive assessment of individual heat tolerance (Johnson et al., 2013). However, the majority of HA literature reports average responses and fails to recognise the differences in adaptive responses. The data demonstrated large differences in the magnitude of adaptation between individuals. For example, one participant in the current study demonstrated a large adaptive response following STHA with peak PSI reducing from high (8.65) to moderate (6.98) strain, combined with a 150% increase in SWR. However, another participant experienced no alterations in peak PSI and had only a 23% increase in SWR, demonstrating a small adaptive response.

There are several factors which have been suggested to alter the magnitude of the adaptation response which may explain the variance observed in the current study. Firstly, the closer an individual is to their genetically determined maxima, the greater the adaptation resistance as the adaption reserve narrows with progressively small gains occurring over time (Prud'Homme et al., 1984). Furthermore, within any given population there are high responders and low responders to thermal stimulation (Taylor, 2014). The majority of young sedentary individuals who live in a predominantly temperate climate will be high thermal responders. Whereas, individuals who live, work and train in high ambient conditions will show adaptation resistance (Taylor, 2014).

The exercise elicited during the HA sessions was performed at 65% of $\dot{V}O_2$ peak. There are several limitations observed with setting exercise percentage based on $\dot{V}O_2$ peak which may explain the variance observed in the magnitude of adaptation in current study. The $\dot{V}O_2$ peak values for the participants' in the current study ranged from 33 mL.kg^{-1}.min^{-1} to 69 mL.kg^{-1}.min^{-1}. Thus, those individuals with a lower $\dot{V}O_2$ peak worked at a lower metabolic heat production providing a lower stimulus for sweat production (Cramer and Jay, 2014; Gagnon et al., 2013, 2008) and potentially

constraining adaptation. The BM of the participants' in the current study ranged from 41.3 kg to 89.4 kg. A lower BM entails less heat storage and therefore, a lower exercise intensity is required to increase T_c to 38.5°C (Gagnon et al., 2009). Consequently, the stress imposed during the HA sessions was likely lower in participants with a lower BM compared with those with a higher BM, potentially constraining adaptation and influencing the time course of adaptation due to inadequate endogenous heat strain. Furthermore, changes in T_c are not only a function of the change in body heat content, but also of its mass and tissue composition as defined by the specific heat of the tissue of the human body. The participants' sum of skin fold and thus, body fat percentage ranged from 20 mm to 56 mm. An individual with a lower body fat percentage will have a higher specific heat. Thus, at a given change in body heat content participants with a low body fat percentage will have a lower change in T_c (Jay and Kenny, 2007), potentially constraining adaptation.

Johnson et al., (2013) observed an improved heat tolerance following 9-d HA in a triathlete who had previous incidences of heat illness. Following this assessment, the athlete was deemed safe to race, due to a substantial improvement in heat tolerance, and went on to complete the race with no signs of exertional heat illness. The current study supports this model to evaluate individuals' heat tolerance since there were a variety of adaptive responses between individuals. Although thresholds have not been established, those individuals who demonstrate small adaptive responses, especially when combined with a high physiological strain during the RHTT, will likely experience substantial strain during competition and potentially at a higher risk of experiencing exertional heat illness. Thus, when assessing heat tolerance, practitioners should consider using the RHTT pre and post a heat-alleviating procedure such as HA. This method is considered to provide a more in-depth assessment of heat tolerance with information being provided on the initial exposure and the adaptive capacity of an individual; to support the modification of future preparation strategies prior to training and competing in the heat.

5.6. Limitations

The RHTT may be limited by its applicability when comparing between groups, especially when participants are not matched for biophysical characteristics. Administering a fixed intensity protocol when comparing between groups will elicit exercise at a variety of requirements for evaporative heat loss, resulting in an inaccurate assessment of thermoregulatory strain between participants (Cramer and Jay, 2014; Gagnon et al., 2013, 2008). However, the RHTT is an appropriate procedure to assess heat tolerance when the experimental design is a repeated measure, or to track an athlete over time prior to training and competing in high ambient conditions.

Furthermore, the role of the RHTT to aid in clinical decisions following exertional heat illness in athletes, and predicting individuals' susceptibility to exertional heat illness is not known. Collecting data on individuals who have previously incurred exertional heat illness would reinforce the value of the RHTT. Providing diagnostic and prognostic information would help identify individuals who are at a greater risk of developing exertional heat illness. Future investigations are required to gather this information to improve the preparation of individuals prior to training or competing in the heat; ensuring interventions are implemented to best prepare the individual.

5.7.   Conclusion

In conclusion, this study demonstrates that the RHTT is sensitive to monitor adjustments in the classic markers of heat tolerance and adaptation with reductions in measures of peak T_{re}, peak T_{sk}, peak HR, and SWR. These findings support the use of the RHTT in future investigations to assess chronic heat-alleviating interventions. Furthermore, these findings could be used to support optimal preparation, with individualised strategies developed based upon an individual's initial heat tolerance and their adaptive capacity, prior to training and competing in the heat. The RHTT has the potential to be used to track changes in heat tolerance assisting decision making about an athlete's ability to train and compete in high ambient conditions, whilst adopting a more favourable protocol for endurance runners, due to the running mode of exercise administered.

6. A comparison of male and female temporal patterning to short- and long-term heat acclimation

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Study 1 (Chapter 4) and Study 2 (Chapter 5) have established the RHTT as a reliable, mode specific (Study 1) and sensitive test (Study 2). There is a paucity of data on females' responses to HA. This chapter will provide original data on males and females' responses to HA using the RHTT as a method to evaluate changes in heat tolerance.

6.1.   Abstract

The current study assessed the sex differences in thermoregulatory and physiological adaptation to STHA (5 d) and LTHA (10 d). Sixteen (8M; 8F) participants performed three RHTT, preceding HA (RHTT1), following STHA (RHTT2) and LTHA (RHTT3). The RHTT involved 30-min running (9 km.hr^{-1}, 2% gradient) in 40°C, 40% RH (WBGT = 33°C). Following STHA, T_{re} rest (Males: -0.24 ± 0.16°C, $p \leq$ 0.001; Females: -0.02 ± 0.08°C, p = 0.597), peak T_{re} (Males: -0.39 ± 0.36°C, $p \leq$ 0.001; Females -0.07 ± 0.18°C, p = 0.504), and peak HR (Males: -14 ± 12 beats.min^{-1}, $p \leq$ 0.001; Females: -5 ± 3 beats.min^{-1}, p = 0.164) reduced in males, but not females. Following STHA, SWR$_{BSA}$ increased (428 ± 269 g.hr^{-1}.m^2, p = 0.029) in females, but not males (-11 ± 286 g.hr^{-1}.m^2, p = 0.029). Following LTHA, T_{re} rest (Males: -0.04 ± 0.15°C, p = 0.459; Females: -0.22 ± 0.12°C, $p \leq$ 0.01) and peak T_{re} (Males: -0.05 ± 0.26°C, p = 0.590; Females: -0.41 ± 0.24°C, $p \leq$ 0.01) reduced in females, but not males. Following LTHA, SWR$_{BSA}$ increased in males (308 ± 346 g.hr^{-1}.m^2, p = 0.029), but not females (44 ± 373 g.hr^{-1}.m^2, p = 0.733). Males and females responded to STHA; however, females required LTHA to establish reductions in thermoregulatory and cardiovascular strain. These findings suggest that HA protocols should be designed to target sex differences in thermoregulation for optimal adaptation.

6.2.   Introduction

Increasing ambient temperature is known to have a detrimental effect on endurance performance (Galloway and Maughan, 1997). During prolonged submaximal exercise in high ambient conditions, there is a greater requirement for heat loss due to either a rate of heat gain from the environment, or a lower gradient for dry heat loss, typically resulting in a greater change in body heat content compared to temperate conditions. Many athletes, soldiers, and manual operatives exposed to high ambient conditions are susceptible to heat illnesses; including heat cramps, heat syncope, heat exhaustion, and heat stroke. Prior to a heat illness, individuals vary in their ability to tolerate

exercise heat stress, some demonstrating a decreased capability to dissipate heat under the same exercise heat stress (Epstein, 1990). These individuals are characterized by an earlier and greater rise in T_c, greater storage of metabolic heat, greater physiological strain, and reduced sweating sensitivity when exercising in the heat (Epstein et al., 1983; Moran et al., 2004).

Males and females differ in their thermoregulatory responses to exercise-heat stress, largely due to females having a reduced sudomotor function (Gagnon and Kenny, 2011) thus decreasing evaporative heat loss capacity, with the resultant increase in physiological strain (Moran et al., 1999). It has been shown that males and females display similar rates of heat dissipation at low requirements for heat loss. However, sex differences in sudomotor function have been demonstrated beyond a certain requirement for heat loss (Gagnon and Kenny, 2012). In contrast, when males and females display similar heat loss for a given heat production, females may display a higher change in T_c due to physical characteristics (Gagnon et al., 2009; Havenith, 2001). These results suggest that females may reach hyperthermic levels in a shorter time period than males, consequently females have been more frequently diagnosed as heat intolerant compared with males (Druyan et al., 2012), potentially putting them at greater risk of obtaining a heat-related illness. The observed sex differences in thermoregulation are not always evident, but the difference may become more evident as the heat stress increases (Gagnon and Kenny, 2012). Furthermore, hormonal fluctuations associated with the menstrual cycle are suggested to modify central regulatory mechanisms for thermoregulation (Inoue et al., 2005). Elevated progesterone concentrations during the luteal phase of the menstrual cycle, have been reported to increase resting T_c by ~0.34°C, the T_c onset threshold for sweating by 0.29°C, and the T_c threshold for cutaneous vasodilation by 0.23 - 0.30°C (Inoue et al., 2005).

Heat acclimation improves heat transfer from the body's core to the skin and ultimately to the external environment, serving to attenuate physiological strain and improve exercise capacity (Lorenzo et al., 2010; Sunderland et al., 2008). HA reduces heat storage, partially as a result of adaptations to the sudomotor function causing an increase in whole body evaporative heat loss (Poirier et al., 2015). Additionally, HA increases blood volume preserving stroke volume and reducing HR at a given workload (Frank et al., 2001; Lorenzo and Minson, 2010).

There is a dearth of literature assessing female responses to HA. Previously, the physiological responses of males and females to 10-d fixed intensity HA were assessed with females initially exhibiting lower T_{re} and HR, despite a lower SWR compared with males (Avellini et al., 1980). Following HA, the physiological strain was similar between males and females, although males maintained a greater SWR. This study adopted a traditional HA protocol which resulted in a

progressive decline in the adaptation stimulus over the duration of HA. Controlled hyperthermia ensures consistent potentiating stimuli for adaptation throughout the HA period, eliciting reductions in thermal strain and increases in work capacity during both STHA (Garrett et al., 2012, 2009) and LTHA (Patterson et al., 2004a), potentially promoting more complete adaptation (Taylor and Cotter, 2006). It remains unknown the extent to which females adapt to the controlled hyperthermia model of HA and whether their responses differ to that of males.

Heat acclimation is often separated into STHA (≤ 8 d) and LTHA (≥ 10 d) (Garrett et al., 2011). STHA is a preferred regime, as it provides less disruption of quality training prior to competition. Approximately 70% of adaptations have been demonstrated to occur following STHA, evidenced by reductions in thermoregulatory and cardiovascular strain, combined with an improved sudomotor function (Poirier et al., 2015). Acknowledging previous observations that males typically have a superior sudomotor function compared with females (Gagnon and Kenny, 2011; Inoue et al., 2005), it may be expected that females achieve superior sudomotor adaptation following STHA compared with males. However, Sunderland and colleagues (2008) only achieved partial HA in trained females with a 33% increase in intermittent sprint performance in the heat, despite no alterations in classic indicators of HA including HR, T_{re}, and SWR, following STHA. These typical adaptive responses have been previously observed in trained males following STHA (Buono et al., 1998; Fujii et al., 2012; Garrett et al., 2011; Poirier et al., 2015; Racinais et al., 2012); suggesting females may require LTHA to achieve adaptation. Due to the self-paced exercise administered pre and post HA, participants were exercising at a higher absolute intensity following HA, suggesting an increase in metabolic heat production; potentially negating any improvements in thermoregulation achieved through HA. Research is required to determine the extent to which females adapt to STHA when using a fixed intensity HTT to monitor adaptations.

The primary aim of this study was to compare males and females' thermoregulatory, cardiovascular, and sudomotor adaptation to STHA and LTHA using the controlled hyperthermia model of HA. The primary hypothesis is that males will achieve greater reductions in T_{re} and HR following STHA compared with females. The secondary hypothesis is that there will be no differences in the reduction in T_{re} and HR between males and females following LTHA. The tertiary hypothesis is that females will achieve a greater increase in SWR following STHA and LTHA compared with males.

6.3. Materials and methods

Participants

Sixteen (8M, 8F) athletes (3.2) (table 6.1) provided written informed consent to participate in the study, which was approved by the University of Brighton Research Ethics & Governance Committee and conducted in line with health and safety guidelines (3.1.) and in accordance with the Declaration of Helsinki, 2013.

Table 6.1 Participant characteristics. Mean ± SD.

	Males	Females
Number	8	8
Age (yrs.)	22 ± 6	20 ± 1
Stature (m)	1.78 ± 0.06	1.64 ± 0.07*
BM (kg)	74.16 ± 6.92	58.89 ± 7.70*
Sum of 4 skin folds (mm)	34 ± 5	45 ± 13*
Absolute $\dot{V}O_2$ peak (L.min^{-1})	3.63 ± 0.69	2.69 ± 0.30*
Relative $\dot{V}O_2$ peak (mL.kg^{-1}.min^{-1})	48.54 ± 5.68	46.10 ± 5.82
End Power output (W)	299 ± 33	200 ± 25*

Notes: BM, body mass; $\dot{V}O_2$ peak, peak oxygen consumption. *denotes significant difference between sexes ($p \leq 0.05$).

Preliminary testing

During the first visit to the laboratory standardised anthropometric assessment was conducted (3.5.) followed by a graded exercise test. A graded exercise test was performed to determine $\dot{V}O_2$ peak (3.14.). Breath-by-breath expired metabolic gas was measured using online gas analysis (3.9.) and a capillary lactate sample was taken on completion of the test (3.11.). A regression equation was computed from the data obtained to calculate the required intensity (65% $\dot{V}O_2$ peak) for the experimental controlled hyperthermia HA exercise sessions (Appendix 4).

Experimental design

Testing was completed over a 17-d period. The timing of testing for female participants was conducted in line with the menstrual cycle standardisation guidelines (3.6.). Three participants were using oral contraceptive (Yasmin (30 μg ethinylestradiol/ 3mg drospirenone). All trials were conducted in line with pre-trial diet and exercise standardisation (3.4.). Participants provided a urine sample on arrival to ensure adequate hydration (3.4.). Participants performed 10 HA sessions (3.14.) separated by three RHTT (3.16.). The first RHTT was performed 48-hrs prior to beginning HA (RHTT1), the second 48-hrs following 5-d HA (RHTT2) and the third 48-hrs following ten HA sessions (RHTT3). All experimental trials were performed in a hot environment (3.7.). Exercise was terminated in line with University guidelines (3.1.). There were no incidences of early termination of exercise during the RHTT within this study.

Running heat tolerance test

The RHTT (3.16.) was performed on three occasions. Following a 20-min stabilisation period in a temperate environment (3.7.), measures of T_{re}, $T_{sk,}$ (3.8.) and HR (3.9.) were recorded, and participants entered the environmental chamber. Towel dried nude BM was recorded to calculate SWR (3.12.). Throughout the test T_{re}, $T_{sk,}$ and HR were recorded at 5-min intervals. Mean T_{sk} (Equation 2.4) was subsequently calculated.

Heat acclimation

HA involved two blocks of five consecutive controlled hyperthermia sessions separated by 48-hrs (3.14.).

Statistical analyses

All data were first checked for normality using Shapiro-Wilk and corrected for sphericity using the Greenhouse Geisser method. An independent samples t-test was used to identify differences between male and female characteristics. A two-way (3 x 2) mixed design ANOVA was performed to identify differences between the performance and physiological characteristics during STHA and LTHA, the physiological responses on D1, D5 and D10 of HA and the physiological responses during RHTT1, RHTT2 and RHTT3 (3.17.4.). When a main effect or interaction effect was found, results were followed up using a Bonferroni corrected post hoc comparison (3.17.6.). Effect sizes (partial Eta squared (np^2)) were calculated to analyse the magnitude and trends of the interventions

(3.17.5.). All data was analysed using a standard statistical package (SPSS version 20.0), and reported as mean ± SD. Statistical significance was accepted at the level of $p \leq 0.05$.

6.4.   Results

Performance responses during heat acclimation (D1 - D5 and D5 - D10)

Table 6.2 presents the mean ± SD data for the performance and physiological responses during STHA and LTHA. All participants completed ten, 90-min HA sessions. ANOVA revealed a main effect of HA phase on exercise duration ($F_{(1, 14)}$ = 7.728, p = 0.015, np^2 = 0.356). Exercise duration was lower in STHA (70 ± 8 min) compared with LTHA (75 ± 7 min). There was no interaction effect between HA phase and sex for exercise duration ($F_{(1, 14)}$ = 0.340, p = 0.569, np^2 = 0.024).

There was a main effect of HA phase on exercise intensity ($F_{(1, 14)}$ = 4.710, p = 0.048, np^2 = 0.252). Exercise intensity was lower in STHA (57 ± 6% $\dot{V}O_2$ peak) compared with LTHA (59 ± 5% $\dot{V}O_2$ peak). There was no interaction effect between HA phase and sex for exercise intensity ($F_{(1, 14)}$ = 0.587, p = 0.456, np^2 = 0.04).

There was a main effect of HA phase on total work ($F_{(1, 14)}$ = 16.272, p < 0.001, np^2 = 0.538). Total work was lower in STHA (484 ± 105 kJ) compared with LTHA (570 ± 124 kJ). There was no interaction effect between HA phase and sex for total work ($F_{(1, 14)}$ = 0.186, p = 0.673, np^2 = 0.013).

ANOVA revealed a main effect of HA phase on duration of $T_{re} \geq 38.5°C$ ($F_{(1, 14)}$ = 4.982, p = 0.042, np^2 = 0.262). The duration of $T_{re} \geq 38.5°C$ was higher in STHA (49 ± 8 min) compared with LTHA (46 ± 8 min). There was no interaction effect between HA phase and sex for duration of $T_{re} \geq 38.5°C$ ($F_{(1, 14)}$ = 0.513, p = 0.486, np^2 = 0.035).

Table 6.2 Performance and physiological responses during short-term heat acclimation and long-term heat acclimation. Mean ± SD.

	STHA		LTHA	
	Males	**Females**	**Males**	**Females**
Exercise Duration (min)	69 ± 7	70 ± 9	76 ± 7*	74 ± 7*
Mean Exercise Intensity (% $\dot{V}O_2$ peak)	55 ± 7	59 ± 5	58 ± 6*	60 ± 4*
Total Work Done (kJ)	562 ± 70	413 ± 79	653 ± 116*	487 ± 63*
Duration of T_{re} > 38.5°C (min)	48 ± 9	51 ± 7	45 ± 8*	47 ± 9*
T_{re} rest (°C)	37.12 ± 011	37.33 ± 0.46	36.72 ± 0.24*	37.00 ± 0.25*
Mean T_{re} (°C)	38.20 ± 0.10	38.19 ± 0.21	38.17 ± 0.14	38.23 ± 0.10
HR rest (beats.min^{-1})	74 ± 3	80 ± 5	62 ± 6*	67 ± 4*
Mean HR (beats.min^{-1})	150 ± 9	151 ± 9	151 ± 9	154 ± 8
SWR_{BSA} (g.hr^{-1}.m^2)	276 ± 65	278 ± 53	317 ± 65*	341 ± 194*

Notes: LTHA, long-term heat acclimation; HR, heart rate; SWR_{BS}, Sweat rate relative to body surface area; STHA, short-term heat acclimation; T_{re}, rectal temperature. * denotes significantly difference to STHA ($p \leq 0.05$).

Physiological responses during heat acclimation (D1 - D5 and D5 - D10)

Thermoregulatory responses

There was a main effect of HA day on T_{re} rest ($F_{(2, 28)}$ = 37.281, $p \leq 0.001$, np^2 = 0.727). There was a reduction in T_{re} rest from D1 to D5 (-0.26 ± 0.19 °C, $p \leq 0.001$), from D5 to D10 (-0.21 ± 0.28, p = 0.002), and from D1 to D10 (-0.47 ± 0.20, $p \leq 0.001$). There was no interaction effect between HA day and sex on T_{re} rest ($F_{(2, 28)}$ = 1.732, p = 0.195, np^2 = 0.110).

There was no main effect of HA phase on mean T_{re} ($F_{(1, 14)}$ = 0.000, p = 0.988, np^2 = 0.000). Furthermore, there was no interaction effect between HA phase and sex on mean T_{re} ($F_{(1, 14)}$ = 0.872, p = 0.366, np^2 = 0.059).

Cardiovascular responses

There was a main effect of HA day on HR rest ($F_{(2, 28)}$ = 24.137, $p \leq 0.001$, np^2 = 0.633). There were no changes in HR rest from D1 to D5 (-4 ± 6 beats.min^{-1}, p = 0.070). There was a reduction in HR rest from D5 to D10 (-6 ± 4 beats.min^{-1} $p \leq 0.001$) and from D1 to D10 (-10 ± 7 beats.min^{-1}, $p \leq 0.001$). There was no interaction effect between HA day and sex on HR rest ($F_{(2, 28)}$ = 2.117, p = 0.139, np^2 = 0.131).

There was no main effect of HA phase on mean HR ($F_{(1, 14)}$ = 3.059, p = 0.102, np^2 = 0.179). Furthermore, there was no interaction effect between HA phase and sex on mean HR ($F_{(1, 14)}$ = 0.716, p = 0.412, np^2 = 0.049).

Sudomotor responses

There was a main effect of HA day on SWR$_{BSA}$ ($F_{(2, 28)}$ = 16.266, $p \leq 0.001$, np^2 = 0.537). There was an increase from D1 to D5 (89 ± 144 g.hr^{-1}.m^2, p = 0.043), from D5 to D10 (87 ± 114 g.hr^{-1}.m^2, p = 0.014) and from D1 to D10 (177 ± 134 g.hr^{-1}.m^2, $p \leq 0.001$). There was no interaction effect between HA day and sex on SWR$_{BSA}$ ($F_{(2, 28)}$ = 2.806, p = 0.077, np^2 = 0.167).

There was a main effect of HA phase on SWR$_{BSA}$ ($F_{(1, 14)}$ = 21.737, $p \leq 0.001$, np^2 = 0.608). SWR$_{BSA}$ was lower in STHA (277 ± 58 g.hr^{-1}.m^2) compared with LTHA (329 ± 79 g.hr^{-1}.m^2). There was no interaction effect between HA phase and sex on SWR$_{BSA}$ ($F_{(1, 14)}$ = 0.987, p = 0.337, np^2 = 0.066).

Thermoregulatory response to short-term and long-term heat acclimation

Table 6.3 presents the mean ± SD data for males and females physiological responses during RHTT1, RHTT2, and RHTT3. There was a main effect of RHTT for T_{re} rest ($F_{(2, 28)}$ = 26.084, $p \leq 0.001$, np^2 = 0.651). T_{re} rest reduced following STHA $_{(RHTT1 \text{ to } RHTT2)}$ (-0.13 ± 0.16°C, p = 0.002), LTHA $_{(RHTT2 \text{ to } RHTT3)}$ (-0.13 ± 0.16°C, p = 0.006) and LTHA $_{(RHTT1 \text{ to } RHTT3)}$ (-0.26 ± 0.16°C, $p \leq 0.001$). There was an interaction effect between RHTT and sex for T_{re} rest ($F_{(2, 28)}$ = 5.282, p = 0.011, np^2 = 0.274). T_{re} rest reduced following STHA $_{(RHTT1 \text{ to } RHTT2)}$ in males (-0.24 ± 0.16°C, $p \leq 0.001$), but no differences were observed in females (-0.02 ± 0.08°C, p = 0.597). T_{re} rest reduced following LTHA $_{(RHTT2 \text{ to } RHTT3)}$ for females (-0.22 ± 0.12°C, $p \leq 0.001$), but no differences were observed in males (-0.04 ± 0.15°C, p = 0.459). T_{re} rest reduced following LTHA $_{(RHTT1 \text{ to } RHTT3)}$ for both males (-0.28 ± 0.17°C, $p \leq 0.001$) and females (-0.24 ± 0.17°C, $p \leq 0.001$).

There was a main effect of RHTT for peak T_{re} ($F_{(2, 28)}$ = 17.972, $p \leq 0.001$, np^2 = 0.532). Peak T_{re} reduced following STHA $_{(RHTT1 \text{ to } RHTT2)}$ (-0.23 ± 0.32°C, p = 0.018), LTHA $_{(RHTT2 \text{ to } RHTT3)}$ (-0.26 ± 0.30°C, p = 0.008) and LTHA $_{(RHTT1 \text{ to } RHTT3)}$ (-0.46 ± 0.36°C, $p \leq 0.001$). There was an interaction effect between RHTT and sex for peak T_{re} ($F_{(2, 28)}$ = 3.339, p = 0.050, np^2 = 0.193). Peak T_{re} reduced following STHA $_{(RHTT1 \text{ to } RHTT2)}$ for males (-0.39 ± 0.36°C, $p \leq 0.001$), but no differences were observed in females (-0.07 ± 0.18, p = 0.504). Peak T_{re} reduced following LTHA $_{(RHTT2 \text{ to } RHTT3)}$ for females (-0.41 ± 0.24°C, $p \leq 0.001$), but no differences were observed in males (-0.05 ± 0.26°C, p = 0.590). Peak T_{re} reduced following LTHA $_{(RHTT1 \text{ to } RHTT3)}$ for both males (-0.44 ± 0.45°C, p = 0.005) and females (-0.48 ± 0.27°C, p = 0.003).

Figure 6.1 presents T_{re} data at 5-min intervals during the RHTT for both males and females. There was a main effect of RHTT and time on T_{re} ($F_{(12, 168)}$ = 2.343, p = 0.008, np^2 0.143). Following STHA $_{(RHTT1 \text{ to } RHTT2)}$ there was a reduction in T_{re} at 5 ($p \leq 0.001$), 10 (p = 0.008), 15 (p = 0.027), 20 (p = 0.007) and 25 (p = 0.018) min. Following LTHA $_{(RHTT2 \text{ to } RHTT3)}$ there were no differences in T_{re} at 5 (p = 0.265), 10 (p = 0.347), 15 (p = 0.138), 20 (p = 0.346) and 25 (p = 113) min. Following LTHA $_{(RHTT1 \text{ to } RHTT3)}$ there was a reduction in T_{re} at 5 (p = 0.002), 10 (p = 0.005), 15 (p = 0.006), 20 (p = 0.002) and 25 ($p \leq 0.001$) min. There was no interaction effect between RHTT and time and sex for T_{re} ($F_{(12,168)}$ = 1.055 p = 0.402, np^2 = 0.070).

There was no main effect of RHTT for T_{re} change ($F_{(2, 28)}$ = 2.502, p = 0.100, np^2 = 0.152). There was no interaction effect between RHTT and sex observed for T_{re} change ($F_{(2, 28)}$ = 0.513, p = 0.604, np^2 = 0.035).

Table 6.3 Physiological variables during baseline testing (RHTT1), following STHA (RHTT2) and following LTHA (RHTT3). Mean ± SD.

	RHTT1		RHTT2		RHTT3	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
T_{re} **rest (°C)**	37.16 ± 0.11	37.23 ± 0.29	36.92 ± 0.19*	37.21 ± 0.34	36.88 ± 0.18*	36.94 ± 0.29*†
T_{re} **peak (°C)**	38.67 ± 0.25	39.18 ± 0.39	38.28 ± 0.24*	39.12 ± 0.44	38.24 ± 0.40*	38.71 ± 0.42*†
T_{re} **change (°C)**	1.51 ± 0.26	1.95 ± 0.31	1.37 ± 0.25	1.91 ± 0.27	1.35 ± 0.41	1.72 ± 0.38
T_{sk} **peak (°C)**	37.30 ± 0.53	37.66 ± 0.95	36.80 ± 0.36	37.25 ± 0.55	36.27 ± 0.77	36.58 ± 0.72*†
HR rest (beats.min^{-1})	71 ± 9	78 ± 10	66 ± 8	71 ± 7	63 ± 4	62 ± 7
HR peak (beats.min^{-1})	185 ± 11	190 ± 11	170 ± 12*	185 ± 11	172 ± 10*	180 ± 11 *
SWR (g.hr^{-1})	1610 ± 435	524 ± 244	1588 ± 359	1215 ± 334*	2170 ± 609*†	1300 ± 353*
SWR$_{BSA}$ (g.hr^{-1}.m^2)	838 ± 215	326 ± 156	827 ± 168	754 ± 260*	1135 ± 324*†	798 ± 229*

Notes: HR, heart rate; RHTT, running heat tolerance test; SWR, Sweat rate; SWR$_{BSA}$, Sweat rate relative to body surface area; T_{re}, rectal temperature; T_{sk}, skin temperature. *denotes significant difference to RHTT1 ($p \leq 0.05$). †denotes significant difference to RHTT2 ($p \leq 0.05$).

There was a main effect of RHTT for peak T_{sk} ($F_{(2, 28)}$ = 19.085, $p \leq 0.001$, np^2 = 0.577). Peak T_{sk} reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ (-0.45 ± 0.62°C; p = 0.038), LTHA $_{(RHTT2\ to\ RHTT3)}$ (-0.60 ± 0.62°C, p = 0.007) and LTHA $_{(RHTT1\ to\ RHTT3)}$ (-1.05 ± 0.74°C, $p \leq 0.001$). There was no interaction effect between RHTT and sex observed for peak T_{sk} ($F_{(2, 28)}$ = 0.088, p = 0.916, np^2 = 0.006).

Figure 6.1 Rectal temperature (T_{re}) at 5-min intervals during the running heat tolerance test for males (left) and females (right). Grey markers represent the RHTT1, black markers RHTT2 and white marker RHTT3. Mean ± SD. N = 16 (8 M, 8F).

*denotes significant difference in STHA $_{(RHTT1\ to\ RHTT2)}$ ($p \leq 0.05$). + denotes significant difference in LTHA $_{(RHTT2\ to\ RHTT3)}$ ($p \leq 0.05$). † denotes significant difference in LTHA $_{(RHTT1\ to\ RHTT3)}$ ($p \leq 0.05$).

Cardiovascular response to short-term and long-term heat acclimation

There was a main effect of RHTT for HR rest ($F_{(2, 28)}$ = 11.177, $p \leq 0.001$, np^2 = 0.444). Following STHA $_{(RHTT1\ to\ RHTT2)}$ there were no observed differences in HR rest (-6 ± 11 beats.min^{-1}, p = 0.117). HR rest reduced following LTHA $_{(RHTT2\ to\ RHTT3)}$ (-6 ± 8 beats.min^{-1}, p = 0.027) and LTHA $_{(RHTT1\ to\ RHTT3)}$ (-12 ± 12

beats.min^{-1}, p = 0.003). There was no interaction effect between RHTT and sex observed for HR rest ($F_{(2, 28)}$ = 0.942, p = 0.402, np^2 = 0.063).

There was a main effect of RHTT for peak HR ($F_{(2, 28)}$ = 19.916, $p \leq 0.001$, np^2 = 0.587). Peak HR reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ (-9 ± 10 beats.min^{-1}, p = 0.002), showed no differences following LTHA $_{(RHTT2\ to\ RHTT3)}$ (-2 ± 8 beats.min^{-1}, p = 1.000), but reduced following LTHA $_{(RHTT1\ to\ RHTT3)}$ (-11 ± 6 beats.min^{-1}, $p \leq 0.001$). There was an interaction effect between RHTT and sex for peak HR ($F_{(2, 28)}$ = 3.598, p = 0.041, np^2 = 0.204). Peak HR reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ in males (-14 ± 12 beats.min^{-1}, $p \leq 0.001$), no differences were observed in females (-5 ± 3 beats.min^{-1}, p = 0.164). There were no differences observed for peak HR following LTHA $_{(RHTT2\ to\ RHTT3)}$ in both males (2 ± 10 beats.min^{-1}, p = 0.505) and females (-5 ± 5 beats.min^{-1}, p = 0.076). Peak HR reduced following LTHA $_{(RHTT1\ to\ RHTT3)}$ for both males (-13 ± 7 beats.min^{-1}, $p \leq 0.001$) and females (-10 ± 6 beats.min^{-1}, $p \leq 0.001$).

Figure 6.2 presents the HR 5-min interval data for both males and females. There was no main effect of RHTT and time on HR ($F_{(12, 168)}$ = 0.845, p = 0.604, np^2 = 0.057). There was no interaction effect between RHTT and time and sex for HR ($F_{(12,168)}$ = 1.055 p = 0.401, np^2 = 0.070).

Sudomotor response to short-term and long-term heat acclimation

There was a main effect of RHTT for SWR ($F_{(2, 28)}$ = 12.207, $p \leq 0.001$, np^2 = 0.466). SWR increased following STHA $_{(RHTT1\ to\ RHTT2)}$ (334 ± 590 g.hr^{-1}, p = 0.042). There were no differences observed for LTHA $_{(RHTT2\ to\ RHTT3)}$ for SWR (334 ± 636 g.hr^{-1}, p = 0.131) however, an increase was observed following LTHA $_{(RHTT1\ to\ RHTT3)}$ (668 ± 529 g.hr^{-1}, $p \leq 0.001$). There was an interaction effect between RHTT and sex for SWR ($F_{(2, 28)}$ = 3.661, p = 0.039, np^2 = 0.270). SWR increased following STHA $_{(RHTT1\ to\ RHTT2)}$ in females (691 ± 412 g.hr^{-1}, $p \leq 0.001$), but no differences were observed in males (-22 ± 533 g.hr, p = 0.896). SWR increased following LTHA $_{(RHTT2\ to\ RHTT3)}$ for males (583 ± 638 g.hr^{-1}, p = 0.016), but no differences were observed in females (85 ± 564 g.hr^{-1}, p = 0.696). SWR increased following LTHA $_{(RHTT1\ to\ RHTT3)}$ for both males (560 ± 594 g.hr^{-1}, $p \leq 0.001$) and females (776 ± 470 g.hr^{-1}, $p \leq 0.001$).
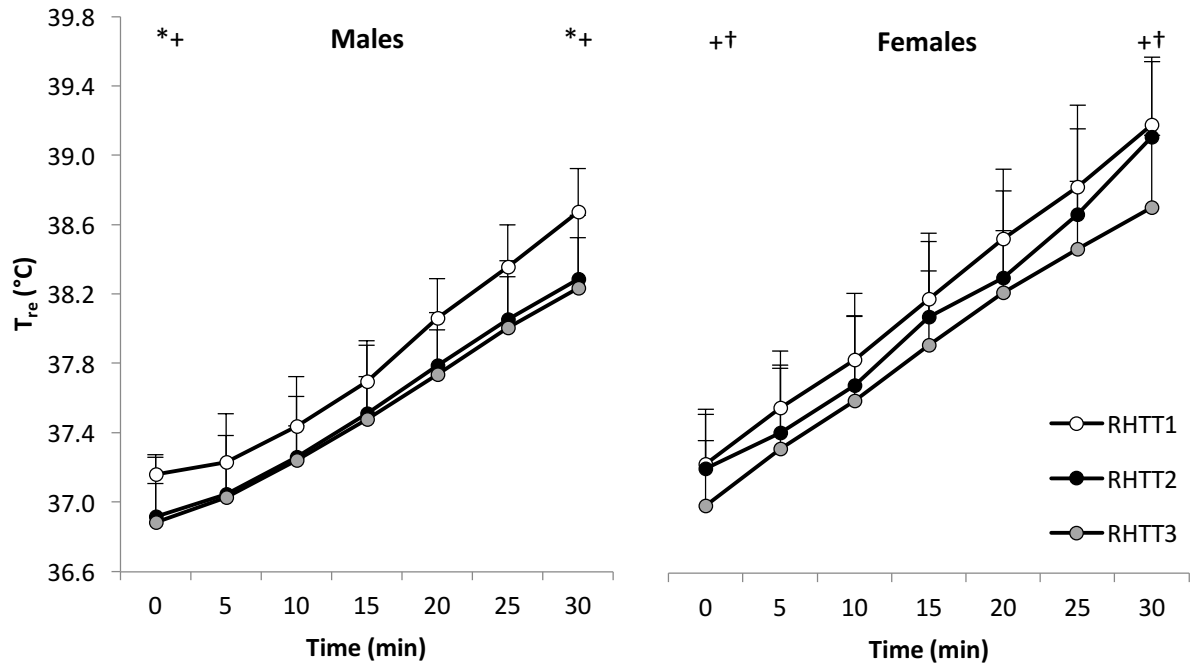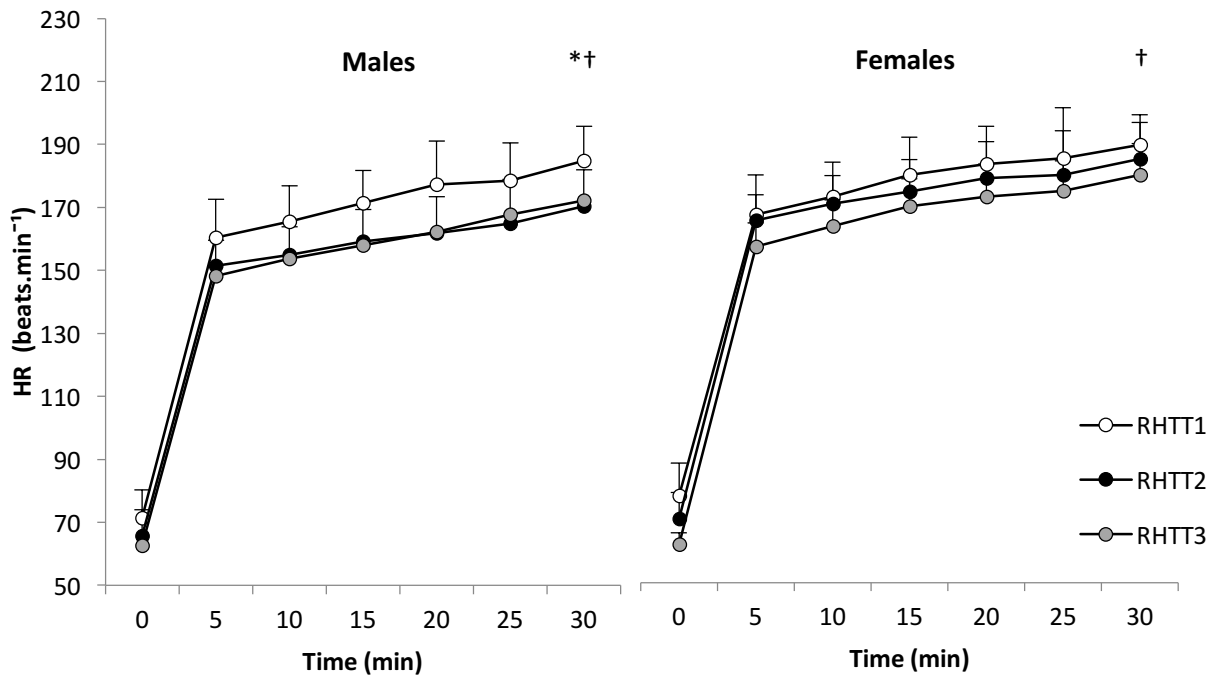
Figure 6.2 Heart rate (HR) at 5-min intervals during the running heat tolerance test for males (left) and females (right). Grey markers represent the RHTT1, black markers RHTT2 and white marker RHTT3. Mean ± SD. N = 16 (8M, 8F).

*denotes significant difference in STHA $_{(RHTT1\ to\ RHTT2)}$ ($p \leq 0.05$). † denotes significant difference in LTHA $_{(RHTT1\ to\ RHTT3)}$ ($p \leq 0.05$).

There was a main effect of RHTT for SWR$_{BSA}$ ($F_{(2, 28)}$ = 11.947, $p \leq 0.001$, np^2 = 0.460). SWR$_{BSA}$ increased following STHA $_{(RHTT1\ to\ RHTT2)}$ (334 ± 590 g.hr^{-1}.m^2; p = 0.029). There were no differences observed for LTHA $_{(RHTT2\ to\ RHTT3)}$ (334 ± 636 g.hr^{-1}.m^2, p = 0.210), however, an increase was observed following LTHA $_{(RHTT1\ to\ RHTT3)}$ (668 ± 529 g.hr^{-1}.m^2, $p \leq 0.001$). There was an interaction effect between RHTT and sex on SWR$_{BSA}$ ($F_{(2, 28)}$ = 3.939, p = 0.031, np^2 = 0.220). SWR$_{BSA}$ increased following STHA $_{(RHTT1\ to\ RHTT2)}$ for females (428 ± 269 g.hr^{-1}.m^2, $p \leq 0.001$), but no differences were observed in males (-11 ± 286 g.hr^{-1}.m^2, p = 0.909). SWR$_{BSA}$ increased following LTHA $_{(RHTT2\ to\ RHTT3)}$ for males (308 ± 346 g.hr^{-1}.m^2, p = 0.029), but no differences were observed in females (44 ± 373 g.hr^{-1}.m^2, p = 0.733). SWR$_{BSA}$ increased following LTHA $_{(RHTT1\ to\ RHTT3)}$ for both males (297 ± 314 g.hr^{-1}.m^2, p = 0.015) and females (472 ± 291 g.hr^{-1}.m^2, $p \leq 0.001$).

6.5.   Discussion

The understanding of HA on experimentation using humans is primarily based upon male participants. The application of HA to females may be inappropriate due to sex differences in thermoregulation. This study examined the sex differences in the temporal patterning to STHA and LTHA. The data demonstrates that both males and females achieve partial adaptation following STHA; with males demonstrating a reduction in thermoregulatory and cardiovascular strain and females demonstrating an increased sudomotor function. Following LTHA, both males and females achieved additional adaptation; with females demonstrating a reduction in thermoregulatory strain and males an increased sudomotor function. These results suggest that both males and females respond to STHA however, females require LTHA to establish reductions in thermoregulatory and cardiovascular strain. These findings support the aim of the study with the primary, secondary and tertiary hypothesis also being accepted.

Short-term heat acclimation

Following STHA, approximately 70% of maximal adaptations has been reported to be achieved (Poirier et al., 2015). In the current study, males demonstrated more adaptation following STHA compared with females, with a reduction in T_{re} rest (-0.24 ± 0.16°C) and peak T_{re} (-0.32 ± 0.36°C); these changes did not alter T_{re} change. The magnitude of reduction in T_{re} is very similar to the 0.3°C observed by Garrett and colleagues (2012) following 5-d controlled hyperthermia HA. Endurance performance is markedly impaired in hot compared to temperate environment due to an increase in T_c causing a decrease in central activation (Nybo and Nielsen, 2001). Attenuation of the peak T_{re} may lessen or delay the likelihood of individuals obtaining or expressing signs of heat-related illnesses when training, working or competing in the heat, demonstrating the effectiveness of STHA in males.

In the current study, exercise intensity, exercise duration, and total work performed was higher during LTHA compared with STHA. This increased exercise intensity was administered to elicit and maintain the target T_c of 38.5°C. The higher exercise intensity during LTHA would result in a higher metabolic heat production compared with STHA. Since total heat loss during exercise is predominantly a function of evaporative heat loss, a greater rate of metabolic heat production in LTHA, with comparable T_{re} values achieved, suggests an increase in evaporative heat loss and thus reduced heat storage. These findings support that partial heat adaptation was achieved during STHA.

A reduction in cardiovascular strain was achieved following STHA in male participants, evidenced by a reduction in peak HR. The 14 ± 12 beats.min^{-1} reduction in peak HR is in accordance with previous observations following STHA using controlled hyperthermia (Garrett et al., 2012; Patterson et al., 2004a). The reduction in cardiovascular strain is potentially due to an increase in blood volume, preserving stroke volume and reducing heat transfer from the body's core to the skin and ultimately, to the external environment.

Sunderland and colleagues (2008) assessed the effect of 4-d HA on female games players' intermittent sprint performance in the heat. Intermittent sprint performance increased by 33%, following 4-d HA, however there were no differences in peak T_{re}, peak HR and SWR. The self-regulated nature of the intermittent sprint protocol used provides no standardised endogenous thermal load, potentially constraining adaptation in some individuals (Taylor and Cotter, 2006). Furthermore, any reduction in thermoregulatory and cardiovascular strain may have been negated due to participants performing more work following HA. The findings in the current study are in agreement with these previous reports, with no reductions in cardiovascular and thermoregulatory strain during STHA in female participants.

Increased SWR have been reported to occur following STHA (Buono et al., 2009; Patterson et al., 2004; Poirier et al., 2015). Patterson and colleagues (2004a) reported that only six HA sessions were required to elicit partial sudomotor adaptation, evidenced by an elevated local SWR. The findings in the current study support previous findings with a 428 ± 269 g.hr^{-1}.m^2 increase in SWR in female participants following STHA. Although, females in the current study had a lower stimulus for sweat production, due to exercising at a lower metabolic heat production potentially, they nonetheless improved sweat production to a greater extent compared to males, particularly following STHA. This is surprising, based on previous findings which suggest that the production of sweat is the main driver for improvements in sweating during HA (Buono et al., 2009). As such, it could be argued that females adapt during STHA to a greater extent than males. However, the observed increase in SWR did not result in a reduced thermal strain in female participants. No plateau was observed in T_{re} during the RHTT in female participants, thus the enhanced SWR did not offset the uncompensatable rate of metabolic heat production observed.

An increase in whole body SWR observed in females following STHA in the current study, suggests either an altered afferent neural activity from the peripheral or central thermo-receptors causing different integration of thermal information, an altered efferent neural activity for a given level of afferent input, or an altered effector response. Sex modulates peripheral control of the sudomotor function, this is evidenced by a reduced thermosensitivity; resulting in females having a reduced

SWR compared with males (Gagnon and Kenny, 2011). Consequently, it may be hypothesised that the enhanced sudomotor function in the female participants following STHA in the current study is as a result of peripheral changes to the thermosensitivity of the eccrine sweat glands. The potential mechanisms for this include an increased cholinergic sensitivity of the eccrine sweat gland and increase glandular hypertrophy (Buono et al., 2009; Lorenzo and Minson, 2010).

In the current study, there were no observed changes in SWR during STHA $_{(RHTT1 \text{ to } RHTT2)}$ in male participants. These findings may be due to a lower peak T_{re} in RHTT2 compared with RHTT1. Specifically, in male participants the peak T_{re} in RHTT1 was 38.67 ± 0.25°C which produced a SWR$_{BSA}$ of 838 ± 215 g.hr^{-1}.m^2. The peak T_{re} in RHTT2 was 38.28 ± 0.24°C which produced a SWR$_{BSA}$ of 827 ± 168 g.hr^{-1}.m^2. It is well reported that an increase in T_c stimulates sudomotor function during exercise in the heat (Sawka et al., 1989). Recent findings, suggest that whole body SWR may underestimate the true adaptation that occurred to the sweat gland function following HA (Buono et al., 2009a; Inoue et al., 1999). Buono et al. (2009) reported 20% increase in whole body SWR, while pilocarpine induced SWR increases by 63% following 8-d HA. Furthermore, Inoue et al. (1999) reported no changes in whole body SWR, while a significant improvement was observed for methycholine induced SWR following 8-d HA.

Long-term heat acclimation

The adaptive effects of LTHA are well established, such that the extent to which an individual physiologically adapts to HA is dependent upon the length of exposure to heat stress conditions. In the current study, there was a reduction in the combined thermoregulatory and cardiovascular strain and an enhanced sudomotor function following LTHA in all participants. These findings are in accordance with the results reported by Avellini and colleagues (1980) when assessing sex differences in adaptation to 10-d fixed intensity HA. Males and females were reported to express a similar adaptive response, evidenced by a reduction in T_{re}, an increased SWR and an improved exercise capacity. Participants worked at the same absolute exercise intensity during the HA session, therefore, there may have been variety in the physiological strain placed upon participants. When work rate remains constant, thermal strain during sequential exposures progressively declines, constraining adaptation (Taylor and Cotter, 2006).

Since controlled hyperthermia HA ensures equal thermal strain during every session, it can be assumed that adaptation was not constrained during sequential sessions in the current study, establishing more complete adaptation (Taylor and Cotter, 2006). Additional adaptations were observed during LTHA for the female participants, evidenced by reductions in measures of

thermoregulatory and cardiovascular strain from RHTT2 to RHTT3. These observed differences were not present in the male participants, thus females require LTHA to establish reductions in thermoregulatory and cardiovascular strain.

LTHA established an improved sudomotor response in the current study. Increased SWR is not unique to the current study with HA known to improve peripheral and central mechanisms involved in sudomotor function, via enhanced sweat gland sensitivity (Buono et al., 2009a) and reductions in the onset threshold for sweating, enhancing evaporative heat loss (Yamazaki and Hamasaki, 2003). Complete sudomotor adaptation has been suggested to take between 10 - 14 d to establish (Armstrong and Maresh, 1991), but this biphasic adaptation may be more protocol dependent. In the current study females obtained no additional benefit to the sudomotor function as a result of LTHA, however, an increase in SWR was observed from RHTT2 to RHTT3 in male participants.

6.6.   Limitations

The exercise elicited during the HA sessions was performed at 65% of $\dot{V}O_2$ peak. Females in the current study had a lower absolute $\dot{V}O_2$ peak compared with the male participants, consequently they worked at a lower metabolic heat production providing a lower stimulus for sweat production (Cramer and Jay, 2014; Gagnon et al., 2013, 2008). Furthermore, females in the current study had a lower BM compared with the male participants which entails less heat storage, and therefore a lower exercise intensity is required to increase their T_c to 38.5°C (Gagnon et al., 2009). Consequently, the stress imposed during the HA sessions was likely lower in female participants, potentially constraining adaptation and influencing the time course of adaptation, due to inadequate endogenous heat strain. Future research is warranted to quantify these potential differences between males and females. Future work should involve the implementation of a controlled hyperthermia HA protocol where workload is administered using relative heat production. This may further optimise adaptation to HA by reducing individual variability associated with metabolic heat production (Cramer & Jay 2014)

Future work should implement greater control over hormonal alterations which alter thermoregulatory responses associated with the menstrual cycle between repeated trials. An elevation in progesterone concentration, associated with the luteal phase of the menstrual cycle, alters resting T_c, the threshold for sweating, and cutaneous vasodilation and consequently, tolerance to exercise heat stress (Inoue et al., 2005). In the current study, the minimum number of days the protocol required for completion was 16, thus crossing over two menstrual cycle phases. For those participants not using oral contraception, RHTT1 and RHTT2 were performed during the

follicular phase of their self-reported menses, when resting T_c and the threshold for the onset of sweating and cutaneous vasodilation is lower compared with the luteal phase. However, RHTT3 was performed during the luteal phase of their self-reported menses. Consequently, the extent of the adaptation reported in females may have been smaller due to alterations in hormone concentrations of progesterone associated with the menstrual cycle (Inoue et al., 2005). Controlling for the hormonal alteration associated with the menstrual cycle throughout HA would provide a greater understanding into the true adaptation present.

Furthermore, in the current study, changes in PV and fluid regulation were not measured, both of which assist in the maintenance of an elevated SWR and a reduced cardiovascular strain associated with HA (Taylor, 2014). Consequently, the effect of these adaptations on the improvement in SWR and reduction in cardiovascular strain in the current study is not known. Future research should be conducted observing these variables whilst accounting for hormonal fluctuations associated with the menstrual cycle, due to their known effect on fluid balance and PV.

6.7.   Conclusion

In the current study, HA was effective in attenuating physiological strain and improving exercise-heat tolerance in both males and females and thus may reduce the likelihood of obtaining a heat-related illness during training or competition in the heat. STHA is a preferred regime for athletes since it is easier to adopt when sustaining quality training and tapering performance in the weeks before competition. These findings suggest that whilst STHA may be effective in achieving partial adaptation in males and females, females require LTHA to establish reductions in cardiovascular and thermoregulatory strain.

These findings provide original data which will support the implementation of HA protocols that are tailored to target sex differences in the temporal patterning of adaptation. An additional element of HA is an acquired cellular thermotolerance. Study 4 (Chapter 7) will compare males and females' cellular adaptation, specifically Hsp72 mRNA across the course of STHA and LTHA.

7. Sex comparison of leukocyte Hsp72 mRNA transcription during heat acclimation

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In Study 3 (Chapter 6) it became apparent that females' phenotypic responses to HA differed from those of males. An additional element to HA in an acquired cellular thermotolerance. This study will explore the differences between males and females' cellular adaptation to STHA and LTHA using 10 out of the 16 participants presented in Study 3 (Chapter 6).

7.1.   Abstract

Thermotolerance is an acquired state of increased cytoprotection achieved following single or repeated exposures to heat stress, in part characterised by changes in the intracellular 72kda heat shock protein (HSP72; HSPA1A). Females have demonstrated reduced exercise induced HSP72 in comparison to males. This study examined sex differences in Hsp72 mRNA transcription during heat acclimation (HA) to identify whether sex differences were a result of differential gene transcription. Ten participants (5M, 5F) performed 10, 90-min controlled hyperthermia HA sessions over 12-d. Leukocyte Hsp72 mRNA was measured pre and post D1, D5, and D10, via RT-QPCR. HA was evidenced by a reduction in resting T_{re} (-0.36 ± 0.53°C) and resting heart rate [(HR); -13 ± 7 beats.min^{-1}] following HA (p ≤ 0.05). During HA no difference ($p > 0.05$) was observed in ΔT_{re} between males (D1 = 1.52 ± 0.19°C; D5 = 1.56 ± 0.38°C; D10 = 1.80 ± 0.28°C) and females (D1 = 1.52 ± 0.47°C; D5 = 1.40 ± 0.22°C; D10 = 1.80 ± 0.28°C). This was also true of mean T_{re} demonstrating equality of thermal stimuli for mRNA transcription and HA. There were no differences ($p > 0.05$) in Hsp72 mRNA expression between HA sessions or between males (D1 = +1.8 ± 1.5 fold; D5 = +2.0 ± 1.0 fold; D10 = +1.1 ± 0.4 fold) and females (D1 = +2.6 ± 1.8 fold; D5 = +1.8 ± 1.4 fold; D10 = +0.9 ± 1.9 fold). This experiment demonstrates that there is no difference in Hsp72 mRNA increases during HA between sexes when controlled hyperthermia HA is utilised. Gender specific differences in exercise-induced HSP72 reported elsewhere likely result from post-transcriptional events.

7.2.   Introduction

Repeated exposure to stressful thermal environments initiates heat adaptation in humans (Taylor, 2014). Heat adaptation incorporates the interrelated acclimation and thermotolerance (Horowitz, 2014; Sawka et al., 2011a). A heat acclimated phenotype describes enhanced heat loss effector responses and hypervolemia which mitigate physiological, perceptual, and functional detriments

to heat exposure (Périard et al., 2015; Taylor, 2014). Thermotolerance or acquired cellular thermotolerance is the nomenclature used to describe cellular adaptations caused by a single, or repeated severe, but non-lethal heat exposure [e.g. heat acclimation (HA)] (Moseley, 1997).

HA repeatedly initiates the heat shock response (HSR), typically increasing various basal heat shock proteins (HSP), including HSP72 (HSP72). In response to 10-d HA, baseline intracellular HSP72 has been shown to increase by 18% (McClung et al., 2008) whilst Hsp72 mRNA demonstrates a pattern whereby transcription occurs within each HA session (+195%) before returning to baseline 24-hrs later (Gibson et al., 2015a, 2015c). These transient HA mediated cellular adaptations to iHSP72 can confer cytoprotection to subsequent thermal (McClung et al., 2008) and non-thermal (Gibson et al., 2015c) stressors *in vitro* (McClung et al., 2008) and *in vivo* (Lee et al., 2016). Eloquent in-vitro data demonstrates that cytoprotection to stress (thermal or otherwise) is abolished when HSP72 is knocked out (Drew et al., 2014; Lee et al., 2004; Senf et al., 2013) or blocked (Kuennen et al., 2011). HA mediated in-vivo cytoprotection is dependent upon sufficient Hsp72 mRNA transcription (Moran et al., 2006) and subsequent HSP72 protein translation (Silver and Noble, 2012).

Controlled hyperthermia HA results in a greater Hsp72 mRNA compared with matched training in cool conditions as a result of greater endogenous stimuli for transcription (Gibson et al., 2015c). Due to the consistent endogenous thermal stimulus there is an equality of Hsp72 mRNA transcription during 10-d controlled hyperthermia HA (Gibson et al., 2015a). Thus, controlled hyperthermia is a preferred HA method compared with traditional exogenously prescribed HA since it induces robust Hsp72 mRNA responses, ensuring sufficient and consistent increases in endogenous stimuli throughout an *in vivo* chronic intervention, particularly when comparing independent groups.

Morton and colleagues (2009) reported a sex specific HSP adaptation in human skeletal muscle following 6-wks. of continuous and interval training. Specifically, HSP70 increased by 38 ± 41% and 23 ± 36% following continuous and interval training respectively in males (n = 5); however females (n = 5) had no changes (3 ± 37% and 4 ± 14% increase respectively), despite similar training status, training prescription and training adaptations ($\dot{V}O_2$ max) (Morton et al., 2009). Differential sex responses reported by Morton et al. (2009) may be attributed to cytoprotective effects of oestrogen. Elevated oestrogen has been shown to afford cellular protection (Shinohara et al., 2004), accordingly increased oestrogen in females versus males may provide a mechanism for inhibited changes in HSP72 expression (Bombardier et al., 2009). Oestrogen binds to the oestrogen receptor, which is a member of the steroid family of nuclear receptors and is the oestrogen response element in target genes, leading to the transcriptional regulation of many genes (Ogita et al., 2003). Gillum

et al. (2013) reported higher intracellular HSP72 concentrations following a single bout of exercise in the heat in males compared with females (in both the follicular and luteal phase of the menstrual cycle), despite similar baseline values and identical endogenous stimuli for Hsp72 mRNA transcription (Gillum et al., 2013). Differential sex responses were also suggested to be a result of oestrogen providing cellular protection and thus, decreasing the necessity for translation of HSP72 in females. Although, stress-mediated sex specific differences in the HSP72 have been seen (Gillum et al., 2013; Morton et al., 2009), they have not been examined at an mRNA level across the course of controlled hyperthermia HA.

Determination of Hsp72 mRNA transcription in females would facilitate identification of whether the inhibited HSP72 response resulted from absent gene signalling, or mitigated protein translation, potentially due to elevated oestrogen (Gillum et al., 2013; Morton et al., 2009). Absence of data in female populations could be problematic for practitioners who may adopt HA protocols that are informed by mechanistic cellular adaptations from male only cohorts (Gibson et al., 2015a; Mee et al., 2015). This may reduce the magnitude to which females are protected against heat injury (Moran et al., 2006).

The aim of the current study was to determine whether the Hsp72 mRNA response during controlled hyperthermia HA, differed between males and females. It was hypothesised that the Hsp72 mRNA response will be lower in females compared to males across the course of controlled hyperthermia HA.

7.3.   Materials and methods

Participants

Based on *a priori* power analyses (3.17.1), four participants in each group would result in 95% probability of detecting a difference in Hsp72 mRNA across the course of controlled hyperthermia HA (Gibson et al., 2015a). In line with power analysis, and previous work in the area (Morton et al., 2009), ten (5M, 5F) athletes (3.2) (table 7.1) provided written informed consent to participate. This data was collected alongside Study 3 (Chapter 6), however participants were pre-determined prior to data collection for their involvement in both studies. The study was approved by the University of Brighton Research Ethics & Governance Committee and conducted in line with health and safety guidelines (3.1) and in accordance with the Declaration of Helsinki, 2013.

Table 7.1 Participant characteristics. Mean ± SD.

	Age	Stature	BM	Relative $\dot{V}O_2$ peak
	(yrs.)	(m)	(kg)	(mL.kg^{-1}.min^{-1})
Males (N = 5)	24 ± 7	1.75 ± 0.03	70.1 ± 5.1	46 ± 4
Females (N = 5)	20 ± 1	1.63 ± 0.09	57.1 ± 4.9	46 ± 4

Notes: BM, body mass; $\dot{V}O_2$ peak, peak oxygen uptake

Preliminary testing

During the first visit to the laboratory standardised anthropometric assessment was conducted (3.5.) followed by a graded exercise test. A graded exercise test was performed to determine $\dot{V}O_2$ peak (3.14.). Breath-by-breath expired metabolic gas was measured using online gas analysis (3.9.) and a capillary lactate sample was taken on completion of the test (3.11.). A regression equation was computed for the data obtained to calculate the required intensity (65% $\dot{V}O_2$ peak) for the experimental controlled hyperthermia HA exercise sessions (Appendix 4).

Experimental design

Testing was completed over a 12-d period. The timing of testing for female participants was conducted in line with the menstrual cycle standardisation guidelines (3.6.). Two participant were taking oral contraceptive: Yasmin (30 µg ethinylestradiol/ 3mg drospirenone). All trials were conducted in line with pre-trial diet and exercise standardisation (3.4.). In addition, confounding variables including smoking, caffeine, glutamine, alcohol, generic supplementation, prior thermal, hypoxic, and hyperbaric exposures were all controlled in line with previous work in the field (Taylor et al., 2011). Participants provided a urine sample upon arrival to ensure adequate hydration (3.4.). Volunteers performed two blocks of five consecutive days of controlled hyperthermia HA (3.14.) in a hot environment (3.7.) separated by 48 hr. Exercise was terminated in line with university guidelines (3.1.). There were no incidences of early termination of exercise within this study. Following a 20-min stabilisation period, measures of T_{re} (3.8.) and HR (3.9.) were recorded, and

participants entered the environmental chamber. Towel dried nude BM was also recorded to calculate SWR (3.12.). Throughout the HA sessions T_{re}, HR, were recorded at 5-min intervals.

Blood sampling, RNA extraction, and one-step reverse transcription quantitative polymerase chain reaction (RT-qPCR)

In line with previous work in the field (Gibson et al., 2015; Tuttle et al., 2015), venous blood samples were taken immediately before and immediately post exercise heat exposure on D1, D5 and D10 of controlled hyperthermia HA. All blood samples were drawn from the antecubital vein into 6 mL EDTA Vacuette tubes (Grenier BIO-One, Stonehouse, UK). 1 mL of venous blood was pipetted into 10 mL of 1 in 10 red blood cell lysis solution (10X red blood Cell Lysis Solution; Miltenyi Biotech, Bisley, UK). Samples were incubated for 15-min at room temperature then isolated via centrifugation at 400 g for 5-min and washed twice in 2 mL phosphate-buffered saline at 400 g for 5-min to isolate all leukocytes. Samples were then stored in a -83°C freezer and transported to Bedfordshire University for the remainder of the analysis which was performed by a trained laboratory technician. RNA was extracted via the previously validated acid guanidium thiocyanate–phenol–chloroform extraction method (Chomczynski and Sacchi, 1987). A standard RNA concentration was produced prior to PCr by adding a fixed amount of RNA to cell culture grade water to give an equal dilution. Quantity was determined at an optical density of 260 nm while quality was determined via the 260/280 and 260/230 ratios using a nanodrop spectrophotometer (NanoDrop 2000c; Thermo Scientific, Waltham, MA, USA).

Hsp72 mRNA was quantified using RT-QPCR. Primers (table 7.2) were designed using primer design software (Primer Quest and Oligoanalyzer; Integrated DNA Technologies, Coralville, IA, USA). 20 µL reactions containing 10 µL SYBR-Green RT-PCR Mastermix (Quantifast SYBRgreen Kit; Qiagen, Manchester, UK), 0.15 µL forward primer, 0.15 µL reverse primer, 0.2 µL reverse transcription mix (Quantifast RT Mix; Qiagen) and 9.5 µL sample (70 ng RNA/µL) were prepared in separate tubes. Each PCR reaction (Rotorgene Q; Qiagen) was then performed as follows: 10-min, 50°C (reverse transcription), 5-min 95°C (transcriptase inactivation and initial denaturation); followed by: 10-s, 95°C (denaturation), 30-s, 60°C (annealing and extension) for 40 cycles. Fluorescence was measured following each cycle as a result of the incorporation of SYBR green dye into the amplified PCR product. Melt curves (50 to 95°C; ramp protocol 5-s stages) were analysed for each reaction to ensure only the single gene of interest was amplified. The relative quantification of mRNA expression for each sample was assessed by determining the ratio between the cycle threshold value of the target mRNA and the cycle threshold values for β2-microglobuin fold change in relative mRNA expression was calculated using the 2-ΔΔCT method (Schmittgen and Livak, 2008).

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Table 7.2 Hsp72 mRNA primer sequences.

Gene	NCBI Accession #	Primer	Sequence (5'→3')	Amplitude length
B2- Microglobulin **(β2-M)**	NM_004048	Forward	CCGTGTGAACCATGTGACT	91
		Reverse	TGCGGCATCTTCAAACCT	
Hsp72	NM_005345	Forward	CGCAACGTGCTCATCTTTGA	198
		Reverse	TCGCTTGTTCTGGCTGATGT	

Notes: NCBI National Centre for Biotechnology Information

Statistical analysis

All data were first checked for normality using Shapiro-Wilk and corrected for sphericity using the Greenhouse Geisser method. A two way (3 x 2) mixed design analysis of variance (ANOVA) was performed to determine differences between the physiological and performance characteristics between D1, D5 and D10 in males and females (3.17.4.). A three-way (3 x 2 x 2) mixed design ANOVA was performed to identify differences between the Hsp72 mRNA, pre and post, on D1, D5, and D10 of controlled hyperthermia HA between males and females. When a main effect or interaction effect was found, results were followed up using a Bonferroni corrected post hoc comparison (3.17.6). Effect sizes [partial eta squared (np^2)] were calculated to analyse the magnitude and trends of the interventions (3.17.5.). All data were analysed using a standard statistical package (SPSS version 20.0, IBM, Armonk, New York, USA) and reported as mean ± SD. Statistical significance was accepted at the level of $p \leq 0.05$.

7.4. Results

Evidence of heat acclimation

Figure 7.1 presents the resting T_{re} and resting HR data for D1, D5 and D10 of controlled hyperthermia HA. There was a main effect of day on T_{re} rest ($F_{(2, 16)} = 11.219$, $p \leq 0.001$, $np^2 = 0.584$). No differences were observed from D1 to D5 ($p = 0.563$). However, T_{re} rest reduced from D1 to D10 ($p = 0.027$) and from D5 to D10 ($p = 0.003$). There was no interaction effect between day and sex

on T_{re} rest ($F_{(2, 16)}$ = 3.287, p =0.064, np^2 = 0.291). However, the mean reduction in T_{re} rest from D1 to D5 was -0.28 ± 0.21°C in males whereas in females there were no changes (+0.08 ± 0.24°C). The mean reduction in T_{re} rest from D5 to D10 was -0.44 ± 0.22°C in females whereas in males there were no changes (-0.09 ± 0.06°C).
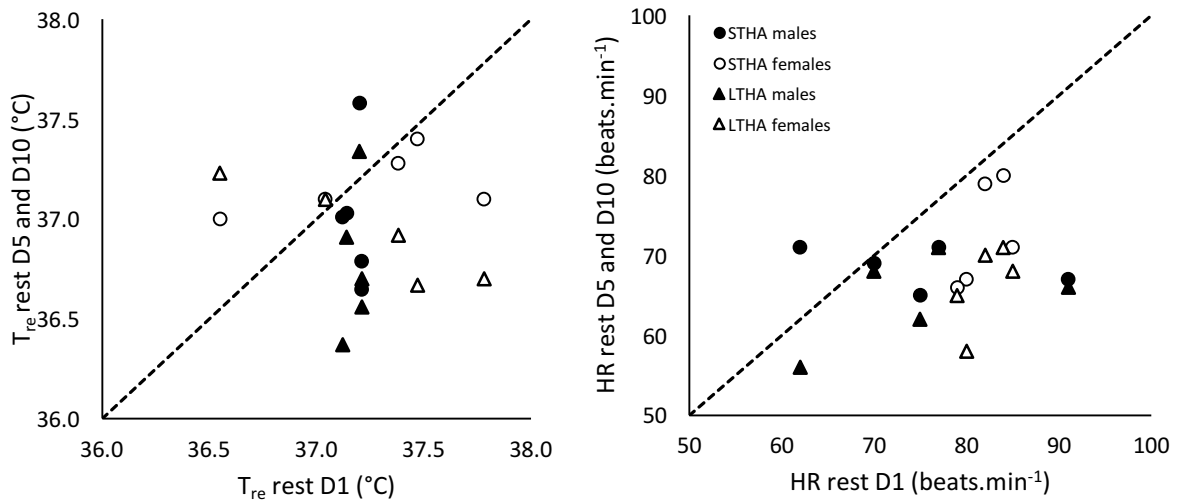
Figure 7.1 Resting rectal temperature (T_{re}) (left) and resting heart rate (HR) (right) on D1, D5 and D10 of heat acclimation. Dotted line represents line of equality. N = 10 (5M, 5F).

Notes: Males (closed markers) Females (open markers) STHA (D1 to D5) (o) LTHA (D1 to D10) (Δ).

There was a main effect of day on HR rest ($F_{(2, 16)}$ = 15.227, p ≤ 0.001, np^2 = 0.656). HR rest reduced from D1 to D5 (p = 0.040) from D1 to D10 (p = 0.008) and from D5 to D10 (p = 0.008). There was no interaction effect between day and sex on HR rest ($F_{(2, 16)}$ = 0.383, p = 0.688, np^2 = 0.046). However, the mean reduction in HR rest from D1 to D5 was greater in male participants (9 ± 10 beats.min^{-1}) compared with females (6 ± 5 beats.min^{-1}). The mean reduction in HR rest from D5 to D10 was greater in female participants (6 ± 4 beats.min^{-1}) compared with males (3 ± 2 beats.min^{-1}).

Hsp 72 mRNA responses to heat acclimation between sexes

Figure 7.2 presents the means ± SD for Hsp72 mRNA, pre and post on D1, D5, and D10 of controlled hyperthermia HA between males and females. There was a main effect of time on Hsp72 mRNA response ($F_{(1,8)}$ = 32.998, p ≤ 0.001, np^2 = 0.805). Hsp 72 mRNA increased pre to post on D1 (1.7 ±

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0.8 fold, 3.9 ± 1.8 fold; p = 0.003), D5 (1.6 ± 0.8 fold, 3.5 ± 1.7 fold; $p \leq 0.001$), and D10 (2.0 ± 0.7 fold, 3.0 ± 1.4 fold; p = 0.050). There was no interaction effect between time and sex ($F_{(2, 16)}$ = 1.027, p = 0.381, np^2 = 0.114). Figure 7.3 presents the individual Hsp72 responses pre and post D1, D5, and D10 relative to D1 pre of controlled hyperthermia HA. The increase in Hsp72 mRNA from pre to post on controlled hyperthermia HA D1, D5, and D10 was similar between males (D1, 1.8 ± 1.5 fold; D5, 2.0 ± 1.0 fold; D10, 1.1 ± 0.4 fold) and females (D1, 2.6 ± 1.8 fold; D5, 1.8 ± 1.4 fold; D10, 0.9 ± 1.9 fold).

There was no main effect of day on Hsp72 mRNA response ($F_{(1, 8)}$ = 0.052, p = 0.826, np^2 = 0.006). There was no interaction effect between day and sex on Hsp72 mRNA response ($F_{(2, 16)}$ = 1.027, p = 0.381, np^2 = 0.114). There was no interaction effect between time, day and sex on Hsp72 mRNA response ($F_{(2, 16)}$ = 0.479, p = 0.628, np^2 = 0.057).
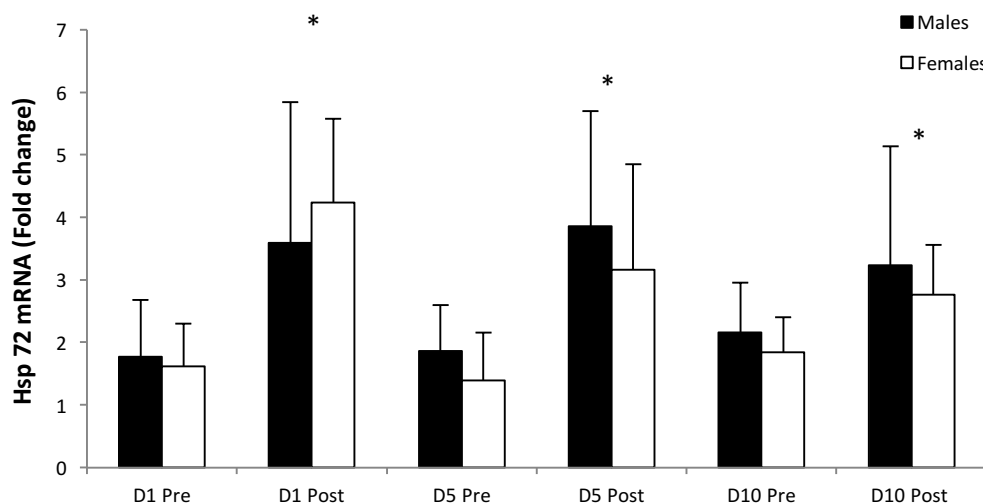
Figure 7.2 Hsp72 mRNA pre and post session on D1, D5, and D10 of heat acclimation in males and females. Mean ± SD. N = 10 (5M, 5F).

Notes: *Denotes significant pre to post difference within session in all participants ($p \leq 0.05$)
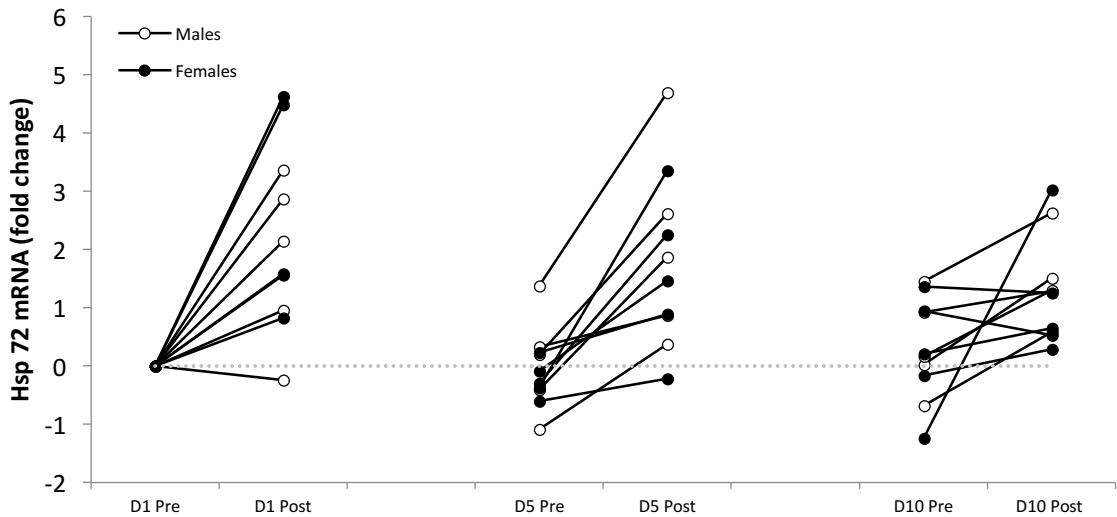
Figure 7.3 Hsp72 mRNA pre to post session on D1, D5 and D10 relative to D1 pre of heat acclimation in males (open circles) and females (closed circles). N = 10 (5M, 5F).

Comparable heat acclimation sessions

Table 7.3 presents the mean ± SD for performance and physiological variables during D1, D5, and D10 of controlled hyperthermia HA for males and females. There was no main effect of day on mean T_{re} ($F_{(2, 16)}$ = 2.536, p = 0.143, np^2 = 0.241). Furthermore, there was no interaction effect between day and sex on mean T_{re} ($F_{(2, 16)}$ = 1.880, p = 0.185, np^2 = 0.190). There was no main effect of day on T_{re} change ($F_{(2, 16)}$ = 3.042, p = 0.076, np^2 = 0.275). Furthermore, there was no interaction effect between day and sex on T_{re} change ($F_{(2, 16)}$ = 0.234, p = 0.794, np^2 = 0.028). There was no main effect of day on mean HR ($F_{(2, 16)}$ = 0.488, p = 0.623, np^2 = 0.057). Furthermore, there was no interaction effect between day and sex on mean HR ($F_{(2, 16)}$ = 0.242, p = 0.788, np^2 = 0.029).

There was a main effect of day on mean power ($F_{(2, 16)}$ = 11.608, $p \leq 0.001$, np^2 = 0.592). Mean power was higher on D5 ($p \leq 0.001$) and D10 (p = 0.015) compared to D1. There were no differences between D5 and D10 (p = 1.000). There was no interaction effect between day and sex on mean power ($F_{(2, 16)}$ = 0.663, p = 0.529, np^2 = 0.076).

Table 7.3 Physiological and performance measures on D1, D5 and D10 of controlled hyperthermia heat acclimation. Mean ± SD.

	D1		D5		D10	
	Males	**Female**	**Males**	**Females**	**Males**	**Females**
Mean T_{re} (°C)	38.18 ± 0.20	38.32 ± 0.17	38.08 ± 0.18	38.11 ± 0.12	38.17 ± 0.10	38.26 ± 0.90
T_{re} change (°C)	1.52 ± 0.19	1.52 ± 0.47	1.56 ± 0.38	1.40 ± 0.22	1.80 ± 0.28	1.80 ± 0.28
Mean HR (beats.min^{-1})	146 ± 14	152 ± 9	145 ± 8	146 ± 10	143 ± 12	148 ± 9
Power (W)	87 ± 16	62 ± 13	121 ± 16	81 ± 20	127 ± 25	87 ± 13
Intensity (% $\dot{V}O_2$ peak)	38 ± 5	35 ± 8	50 ± 6	44 ± 7	52 ± 11	53 ± 14

Notes: HR, heart rate; T_{re}, rectal temperature; $\dot{V}O_2$ peak, peak oxygen uptake

There was a main effect of day on relative exercise intensity ($F_{(2, 16)}$ = 21.593, $p \leq 0.001$, np^2 = 0.730). Relative exercise intensity was higher on D5 ($p \leq 0.001$) and D10 ($p \leq 0.001$) compared to D1. There were no differences between D5 and D10 (p = 0.221). There was no interaction effect between day and sex on relative exercise intensity ($F_{(2, 16)}$ = 0.1.034, p = 0.378, np^2 = 0.114).

7.5.   Discussion

This is the first study to compare Hsp72 mRNA expression in males and females across the course of controlled hyperthermia HA. In contrast to our hypothesis, this experiment demonstrates that the Hsp72 mRNA response is similar between males and females on D1, D5, and D10 of a controlled hyperthermia HA. This suggests that sex differences in HSP following acute (Gillum et al., 2013) and chronic (Morton et al., 2009) *in vivo* exercise bouts are due to post transcriptional events. Controlled hyperthermia HA resulted in typical phenotypic adaptations, evidenced by reduction in resting T_{re} and HR across the course of controlled hyperthermia HA. Males and females demonstrated equal physiological responses (ΔT_{re}, mean T_{re} and HR) to each HA session where Hsp72 mRNA was measured. Accordingly, equality of these endogenous stimuli, both between groups, and throughout HA elicited equal increases in Hsp72 mRNA transcription. Comparable transcription of Hsp72 mRNA between males and females, suggests endogenous stimuli which induce the HSR are the most important criteria for increasing Hsp72 mRNA (Gibson et al., 2015a), with no sex dependent inhibition or amplification in transcription.

In the current study, HA produced a significant increase in Hsp72 mRNA providing further evidence that the controlled hyperthermia HA method presents a sufficient endogenous stress to surpass the minimum requirement to elicit increased transcription of Hsp72 mRNA, in both males and females, at the onset and culmination of discrete and repeated bouts of exercise-heat stress. Gibson et al. (Gibson et al., 2015a) reported an increase in Hsp72 mRNA pre to post on D1 (1.9 ± 0.6 fold, 4.9 ± 1.1 fold), D5 (2.3 ± 0.8 fold, 5.3 ± 2.5 fold) and D10 (2.1 ± 0.7 fold, 4.3 ± 1.3 fold) during a 10-d controlled hyperthermia HA protocol in male participants. The data in the current study demonstrates females have a comparable magnitude of response to males. Accordingly, this data provides mechanistic support for practitioners prescribing controlled hyperthermia HA for female athletes. Sustained increases in Hsp72 mRNA throughout the HA, further demonstrates the continued stimulation of the pathways responsible for thermotolerance, i.e. the equality of HSR, in both males and females.

Previously, a greater HSP72 increase has been reported in males compared to females (Gillum et al., 2013; Morton et al., 2009); however, these studies measured the protein (HSP) whereas the

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current study measured the gene (Hsp mRNA). Interestingly, the current data contradicts Paroo et al. (Paroo et al., 2002) findings, who reported a sex specific HSR at the level of protein and mRNA; with male rats having a significantly higher HSP70 (200% of control) and Hsp70 mRNA (+900% of control) response following 60-min of exercise at 70% $\dot{V}O_2$ max when compared with females (HSP70 = 100% of control; Hsp70 mRNA = 450% of control). Paroo et al. (Paroo et al., 2002) did however provide mechanistic evidence for an interaction between HSP70, Hsp70 mRNA and oestrogen. Ovariectomized female animals treated with a placebo demonstrated equivalent increases in HSP72 (+150% of control) and Hsp72 mRNA (+1,200% of control) to males, whilst endogenous oestrogen returned the typical inhibited female HSR response (HSP70 100% of control; Hsp70 mRNA 300% of control). Methodologically, Paroo et al. (Paroo et al., 2002) implemented the northern blotting technique which is less sensitive than the RT- QPCR technique used in the current study, potentially explaining the non-significantly increased Hsp72 mRNA.

Elevated oestrogen affords cellular protection (Shinohara et al., 2004) and thus, this cytoprotective pathway may inhibit changes in HSP72 translation (Bombardier et al., 2009). It is likely, that oestrogen most greatly mediates post Hsp72 mRNA transcriptional changes which inhibit the translation of HSP72. The mechanism by which oestrogen attenuates HSR may be mediated through its indirect antioxidant properties by stabilising cellular membranes and attenuating oxidative stress; such an effect could protect thermal sensitive cells against exercise-induced damage, and thereby result in a blunted HSP72 response (Paroo et al., 2002). The lack of observed difference between males and females in the current study, may be a result of low oestrogen concentrations, which may not have been sufficient to exert an antioxidant effect.

7.6.   Limitations

Future work should involve the measurement of HSP72 protein alongside Hsp72 mRNA across the course of controlled hyperthermia HA in males and females, to help underpin the true effect of sex on the HSR; the absence of which is a limitation of the present study. Furthermore, oestrogen is reported to have a dose dependent inhibition of HSP72 expression at the transcription level (Shinohara et al., 2004). Future work should investigate the HSR, and subsequent HSP72 and Hsp72 mRNA response to discrete and repeated bouts of exercise-heat stress in high and low oestrogen conditions and in post-menopausal women, who naturally have lower oestrogen concentrations. This information would help practitioners implement controlled hyperthermia HA strategies that ensure an optimal stimulus for cellular adaptation.

7.7. Conclusion

Males and females have equal Hsp72 mRNA expression throughout 10-d of controlled hyperthermia HA. This suggests that there are no differences in the endogenous criteria to transcribe Hsp72 mRNA via the HSR between males and females. Differences in basal HSP72 observed elsewhere are therefore likely to result from inhibited protein translation, potentially due to the influence of oestrogen.

This study explored the cellular adaptations in males and females across the course of HA. Since no sex differences in the cellular adaptation were observed, Chapter 8 will take a more applied approach to investigate whether the phenotypic adaptations can be accelerated during STHA.

8. Passive heat exposure preceding short-term heat acclimation accelerates adaptation in females

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The initial experiments have established the RHTT is repeatable (Study 1), mode specific and sensitive to changes in thermoregulatory responses (Study 2). In Study 3 (Chapter 6) it became apparent that females' responses to HA differed from those of males, but this was not due to differences in thermotolerance (Study 4). This study will explore a potential method to accelerate females' response to STHA using the RHTT to monitor changes.

8.1. Abstract

The current study assessed whether STHA preceded by a passive heat accelerated heat adaptation in females. Nine females performed two HA interventions in a randomised order separated by 7-wks. A RHTT was performed 24-hr pre (RHTT1) and 24-hr post (RHTT2) STHA. During the HA_{CH} intervention participants preceded the HA session by 20-min seated rest in a temperate environment wearing shorts and a sports bra (20°C, 40% RH; WBGT = 19°C). During the HA_{sauna} intervention participants preceded the HA session by 20-min seated rest in a hot environment (50°C, 30% RH; WBGT > 40°C), wearing shorts and sports bra and a 100% vinyl sauna suit. T_{re} rest (-0.28 ± 0.16°C), peak T_{re} (-0.42 ± 0.22°C), HR rest (-10 ± 4 beats.min^{-1}), peak HR (-12 ± 7 beats.min^{-1}), T_{re} at the onset of sweating (-0.29 ± 0.17°C) reduced ($p \leq 0.001$) from RHTT1 to RHTT2 in HA_{sauna}; but not HA_{CH}. TS ($p = 0.002$) and RPE ($p \leq 0.001$) reduced from RHTT1 to RHTT2 in HA_{sauna}; but not HA_{CH}. Plasma volume expansion was greater following HA_{sauna} (9.3 ±7.6%) compared to HA_{CH} (1.3 ± 5.0%) ($p = 0.013$). Sweat rate increased ($p \leq 0.001$) and sweat sodium chloride (NaCl) concentration reduced ($p = 0.006$) in both conditions. HA_{sauna} was effective in attenuating thermoregulatory, cardiovascular and perceptual strain; this was not achieved following HA_{CH}. This novel HA strategy provides a time-efficient method to stimulate HA in females.

8.2. Introduction

Heat acclimation improves thermal comfort (Gonzalez and Gagge, 1976), submaximal exercise performance, and increases maximal aerobic capacity in the heat (Lorenzo et al., 2010). The benefits arise from enhanced sudomotor and skin blood flow responses, PV expansion, cardiovascular stability and an improved fluid balance (Poirier et al., 2015). As a result, HA is the consensus recommendation strategy to attenuate the physiological strain associated with training

and competing in the heat (Racinais et al., 2015). However, there remains a paucity of data concerning best practice for HA in females; consequently, female athletes are implementing HA strategies based on data collected from male participants. The implications of this are that females may be adopting sub-optimal strategies that provide little benefit to alleviating physiological strain.

Heat acclimation is often separated into STHA (≤ 8-d) and LTHA (≥ 18-d). Approximately 70% of maximal adaptations occur following STHA evidenced by reductions in thermoregulatory and cardiovascular strain (Poirier et al., 2015), combined with an improved sudomotor function (Buono et al., 2009b). Study 3 (Chapter 6) presented evidence to suggest that females achieve only partial adaptation following STHA, evidenced with an increase in whole body SWR despite no changes in other classic markers of HA. Moreover, females required LTHA to establish reductions in cardiovascular and thermoregulatory strain. These findings are further supported by the work of Sunderland et al. (2008) who reported no difference in T_{re} and HR following 4-d HA in females. The absence of evidence concerning effective STHA protocols in females is problematic for athletes, since STHA is a preferred regime as it provides less disruption from quality training prior to competition than more traditional HA protocols (Gibson et al., 2015b).

Recently, the concept of combining passive heat exposures with temperate training has received considerable interest. Scoon et al. (2007) and Stanley et al. (2014) reported a 7.1% and 17.8% PV expansion, respectively, when temperate training was followed by a 30-min sauna (87°C) exposure. Furthermore, Stanley et al. (2014) reported that PV reached peak expansion (+17.8%) following four exposures; highlighting the effectiveness of short-term sauna exposure. Multiple sauna exposures appear to specifically augment PV expansion, which may contribute to improved myocardial efficiency (Horowitz et al., 1986b), increased ventricular compliance (Horowitz et al., 1986a) and improved maximal cardiac output (Lorenzo et al., 2010); all of which may translate into improved physical performance. Thus, it seems plausible that combining passive heat exposure with HA may result in an accelerated adaptation in females through targeting PV expansion.

Despite observing an increase in SWR in females following STHA in Study 3 (Chapter 6), SWR remained 10% lower than males' baseline SWR. These findings were evident even with females potentially working at a higher metabolic heat production and thus evaporative requirement, due to the fixed intensity protocol. During uncompensable heat stress, the evaporative requirement for heat balance exceeds the maximum possible heat loss (Montain et al., 1994). Protective clothing exacerbates the challenge of thermoregulation because of limited water vapour permeability across the clothing layers, decreasing the rate of heat exchange (Cheung et al., 2000). When wearing clothing that restricts sweat evaporation, much of the sweat may become absorbed or

trapped within the clothing, altering the rate of heat transfer. Thus, it seems plausible that stimulating sudomotor function whilst restricting sweat evaporation prior to a HA session through the use of protective clothing may provide more complete sudomotor adaptation due to greater thermal burden, without additional training load.

STHA offers a more ecologically valid method for athletes compared with LTHA, thus it is essential to establish effective STHA methods for a female population whilst limiting the exercise stimulus. This study aimed to investigate whether STHA, preceded by a passive heat exposure, designed to target PV expansion and sudomotor adaptation, accelerated heat adaptation in females. The primary hypothesis was that STHA preceded by a passive heat exposure (HA_{sauna}) will result in a greater plasma volume expansion in females compared with HA alone. The secondary hypothesis is that HA_{sauna} will produce a greater sudomotor adaptation in females compared with HA alone.

8.3.   Materials and methods

Participants

Nine female athletes (3.2) (Mean ± SD; age, 22 ± 4 yrs.; BM, 59.33 ± 11.55 kg; stature, 1.64 ± 0.01 m; BSA, 1.6 ± 0.2 m^2; sum of 4 skin folds, 49 ± 11 mm; $\dot{V}O_2$ peak, 50 ± 4 $mL.kg^{-1}.min^{-1}$) provided written informed consent to participate in the current study which was approved by the University of Brighton Research Ethics & Governance Committee and conducted in line with health and safety guidelines (3.1) and in accordance with the Declaration of Helsinki, 2013.

Preliminary testing

During the first visit to the laboratory standardised anthropometric assessment was conducted (3.5.). A graded exercise test using a motorised treadmill was performed to determine lactate threshold and $\dot{V}O_2$ peak (3.15.). In addition, $\dot{V}O_2$ peak was determined using a cycle ergometer (3.14.). A regression equation was computed for the data obtained to calculate the required intensity (65% $\dot{V}O_2$ peak) for the experimental controlled hyperthermia HA exercise sessions (Appendix 4). Breath-by-breath expired metabolic gas was measured using online gas analysis (3.9.). A capillary lactate sample was collected and analysed at the end of each stage during the lactate threshold test and on completion of the $\dot{V}O_2$ peak test (3.11.).

Experimental design

The timing of testing for female participants was conducted in line with the menstrual cycle standardisation guidelines (3.6.). Four participants were taking oral contraceptive: two used Yasmin (30 μg ethinylestradiol/ 3mg drospirenone) and two used Microgynon (30 μg ethinylestradiol/ 150 μg levonorgestrel). Hormonal status was confirmed by taking a venous blood sample on the first and final day of each experimental intervention. All trials were conducted in line with pre-trial diet and exercise standardisation (3.4.). Participants provided a urine sample upon arrive to ensure adequate hydration (3.4.). All experimental trials were performed in a hot environment (3.7.). Exercise was terminated in line with university guidelines (3.1). There were no incidences of early termination of exercise during the RHTT within this study.

Running heat tolerance test

Participants performed two RHTT (3.16.) on a motorised treadmill (3.15.) separated by five HA sessions. The first RHTT was performed 24-hrs prior to beginning HA (RHTT1) and the second 24-hrs following 5-d HA (RHTT2). Following a 20-min stabilisation period in a temperate environment (3.7.), measures of T_{re}, T_{sk} (3.8.), HR (3.9.), RPE, and TS (3.13.) were recorded, and participants entered the environmental chamber. Towel dried nude BM was also recorded to calculate SWR (3.12.). Throughout the test T_{re}, T_{sk}, and HR were recorded at 5-min intervals and RPE and TS at 10-min intervals. Mean T_{sk} (Equation 2.4) was subsequently calculated. Breath-by-breath expired metabolic gas was measured using online gas analysis throughout the RHTT (3.9.). Relative metabolic heat production ($W.kg^{-1}$) was calculated in accordance with the guidelines of Cramer and Jay (2014).

Forearm sweat samples were collected during each of the RHTT using a Macroduct sweat collector (Wescor, Logan, UT). Immediately prior to entering the chamber the participants' skin was cleaned with deionized water and dried immediately before securing the Macroduct sweat collector. The collectors were held in place on the midpoint of the anterior forearm by a Velcro strap, which prevented leakage and sample contamination. The Macroduct collector has a surface area of 5.2 cm^2. Sweat secreted by the sweat glands is forced from the ducts under hydraulic pressure and flows between the skin and the concave under surface of the Macroduct collector and into the micro bore tubing spiral. A small amount of blue dye is mixed with the secreted sweat prior to entering the spiral tubing to allow for visual identification of the onset of sweating. The forearm sweat samples were analysed in duplicate for sweat NaCl using a sweat conductivity analyser (Sweat Chek 3120, Wescor, UK).

Heat acclimation

In a randomised, cross-over design, participants completed two 5-d HA interventions (figure 8.1). The two interventions were separated by 7-wks. to ensure the decay of adaptation (Pandolf, 1998; Poirier et al., 2015). Following a 20-min stabilisation period, measures of T_{re} (3.8.), HR (3.9.), RPE and TS (3.13.) were recorded, and participants entered the environmental chamber. For the first intervention (HA$_{CH}$) participants entered into a temperate environment (3.7.) whilst wearing shorts and a sports bra, for a further 20-min seated rest. For the second intervention (HA$_{sauna}$) participants entered into a hot environment (50°C, 30% RH; WBGT = 40°C) whilst wearing a 100% vinyl sauna suit to perform a further 20-min seated rest. The vinyl sauna suit covered the majority of the body with only the hands, feet, and head exposed. Following this, participants in both interventions completed a controlled hyperthermia HA session (3.14.) wearing shorts and a sports bra in a hot environment (3.7.). All HA sessions were completed on a cycle ergometer (3.14.).
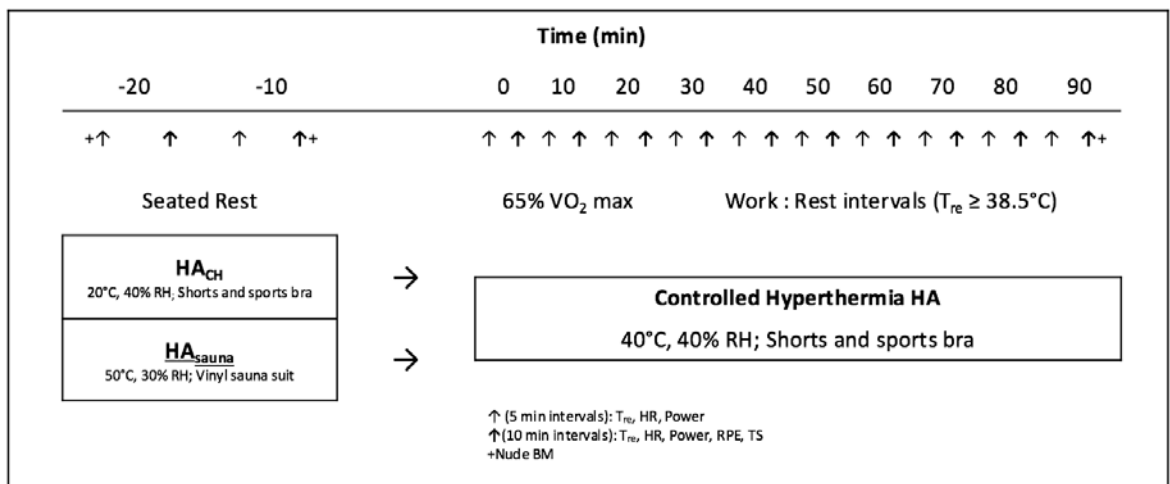
Figure 8.1 Schematic of the experimental design.

Notes: BM, body mass; HA, heat acclimation; HR, heat rate; RH, relative humidity; T_{re}, rectal temperature.

Blood sampling and analysis

Following 20-min of seated rest a capillary blood sample was taken from the fingertip prior to each of the RHTTs to estimate PV expansion using Equation 8.1 (Dill and Costill, 1974). Capillary blood samples were collected in a microcuvette in duplicate for the analysis of haemoglobin and analysed using B- Haemoglobin Photometer (Hemocue Ltd, Sweden). Capillary blood samples were collected in glass capillary tubes in duplicate for the analysis of haematocrit after being centrifuged at 11,800 rpm for 3-min (Haemotospin 1300 Centrifuge, Hawksley & Sons Ltd, UK).

Equation 8.1 Calculation for change in Plasma Volume (ΔPV)

$$\Delta PV, \% = 100 \, (PV_A - PV_B)/ \, PV_B$$

Where: ΔPV, change in plasma volume; PVA, pre HA plasma volume; PVB, post HA plasma volume

Venous blood samples were taken in a rested state prior to the RHTTs and on D22 of the menstrual cycle. A 6 mL whole blood sample was drawn from the antecubital fossa. Each sample was divided into two EDTA tubes (Starstedt, Germany). Whole blood samples were centrifuged (Eppendorf 5702R Centrifuge, Eppendorf UK Ltd, UK) at 4400 rpm for a period of 10-min. Plasma was pipetted (Eppendorf research pipettes, Eppendorf UK Ltd, UK) into 1.5 mL microtubes (Western laboratory science, UK) and stored at -86°C (Sanyo VIP series, Sanyo Electric Biomedical Co Ltd, Japan) until analysis. To perform the analysis, commercially available 17β-estradiol (ab108667) and Progesterone (ab108670) immunoenzymatic assay kits (Abcam plc, UK) were used. Quantitative determination of plasma concentrations of 17β-estradiol and Progesterone were performed according to the manufacturer's guidelines. Incubation of the 96 well kit, including the required quality control standards was performed on an orbital platform shaker (Titramax 1000, Heidolp UK, UK) at 1.5 mm orbital vibration and read by a microplate reader using absorption at 450 nm (elx800, BioTek UK, UK). The intra-assay and inter-assay variability, as described by the manufacturer was 9% and 10% for 17β-estradiol and 4% and 9.3% for progesterone respectively. The lowest detectable concentration of 17β-estradiol and progesterone, as described by the manufacturer was 8.68 pg.mL^{-1} and 0.05 ng.mL^{-1}, respectively.

Statistical analyses

All data were first checked for normality using the Shapiro Wilk test and corrected for sphericity using the Greenhouse Geisser method. A two way (2 x 2) repeated measures ANOVA was performed to identify differences between the physiological and perceptual responses during RHTT1 and RHTT2 between the HA$_{CH}$ and HA$_{sauna}$ conditions and the physiological and HA method characteristics during D1 and D5 of HA$_{CH}$ and HA$_{sauna}$ (3.17.4.). When a main effect or interaction effect was found, results were followed up using a Bonferroni corrected post hoc comparison (3.17.6.). Partial eta squared (np^2) was used as a measure of effect size (3.17.5.). A paired samples t-test was performed on the PV data (3.17.4.). All data were analysed using a standard statistical package (SPSS version 20.0, IBM, Armonk, New York, USA) and reported as mean ± SD. Statistical significance was accepted at the level of $p \leq 0.05$.

8.4.   Results

HA protocol during HA$_{CH}$ and HA$_{sauna}$ (D1 - D5)

Table 8.1 presents the mean ± SD data for the HA protocol and physiological responses for both conditions. All participants completed five HA sessions on consecutive days in both conditions. Duration spent with a T$_{re}$ ≥ 38.5°C was on average 52 ± 11 min across all sessions in both conditions. Duration with a T$_{re}$ ≥ 38.5°C reduced in both conditions from D1 to D5 ($F_{(1, 8)}$ = 8.147, p = 0.021, np^2 = 0.505), however, no interaction effect between day and condition on duration with a T$_{re}$ ≥ 38.5°C was observed ($F_{(1, 8)}$ = 1.488, p = 0.257, np^2 = 0.157). No main effect for day ($F_{(1, 8)}$ = 5.139, p = 0.053, np^2 = 0.391), or interaction effect between day and condition on exercise duration was observed ($F_{(1, 8)}$ = 2.250, p = 0.172, np^2 = 0.220).

Relative exercise intensity increased in both conditions from D1 to D5 ($F_{(1, 8)}$ = 5.586, p = 0.046, np^2 = 0.411), however, no interaction effect between day and condition was observed ($F_{(1, 8)}$ = 2.967, p = 0.123, np^2 = 0.271). Total work increased in both conditions from D1 to D5 ($F_{(1, 8)}$ = 5.593, p = 0.046, np^2 = 0.411), however, no interaction effect between day and condition was observed ($F_{(1, 8)}$ = 3.215, p = 0.111, np^2 = 0.287).

Physiological responses during HA$_{CH}$ and HA$_{sauna}$ (D1 – D5)

There was an interaction effect between day and condition on T$_{re}$ rest ($F_{(1, 8)}$ = 5.395, p = 0.049, np^2 = 0.403). No differences were observed between conditions on T$_{re}$ rest on D1 (p = 0.57). T$_{re}$ rest reduced from D1 to D5 (p = 0.006) in HA$_{sauna}$, but not in HA$_{CH}$ (p = 0.268). There was an interaction effect between day and condition on mean T$_{re}$ ($F_{(1, 8)}$ = 6.380, p = 0.035, np^2 = 0.444). No differences were observed between conditions for mean T$_{re}$ on D1 (p = 0.167). Mean T$_{re}$ reduced from D1 to D5 (p = 0.016) in the HA$_{CH}$ condition, but not in HA$_{sauna}$ (p = 0.132). There was an interaction effect between day and condition on HR rest ($F_{(1, 8)}$ = 5.924, p = 0.041, np^2 = 0.425). No differences were observed between conditions for HR rest on D1 (p = 0.251). HR rest reduced to a greater extent from D1 to D5 in HA$_{sauna}$ (p ≤ 0.001) compared to HA$_{CH}$ (p = 0.009). No main effect was observed for day on mean HR ($F_{(1, 8)}$ = 2.837, p = 0.131, np^2 = 0.262), furthermore, no interaction effect between day and condition was observed ($F_{(1, 8)}$ = 0.059, p = 0.814, np^2 = 0.007).

SWR was higher in the HA$_{sauna}$ condition on D1 and D5 compared with HA$_{CH}$ ($F_{(1, 8)}$ = 21.719, p = 0.002, np^2 = 0.731). Similarly, SWR$_{BSA}$ was higher in the HA$_{sauna}$ condition on D1 and D5 compared with HA$_{CH}$ ($F_{(1, 8)}$ = 23.710, p ≤ 0.001, np^2 = 0.748) (table 8.1).

Table 8.1 Heat acclimation protocol and physiological responses during D1 and D5 of HA_{CH} and HA_{sauna}. Mean ± SD.

	HA_{CH} D1	HA_{CH} D5	HA_{sauna} D1	HA_{sauna} D5
Duration ≥ 38.5°C (min)	54 ± 13	47 ± 8*	61 ± 7	47 ± 13*
Exercise Duration (min)	63 ±14	69 ± 14	59 ± 15	71 ± 12
Mean Exercise Intensity (%$\dot{V}O_2$ peak)	51 ± 14	61 ± 4*	63 ± 5	65 ± 10*
Total work (kJ)	341 ± 108	365 ± 134*	346 ± 123	423 ± 127*
T_{re} rest (°C)	37.30 ± 0.13	37.22 ±0.21	37.33 ± 0.13	37.07 ± 0.19*
Mean T_{re} (°C)	38.41 ± 0.17	38.19 ± 0.10*	38.31 ± 0.14	38.20 ± 0.19
HR rest (beats.min^{-1})	70 ± 5	66 ± 7*	73 ± 5	64 ± 5*
Mean HR (beats.min^{-1})	143 ± 9	140 ± 11	145 ± 7	142 ± 8
SWR (g.h^{-1})	702 ± 116	782 ± 222	928 ± 226[+]	1009 ± 290 [+]
SWR_{BSA} (g.hr^{-1}.m^2)	428 ± 45	474 ± 103	568 117[+]	624 190[+]

Notes: HA, heat acclimation; HR, heart rate; SWR, sweat rate; SWR_{BSA}, sweat rate relative to body surface area; T_{re}, rectal temperature; $\dot{V}O_2$ peak; peak oxygen uptake. *Denotes significant difference within condition from D1 to D5. +Denotes significant difference between conditions on D1 or D5.

Plasma concentration of 17β Estradiol and Progesterone

Figure 8.2 presents the plasma concentrations of 17β-estradiol and progesterone. There was a main effect of day on plasma concentrations of 17β-estradiol ($F_{(4, 32)}$ = 55.484, $p ≤ 0.001$, np^2 = 0.874). There were no differences between the RHTT1 and RHTT2 in both conditions. However, 17β-estradiol was higher on D22 compared with RHTT1 and RHTT2 in both conditions. There was a main effect of day on plasma concentrations of progesterone ($F_{(4, 32)}$ = 5.983., $p = 0.04$, np^2 = 0.428). There were no differences between the RHTT1 and RHTT2 in both conditions. However, progesterone was higher on D22 compared with RHTT1 and RHTT2 in both conditions. None of the experimental sessions had to be withdrawn or repeated based on blood sample results.
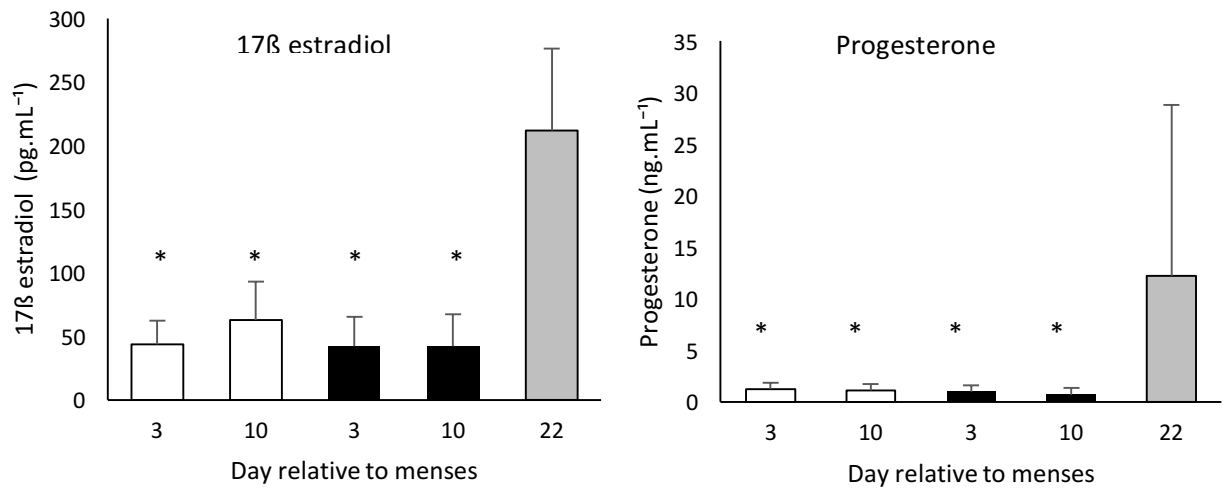
Figure 8.2 Plasma concentrations of 17ß-estradiol and progesterone prior to RHTT1 (D3) and RHTT2 (D10) in the HA$_{CH}$ intervention (white bars) and HA$_{sauna}$ intervention (black bars) and D22 of the menstrual cycle (grey bar). Mean ± SD. N = 9.

Notes: * denotes significant difference to D22.

Thermoregulatory responses

Table 8.2 presents the mean ± SD for the thermoregulatory, cardiovascular and perceptual variables during RHTT1 and RHTT2 in both conditions. There was an interaction effect between RHTT and condition on T_{re} rest ($F_{(1, 8)}$ = 24.636, $p ≤ 0.001$, np^2 = 0.755). No differences were observed in T_{re}

rest between conditions during RHTT1 ($p = 0.528$). T_{re} rest reduced from RHTT1 to RHTT2 in HA_{sauna} (-0.28 ± 0.16°C; $p \leq 0.001$); but not HA_{CH} (-0.07 ± 0.17°C; $p = 0.277$) (figure 8.3). There was an interaction effect between RHTT and condition on peak T_{re} ($F_{(1, 8)} = 18.951$, $p = 0.002$, $np^2 = 0.703$). No differences were observed in peak T_{re} between conditions during RHTT1 ($p = 0.888$). Peak T_{re} reduced from RHTT1 to RHTT2 in HA_{sauna} (-0.42 ± 0.23°C $p \leq 0.001$), but not HA_{CH} (-0.05 ± 0.17°C; $p = 0.380$). There was no interaction effect between RHTT and condition on T_{re} change ($F_{(1, 8)} = 2.274$, $p = 0.170$, $np^2 = 0.221$).

There was an interaction effect between RHTT and condition for peak T_{sk} ($F_{(1, 8)} = 7.409$, $p = 0.026$, $np^2 = 0.481$). No differences were observed in peak T_{sk} between conditions during RHTT1 ($p = 0.091$). Peak T_{sk} reduced from RHTT1 to RHTT2 in HA_{sauna} (-0.89 ± 0.86°C; $p = 0.015$), but no HA_{CH} (+0.03 ± 0.58°C; $p = 0.893$).
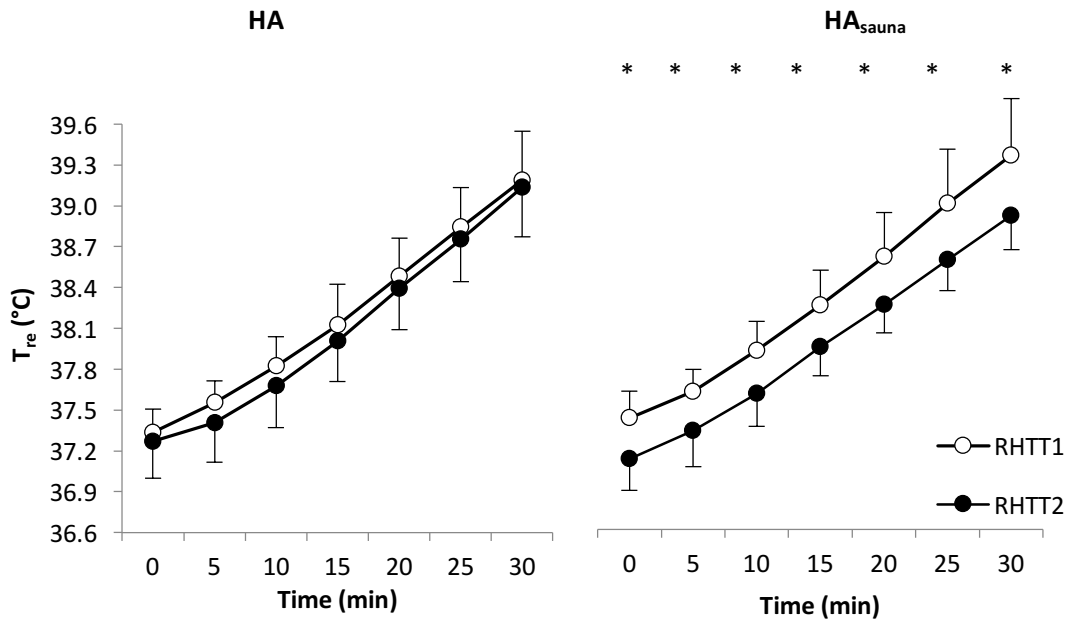
Figure 8.3 Rectal temperature (T_{re}) at 5-min intervals during the RHTT for the HA_{CH} intervention (left) and the HA_{sauna} intervention (right). Open markers represent RHTT1 and the closed markers represent RHTT2. Mean ± SD. N = 9.

Notes: * Denotes significant difference between RHTT1 and RHTT2

Table 8.2 Physiological variables during baseline testing (RHTT1), and following 5-d HA_{CH} or HA_{sauna} (RHTT2). Mean ± SD.

	HA_{CH} RHTT1	HA_{CH} RHTT2	HA_{sauna} RHTT1	HA_{sauna} RHTT2
T_{re} rest (°C)	37.34 ± 0.17	37.27 ± 0.27	37.37 ± 0.19	37.09 ± 0.22*
T_{re} peak (°C)	39.19 ± 0.36	39.14 ± 0.36	39.20 ± 0.39	38.78 ± 0.24*
T_{re} change (°C)	1.85 ± 0.36	1.87 ± 0.32	1.83 ± 0.40	1.69 ± 0.24
Peak T_{sk} (°C)	37.26 ± 0.42	37.29 ± 0.44	37.62 ± 0.68	36.73 ± 0.69*
HR rest (beats.min^{-1})	70 ± 4	66 ± 8	73 ± 5	64 ± 5*
HR peak (beats.min^{-1})	191 ± 8	188 ± 9	192 ± 10	180 ± 8*
SWR (g.hr^{-1})	847 ± 354	1253 ± 388*	882 ± 378	1447 ± 287*
SWR_{BSA} (g.hr^{-1}.m^2)	520 ± 222	773 ± 263*	542 ± 242	890 ± 193*
TS	7.5 ± 0.5	7.5 ± 0.5	7.5 ± 0.5	7.0 ± 0.5*
RPE	19 ± 1	18 ± 2	19 ± 1	16 ± 2*

Notes: HR, heart rate; RHTT, running heat tolerance test; RPE, ratings of perceived exertion; SWR, sweat rate; SWR_{BSA}, sweat rate relative to body surface area; T_{re}, rectal temperature; T_{sk}, skin temperature; TS, thermal sensation. *Denotes significant difference to RHTT1.

Cardiovascular responses to HA$_{CH}$ and HA$_{sauna}$

There was an interaction effect between RHTT and condition on HR rest ($F_{(1, 8)}$ = 6.545, p = 0.035, np^2 = 0.447). No differences were observed in HR$_{rest}$ between conditions during RHTT1 (p = 0.172). HR rest reduced from RHTT1 to RHTT2 in HA$_{sauna}$ (-10 ± 4 beats.min^{-1}; p ≤ 0.001), but not HA$_{CH}$ (-4 ± 5 beats.min^{-1}; p = 0.464) (figure 8.4). There was an interaction effect between RHTT and condition on peak HR ($F_{(1, 8)}$ = 8.983, p = 0.017, np^2 = 0.406). No differences were observed in peak HR between conditions during RHTT1 (p = 0.657). Peak HR reduced from RHTT1 to RHTT2 in HA$_{sauna}$ (-12 ± 7 beats.min^{-1}; p ≤ 0.001), but not HA$_{CH}$ (-3 ± 4 beats.min^{-1}; p = 0.380).

PV expansion was greater in the HA$_{sauna}$ condition (9.3 ± 7.6 %) compared with the HA$_{CH}$ condition (1.3 ± 3.1%) ($t_{(8)}$ = -3.163, p = 0.013).
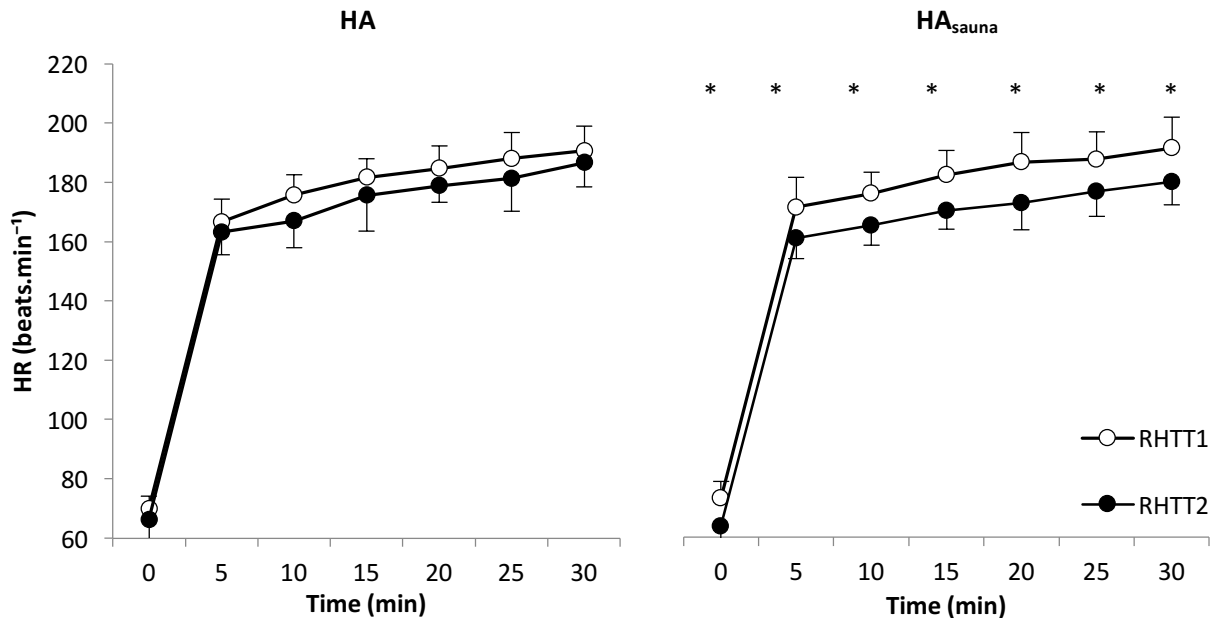
Figure 8.4 Heart rate (HR) at 5-min intervals during the RHTT for the HA$_{CH}$ intervention (right) and the HA$_{sauna}$ intervention (left). Open markers represent RHTT1 and the closed markers represent RHTT2. Mean ± SD. N = 9.

Notes: * Denotes significant difference between RHTT1 and RHTT2.

Metabolic responses

V̇O$_2$ reduced from RHTT1 to RHTT2 in both conditions (HA$_{CH}$: -0.05 ± 0.14 L.min^{-1}, HA$_{sauna}$: -0.12 ± 0.18 L.min^{-1}) ($F_{(1, 8)}$ = 10.896, p = 0.011, np^2 = 0.577), however there was no interaction effect

observed between RHTT and condition ($F_{(1, 8)}$ = 0.634, p = 0.449, np^2 = 0.073). There was no main effect of RHTT on RER ($F_{(1, 8)}$ = 0.139, p = 0.719, np^2 = 0.017) or interaction effect between RHTT and condition on RER ($F_{(1, 8)}$ = 0.177, p = 0.685, np^2 = 0.022). Metabolic heat production reduced in both conditions from RHTT1 to RHTT2 (HA_{CH}: -0.3 ± 0.7 W.kg^{-1}, HA_{sauna}: -0.8 ± 1.0 W.kg^{-1}; $F_{(1, 8)}$ = 10.896, p = 0.011, np^2 = 0.577). No interaction effect between RHTT and condition on metabolic heat production was observed ($F_{(1, 8)}$ = 0.901, p = 0.370, np^2 = 0.101).

Sudomotor responses

Sweat rate ($F_{(1, 8)}$ = 49.982, $p \leq$ 0.001, np^2 = 0.862) and SWR$_{BSA}$ ($F_{(1, 8)}$ = 52.796, $p \leq$ 0.001, np^2 = 0.868), increased in both conditions from RHTT1 to RHTT2. No interaction effect between RHTT and condition was observed for SWR ($F_{(1, 8)}$ =3.893, p = 0.084, np^2 = 0.327) and SWR$_{BSA}$ ($F_{(1, 8)}$ =3.605, p = 0.094, np^2 = 0.311) (figure 8.5).

There was an interaction effect between RHTT and condition for sweat NaCl ($F_{(1, 8)}$ = 13.932, p = 0.006, np^2 = 0.635). Sweat NaCl reduced from RHTT1 to RHTT2 to a greater extent in HA_{sauna} (-16 ± 10 mmol.L^{-1}) compared with HA_{CH} (-5 ± 2 mmol.L^{-1}). There was an interaction effect between RHTT and condition for T_{re} at onset of sweating ($F_{(1, 8)}$ = 12.386, p = 0.008, np^2 = 0.608). No differences were observed in T_{re} at onset of sweating between conditions during RHTT1 (HA_{CH} 37.55 ± 0.20°C, HA_{sauna} 37.54 ± 0.16°C; p = 0.989). The T_{re} at onset of sweating reduced from RHTT1 to RHTT2 in HA_{sauna} (-0.29 ± 0.15°C; $p \leq$ 0.001), but not HA_{CH} (-0.08 ± 0.17°C; p = 0.137) (figure 8.5).
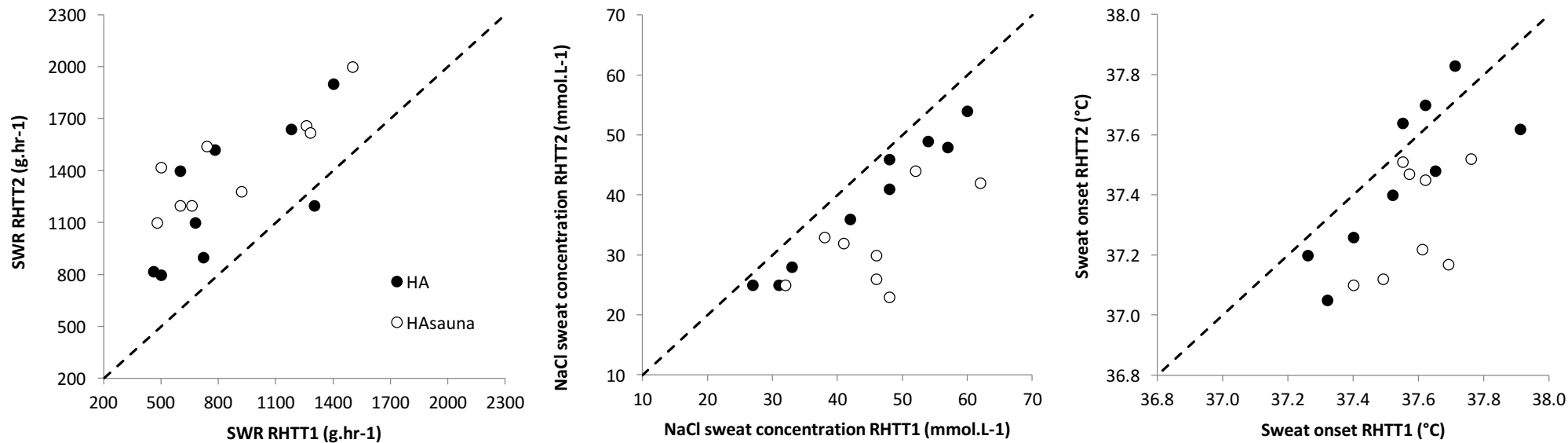
Figure 8.5 Sweat rate (SWR) (left), sodium chloride (NaCl) sweat concentration (middle), and rectal temperature at onset of sweating (right) during RHTT1 (x axis) and RHTT2 (y axis) in the HA_{CH} intervention (closed markers) and HA_{sauna} intervention (open markers). Dotted line represents line of equality. N = 9.

Perceptual responses

There was an interaction effect between RHTT and condition on TS ($F_{(1, 8)}$ = 67.600, $p \leq 0.001$, np^2 = 0.894). No differences were observed in TS between conditions during RHTT1 (p = 1.000). TS reduced from RHTT1 to RHTT2 in HA_{sauna} (p = 0.002), but not HA_{CH} (p = 0.729). There was an interaction effect between RHTT and condition on RPE ($F_{(1, 8)}$ = 24.143, p = 0.001, np^2 = 0.751). No differences were observed in RPE between condition during RHTT1 (p = 0.347). RPE reduced from RHTT1 to RHTT2 in HA_{sauna} ($p \leq 0.001$), but not HA_{CH} (p = 0.081).

8.5.   Discussion

The current study demonstrated that preceding STHA with a passive heat exposure accelerates the adaptation in females, with HA_{sauna} resulting in PV expansion and superior adaptation to the sudomotor function compared with HA alone. These findings are in support of the aim of the study. Specifically, the primary and secondary hypothesis were both supported. In addition, these data confirmed the findings from Study 3 (Chapter 6) with females failing to achieve some of the classic markers of controlled hyperthermia STHA, including a reduced thermoregulatory and cardiovascular strain. However, following HA_{sauna} reductions in thermoregulatory, cardiovascular and perceptual strain were observed. The adaptation pathway was likely mediated in part by PV expansion and an improved thermoeffector and thermosensitivity response of the sudomotor function. The importance of these findings is that HA_{sauna} may accelerate HA without increasing the exercise stimulus. However, it remains unknown if there are additional modifications to the HA protocol that may offer the potential for further accelerating the adaptations within a female population.

HA_{CH} and HA_{sauna} were successfully matched for the prescribed training parameters of duration T_{re} $\geq 38.5°C$, exercise duration, relative exercise intensity and total work completed (table 8.1). This provides confidence that the additional adaptations induced following HA_{sauna} compared with HA_{CH} were a result of the 20-min pre HA session exposure to a hot whilst wearing a sauna suit compared with temperate conditions. The data indicate that the HA_{sauna} is an effective HA strategy, evidenced by a reduction in T_{re} rest, peak T_{re}, HR rest, peak HR, TS and RPE combined with PV expansion and an enhanced thermoeffector (T_{re} at onset of sweating) and thermosensitivity (SWR) of the sudomotor function. These findings are largely in keeping with other research employing similar STHA regimes in males (Garrett et al., 2012; Gibson et al., 2015b; Neal et al., 2015).

Plasma volume was unchanged in the HA_{CH} condition. These findings are in accordance with other controlled hyperthermia STHA investigations using male participants (Garrett et al., 2009; Neal et al., 2015). It has been suggested that sauna exposure acts as an independent stimulus for HA by augmenting PV expansion (Scoon et al., 2007; Stanley et al., 2015). In the current study, there was a 9.3 ± 7.6% PV expansion following the HA_{sauna} condition. The magnitude of PV expansion is very similar to that observed by others following LTHA and long-term sauna exposure (Burk et al., 2012; Gibson et al., 2015c; Stanley et al., 2015); but exceeded the 6.5% expansion reported by Lorenzo & Minson (2010) following LTHA and 7.1% expansion reported by Scoon et al. (2007) following long-term sauna exposure. PV expansion results in an increased vascular filling to support cardiovascular stability, increased specific heat capacity of blood and an improved skin blood flow responses (Sawka et al., 2011a). Thus, the reduced thermoregulatory and cardiovascular responses observed in the current study were likely in part mediated by the PV expansion.

Enhanced thermosensitivity of the sudomotor function was observed, evidenced by a 407 $g.hr^{-1}$ and 564 $g.hr^{-1}$ increase in SWR during the RHTT following the HA_{CH} and HA_{sauna} condition, respectively. These findings are in accordance with the 691 $g.hr^{-1}$ increases in SWR previously observed in females following STHA (Study 3, Chapter 6). Furthermore, in the current study, there was an enhanced thermoeffector response, evidenced by a reduction in the T_{re} at the onset of sweating in the HA_{sauna} condition. Thus, the observed increase in SWR may in part be mediated by an increased cholinergic sensitivity of the eccrine sweat gland causing an altered afferent neural activity from the central thermoreceptors that altered the integration of thermal information (Charkoudian, 2010). Despite an enhanced thermosensitivity following the HA_{CH} condition there were no alterations in the thermoeffector response, thus, these findings may in part be explained by an increase in glandular hypertrophy (Buono et al., 2009b; Lorenzo and Minson, 2010). The reduced thermoregulatory strain observed in the current study was likely in part mediated by an enhanced evaporative heat loss combined with a reduction in metabolic heat production.

This study is the first study to provide data on sweat mineral concentrations and losses following controlled hyperthermia STHA in a female population. Both the HA_{CH} and HA_{sauna} condition resulted in a reduction in sweat NaCl. The magnitude of reduction for the HA_{sauna} condition exceeded that reported previously in males following STHA (Neal et al., 2015). The mechanism of sweat mineral conservation is unclear, although previous data suggests sweat sodium conservation following HA involves increased sodium ion reuptake within the re-absorptive duct of the sweat gland (Sato and Dobson, 1970; Sato et al., 1971). This is a novel finding presented within the current study has practical relevance to female athletes who may experience chronic perfuse sweating during training which may raise the potential for mineral deficiencies (Chinevere et al., 2008).

HA$_{sauna}$ resulted in a reduction pre to post in TS and RPE. This may have implications for self-paced exercise in the heat, as a consequence of TS being a main driver of behavioural thermoregulation (Flouris and Schlader, 2015). The observed reductions in TS and RPE following HA$_{sauna}$ is a novel finding with practical relevance for athletes preparing to compete under hot environmental conditions. However, further investigations are required to determine the effect of STHA, with or without additional sauna stress on pacing and time trial performance in females.

8.6. Limitations

Typically, it is suggested that phenotypic adaptations decay at a rate of a 1-d loss of acclimation status for every 2-d spent without heat exposure (Givoni and Goldman, 1973), supporting the 7-wks between the two HA conditions adopted in the current study. However, even with 7-wks. between the two HA conditions it is possible that the molecular memory from the prior condition was different between participants (Horowitz, 2014). The current study controlled for this by using a randomised design however, further investigation is required to quantify the molecular memory established during the two conditions.

Changes in PV were estimated via changes in haematocrit and haemoglobin concentrations. This method has been reported to reflect small-to-moderate changes in PV accurately, however, it may underestimate large changes in PV (Lundvall and Lindgren, 1998). Nevertheless, a typical error as a coefficient of variation of 4.0% and 3.9% for the measurement of haematocrit and haemoglobin respectively was observed between duplicate samples providing confidence that the change observed is a meaningful change.

The current study recruited physically active females who performed both running and cycling in their weekly training regimen and had an average $\dot{V}O_2$ peak of 50 mL.kg^{-1}.min^{-1}. Thus, these results are constrained to a physically active, female population. HA is a strategy typically adopted by highly trained athletes prior to training and competing in the heat. Further investigations are required to establish the magnitude of adaptation in a highly trained group of females who may experience smaller marginal gain from HA.

8.7. Conclusion

This is the first study to investigate methods to optimise STHA specifically for females. The HA$_{sauna}$ condition established reductions in thermoregulatory, cardiovascular and perceptual strain, and an improved thermosensitivity and thermoeffector sudomotor function. These findings provide evidence that preceding a HA session with a passive heat exposure may offer a time-efficient means

by which to further enhance acclimation in a female population. Further investigations are required to establish the optimal exposure to establish the greatest magnitude of adaptation whilst minimising the exercise stimulus and exposure duration.

9. General discussion

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This chapter will be presented in four main sections. Firstly, the principle findings for each of the studies presented within this thesis will be discussed with a table of hypotheses from each study and whether they were accepted or rejected (table 9.1). Secondly, a mechanistic overview will be provided, for the main physiological issues arising from the experimental studies, using pooled data from across all the studies presented within this thesis. Possible directions for future research will then be considered prior to discussing the practical application and impact of the data presented within this thesis.

9.1. Principle Findings

9.1.1. Heat tolerance test

The first experimental study (Chapter 4) within this thesis introduced the first HTT which adopts a running mode of exercise and assessed its repeatability. The data from this study supported the primary and secondary hypothesis (table 9.1). Physiological responses during the RHTT had strong, positive correlations and no significant differences in physiological responses between repeated trials. Following Chapter 4, the sensitivity of the RHTT to changes in heat tolerance following a chronic, heat-alleviating strategy was assessed. The data from this study (Study 2, Chapter 5) also supported the primary and secondary hypothesis (table 9.1). Thus, the RHTT was sensitive to changes in heat tolerance and to individual differences in the magnitude of adaptation. The results from these two studies indicate that the RHTT is a sensitive tool which can be used to quantify the changes in tolerance following chronic heat-alleviating procedures when using a repeated measures design. In addition, the results suggest that the RHTT may be an appropriate tool for physiologists, coaches and medical staff to use to help inform decisions regarding athletes' safety, and to inform the severity of the heat-alleviating procedures required prior to training and competing in the heat.

9.1.2. Sex comparisons to heat acclimation

The data from the third (Chapter 6) and fourth (Chapter 7) studies presented within this thesis were collected together, but reported as two separate chapters since they were addressing very distinct questions. The aim of the third study was to provide the first data comparing males' and females' phenotypic adaptations to STHA and LTHA using the RHTT to quantify the magnitude of adaptation.

HA was effective in attenuating physiological strain and improving exercise heat tolerance. STHA was effective in achieving partial adaptation in both males and females. However, females required LTHA to establish reductions in cardiovascular and thermoregulatory strain. The data from this study supports the primary, secondary and tertiary hypothesis (table 9.1) and provides novel information on females' phenotypic responses to controlled hyperthermia HA.

The aim of the fourth study (Chapter 7) was to provide the first data comparing males' and females' cellular adaptions to STHA and LTHA via the quantification of Hsp72 mRNA. Males and females had similar Hsp72 mRNA expression across the course of controlled hyperthermia HA. There were no differences in the endogenous criteria to elicit the HSR and the adaptation towards thermotolerance between males and females occurs indiscriminately. This data is not in support of the hypothesis that Hsp72 mRNA response will be attenuated in females compared to males across the course of controlled hyperthermia HA (table 9.1). Accordingly, this data provides mechanistic support for practitioners prescribing controlled hyperthermia HA for female athletes.

9.1.3. Accelerated heat acclimation responses in females

The aim of the final study presented within this thesis was to investigate whether it is possible to accelerate the phenotypic adaptions to STHA in females during the follicular phase of the menstrual cycle, confirmed by plasma concentrations of 17ß estradiol and progesterone. To do this, a novel HA method combining a passive sauna like exposure with controlled hyperthermia was used, which combined controlled hyperthermia HA with a passive exposure in 50°C whilst wearing a 100% vinyl sauna suit. This intervention resulted in an expansion of PV and an improved sudomotor function, resulting in a reduced resting and peak thermoregulatory and cardiovascular strain, in addition to a reduction in perceptual responses; all of which were not observed following HA alone. This novel data is in support of the primary and secondary hypotheses (table 9.1). These findings provide new evidence that females can accelerate the phenotypic adaptations during controlled hyperthermia HA.

Table 9.1 Hypotheses for each study chapter presented in this thesis.

Hypotheses	Accepted	Rejected
Study 1 (Chapter 4): Repeatability of a running heat tolerance test		
Physiological responses during the running heat tolerance test will have a strong positive correlation on repeated trials.	X	
There will be no differences in physiological responses during the running heat tolerance test between repeated trials.	X	
Study 2 (Chapter 5): Sensitivity of a running heat tolerance test		
There will be differences in physiological responses during the running heat tolerance test following STHA.	X	
The running heat tolerance test will be sensitive to individual differences in the magnitude of adaptation.	X	
Study 3 (Chapter 6): A comparison of male and female temporal patterning to short- and long-term heat acclimation		
Males will achieve greater reduction in T_{re} and HR following short-term heat acclimation compared with females.	X	
There will be no differences in the reductions in T_{re} and HR between males and females following long-term heat acclimation.	X	
Females will achieve a greater increase in sweat rate following short-term heat acclimation and long-term heat acclimation compared with males.	X	

Study 4 (Chapter 7): Sex comparison of leukocyte Hsp72 mRNA transcription during heat acclimation

Hsp72 mRNA response will be lower in females compared to males across the course of controlled hyperthermia heat acclimation.   **X**

Study 5 (Chapter 8): Passive heat exposure preceding short-term heat acclimation accelerates phenotypic adaptation in females

Short-term heat acclimation preceded by a passive heat exposure will result in greater plasma volume expansion in females compared   **X**

with heat acclimation alone.

Short-term heat acclimation preceded by a passive heat exposure will produce a greater sudomotor adaptation in females compared   **X**

with heat acclimation alone.

9.2.   Mechanistic Overview

9.2.1. Heat Tolerance

This thesis has introduced the first HTT which adopts a running mode of exercise. Data has been presented to demonstrate that the method is repeatable, supporting the use of the RHTT when using a repeated measures design. Interestingly, data has also been presented to demonstrate that individuals' thermoregulatory responses to the RHTT are highly variable with participants' responses along a heat tolerance continuum (figure 4.1). When exercising at a fixed absolute workload as in the RHTT, typically this results in a similar absolute heat production between individuals of a variety of biophysical factors. However, this will typically result in greater heat production relative to BM in fitter individuals with a low percentage body fat, since they often have a smaller body size. Evidence has been provided to support that when matching exercise intensity at a fixed heat production relative to total BM the systematic differences in T_c between groups of different body size (Cramer and Jay, 2014) and fitness (Jay et al., 2011) are eliminated. Thus, the fixed workload of the RHTT may limit applicability of the test when comparing between individuals, especially when they are not matched for biophysical factors. This may potentially challenge the RHTT being able to demonstrate individual differences in heat tolerance with the use of a heat tolerance continuum.

To identify the predominant factors responsible for the observed differences in changes in T_{re} between individuals during the RHTT and to determine whether the protocol has the potential to be used to compare between independent groups, all female data presented within this thesis (N = 30) was pooled to enable retrospective analysis. Part 1 of the analyses involved all pooled data (N = 30). Correlation coefficients (r) were established between changes in T_{re} during the RHTT with BSA, sum of skin folds, $\dot{V}O_2$ peak, relative exercise intensity, and heat production expressed in its absolute form (W), relative to BSA (W.m^2), and BM (W.kg^{-1}).

Part 2 of this analyses, involved 14 females who were selected from the pooled data to enable comparisons of T_{re} change between two independent groups of low BM (N = 7; 49.5 ± 4.5 kg) and high BM (N = 7; 71.7 ± 2.2 kg). The criteria for selection of these participants for the low BM group was a BM of ≤ 55 kg and ≥ 65 kg for the high BM group. Part 3 of the analyses involved a separate 14 females, who were selected from this pooled data to enable the comparison of T_{re} change between two independent groups of low $\dot{V}O_2$ peak (N = 7; 41 ± 2 mL.kg^{-1}.min^{-1}) and high $\dot{V}O_2$ peak (N = 7; 57 ± 5 mL.kg^{-1}.min^{-1}). The criteria for selection of these participants for the low $\dot{V}O_2$ peak was ≤ 48 mL.kg^{-1}.min^{-1} (performance level 1 and 2) and for the high $\dot{V}O_2$ peak was ≥ 52 mL.kg^{-1}.min^{-1} (performance level 4 and 5) (Decroix et al., 2016). Independent sample t tests were used to

established differences between the independent groups' data. Participant characteristics are displayed in table 9.2 and presented as mean, SD, minimum, and maximum values.

Figure 9.1 presents the correlation coefficients for the association between changes in T_{re} and biophysical and exercise intensity variables. Absolute heat production ($r = 0.603$, $p \leq 0.001$; figure 9.2), heat production relative to BSA ($r = 0.592$, $p \leq 0.001$), and heat production relative to BM ($r = 0.549$, $p = 0.002$) had medium positive correlations with change in T_{re}. BSA ($r = 0.349$, $p = 0.058$), sum of skin folds ($r = 0.248$, $p = 0.186$), relative $\dot{V}O_2$ peak ($r = -0.204$, $p = 0.278$), and relative exercise intensity ($r = 0.362$ $p = 0.051$) did not have a significant correlation with change in T_{re} (figure 9.1).

T_{re} changes were similar between the low BM group ($1.7 \pm 0.2°C$) and the high BM group ($2.0 \pm 0.4°C$) ($t_{(12)} = 1.781$, $p = 0.100$) despite a higher heat production in the high BM group (low BM: 515 ± 78 W; high BM: 782 ± 176 W; $t_{(12)} = 3.645$, $p = 0.003$). Interestingly, when heat production was expressed relative to BM these differences were eliminated (low BM: 10.5 ± 1.7 W.kg^{-1}; high BM: 10.9 ± 2.6 W.kg^{-1}; $t_{(12)} = 0.403$, $p = 0.694$).

T_{re} changes were similar between the low $\dot{V}O_2$ peak group ($2.0 \pm 0.4°C$) and the high $\dot{V}O_2$ peak group ($1.8 \pm 0.4°C$) ($t_{(12)} = 0.952$, $p = 0.360$) despite a slightly, although not significantly higher heat production in the low $\dot{V}O_2$ peak group (low $\dot{V}O_2$ peak: 735 ± 189 W; high $\dot{V}O_2$ peak: 547 ± 143 W; $t_{(12)} = 1.617$, $p = 0.132$). Although, when heat production was expressed relative to BM these differences were eliminated (low $\dot{V}O_2$ peak: 10.7 ± 2.4 W.kg^{-1}: high $\dot{V}O_2$ peak: 10.6 ± 2.2 W.kg^{-1}; $t_{(12)} = 0.117$, $p = 0.909$).

On completion of the RHTT absolute heat production (figure 9.2), heat production relative to BSA, and heat production relative to BM were found to be the best predictors of T_{re} change. Furthermore, similar changes in T_{re} were observed in two independent groups with large differences in BM and fitness. These findings are explained by the exercise intensity administered during the RHTT equating to similar heat production relative to BM, permitting unbiased independent group comparisons of T_c. These findings are supported by the work of Cramer and Jay (2014) who reported similar changes in T_{re} between two independent groups with large differences in BM when exercise was administered at a fixed rate of heat production relative to BM. Furthermore, Jay et al. (2011) reported similar changes in T_{re} when exercise was administered at fixed rates of heat production relative to BM between independent groups with large differences in fitness. These findings provide initial evidence to support the use of the RHTT between independent groups since it appears that despite the exercise intensity being fixed, the relative heat production is similar between independent groups of different BM and fitness. These

interpretations are limited to a female population, further consideration should be made when using male participants.

Table 9.2 Participant characteristics. Mean ± SD (Range).

	All Data (N = 30)	Low BM (N = 7)	High BM (N = 7)	Low $\dot{V}O_2$ peak (N = 7)	High $\dot{V}O_2$ peak (N = 7)
Stature (m)	1.66 ± 0.08	1.56 ± 0.07	1.71 ± 0.05 *	1.60 ± 0.10	1.60 ± 0.10
	(1.46–1.78)	(1.46-1.63)	(1.68-1.75)	(14.8–1.70)	(1.54–1.75)
BM (kg)	60.18 ± 8.37	49.51 ± 4.52	71.74 ± 2.21 *	64.42 ± 6.93	54.71 ± 9.33
	(40.03-75.02)	(40.0-53.0)	(68.0-75.0)	(51.6-72.2)	(40–70)
BSA (m^2)	1.97 ± 0.14	1.51 ± 0.12	1.82 ± 0.13 *	1.74 ± 0.11	1.63 ± 0.22
	(1.28-1.90)	(1.28–1.56)	(1.77–1.90)	(1.44-1.80)	(1.50–1.75)
Sum of skin folds (mm)	56.2 ± 23.3	48.1 ± 25.0	73.1 ± 24.2 *	67.8 ± 29.7	55.7 ± 31.7
	(24.1–110.4)	(32.2–91.6)	(52.0-110.2)	(24.0-110.2)	(30.0-105.6)
$\dot{V}O_2$peak (mL.kg^{-1}.min^{-1})	49 ± 6	51 ± 6	47 ± 4	41 ± 2	57 ± 5 *
	(34-67)	(47-57)	(42-52)	(37–44)	(52–67)

Notes: Data presented for 30 females. BM, body mass; BSA, body surface area; $\dot{V}O_2$ peak, peak rate of oxygen uptake. * denotes significant difference between low BM and high BM, or low $\dot{V}O_2$ peak and high $\dot{V}O_2$ peak
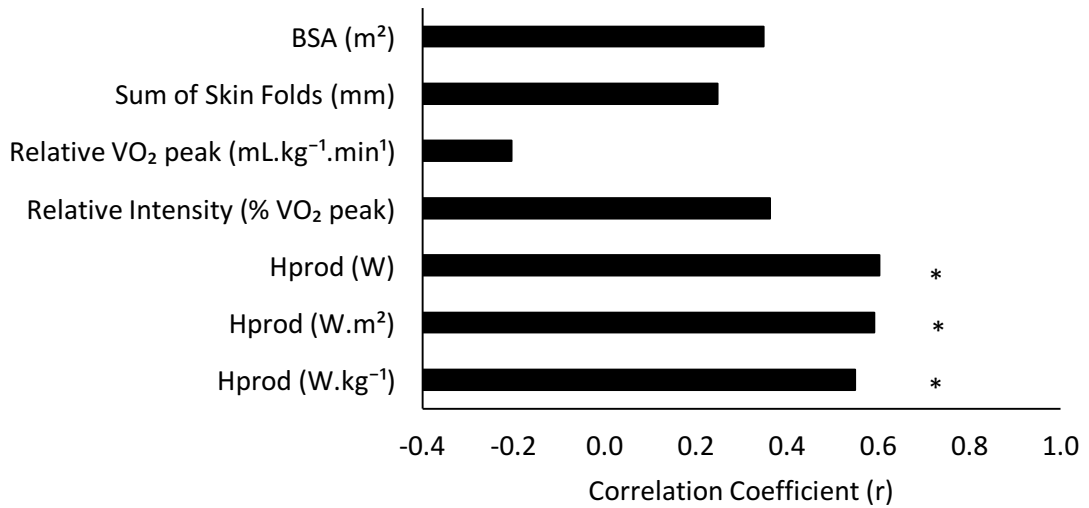
Figure 9.1 Correlation coefficients for association between changes in rectal temperature and body surface area (BSA), sum of skin folds, relative $\dot{V}O_2$ peak, relative exercise intensity, absolute heat production (Hprod, W), heat production relative to BSA (Hprod, $W.m^2$), and heat production relative to BM (Hprod, $W.kg^{-1}$).

Notes: * denotes significant relation ($p \leq 0.05$).
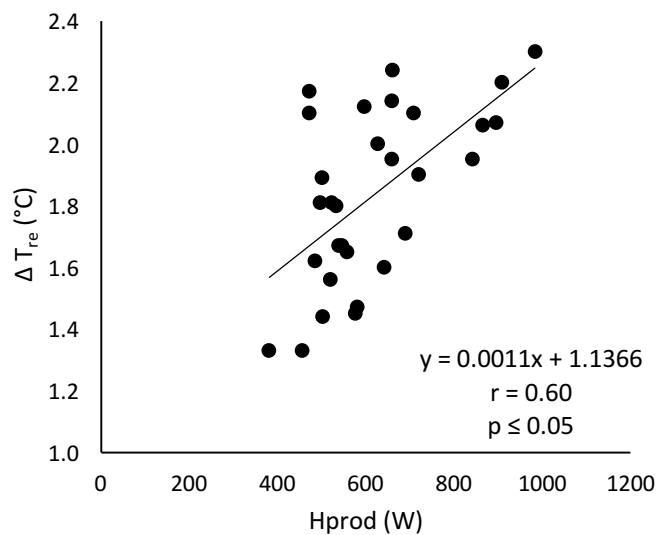
Figure 9.2 Scatter plot illustrating the relationship between the change in rectal temperature (ΔT_{re}) and absolute heat production (Hprod, W) during the RHTT in females. N = 30.

9.2.2. Heat Acclimation

A primary objective of this thesis was to explain females' physiological and cellular responses to controlled hyperthermia HA while also considering the effect of combining HA with passive exposures to accelerate the physiological and perceptual responses to a fixed exercise heat stress. Prior to the production of this thesis a large body of research had investigated males' responses to controlled hyperthermia HA, however, there was no literature published on females' responses. The limited data published on females, has used fixed (Avellini et al., 1980) or self-paced (Sunderland et al., 2008) HA strategies which potentially constrained adaptation. This thesis presents the first data set on females' phenotypic and cellular adaptations to controlled hyperthermia HA.

Temporal patterning of controlled hyperthermia heat acclimation in females

Functionally, HA enhances the capacity of an organism to tolerate heat stress by augmenting heat dissipation and elevating the body temperature that can be sustained in the heat (Horowitz and Kodesh, 2010). Such resistance is a consequence of systemic adaptation, a chronic process dependent upon heat-induced reprogramming of gene expression (Horowitz and Kodesh, 2010). Traditionally, measures of T_{re}, HR, and PV were reported to plateau following $3 - 8$ d of HA, with SWR taking 8 - 14 d to reach a plateau (Armstrong and Maresh, 1991). This data is limited to males' responses. The repeated exposure to a constant overload produces physiological habituation. Thus, the observed plateaus in key markers of HA may be an artefact of constant work rate methods and the inherent decrease in physiological strain observed over the duration of the protocol. To avoid this inherent limitation and optimise adaptation, controlled hyperthermia is used. Controlled hyperthermia HA involves rapidly heating deep tissues to a target T_c, which is then sustained for a fixed duration. Controlled hyperthermia HA ensures a progressive overload approach which induces more complete adaptation while revealing the mechanisms that increase heat tolerance (Taylor, 2014). In contrast to traditional protocols, data presented within this thesis using controlled hyperthermia HA did not result in the classic plateau in physiological responses. Considering the responses to controlled hyperthermia HA in the current thesis, it is likely that this method may alter the temporal patterning of adaptation with the potential for sustained adaptation as the protocol progresses.

Females' cardiovascular responses to controlled hyperthermia heat acclimation

Following controlled hyperthermia HA, reductions in resting HR have been observed in males following LTHA (-12 beats.min^{-1}) compared with STHA (-4 beats.min^{-1}) (Garrett et al. 2012; Gibson; Gibson, Turner, et al. 2015; Patterson et al. 2004a). This was also observed in this thesis in females. Data presented in Study 3 (Chapter 6) demonstrates an enhanced reduction in resting HR following 10-d of controlled hyperthermia HA (-16 beats.min^{-1}) compared with 5-d (-7 beats.min^{-1}) in females. These findings are further supported by the data presented in Study 5 (Chapter 8) with only small reductions in resting HR (-4 beats.min^{-1}) observed following 5-d of controlled hyperthermia HA. In addition to decreased resting HR, controlled hyperthermia also reduced peak HR during the RHTT. Following STHA, peak HR was reduced in both Study 3 (Chapter 6; -5 beats.min^{-1}) and Study 5 (Chapter 8; -3 beats.min^{-1}) in female participants. An enhanced reduction was observed following LTHA in Study 3 (Chapter 6; -10 beats.min^{-1}) and following STHA combined with a passive heat exposure in Study 5 (Chapter 8; -12 beats.min^{-1}). These findings challenge original reports that a reduction in resting HR plateau following 6-d HA (Armstrong and Maresh, 1991). This further demonstrates that the controlled hyperthermia method of HA is capable of augmenting the physiological adaptations to a greater extent compared with more traditional methods.

A reduced cardiovascular strain following HA has been attributed to an expansion of PV. Following controlled hyperthermia HA an enhanced expansion of PV is observed in males following LTHA (14 ± 1%) compared with STHA (7 ± 5%) (Garrett et al., 2014; Gibson et al., 2015c; Patterson et al., 2014, 2004b). In Study 5 (Chapter 8), PV remained unchanged following STHA, however when STHA was combined with a passive heat exposure PV increased by 9.3%, mirroring the changes in HR responses. These findings suggest that following STHA PV has not reached full expansion, challenging the original reports that expansion in PV plateau following 6-d HA (Armstrong and Maresh, 1991).

Females' thermoregulatory responses to controlled hyperthermia heat acclimation

Following controlled hyperthermia HA, an enhanced reduction in resting T_{re} has been observed in males following LTHA (-0.3°C) compared with STHA (-0.2°C) (Garrett et al., 2014; Gibson et al., 2015b; Neal et al., 2015; Patterson et al., 2004a). However, Gibson et al., (2015b) reported no further reductions in resting T_{re} following 10-d (-0.09°C) compared with 5-d (-0.10°C) controlled hyperthermia HA. This was not observed for females in this thesis. Data presented in Study 3 (Chapter 6) demonstrates no changes in resting T_{re} following 5-d controlled hyperthermia HA (-0.02°C) in females. These findings are further supported by the data presented in Study 5 (Chapter

8) with only a very small reduction in resting T_{re} (-0.07 °C) observed following 5-d controlled hyperthermia HA. However, in line with previous data on males, a reduction was observed following LTHA in Study 5 (Chapter 8; -0.29°C) and following STHA combined with passive heat exposure in Study 5 (Chapter 8; -0.28°C). Another classic marker of HA is a reduced exercise T_{re} owing in part, to a reduced resting T_{re} and improved heat dissipation. Following STHA, peak T_{re} remained unchanged in both Study 3 (Chapter 6; -0.06°C) and Study 5 (Chapter 8; -0.05°C). However, a reduction was observed following LTHA in Study 3 (Chapter 6; -0.47°C) and following STHA combined with a passive heat exposure in Study 5 (Chapter 8; -0.42°C). These findings challenge original reports that reductions in resting T_{re} plateau following 8-d HA (Armstrong and Maresh, 1991), with females requiring at least 10-d to achieved reductions in resting T_{re}.

Females' sudomotor responses to controlled hyperthermia heat acclimation

Following controlled hyperthermia HA an enhanced increase in SWR has been observed in males following LTHA (+0.46 L.hr^{-1}) compared with STHA (+0.23 L.hr^{-1}) (Gibson et al., 2015b, 2015c; Neal et al., 2015; Patterson et al., 2004a). This was also observed for females in this thesis. Data presented in Study 3 (Chapter 6) demonstrates a marginally enhanced SWR (+0.78 L.hr^{-1}) following 10 -d controlled hyperthermia HA compared with 5-d (+0.69 L.hr^{-1}) in females. These findings are further supported by the data presented in Study 5 (Chapter 8) with large increases in SWR occurring in the first 5-d of controlled hyperthermia HA (+0.41 L.hr^{-1}). In these female participants, approximately 90% of the SWR adaptation occurred in the first 5-d of controlled hyperthermia HA. These findings challenge original reports that an increase in SWR plateaus between 8 and 14-d of HA (Armstrong and Maresh, 1991) with a plateau potentially occurring much earlier.

The Armstrong and Maresh table for plateau days of physiological adaptation during heat acclimatisation (Armstrong and Maresh, 1991) has evolved to be a temporal patterning model of HA acquisition (table 9.3). To achieve a plateau in responses following 3 - 6 d of HA was previously viewed favourable. However, taking into account the limitations of fixed intensity, traditional methods of HA and the advanced understanding of the controlled hyperthermia method of HA, the plateau may simply reflect incomplete adaptation. Recent literature investigating the controlled hyperthermia method of HA combined with data from this thesis on females' responses to controlled hyperthermia HA adds to the temporal patterning model of HA acquisition. Controlled hyperthermia HA ensures a progressive overload approach which induces more complete adaptation thus, often a plateau in responses is not observed. Table 9.4 presents the days of physiological adaptation during controlled hyperthermia HA in both males and females and uses an adapted format to that of Armstrong and Maresh (1991). The data used to produce this table is a

combination of recent controlled hyperthermia HA studies (Garrett et al., 2014, 2012; Gibson et al., 2015b, 2015c; Neal et al., 2015; Patterson et al., 2014, 2004a, 2004b) and data presented in this thesis.

Table 9.3 Plateau days of physiological adaptations (the point at which approximately 85% of the adaptation occurs) during heat acclimatisation.

Adaptation	Days of heat acclimatisation													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
HR decrease			___	___	___									
PV expansion			___	___	___									
T$_{re}$ decrease				___	___	___								
RPE decrease			___	___	___									
Sweat NaCl decrease					___	___	___	___						
SWR increase									___	___	___	___	___	___
Renal NaCl decrease			___	___	___	___								

Notes: HR, heart rate; NaCl, Sodium Chloride; PV, plasma volume; T$_{re}$, rectal temperature; SWR, sweat rate. Adapted from Armstrong and Maresh, (1991).

In males, the early adaptations primarily involve an improved control of cardiovascular function, including expanded PV and a reduced HR. In females the early adaptations primarily involve an increased SWR with an improved control of cardiovascular function requiring ≥10 d. Taken together, these data would appear to suggest that when using a controlled hyperthermia HA method the temporal patterning is different to that reported when using constant workload protocols. The key observation is that following 10-d controlled hyperthermia HA, there is a sustained adaptation as the protocol progresses. In addition, these data would appear to suggest that females adopt a slightly different temporal patterning to males when using a controlled hyperthermia method of HA.

Table 9.4 Days of physiological adaptation in males and females during controlled hyperthermia heat acclimation.

Adaptation	Sex (M/F)	1	2	3	4	5	6	7	8	9	10
Reduced HR rest	M		————	————	————	————	————	————	————	————	————
	F			– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –
PV expansion	M		————	————	————	————	————	————	————	————	————
	F					– – –	– – –	– – –	– – –	– – –	– – –
Reduced T_{re} rest	M		————	————	————	————	————	————	————	————	————
	F					– – –	– – –	– – –	– – –	– – –	– – –
SWR Increase	M				————	————	————	————	————	————	————
	F		– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –
Reduced Sweat NaCl	M		————	————	————	————	————	————	————	————	————
	F		– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –	– – –

Notes: Solid line represents males and the broken line represents females. HR, heart rate; NaCl, Sodium Chloride; PV, plasma volume; T_{re}, rectal temperature; SWR, sweat rate.

Females' acquired thermotolerance following controlled hyperthermia heat acclimation

In addition to the phenotypic adaptations to controlled hyperthermia discussed, this thesis also presented the first data set on females acquired cellular thermotolerance associated with controlled hyperthermia STHA and LTHA. The data provides confidence that when using a controlled hyperthermia method of HA equal signals for the attainment of thermotolerance are present in both males and females. The equal Hsp72 mRNA increases occurring in both males and females, who were required to complete a range of exercise intensities to achieve the target T_c, suggests that as long as a minimum endogenous criterion (38.5°C) (Gibson et al., 2014) is surpassed, additional exercise or thermal stimulus is not of further benefit. These findings are in support of recent observations that controlled hyperthermia (38.5°C and 39.0°C) and fixed intensity HA methods all elicit similar Hsp72 mRNA responses across the course of LTHA assuming a minimum

endogenous criteria is achieved (Gibson et al., 2015a). Taken together, these data would appear to suggest that exercise per se is not a significant indicator of Hsp72 mRNA response and the level of hyperthermia achieved is a more important driver for the acquired thermotolerance.

9.2.3. Accelerated heat acclimation in females

There was clear evidence of HA in Study 5 (Chapter 8) when controlled hyperthermia STHA was preceded by a sauna-like exposure to 50°C whilst wearing a 100% vinyl sauna suit. This was demonstrated by a lower resting T_{re} (-0.25 ± 0.16°C) and a lower peak T_{re} (-0.42 ± 0.22°C) following the RHTT. Additional HA responses included, PV expansion (+9.3 ± 7.6%), a lower set point for sweating onset (-0.28 ± 0.16°C), a reduction in sweat NaCl (-16 ± 10 mmol.L^{-1}) and an increase in SWR (+0.57 ± 0.20 L.hr^{-1}). Furthermore, resting HR (-10 ± 4 beats.min^{-1}), peak HR (-12 ± 7 beats.min^{-1}), RPE (-3 ± 1), and TS (-0.5 ± 0.5) reduced. The mechanism responsible for establishing this accelerated adaptation exposure requires elucidation.

The type of the thermal loading induced during heat exposure, to elicit adaptation, will influence the vascular adaptation that occurs. The original work by Fox et al. (1964) and later Regan et al. (1994) demonstrated that while HA is dependent upon the degree of T_c elevation, the elevation of T_{sk} is important for complete adaptation. During passive heating cutaneous blood flow is maximised since there is less competition for the available cardiac output. However, when exercising in the heat there is a higher demand from active muscle for blood supply, thus cutaneous blood flow is compromised (Kenney et al., 2014). This difference in blood flow distribution may help explain the importance of elevating T_{sk} for both its direct impact on the blood volume (Maw et al., 2000) and for its role within the heat adaptation process (Taylor, 2014). HA modifies both the central thresholds and the sensitivity of cutaneous blood flow, with the former reflecting altered hypothalamic processing (Bruck, 1986; Horowitz, 2014). This mechanism may in part explain the observed adaptations when controlled hyperthermia HA was preceded by a sauna-like exposure in Study 5 (Chapter 8), with a likely increase in T_{sk} occurring during this exposure.

A mechanism which may explain some of the observed reductions in thermoregulatory strain following controlled hyperthermia HA combined with a sauna-like exposure is PV expansion. An increase in venous return to the heart enhances the sensitivity of skin vasodilation in response to increased T_c during exercise. Nose et al. (1990) reported a 35% increase in skin blood flow with a 150 mL increase in PV following intravenous saline infusion when exercising in a warm environment. However, these findings are challenged by Takeno et al. (2001) who examined the effects of PV expansion on the sensitivity of the skin vasodilatory response to an increase in T_c in a

cool and warm environment. They found that the increase in sensitivity was less in a cool than warm environments despite a similar PV expansion. These results suggest that improvements of the thermoregulatory response after aerobic training are primarily caused by neural adaptation of the thermoregulatory centre in the hypothalamus to repeated heat exposure during training and that increased PV is not a cause of improvement, but a result of adaptation.

An elevated sensitivity of the sweating response also accompanies heat adaptation, and this it is apparent when heat adapted individuals are either heat exposed (Fox et al., 1964, 1963a; Goto et al., 2010; Lorenzo and Minson, 2010; Patterson et al., 2004a), or stimulated pharmacologically (Collins et al., 1966). Fox et al. (1967) suggested this phenomenon was due to sweat gland training since inhibition of local sweating during HA resulted in less pronounced sweat flow during subsequent heat stimulus. Conversely, when skin regions were repeatedly stimulated either by local heating (Fox et al., 1964), or pharmacologically (Collins et al., 1966) without simultaneous whole body heating, then those regions responded to a uniform thermal impulse with greater sweat flows. Lorenzo & Minson (2010) recently verified these local training responses with an increased sensitivity of sweat glands when locally infused with acetylcholine following HA. The higher SWR during the sauna-like exposures compared with the temperate exposure prior to the HA sessions in chapter 8 may in part, explain the observed adaptations. Sensitivity changes are principally a peripheral phenomenon however, it seems possible that an enhanced sweating response following HA could be attributed to a combination of central and peripheral mechanisms.

The responsible mechanism for establishing accelerated adaptation following controlled hyperthermia HA combined with a passive exposure to sauna-like conditions requires elucidation, but likely includes increased central thresholds and sensitivity of cutaneous blood flow, PV expansion and an increase in sweat sensitivity. Additional data is required to establish these mechanisms fully and verify these findings to support the development of optimised HA protocols. Together this knowledge can help support the development of HA for females by ensuring protocols target the forcing functions of HA.

9.3.   Directions for future research

This thesis has introduced the first HTT that adopts a running mode of exercise. The repeatability (Study 1, Chapter 4), and the sensitivity (Study 2, Chapter 5) of the RHTT has been established supporting the use of the RHTT when using a repeated measures design. Furthermore, retrospective analysis within the general discussion (9.2.1.) provided evidence to support the use of the RHTT between independent groups since it appears that despite the exercise intensity being

fixed, the relative heat production is similar between independent groups of different BM and fitness. Future research is required to establish the repeatability and sensitivity of a RHTT that elicits matched heat production between individuals. This would provide confidence in the ability of the procedure to be used when assessing thermoregulatory responses between independent groups.

This thesis has provided the fundamental studies to support future research in developing a more comprehensive understanding into the mechanisms responsible for the thermoregulatory adaptations to controlled hyperthermia HA in females. This thesis has taken a very practical approach with the application to endurance athletes being the predominant focus. Additional research is required to develop more optimised HA strategies for females, to support training and competitions in hot climates.

In the present thesis, controlled hyperthermia HA has been used to investigate females' phenotypic adaptations. From the data presented in Study 3 (Chapter 6), this method was considered effective when conducted over 10-d. However, 5-d was not sufficient to establish thermoregulatory and cardiovascular adjustments, unless combined with an additional passive thermal stimulus (Study 5, Chapter 8). These adaptations are in part explained by PV expansion, thus supporting cardiovascular adjustments, in addition to an increase in evaporative cooling. Lorenzo and Minson, (2010) investigated the mechanisms responsible for phenotypic adaptation by locally stimulating the skin with specific concentrations of the endothelium-dependent vasodilatory acetylcholine, infused via micro-dialysis and by performing a standardised local heating protocol, in resting males. The results from this study provide confidence that adaptations occur within the skins' microcirculation and sweat gland apparatus following HA in males, promoting a superior thermoregulatory response. In addition, no changes were observed in maximal skin blood flow following HA, demonstrating that the observed changes are attributable to improvements in cutaneous vascular function and not structural changes. Developing a greater level of sophistication with the procedures and measures used to assess HA responses in females, would further our understanding into the mechanisms responsible for the adaptations observed in females.

Within this thesis, where possible females have been tested during the follicular phase of the menstrual cycle. Despite the convenience of studying females in the follicular phase of the menstrual cycle, the broader clinical relevance of the findings may be limited since females are only in this part of their cycle for approximately 50% of their reproductive lives. Furthermore, intense physical exercise has been associated with various menstrual irregularities including shortened luteal phase, anovulation and amenorrhea (Loucks and Horvath, 1985). In addition, post-menopausal women display substantially reduced concentration of these female hormones. It

remains unclear whether females' heat adaptation is compromised as a result of ovarian hormonal fluctuations associated with different phases of the menstrual cycle, menstrual irregularities and in post-menopausal women. Future research is required to establish the level of acclimation achieved during the different phases of the menstrual cycle and in females with menstrual cycle irregularities. This information may support the optimisation of protocols and the timing of administering HA strategies.

To date, there is no literature on the time course of the decay and maintenance of heat adaptation in females. This information has a high level of practical importance, to ensure the adaptations achieved remain present during competition. The observation made within the current thesis, that the temporal patterning of the induction is different between males and females, may lead one to speculate that the temporal patterning of the decay may also differ. Traditionally, for every 2-d without heat exposure, 1-d of HA is lost, (Givoni and Goldman, 1973) with the temporal patterning mirroring the induction (Armstrong and Maresh, 1991). Furthermore, the length of decay has also been suggested to depend upon physical fitness of participants and the magnitude of adaptation achieved (Pandolf, 1998). Future research is required to determine whether the type of HA determines the rate of decay since these previous observations were based on traditional HA methods. Furthermore, research is required to explore the decay rate of adaptations achieved following effective STHA protocols in females. In addition, methods to maintain the adaptations or reduce the speed of the decay would have a high level of practical importance for athletes.

Controlled hyperthermia HA used within this thesis, is an exercise intensive model whereby T_c is rapidly increased by an exercise stimulus and maintained by a combination of exercise and seated rest. There are practical disadvantages of using exercise HA protocols. These protocols can be costly and impractical for non-acclimated individuals living in cooler climates, as their completion requires access to an environmental chamber or temporary relocation to a hot climate. Furthermore, the exercise required to ensure an adequate stimulus for adaptation may interfere with athletes tapering prior to competition. Using an intervention involving passive exposures, for example sauna exposures or hot water immersion may overcome a number of the practical limitations with current exercise HA protocols. The use of a sauna exposure and hot water immersion following temperate training have been reported to expand PV, resulting in a reduced thermoregulatory and perceptual strain in males (Stanley et al., 2015; Zurawlew et al., 2015). Research is required to establish the effectiveness of these passive HA strategies in a female population. This body of research may support a future longitudinal study where athletes incorporate passive heat exposures during their typical temperate training. This will establish whether it is possible to maintain complete

acclimation throughout training, avoiding the requirement for intensive heat-alleviating procedures and to offer protection during unexpected heat stress during training.

9.4.   Practical application of findings

The RHTT presented within this thesis offers a practical method to assess thermoregulatory responses to a given heat stress, specifically in runners, but with application to other occupations such as the military. The practical application of the RHTT is twofold. Firstly, data presented within this thesis supports the use of the RHTT to assess thermoregulatory changes that occur due to acute and chronic heat alleviating interventions. Due to the low typical error and coefficient of variation association with the key thermoregulatory variables, researchers can have confidence that the effect observed is a true effect and not due to error within the measure. Secondly, the data presented within this thesis supports the use of the RHTT to observe individuals thermoregulatory responses over time, providing information to physiologists, coaches and medical staff to make decisions regarding athlete's safety prior to training and competing in the heat. The practical importance of the RHTT may stretch as far as supporting medical decisions about athletes' safety to return to training and competition following exertional heat illness and/ or associated symptoms (Johnson et al., 2013).

The practical importance of the data presented within the third experimental chapter is that the temporal patterning to HA is in part mediated by sex differences. STHA is a preferred regime for athletes since it is easier to adopt when sustaining quality training and tapering performance in the weeks prior to competition. However, data presented within this thesis provides compelling evidence that considerations need to be made when using controlled hyperthermia STHA strategies with females. STHA may be effective in achieving partial adaptation, however females require LTHA to establish reductions in cardiovascular and thermoregulatory strain. Based on this evidence, physiologists need to ensure that when supporting female athletes with heat-alleviating interventions, they adopt an adequate number of HA sessions to ensure athletes are fully acclimated, evidenced by a plateau (≤5% change) in responses. Ensuring that an adequate thermal stimulus is achieved will likely support a reduction in the possibility of obtaining a heat-related illness during training or competition in the heat.

The practical importance of the data presented in Study 5 (Chapter 8) is that controlled hyperthermia STHA used in conjunction with a passive heat exposure to sauna-like conditions provides a practical alternative to LTHA in females. This procedure resulted in reduced cardiovascular and thermoregulatory strain similar to that observed following LTHA in Study 3

(Chapter 6). The use of this combined thermal stimulus provides new knowledge regarding techniques which can accelerate STHA. The measurement of central and peripheral sudomotor adaptation in Study 5 (Chapter 8) provides useful practical insight into the sudomotor adaptions that occurs following controlled hyperthermia HA. The use of these techniques over STHA in females provides new knowledge that may permit better understanding of the possible mechanisms to explain the adaptations observed, supporting further optimisation of HA strategies. In addition, this data supports future investigations into the benefits of passive versus exercise heat exposures in females. Passive procedures would be preferable to athletes since there would be less interference with quality training and they could be incorporated more easily into a tapering period prior to competition.

This data has practical importance beyond young, healthy, female athletes. The findings from these studies may be applied in a manner to support a range of females from both an occupational population who are exposed to high ambient conditions combined with a high work load, in addition to the elderly, and those with comorbid medical conditions that may inhibit their thermoregulatory ability. With a total of 389 exertional heat illness cases reported in UK military personnel during an 88 month period (September 2007- December 2014), with 236 occurring in the UK, of these 125 in non-summer months, heat illness has become a major priority of the military (Stacey et al., 2015). Ensuring our military personal are adequately prepared for training in high ambient conditions is of upmost importance. With the current considerations to allow females into ground close combat roles, strategies will need to be implemented to ensure their safety from a heat stress perspective. The information provided within this thesis could support the implementation of beneficial strategies to reduce the likelihood of females experiencing exertional heat illness. Furthermore, the elderly compared with younger individuals, store more heat during short exposures to dry and humid heat (Stapleton et al., 2014), consequently the death rates during heat waves are considerably higher in the elderly. The data presented within this thesis could also be used in a manner to inform future investigations and heat-alleviating interventions for elderly females.

9.5. Conclusion

The RHTT introduced within this thesis, offers a simple practical tool to evaluate changes in thermoregulatory responses. Data presented within this thesis supports athletes using the RHTT to monitor changes in thermoregulation over time. To validate the use of the RHTT further, a longitudinal study is required to assess the effectiveness of the protocol in predicting the occurrence of exertional heat illness and supporting medical decisions regarding returning to

training following an exertional heat illness. Few scientific studies have examined methods to optimise females' adaptation during STHA, despite a number of female endurance athletes suffering from exertional heat illness and/ or experiencing decrements in performance due to additional heat strain. The volume of data presented within this thesis observing females' responses to HA, collectively provide an original contribution to the literature. The thesis provides novel information on the temporal patterning of HA; with females' only achieving partial adaptation following STHA using a controlled hyperthermia protocol. In addition, this thesis offers the first data set to observe reductions in cardiovascular and thermoregulatory strain in females, following a novel STHA protocol. The inclusion of a passive heat exposure to sauna-like conditions, prior to a typical HA protocol is an exciting new method which has been shown to accelerate adaptation responses in females. Further investigations are required to examine other possible methods by which to optimise acclimation protocols for female athletes and develop a greater understanding into the mechanisms and differences that may occur across the menstrual cycle. Whilst exploring these methods, it is imperative that the HA procedure remains practical and accessible to all individuals, whether athletes when tapering for competition in a hot climate, or military when preparing for deployment.

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Appendices

Informed consent

INFORMED CONSENT FORM

Declaration

I _____ hereby volunteer to take part in this research project which investigates

The principal investigator has explained to my satisfaction the purpose of the experiment and the possible risks involved. I have had the principles and the procedure explained to me and I have also read the participant information sheet. I understand the principles and procedures fully.

I am aware that I will be required to:

Have my aerobic capacity and body composition measured

Perform cycling exercise for up to 90-min in hot conditions on occasions

Have core temperature monitored via rectal thermometry on multiple occasions.

I understand how the data collected will be used, and that any confidential information will normally be seen only by the researchers and will not be revealed to anyone else.

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

I agree that should I withdraw from the study, the data collected up to that point may be used by the researcher for the purposes described in the information sheet.

I understand that the results of the study can be made known to me.

Furthermore, if I am a student, I am aware that taking part, or not taking part in this experiment, will neither be detrimental to, nor further my position as a student.

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

Signature of the Subject   **Date**

Signature of the Investigator   **Date**

Medical questionnaire

MEDICAL QUESTIONNAIRE

Name   Age

Are you in good health?   Yes   No

If No, please provide give details

How often do you currently participate in vigorous physical activity?

< Once per month

Once per month

2-3 times per week

4-5 times per week

5+ times per week

Have you suffered from a serious illness or accident?   Yes   No

If yes, please give particulars:

Do you suffer, or have you ever suffered from:

Respiratory Problems (e.g. Asthma, Bronchitis?)   Yes   No

Diabetes?   Yes   No

Epilepsy?   Yes   No

High/Low Blood Pressure   Yes   No

Cardiovascular problems   Yes   No

Are you currently taking medication or dietary supplements?   Yes   No

If yes, please give details:

In the last 3 months, have you consulted your GP for any condition?   Yes   No

If yes, please give particulars

Are you currently/ recently taking part in any other laboratory experiments?   Yes   No

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to participate in the study if they:

Are unsure of the test protocol and the possible risks and discomforts designated on the subject information sheet;

The answers given on the medical questionnaire or informed consent form do not meet the required criteria;

Have suspended training due to an injury

Have a known history of medical disorders including, high blood pressure and heart or lung disease

Have been verified, or documented as having any blood carried infections (Hepatitis, HIV),

Have had a previous incidence of hyperthermia, heat stroke or other heat-related illness

Have symptoms of nausea, light headedness or anaphylactic shock to needles, probes or other medical type equipment

Have chronic or acute symptoms of gastrointestinal bacterial infections

Have a history of infectious diseases

DECLARATION

I   hereby volunteer to be a participant in experiments and investigations conducted during the period commencing

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

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

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

Signature of the Subject   **Date**

**Signature of the Investigator**   **Date**

Menstrual cycle questionnaire

We require information regarding your menstrual cycle timing. Where possible testing will be scheduled in the follicular phase of the menstrual cycle (Day 3 – 10).

We may require a venous blood sample to quantify concentrations of 17-ß estradiol and progesterone. This will enable us to confirm whether the testing was conducted during the correct phase of the menstrual cycle.

It is essential that you take your time to complete this questionnaire to ensure accuracy of the information provided.

The diagram shows a typical 28 day cycle.
Some women's cycles may be longer or shorter
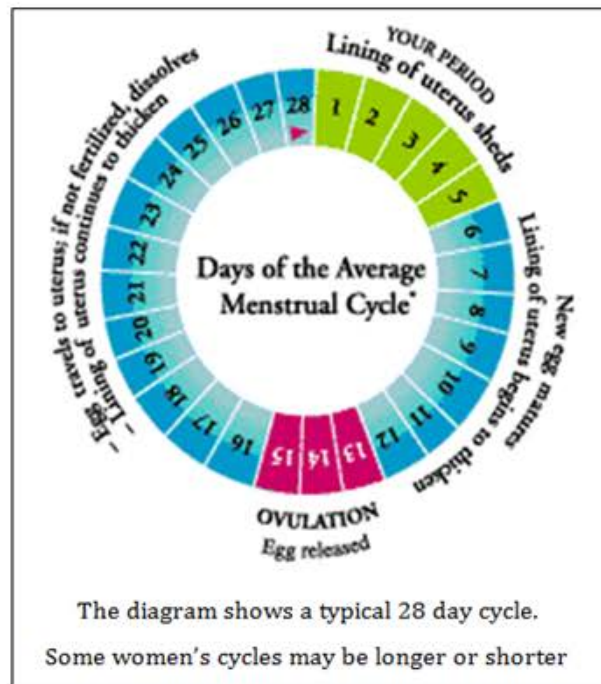
What is the average length of your cycle?

Are your menstrual cycles regular?

Please provide the start date of you previous two menstrual periods?
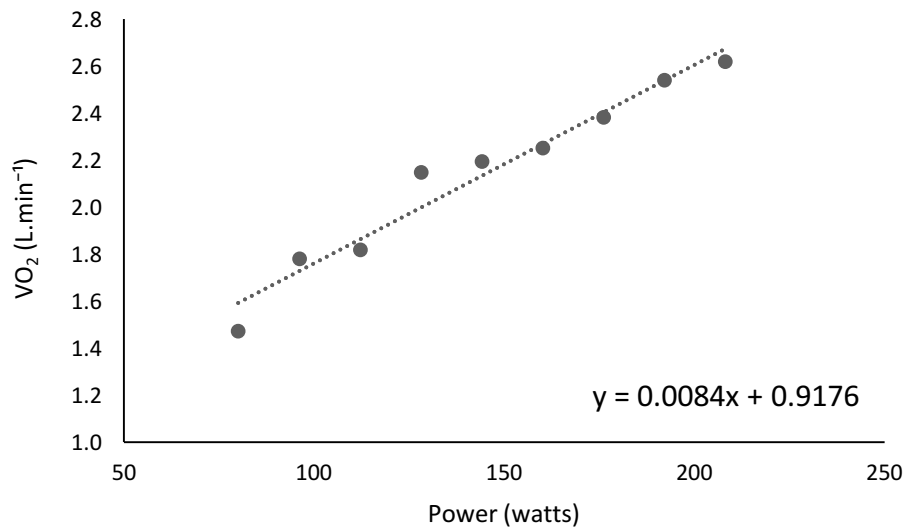
Were these two cycles typical of your average cycle?

Do you take oral contraceptive or use hormone replacement?

If so please provide details:

Please provide any additional notes which may be relevant:

Calculation of 65% $\dot{V}O_2$ peak

Peak $\dot{V}O_2$ = 2.618 L.min^{-1}

$$y = 0.0084x + 0.9176$$

$\dot{V}O_2$ during cycling incremental $\dot{V}O_2$ peak test

Calculation of 65% peak $\dot{V}O_2$

2.618 * 0.65 = 1.7017 L.min^{-1}

Equation of a straight line to calculation power at 65% $\dot{V}O_2$ peak

x = (1.7017 - 0.9176) / 0.0084

= 93.4 watts